Author's response to reviews

Title: Breath acidification in adolescent runners exposed to atmospheric pollution: a prospective, repeated measures observational study

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Author's response to reviews: see over
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Re MS: 1999411375156205

Dear Environmental Health Editorial Team,

On behalf of my co-authors, I thank you and the reviewers for the thoughtful and comprehensive review of our manuscript entitled, “Breath acidification in adolescent runners exposed to atmospheric pollution: A prospective, repeated measures observational study.” We have considered each comment in detail and have attached the following responses, in addition to a revised manuscript reflecting the modifications. The thorough review has clearly strengthened our manuscript, and we are pleased to resubmit it for consideration for publication in Environmental Health.

With regards,

/s/Jill Ferdinands

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Responses to Reviewer #1, Dr. Paul Boyce

Dr. Boyce raises a question regarding our study design; specifically, he asks why we did not include a control group of students. The original goal of our study was to test the hypothesis that vigorous outdoor activity in student athletes exposed to summer air pollution might induce lung inflammation and thereby reduce breath pH. To accomplish this, we used a study design in which the student athletes served as their own controls, a common design in studies of this sort. It was not until after the study sampling was completed and data analysis underway that we observed the interesting pattern of unexpectedly low pH among the student athletes. This surprising finding – while not the intended focus of the study *a priori* – led us to conduct a comparison of the observed breath pH among the student athletes to a group of healthy controls. Due to the *post hoc* nature of this control group, it consisted of CDC and Emory employees rather than a separate group of student athletes. Although our original study was never intended to compare student athletes to healthy controls, we recognize the importance of Dr. Boyce’s point that the comparison to controls is a key element of the results. In keeping with this, we have modified the manuscript to emphasize the control group comparison more strongly by introducing the control group comparison in the methods section and placing the comparison results figure in the main text rather than the supplement.

Dr. Boyce questions why we did not control for running style (i.e., sprinter versus distance runner) in our multivariate models, given that sprinters appeared to have lower EBC pH. We agree that controlling for running style is desirable and would have liked to include “sprinter” as a covariate in the models. Unfortunately, in this sample of student athletes, being a sprinter was strongly correlated with nonwhite race ($\rho = 0.72$, $p<0.001$), and this high degree of correlation forced us to include in the models either race or running style but not both. We chose to include race because we felt that excluding it would omit an important and standard demographic descriptor, and because including race would be more likely to facilitate use of our results in any future meta-analysis of breath pH among health subjects.

Regarding Dr. Boyce’s minor essential revisions, we have modified the manuscript per his suggestions.

Regarding Dr. Boyce’s discretionary revision, in which he questions if ambient air pollution exposure had an effect on pulmonary function, we have added a sentence on p. 11 of the revised manuscript indicating that we saw no effect of either PM$_{2.5}$ or ozone exposure on pulmonary function in this group of student athletes.
Responses to Reviewer #2, Professor John Hunt

Professor Hunt raises an important point regarding the use of personal monitoring of air pollution exposure, commenting that the negative primary result may have differed had personally monitoring been used. Professor Hunt is correct in his assertion that personal monitoring would have been cost-prohibitive. Although personal monitoring was outside the scope of our study, Professor Hunt’s point is well-taken and is reflected in the discussion of important study limitations (p. 17 of revised manuscript). However, the main substantive finding of our study – the unexpectedly low and highly variable breath pH observed among ostensibly healthy student athletes – would likely be unchanged had personal monitoring been used. To qualitatively change these results would require that personal monitoring detect an exposure causally related to the observed acidic breath pH values. We believe it unlikely that such an explanatory exposure – be it in the home or occurring on the weekends when personal monitors would have picked it up but our study could not – would be as prevalent as the low breath pH values we observed (75% student athletes had at least one low breath pH value). Furthermore, the rationale behind the investigation – examining the correlation between ambient air pollution and breath pH – rests partially on the premise that these variables are relatively easily and inexpensively measured (by existing air monitoring stations and the Rtube® exhaled breath collection system, respectively). In other words, we were interested in examining exhaled breath condensate as a biomarker of respiratory morbidity from air pollution exposure specifically using readily-available air quality data precisely because reliance on air quality monitoring data is the norm rather than the exception.

Regarding the comment on the Introduction section: Prof. Hunt states that glutaminase represents one but not the sole controller of airway pH. We agree with Prof. Hunt (indeed, cite his research) and have modified the wording of the sentence to better reflect his comment (p. 5 of revised manuscript).

Re comment on the Results section: We agree with Prof. Hunt and have moved the results of the comparison of breath pH among sedentary controls versus the student athletes to the main results section. Also, per Prof. Hunt’s suggestion, we introduce this comparison in the methods section rather than in the discussion and bring Supplemental Figure 1 into the main results section.

Re comment on the Discussion section: Prof. Hunt’s concern that ingestion of noncarbonated beverages could influence breath pH is a valid one. We, too, were concerned that sports drink consumption could be responsible for the observed acidic pH values. Three things make us doubt that this is the case: (1) the principal investigator was present during each breath sampling period and observed only a small number of students consuming sports beverages, far fewer than the 75% with acidic breath pH values; (2) more sports beverages would have been consumed after practice but we saw essentially similar numbers of acidic breath pH values both before and after practice; (3) in a recent re-analysis of breath pH samples, we identified citric acid – a common ingredient in sports drinks – in only 6 breath samples. This suggests that few of the athletes consumed sports drinks, in concordance with the principal investigator’s impression. Importantly, in
the breath samples containing citric acid, pH was normal. We have considerably expanded the discussion of this point to reflect Prof. Hunt’s comment (pp. 13-14 of revised manuscript).

Per Prof. Hunt’s suggestion, we expanded the normal nonexercising control comparison group to 14 people, as described in the revised methods section. The difference in breath pH between the student athletes and nonexercising controls is statistically significant regardless of whether using the original number of controls or the expanded number of controls.

Professor Hunt brings up an excellent point regarding the possibility that reflux may be responsible for intermittent breath pH acidification events and that vigorous exercise may promote reflux. If this were the case, it would be reasonable to expect more breath acidification events following vigorous exercise. On the contrary, we saw similar frequencies of acidification events both before and after practice. It may be possible, however, that reflux was stimulated by hurrying to practice, as Prof Hunt points out, and we cannot rule out this possibility. However, salivary contamination is unlikely to account for the results because the Rtube collection system includes a saliva trap that has been shown to minimize, if not eliminate, salivary contamination. To address this important issue, we have added a discussion of reflux as a possible explanation on p. 14 of the revised manuscript.

Regarding the figures, we have added the recommended indicators for running style. However, we did not indicate subjects with ETS exposure, asthma, or use of respiratory medication use because there were two or fewer subjects in each of these categories, and we felt that noted these subjects could potentially compromise their anonymity.

Regarding tables 3 and 4, per Prof Hunt’s recommendation, we have placed these tables in the supplement in the revised manuscript.

Regarding the supplemental table and description of sulfate and predicted pH values, this section has been expanded and clarified (page 14 of revised manuscript).

Regarding comment on the Conclusions section, we agree with Prof Hunt’s point and have added emphasis on the higher variance in EBC pH seen in the runners compared to sedentary controls in several locations in the revised manuscript.