Ozone Responses and Diet: Does Sex Determine the Relationship?

Ambient air pollution clearly associates with adverse health effects (1) and is a top 10 contributor to the global disease burden (2). Despite efforts to regulate these exposures, adverse health effects remain. Therefore, in parallel with mitigation efforts, we need to focus on understanding the factors that drive an individual's susceptibility to air pollution. Conceptually, these are defined as gene-by-environment interactions (3). In this framework, an individual's genetic composition influences his or her response to exposures in the environment. However, we are increasingly aware that nongenetic host factors, such as age, obesity, diabetes mellitus, and diet, influence air pollution responses. The interactions between these factors are just beginning to be unraveled. For example, recent literature has identified that ozone (O_3) pulmonary responses have sex-specific effects. Work in several laboratories has shown that male and female mice exhibit different O_3-induced airway hyperresponsiveness (AHR) (4) and that these effects are related to sex hormones (5, 6) or sex-dependent effects on the microbiome (7). Although modification of sex hormones represents an interesting experimental target, as a target strategy to reduce O_3 health effects it may have limited appeal. Alternatively, the microbiome can be modified by diet, and therefore dietary modifications might be a viable strategy.

In this issue of the *Journal*, Tashiro and colleagues (pp. 503–512) explore the effect of dietary modifications on pulmonary responses to acute O_3 exposure (8). Using mice fed diets enriched in different types of dietary fiber (pectin and cellulose) or a fiber-free diet, they made several interesting observations that were sex dependent. In male mice, a pectin-enriched diet elicited a mild increase in O_3-induced AHR at the highest methacholine dose, whereas a cellulose-enriched diet caused a pronounced reduction in AHR. In contrast, female mice exhibited augmented O_3-induced AHR responses to both the pectin- and cellulose-enriched diets. A fiber-free diet did not impact O_3-induced AHR in male mice. Contrary to what might be expected given their augmented responses to pectin and cellulose diets, female mice fed a fiber-free diet also exhibited enhanced AHR, similar to their responses to a fiber-enriched diet. These AHR effects appear to be largely dissociated from O_3-induced injury or inflammatory effects, suggesting that in this model, injury was not driving the AHR responses. To explore potential mechanisms of the diet- and sex-dependent AHR responses, the authors measured sex hormones and also gave the mice fed a fiber-free diet propionate to decrease short-chain fatty acids. Propionate administration did not alter AHR in females fed a fiber-free diet, and sex hormones did not associate with the sex-dependent diet phenotypes. Based on these findings, the authors evaluated sex-dependent, diet-induced changes in the microbiome to explain the observed phenotypes. Greater biodiversity and richness were found in the male mice fed a cellulose diet as compared with a pectin diet, and overall less effect was noted in the female mice fed either diet. However, the use of a fiber-free diet in female mice had a much greater effect on microbiome community structure. A statistical analysis to identify potential associations revealed that four taxa (*Enterococcaceae*, *Lactobacillius*, *Blautia*, and *Streptococcaceae*) associated with the observed sex differences in diet responses to O_3-induced AHR.

The data presented by Tashiro and colleagues build on a body of research, largely developed by this laboratory group, exploring the role of the microbiome in O_3-induced pulmonary responses (9). This includes identifying augmented O_3 responses in obese mice and sex-dependent O_3 responses driven by alterations in the microbiome (7, 10). Collectively, these data support the concept that the microbiome is a central regulator of acute O_3-induced pulmonary responses. Beyond this central observation, various conditions, such as obesity and sex, alter the microbiome, thereby driving these phenotypic responses. By looking at diet as a modifier of the microbiome, the authors begin to explore dietary modifications as a potential therapeutic strategy to mitigate O_3-induced health effects. Provocatively, their data suggest that dietary interventions need to be tailored to the sex of the individual. Based on the body of literature suggesting that sex differences affect a variety of biologic responses, this is perhaps not surprising, but it does suggest that consideration of diet modifications will need to incorporate sex as a response variable.

Questions remain unanswered by this study. The observations of sex-dependent, diet-induced microbiome effects and O_3-induced AHR are associative but not causal. Causality could be inferred from prior studies (7, 9) but requires confirmation. Specifically, their data suggest that specific microbiome taxa drive O_3-induced responses, but taxa-specific effects were not defined. These responses could be assessed in germ-free mice colonized with individual taxa to confirm the effects. In addition, the mechanisms that link alterations in gut microbial responses to O_3 responses and specifically AHR were largely unexplored. For example, it remains unclear whether changes in the gut microbiome are representative of the pulmonary microbiome in this model, and whether changes in the pulmonary microbiome drive the O_3 responses or are principally caused by the gut microbiome. Furthermore, the authors do not define whether the AHR effects are direct (by modifying smooth muscle contraction) or indirect (by affecting immune cell or epithelial cell functions). These questions need to be addressed to better delineate the role of the microbiome in air pollution responses.

An important caveat needs to be raised about the present study, which focuses on responses in C57BL/6 mice. O_3 studies typically use the C57BL/6 strain because of its documented O_3 sensitivity (11). However, the composition of the gut microbiome exhibits strain variations (12). It is therefore possible that different diet- and

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sex-specific effects would be observed in other murine strains. In addition, data suggest that microbiomes and immune responses in mice can vary according to housing conditions and vendors (13). Given the greater genetic and environmental diversity of human populations, the effects of individual genetic variables and environments on microbiome composition and dietary responses will need to be considered. It will be particularly important to consider these effects before initiating human studies focused on diet interventions to mitigate the adverse health effects of air pollution. On that basis, these results need to be interpreted with caution regarding the specific experimental conditions. Nevertheless, this new study continues to demonstrate the importance of the microbiome in air pollution responses and reveals greater complexity in the interactions between humans and their environment, and how these interactions drive the adverse health effects of air pollution. ■

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