

Breath acidification in adolescent runners exposed to atmospheric pollution:

A prospective, repeated measures observational study

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ABSTRACT

Background: Vigorous outdoors exercise during an episode of air pollution might cause airway inflammation. The purpose of this study was to examine the effects of vigorous outdoor exercise during the peak smog season on breath pH, a biomarker of airway inflammation, in adolescent athletes.

Methods: We measured breath pH both pre- and post-exercise on ten days during peak smog season in 16 high school athletes engaged in daily long-distance running in a downwind suburb of Atlanta. The association of post-exercise breath pH with ambient ozone and particulate matter concentrations was tested with linear regression.

Results: We collected 144 pre-exercise and 146 post-exercise breath samples from 16 runners (mean age 14.9 years, 56% male). Median pre-exercise breath pH was 7.58 (interquartile range: 6.90 to 7.86) and did not change significantly after exercise. We observed no significant association between ambient ozone or particulate matter and post-exercise breath pH. However both the pre- and post-exercise breath pH was strikingly low in these athletes when compared to a control sample of sedentary healthy adults and to published values of breath pH in healthy subjects.

Conclusions: Although we did not observe an acute effect of air pollution exposure during exercise on breath pH, breath pH was surprisingly low in this sample of otherwise healthy long-distance runners. We speculate that repetitive vigorous exercise might induce airway acidification.

BACKGROUND

Ground-level ozone and particulate matter (PM) are the primary ambient air pollutants of smog. Exposure to ozone causes airway irritation, wheezing, coughing, pain upon inspiration, and breathing difficulties, and repeated exposure may impair lung growth and cause permanent lung damage (1). Exposure to PM has been repeatedly associated with increased mortality, though how PM causes death is not well understood (2). For both ozone and PM, health risks rise as exposure rises.

Student athletes often exercise vigorously in smoggy outdoor environments, and athletic practices frequently occur in the mid- to late-afternoon when diurnal ozone levels are highest. Vigorous exercise increases minute ventilation and inspiratory flow rate, intensifying exposure of the distal lung to airborne pollutants. Although several studies have documented short- and long-term effects of ozone related to exposure during exercise (3-15), few have examined children or young adults, and little is known about effects of repeated exposures to ambient ozone or PM among student athletes.

Breath condensate contains a number of constituents derived from the respiratory surfaces that hold promise as indices of inflammation. The pH of exhaled breath condensate (EBC) has been proposed as a biomarker of inflammation reflecting the acid-base balance of the airways, which is regulated primarily by a glutaminase-based buffering system of the airway epithelium (16). Several studies suggest that pH of exhaled breath condensate is low in various inflammatory lung diseases including poorly controlled asthma, COPD, cystic fibrosis, and acute lung injury (17-27). Studies

reporting negative correlations between EBC pH and pro-inflammatory cytokines in the airways (17,18) further suggest that EBC pH has promise as a biomarker of the early airway inflammation preceding frank symptoms or lung function impairment. Because it is relatively easy to collect and measure, breath pH has potential as an effective way to assess inflammatory effects of air pollution in high risk populations. Two studies have explored EBC constituents as biomarkers of respiratory morbidity from ozone exposure, including one that examined breath pH, finding no significant change in breath pH after ozone exposure (5,19).

The aim of the present 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 collected EBC pre- and post-exercise on ten days during peak smog season in 16 high school athletes engaged in daily long-distance running in a down-wind suburb of Atlanta.

METHODS

Study design. This was a prospective observational study with a repeated measures design in which atmospheric concentrations of ozone and PM_{2.5} were the independent variables and pH of exhaled breath condensate (referred to as breath pH) was the primary outcome variable.

Subjects. Sixteen members of a high school cross country team participated in the study. Participants had no history of respiratory infection in the four weeks prior to the study.

Informed consent from parents of the participants and informed assent from the participants were obtained according to a protocol approved by the Institutional Review Boards of the Centers for Disease Control and Prevention and Emory University.

Study Procedures. Runners completed a health questionnaire at baseline and trained outdoors between 4 and 5 pm for ten days during a 15-day study period. Before and after training, study coordinators asked runners about respiratory symptoms, performed spirometry (EasyOne spirometer, ndd, Andover, MA), measured on-line exhaled nitric oxide at a flow rate of 50 mL/second (DENOX 88, EcoMedics, MI), and collected 5-minute breath condensate samples (Rtube®, Respiratory Research, Charlottesville, VA) from each participant. Breath condensate samples were collected during tidal breathing without use of noseclips. Spirometric results were expressed as the percent-predicted forced expiratory volume in 1 second (FEV_1) according to population reference standards from NHANES III (20) and submitted to a clinician-investigator (WGT) daily for interpretation and safety monitoring.

Breath condensate analysis. Breath condensate samples were placed on dry ice immediately post-collection, transported to the laboratory daily, and stored at -70°C until analysis. To measure breath pH, 100 μL of EBC was thawed to room temperature and, to remove carbonate, de-gassed with argon for 10 minutes until pH (Orion Instruments, Baton Rouge, LA) was constant. De-aerated breath pH values in healthy subjects are believed to range from 7.4 to 8.8 (21). However, because there remains uncertainty about

the range of normal breath pH, we conservatively defined as low any breath pH value less than 7.0.

Exposure assessment. The study took place from August 16 to 31, 2004, during the peak “smog season” known *a priori* as a time of expected poor air quality for this region. Maximum 1- and 8-hour ozone concentrations and hourly concentrations of particulate matter ≤ 2.5 microns in diameter (PM_{2.5}) were obtained from the Georgia Ambient Air Monitoring System. The nearest monitoring station was <1 mile from the study site for ozone and approximately 12 miles for PM_{2.5}.

Variable definition. We defined “number of symptoms in past 24 hours” as the number of positive responses to questions asking if the subject experienced wheeze, cough, watery eyes, runny nose, itchy or scratchy throat, or sneezing within 24 hours prior to the start of practice. Each day, athletes rated their perceived exertion during running on a scale from 1 (least vigorous) to 10 (most vigorous).

Statistical analysis. Nonparametric rank-sum tests were used to examine differences in breath pH between groups. Mean breath pH values were adjusted for within-subject correlation, age, sex, race, and body mass index (BMI). For ease of interpretation, results are presented as raw breath pH values; however, statistical tests were performed using a nonparametric transform of the breath pH variable (details in Supplement). A general linear mixed model was used to examine the association between ambient air pollutants and post-exercise breath pH, controlling for pre-exercise breath pH. Same-day 1-hr

maximum ozone (ppb) and PM_{2.5} at 5pm ($\mu\text{g}/\text{m}^3$) were log-transformed for statistical analysis. Ozone and PM_{2.5} concentrations were highly correlated, with raw and transformed Pearson correlation coefficients of 0.9 and 0.8, respectively. To avoid the difficulty of fitting a regression model with highly correlated independent variables, we ran separate models for ozone and PM_{2.5}. We compared breath condensate variables in runners collected at rest on day one of the protocol to that in healthy adult controls with Student's t test for un-paired samples. Analyses were conducted in SAS version 9.1 (SAS Corporation, Cary NC) and SPSS for Windows (Version 14).

RESULTS

Mean age of participants (n=16) was 14.9 years, 56% were male, and 69% were white (Table 1). Thirteen of 16 (81%) participants were distance runners and three were sprinters. Two of 16 (13%) reported having asthma, one of whom participated in only the first three days of the study. None were smokers. From 16 participants, we collected 144 pre-exercise and 146 post-exercise breath samples.

Mean (\pm SD) ambient 1-hour maximum ozone concentration was 71 (18) ppb. Median (interquartile range, IQR) was 61 (54-67) ppb. Mean (\pm SD) PM_{2.5} measured at 5pm was 27.2 (11.9) $\mu\text{g}/\text{m}^3$. Median (IQR) was 23.2 (21.7 – 34.7) $\mu\text{g}/\text{m}^3$. Four of 10 study days were air quality alert days, three of these triggered by high ozone levels and one by high PM levels. On three study days ozone concentrations exceeded health-based standards.

Pre-exercise breath pH. Median pre-exercise breath pH was 7.6; range was 4.4 – 8.1; IQR was 6.9 – 7.9. Crude pre-exercise pH was lower among participants with home

tobacco smoke exposure, those 16 to 17 years old, and sprinters (Table 2). Pre-exercise breath pH was bimodally distributed with a prominent peak around 7.5 and a smaller, unexpected peak around 5.0 (Figure 1). Individual median pre-exercise breath pH ranged from 4.9 to 7.9, with four subjects having median pre-exercise breath pH less than 7.0. Pre-exercise breath pH values varied considerably within subject over the ten study days (Figure 2), with day to day within-subject coefficient of variation ranging from 2 to 25%. Overall within-subjection coefficient of variation was 16%. Of 16 participants, 12 (75%) had at least one pre-exercise breath pH ≤ 6.0 , and 10 (63%) had at least one pre-exercise breath pH ≤ 5.0 .

Lower-than-expected values of pre-exercise breath pH (<7.0) were observed on all study days. At least one subject had pre-exercise breath pH ≤ 5.5 each day, although the particular subject varied day to day. On eight of ten study days, at least one participant had pre-exercise breath pH ≤ 5.0 . Breath pH was not associated with date of assay or elapsed time between sample collection and assay.

Post-exercise breath pH. Median post-exercise breath pH was 7.7; range was 3.8 – 8.2; IQR was 7.1 – 7.9. Breath pH did not change significantly after exercise ($p=0.31$ by paired t -test or t test of difference).

We observed no statistically significant association between post-exercise breath pH and same-day 1-hr maximum ozone concentration, controlling for race, home tobacco smoke exposure, perceived exertion during running, and pre-exercise breath pH (Table 3). Similarly, we observed no statistically significant association between post-exercise breath pH and same-day PM_{2.5} at 5pm, controlling for the same factors (Table 4). No

significant associations were observed when ozone and PM_{2.5} concentrations were lagged by 1 or 2 days. Results were not qualitatively different in models that used transformed breath pH or transformed hydrogen ion concentration variables instead of raw breath pH variables. Adjusted post-exercise breath pH was lower among nonwhite subjects compared to white subjects ($p < 0.01$ in both ozone and particulate models) and among subjects reporting no home tobacco smoke exposures ($p < 0.001$ in ozone model, $p < 0.01$ in PM_{2.5} model). As expected, post-exercise breath pH was positively associated with pre-exercise breath pH ($p < 0.05$ in all models). Resting lung function (measured as percent-predicted FEV₁), and exhaled nitric oxide (ppb) were not significantly correlated with resting breath pH (detailed analysis of exhaled nitric oxide will be reported separately).

DISCUSSION

In this study, we sought to examine the association of exhaled breath pH with ambient ozone and particulate matter concentrations among 16 adolescent athletes exposed to these air pollutants during vigorous outdoor exercise. Although there was considerable variation in air quality over the 10 study days, we did not observe a relationship between air pollution levels and post-exercise breath pH. Overall, post-exercise breath pH was indistinguishable from pre-exercise breath pH, suggesting no acute effect of air pollution exposure during vigorous outdoor exercise on breath pH compared to resting values.

This finding is similar to that of Corradi et al (5), who found no change in EBC pH after short-term exposure to ozone during light exercise and is the only study apart from ours that examined the association between air pollution exposure during exercise and EBC pH.

Although we did not observe an acute effect of outdoor exercise, we found that both resting and post-exercise breath pH values were intermittently lower than expected in a majority of subjects. Although airway pH in healthy individuals is believed to be slightly alkaline with pH of 7-8 (21,22), we observed in a majority of subjects intermittent breath pH values comparable to levels seen in severe inflammatory conditions such as acute asthma and sickle cell anemia. Day-to-day variability in breath pH was common and similar in magnitude to that seen in children with persistent asthma. We observed much greater within-subject variation in breath pH than previously reported (17;22-25). The intermittently low breath pH values we observed were not correlated with subject, study day, or assay date, and resampling of banked samples suggested that they could not be attributed to random laboratory error.

Potential explanations for the low breath pH values that we observed include the possibility that salivary contamination or other artifact of sample collection (such as recent eating or drinking) artificially lowered breath pH. In addition, because EBC samples were collected outdoors, it is possible that inhaled atmospheric sulfates reduced EBC pH. From a mechanistic standpoint, it may be possible that routine vigorous exercise and its concurrent effect on systemic lactate production influenced airway pH. Alternatively, it is possible that these otherwise healthy athletes experienced significant intermittent airway acidification events that were accurately reflected by the observed low breath pH values. We address each of these possibilities in turn.

Our use of a one-way valve system with saliva trap during EBC collection makes it unlikely that salivary contamination was responsible for the low pH values (21), and a previous study of the potential for salivary contamination using the same collection system showed no such contamination (22). Although we cannot rule out that the athletes had eaten in the hour prior to collection of the resting breath samples, it is unlikely because samples were collected immediately after the students were dismissed from final classes of the day. Furthermore, the subjects did not eat during running, so we can be confident that the low post-exercise breath pH values are not attributable to having eaten. Exposure to ingested liquids was restricted to water and non-carbonated sports beverages and is unlikely to have reduced breath pH values.

Because EBC samples were collected outdoors, we explored the possibility that inhaled atmospheric sulfates were captured in the EBC, thereby lowering its pH. To do this, we estimated the amount of sulfate expected in the breath condensate samples based on the concentration of sulfate and other relevant atmospheric ions in the ambient air and the rate at which subjects inhaled during collection of the breath samples (details provided in the supplement). We compared predicted sulfate levels in the breath condensate with actual breath sulfate levels from the EBC samples measured by ion chromatography. Based on the predicted breath sulfate concentrations, we also estimated the predicted breath pH and compared it to measured breath pH. These comparisons were conducted on a subsample of five runners on two study days, one chosen to represent a day with high ambient sulfate levels and the other a day with low ambient sulfate levels. The results of this modeling (Supplemental Table 1) suggest that sulfate anions inhaled from

the ambient air and then immediately exhaled and collected in the breath condensate are likely to lower breath pH values to a small degree, but the effect of ambient sulfates on the breath pH is minimal and unlikely to explain entirely the surprisingly low breath pH values we observed in the student athletes.

To examine the possibility that the observed low breath pH values might reflect underlying acidification events in the airways of ostensibly healthy subjects, we undertook a *post-hoc* comparison of resting breath pH values among the student athletes to those observed among a convenience sample of seven healthy, sedentary adults recruited from the faculty and staff of the CDC and Emory University. These adult subjects provided resting breath condensate samples both indoors and outdoors at 4:00 pm on a day with relatively low concentrations of air pollutants. No unusually low breath pH values were observed and median breath pH values were significantly higher among these adults than among the student athletes (Supplemental Figure 1). Furthermore, in the *post-hoc* adult samples, we observed no clear pattern of differences between indoor and outdoor breath pH levels, suggesting that ambient sulfate had little influence on the samples collected outdoors.

Why we observed low breath pH values in this group of otherwise healthy athletes is unclear. One possibility is that the routine vigorous exercise reported by these athletes caused systemic lactic acid production that altered resting airway pH. The mechanism by which the respiratory and systemic acid-base buffering systems may interact to cause this

effect is poorly understood and requires more study. Another potential explanation is that long-term exposure to outdoor air pollution – such as may be experienced by children who routinely exercise outside during polluted summers – may trigger intermittent endogenous airway acidification events indicative of pollution-related lung inflammation. This idea is broadly consistent with results of several studies that suggest that long-term exposure to urban air pollution may cause lung damage or lung growth impairment in children (4;26-28) and could partially explain why we did not see similar values among a comparison group of healthy but sedentary adults. Another possibility is that low pH values representing endogenous intermittent airway acidification events are normal rather than pathologic phenomena. Supporting this speculation are several recent studies that measured breath pH among healthy controls (Table 5) and found surprisingly low breath pH in a subset of subjects (25;29), including one large population-based study that found breath pH values as low as 4.4 in healthy children (30). The possibility that intermittent low breath pH values reflect normal biologic processes calls into question the utility of breath pH measurement – especially one-time breath pH measurement – as a reliable biomarker of lung inflammation or respiratory health effects.

This analysis has several limitations. The small number of subjects (n=16) may have limited statistical power to detect relationships between ambient air pollutants and post-exercise breath pH. We had no data on long-term exercise patterns or residential histories of the subjects and, thus, were unable to examine if ambient air pollution or low breath pH values were associated with cumulative exposure to polluted environments. That we did not see similar low breath pH values in a group of healthy, sedentary adults

with breath samples taken outdoors suggests that the low breath pH values observed among the student athletes may be attributable to characteristics of their lungs that we were unable to measure in this study. Because characteristics of our study subjects may have varied from those of student athletes in general, we caution against generalizing our results.

CONCLUSION

In summary, we found no acute effect on breath pH from vigorous outdoor exercise on days with significant ozone and PM pollution in this group of 16 student athletes. We did, however, observe highly variable and surprisingly low breath pH values in a majority of these student athletes. These intermittent, low breath pH values are unexplained and may represent endogenous airway acidification. Additional studies with greater statistical power are necessary to rigorously examine the potential effect of outdoor exercise in polluted environments on the lungs of young athletes. However, before additional studies propose to use the pH of exhaled breath condensate as a biomarker of airway inflammation or respiratory morbidity, it will be necessary to determine if the low breath pH values we and others have observed among healthy individuals are indicative of respiratory pathology or normal biologic phenomena.

ABBREVIATIONS

AIC	Akaike's Information Criterion
BMI	body mass index
CDC	Centers for Disease Control and Prevention
EBC	exhaled breath condensate
ETS	environmental tobacco smoke

FEV ₁	forced expiratory volume in 1 second
IQR	interquartile range
NHANES	National Health and Nutrition Examination Survey
NR	not reported
PM	particulate matter
PM _{2.5}	particulate matter ≤ 2.5 microns in diameter
ppb	parts per billion
SD	standard deviation
SE	standard error
SEM	standard error of the mean

COMPETING INTERESTS

The authors declare no competing financial interests.

AUTHORS' CONTRIBUTIONS

JMF and WGT conceived of the study, managed and performed field data collection, conducted statistical analysis, and wrote and reviewed the manuscript. CGC provided extensive statistical consultation, assisted with interpretation of analytic results, and reviewed the manuscript. RG conceived and performed breath sulfate analyses, wrote sections of the manuscript, and reviewed the manuscript. ET conducted extensive laboratory analyses, assisted with interpretation of results, and reviewed the manuscript. DV coordinated field data collection, assisted with statistical analysis, and reviewed the manuscript.

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FIGURE LEGENDS

Figure 1. Distribution of pre- and post-exercise breath pH values in runners ($p=0.63$ for nonparametric test of difference in pre- and post-exercise breath pH distributions).

Figure 2. Pre-exercise breath pH by subject ($n=16$ subjects, 144 samples)

Table 1. Selected characteristics of study participants (n=16 subjects)

Variable	Value
Age, yr	
Mean (SD)	14.9 (0.9)
Range	14 -17
Body mass index, kg/m ²	
Mean (SD)	19.8 (1.7)
Range	17.5 – 23.5
Sex, N(%)	
Male	9 (56)
Female	7 (44)
Race, N(%)	
White	11 (69)
Nonwhite	5 (31)
Self-reported asthma diagnosis, N(%)	
Yes	2 (13)
No	14 (88)
Self-reported allergy diagnosis, N(%)	
Yes	4 (25)
No	12 (75)
Wheeze or cough in past month, N(%)	
Yes	4 (25)
No	12 (75)
Home ETS exposure, N(%)	
Yes	2 (12)
No	14 (88)
Running style, N(%)	
Sprinter	3 (19)
Distance	13 (81)

Table 2. Median and interquartile range of pre-exercise breath pH values for selected sample characteristics (n=144 observations from 16 subjects)

Variable	Obs (n)	Median (IQR) (<i>p</i> value)†
Age, yr		
14	59	7.56 (7.05-7.87)
15	59	7.70 (7.34-7.86)
16-17	26	7.22 (5.12-7.58) (0.01)
BMI, kg/m ²		
<20	90	7.68 (7.06-7.87)
≥20	54	7.41 (6.05-7.77) (0.17)
Sex		
Male	86	7.58 (7.05-7.85)
Female	58	7.56 (6.37-7.86) (0.71)
Race		
White	103	7.60 (7.10-7.87)
Nonwhite	41	7.44 (5.49-7.78) (0.13)
Asthma		
Yes	13	7.41 (5.00-7.51)
No	131	7.60 (7.05-7.86) (0.10)
Allergy		
Yes	40	7.51 (7.27-7.82)
No	104	7.60 (6.01-7.86) (0.20)
Wheeze or cough in past month		
Yes	33	7.51 (7.25-7.78)
No	111	7.59 (6.37-7.86) (0.68)
Number of symptoms in past 24 hours*		
0	116	7.55 (6.83-7.86)
1	22	7.68 (5.96-7.87)
2 or more	6	7.57 (7.27-7.66) (0.58)
Home ETS exposure		
Yes	20	7.06 (4.99-7.74)
No	124	7.60 (7.10-7.86) (0.009)
Running style		
Sprinter	21	6.05 (5.12-7.59)
Distance	123	7.64 (7.10-7.86) (0.0004)

* Symptoms included wheeze, cough, scratchy or itchy throat, runny nose, sneezing, and watery eyes (details in supplement)

† Nonparametric Wilcoxon rank-sum tests used to examine differences in raw breath pH between groups.

Table 3. Estimated regression coefficients from mixed linear models[†] with post-exercise breath pH as outcome and 1-hour ozone concentration as ambient air pollutant predictor (n=143 observations from 16 subjects)

	Model 1	Model 2	Model 3	Model 4
Outcome variable	Raw post-exercise breath pH	Normal score transform of post-exercise breath pH	Raw post-exercise breath pH	Raw post-exercise breath pH
Lag time	Same day	Same day	1 day lag	2 day lag
AIC criterion	409.1	371.5	411.4	412.7

Estimated regression coefficient, β (standard error):

Race				
Nonwhite	-1.06	-0.64 (0.17)**	-1.05	-1.05
White	(0.20)***	Ref	(0.21)***	(0.21)***
	Ref		Ref	Ref
Home ETS exposure				
Yes	1.15	0.99 (0.23)***	1.15 (0.27)**	1.15
No	(0.27)***	Ref	Ref	(0.27)***
	Ref			Ref
Exertion (1 to 10 scale)	0.04 (0.05)	0.08 (0.04)*	0.06 (0.05)	0.06 (0.05)
Raw pre-exercise breath pH	0.18 (0.08)*	----	0.19 (0.08)*	0.19 (0.08)*
Normal score transform of pre-exercise breath pH	----	0.18 (0.08)*	----	----
Same-day log max 1-hr ozone (log ppb)	0.67 (0.37)	0.50 (0.32)	----	----
1-day lagged log max 1-hr ozone (log ppb)	----	----	0.34 (0.28)	----
2-day lagged log max 1-hr ozone (log ppb)	----	----	----	0.04 (0.31)

[†] All models use a first-order autoregressive correlation structure

* p<0.05

** p<0.01

*** p<0.001

Table 4. Estimated regression coefficients from mixed linear models[†] with post-exercise breath pH as outcome and PM_{2.5} concentration as ambient air pollutant predictor (n=143 from 16 subjects)

	Model 1	Model 2	Model 3	Model 4
Outcome variable	Raw post-exercise breath pH	Normal score transform of post-exercise breath pH	Raw post-exercise breath pH	Raw post-exercise breath pH
Lag time	Same day	Same day	1 day lag	2 day lag
AIC criterion	413.7	375.0	412.5	413.2

Estimated regression coefficient, β (standard error):

Race				
Nonwhite	-1.05	-0.63 (0.18)**	-1.05	-1.05
White	(0.21)***	Ref	(0.21)***	(0.21)***
	Ref		Ref	Ref
Home ETS exposure				
Yes	1.15	0.97(0.24)**	1.15 (0.28)**	1.15
No	(0.27)***	Ref	Ref	(0.27)***
	Ref		Ref	Ref
Exertion (1 to 10 scale)	0.06 (0.05)	0.10 (0.04)*	0.07 (0.05)	0.06 (0.05)
Raw pre-exercise breath pH	0.19 (0.08)*	----	0.19 (0.08)*	0.19 (0.08)*
Normal score transform of pre-exercise breath pH	----	0.18 (0.08)*	----	----
Same-day log PM _{2.5} at 5pm (log $\mu\text{g}/\text{m}^3$)	0.05 (0.18)	-0.10 (0.16)	----	----
1-day lagged log PM _{2.5} at 5pm (log $\mu\text{g}/\text{m}^3$)	----	----	0.21 (0.18)	----
2-day lagged log PM _{2.5} at 5pm (log $\mu\text{g}/\text{m}^3$)	----	----	----	0.14 (0.18)

[†] All models use a first-order autoregressive correlation structure

* p<0.05

** p<0.01

*** p<0.001

Table 5. Studies reporting breath pH among healthy subjects

Reference	Subjects	Central tendency	Range	Coefficient of variation	Comments
<u>Children:</u>					
Carpagnano et al 2004(23)	15 healthy children with mean age 7 yr	Mean (SE): 7.85 (0.02)	NR; estimated 7.6 – 8.2 from Figure 1A	CV=0.04% from 6 adult controls	Samples de-aerated
Carraro et al 2005(31)	10 healthy children with mean age 10 yr	Median (IQR): 7.85 (7.80 – 7.90)	NR; estimated 7.7 – 8.0 from Fig 2	NR	Samples de-aerated
MacGregor et al 2005(32)	47 healthy control children of mean age 16 yr	Median: 5.90	5.00-7.30	NR	Samples not de-aerated
Nicolaou et al 2006(30)	562 8-year old children from unselected population-based birth cohort	Median (IQR): 7.77 (7.59-7.87)	Range for normal subjects not reported. For all subjects (including 54 asthmatics and 562 normals): 4.40-8.29	NR	Bimodal distribution that “could not be normalized”; samples de-aerated
Rosias et al 2004(33)	9 control children with mean age 9 yr	Median (SEM): 8.11 (0.07) de-aerated 6.64 (0.05) non-de-aerated	NR	NR	pH reported both before and after de-aeration
<u>Adults:</u>					
Borrill et al 2005(34)	12 healthy adults with mean age 26 yr	Mean (95%CI): 7.61 (7.52 – 7.70)	NR	NR ^a	Samples de-aerated
Carpagnano et al 2005a(24)	15 healthy adults with mean age 35 yr	Mean (SD): 7.85 (0.14)	NR; estimated 7.6-8.2 from Fig IC	CV=0.4% in 10 healthy adults	Samples de-aerated
Carpagnano et al 2005b(17)	7 healthy adults with mean age 42 yr	Mean (SEM): 7.9 (0.1)	NR; Estimated 7.8-8.2 from Fig 3C	CV=0.4% in 10 healthy adults ^b	Samples de-aerated
Corradi et al 2002(5)	22 healthy adults with mean age 30 yr, grouped by NQO1 and GSTM1 genotype	Mean ^c : Group 1 (NQO1 wild type and GSTM1 null): 7.91 Group 2 (all other genotypes):	Group 1 (NQO1 wild type and GSTM1 null): 7.70 – 8.08 Group 2 (all other genotypes): 7.80-8.11	NR	Samples apparently de-aerated following procedures in Hunt 2001

Gessner et al 2003(35)	12 healthy adults with mean age 57	Mean (SD): 7.46 (0.48)	NR	NR	Samples de-aerated
Hunt et al 2000(25)	19 healthy subjects with mean age 20.5	Mean (SE) 7.65 (0.20)	NR; estimated 4.6 – 8.5 from Fig 1	CV=3.3% from 6 normals and 3 asthmatics; CV in normals not reported	Samples de-aerated
Kostikas et al 2002(18)	10 healthy adult controls with mean age 34 yr	Mean (95%CI): 7.57 (7.51-7.60)	NR; estimated 7.4 – 7.75 from Fig 1A	NR	Samples de-aerated
Niimi et al 2004(36)	16 healthy adults with mean age 43 yr	Mean (SD): 8.26 (0.20)	NR; estimated 7.8 – 8.6 from Fig 1	NR	Samples de-aerated
Ojoo et al 2005(37)	15 healthy adults with mean age 39 yr	Median (IQR): 6.08 (5.58-6.64)	NR; Estimated 5.6 – 6.7 from Fig 2	NR	Samples not de-aerated
Paget-Brown et al 2006(29)	404 healthy children and adults from 0 to >71 yr of age	Mean: 7.85 Median (IQR): 8.0 (7.8 – 8.1); In 11 to 20 yr olds (n=163): median (IQR): 8.0(7.8-8.1) mean (SD): 7.8 (0.7)	4.5-8.4	NR	Samples de-aerated
Tate et al 2002(38)	12 healthy adults with mean age 33 yr	Mean (SD): 6.15 (0.16)	NR; estimated 5.8 – 6.5 from Fig. 1	NR ^d	Samples not de-aerated
Vaughan et al 2003(22)	76 healthy adults with mean age	Mean (SD): 7.70 (0.49)	NR	Mean CV=4.5%;	Samples de-aerated

21 yr

by subject,
CV= 0.9 –
20%

NR= not reported

^a reports “limits of agreement” using Bland-Altman methods to assess reproducibility

^b appears to be reporting the same CV estimate as in Carpagnano 2005a

^c breath pH measurement at baseline (before exposure to ozone)

^d reports “coefficient of acceptability” as 0.08 pH units

DESCRIPTION OF ADDITIONAL FILES

A supplemental file includes detailed descriptions of statistical methods, regression model selection process, and breath sulfate analysis and results.

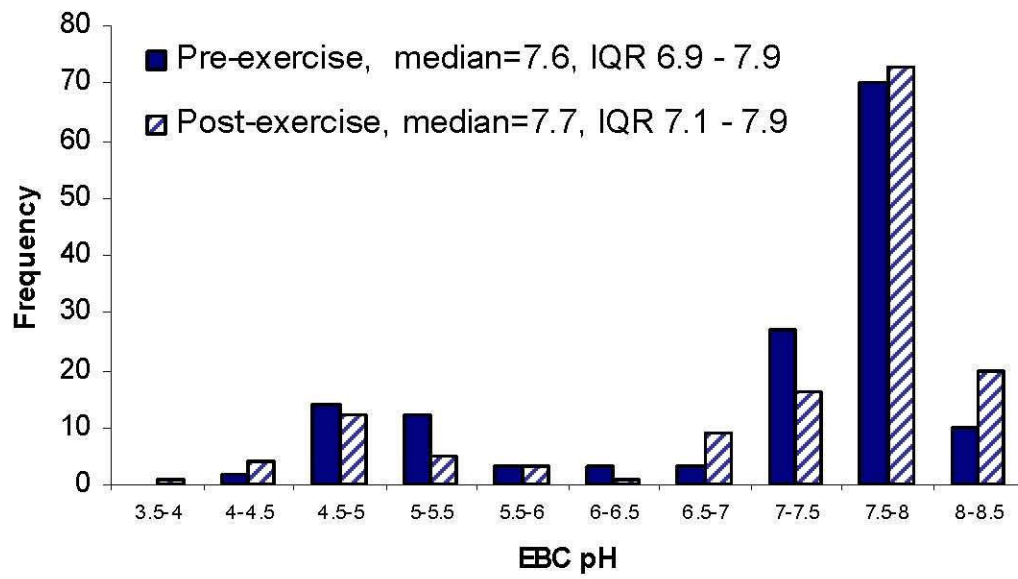


Figure 1

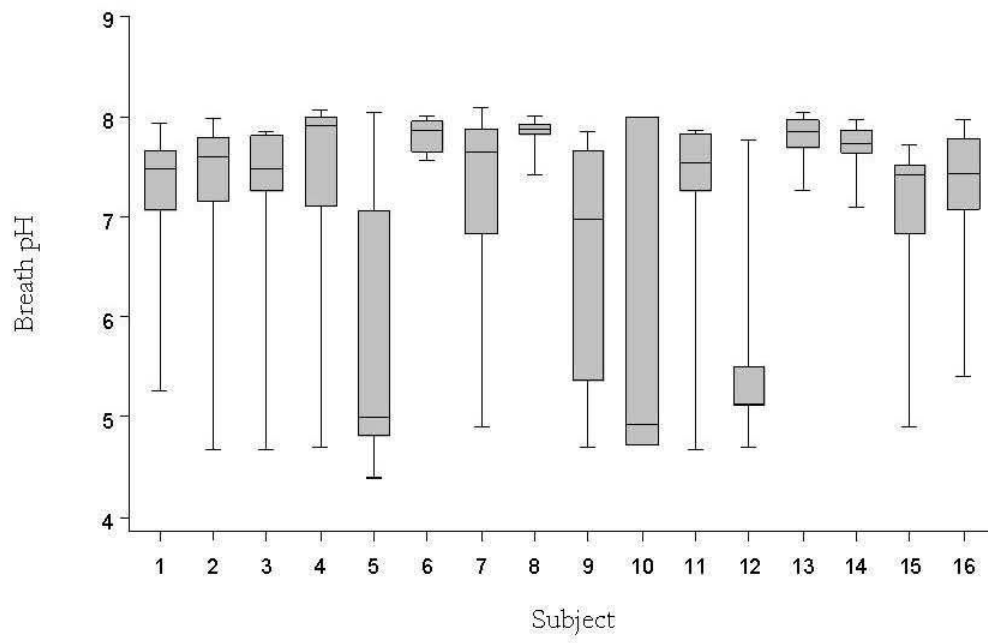


Figure 2

Additional files provided with this submission:

Additional file 1: ferdinands breath acidification aug 17 suppl with figs.doc, 58K
<http://www.ehjournal.net/imedia/8603480381562056/supp1.doc>