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Changes in the concentration of breath ammonia in response to exercise: a preliminary investigation

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Abstract

Breath ammonia has proven to be a difficult compound to measure accurately. The goal of this study was to evaluate the effects that the physiological intervention, exercise, had on the levels of breath ammonia. The effects of vigorous exercise (4000 m indoor row) in 13 participants were studied and increases in breath ammonia were observed in all participants. Mean pre-exercise concentrations of ammonia were 670 pmol ml^{-1} CO_2 (SD, 446) and these concentrations increased to post-exercise maxima of 1499 pmol ml^{-1} CO_2 (SD, 730), $p < 0.0001$. The mean increase in ammonia concentrations from pre-exercise to maximum achieved in conditioned (1362 pmol ml^{-1} CO_2) versus non-conditioned rowers (591 pmol ml^{-1} CO_2) were found to be statistically different, $p = 0.029$. Taken together, these results demonstrate our ability to repeatedly measure the influence of exercise on the concentration of breath ammonia.

Keywords: breath ammonia, exercise, photoacoustic spectroscopy, ammonia production

(Some figures may appear in colour only in the online journal)

1. Introduction

Gaseous ammonia is a base and when dissolved in water it forms its conjugated acid, the ammonium ion (NH_4^+, pKa 9.3). At physiological conditions (pH ≈ 7.40) the ammonium ion is the dominant species [1]. Ammonia is an important molecule relevant to multiple disease and wellness states. However, its physiology is complex and fluid, and there are multiple sources and disposal sites. For example, the kidneys, muscles, and liver may generate or dispose of ammonia depending upon various metabolic conditions [2].

Due to its inherent volatility, ammonia has always been difficult to measure *in vivo* by any method, including blood assays [3, 4]. Breath ammonia measurements are no different. Indeed, breath measurements may even be more difficult, since they introduce new technical issues (e.g. effect of oral pH, mode of breathing–mouth versus nose breathing) even though they avoid others (e.g. tourniquet time). But since they eliminate phlebotomy, breath measurements are ideally suited to repeated measures, and therefore have the unique potential as a method to study the dynamic physiology of ammonia. Determination of breath ammonia introduces additional controversies: should it be collected via the nose or mouth [5, 6] and whether there may be a kinetic component to its excretion similar to breath nitric oxide [7].

Unfortunately, it is presently unclear whether exhaled breath ammonia can, by any method, accurately reflect systemic ammonia. Breath ammonia may, for example, only

reflect protein metabolism, physiological pH of the blood [2, 8] or the metabolic activities of mouth bacteria [5, 6]. In fact, the very nature of ammonia (i.e. its reactivity, volatility and physiologic fluidity) makes this a challenging hypothesis to test. Nevertheless, we remain optimistic that exhaled breath ammonia may reflect systemic levels, and have previously reported some factors important in the measurement process. In order to further evaluate our hypothesis that breath ammonia accurately reflects systemic ammonia, we considered a variety of physiologic interventions designed to either raise or lower systemic ammonia. The ideal intervention would have a generally accepted short-term effect on systemic ammonia levels with the fewest number of possible confounders. Examples of possible short-term interventions to raise ammonia include: a high protein meal or exercise, whereas examples of short-term interventions to lower ammonia include: dialysis, administration of lactulose, or colon purge [9–11]. Amongst these candidate interventions, we chose vigorous exercise in healthy subjects, which has been studied previously [6, 8, 12, 13]. The results of these studies were contradictory but some of these differences may be due to experimental design. Studies reported by Senthilmohan *et al*, Schubert *et al*, and Smith *et al* involve measuring breath ammonia directly (SIFT-MS, PTR-MS) during exercise whereas Ament *et al* measured breath ammonia colorimetrically and blood ammonia off-line at 3.5 min intervals during exercise, Ament *et al*, Senthilmohan *et al*, Schubert, *et al*, measured the excretion of ammonia in breath whereas Smith *et al* measured the excretion of ammonia via the nose.

Ament *et al* studied eleven subjects and found that breath and blood ammonia increased with exercise but breath ammonia returned to baseline within minutes after recovery whereas blood ammonia decreased more slowly in recovery and remained elevated even after 30 min of recovery. Senthilmohan *et al* reported data on eight subjects. The breath ammonia on six subjects initially decreased with exercise and after 20–30 min breath ammonia increased in all the subjects. This group did not study the subjects in recovery. Schubert *et al* studied 21 subjects and reported that breath ammonia increased rapidly until approximately 45% of the full workload and for the remainder of the exercise the concentration of breath ammonia increased more slowly. This group did not study the subjects in recovery. Smith *et al* 2013 reported data on eight subjects who were studied pre-during exercise and post exercise. For this exercise protocol, which was less rigorous than the previous exercise protocols the concentration of ammonia decreased slightly with exercise for six of the study subjects. The remaining two subjects did not follow this trend; in one subject the ammonia concentration increased significantly during and post exercise, whereas the other subject did not appear to change nose ammonia over the protocol.

The results of these studies suggest that the effects of exercise on the excretion of molecules via the breath are complex. Exercise increases minute ventilation more rapidly than it increases cardiac output, therefore as a result of exercise, the concentration of any volatile species in the blood will decrease unless exercise causes an induction of the production of the volatile species. Concomitantly the concentration of any molecule in the breath under relaxed conditions will decrease in concentration with exercise since it will be diluted by increased ventilation. In the case of excretion of ammonia in breath, exercise will also result in the reduction of the concentration of blood ammonium ion since the ratio of ammonia to ammonium ion is defined by the pKa and the pH of the blood. Exercise also affects the blood bicarbonate buffering system as a result of increased excretion of carbon dioxide leading to a change in the pH of the blood and hence the equilibrium concentration of gaseous ammonia. The source and metabolism of ammonia in exercise and many other physiologic interventions and pathophysiologic states remain incompletely understood.

We hypothesize that exercise would temporarily increase exhaled breath ammonia levels compared to pre-intervention measurements due to the observation that ‘normal skeletal muscle releases ammonium ion during exercise [2]’. It is reasonable to propose that vigorous exercise will increase protein metabolism in muscle tissues [8]. Furthermore, we hypothesize that subjects capable of more vigorous exercise would demonstrate greater increases than less conditioned subjects since the differences between the rates of increase of their minute ventilation versus the rates of increase of their cardiac output would be less. Smaller changes in minute ventilation as a result of exercise will produce bigger changes in the concentration of breath ammonia. An alternate explanation is based upon the work of Adeva *et al* who propose that AMP (adenosine monophosphate) deaminase is partly responsible for the production of free ammonium ions in skeletal muscle, thus more muscle tissue converting more ATP (adenosine triphosphate) to AMP will yield a larger increase in ammonium production [2]. Results would be informative to describe the range of ammonia values achievable with our system.

2. Methods

2.1. Study methods

The St. Luke’s University Hospital Institutional Review Board (IRB) approved this study. Study subjects provided informed written consent. Five females and eight males ranging from 19 to 23 years of age participated. All participants were physically active and had no identified underlying conditions that are known to affect breath ammonia. The participants were required to fast for three hours preceding the study and not to drink liquids, or use mouthwash, or clean teeth for the hour before the study. The participants were required to exhale for at least 10 s in a defined manner via a restrictor and each exhalation constituted one sample. Each sample had its corresponding profiles for carbon dioxide and mouth pressure measured. Ideal mouth pressure for a sample is 10 cm of water maintained at least ten seconds. This mouth pressure corresponds to a flow rate of 50 ml s^{-1}. Latex gloves were worn when inserting the disposable mouthpiece into the breath samplers in order to prevent contamination with ammonia from the skin and fingers. The mouthpiece was not touched for the remainder of the study.

For breath ammonia, samples were taken from each subject every 10 min for ~150 min. The first three samples were averaged and used as the baseline. An additional 13 breath samples were taken after exercise. Each subject had pulse and blood oxygen saturation levels recorded before and after exercise, using a finger pulse oximeter (SM-110, Santamedical, Tustin, CA, USA). The exercise intervention consisted of a single 4000 m rowing protocol using an indoor rower (Model D, Concept2, Inc Morrisville, VT, USA). Rowing was selected as the exercise intervention since it was expected that this intervention involves a greater number of muscles than a stationary bicycle. The indoor rower was held at the same setting, damper level of five, for each participant; however, female subjects were asked to complete the workout within 22 min and males within 20 min, but shorter times were encouraged. Having each participant 'travel' the same distance within a relatively similar time frame assured that the work done by each participant was similar. All subjects completed the exercise protocol in the mandated time. The average 500 m split time, average power exerted per stroke, and total workout time were recorded.

After exercise, participants rested for approximately 60–90 s in order to achieve a respiration rate that allowed breath sampling. The study subjects were not allowed to drink water or any other beverage during this exercise protocol. In a subset of three participants, heart rate was recorded before the collection of each breath ammonia sample.

2.2. Breath sampling and analysis

Determination of breath ammonia: quantitative and selective measurements of the concentrations of breath ammonia (NH_3) were performed by using a thermoelectrically cooled CW DFB-QCL based sensor system as described previously [14]. Since each study subject was his/her own control, constant breath sampling was critical. Therefore, a specially designed breath sampler (Loccioni, Angeli di Rosora, Italy) was used to monitor breath exhalation in a manner similar to the American Thoracic Society/European Respiratory Society recommended breath collection protocol for analyzing breath nitric oxide (Fe_{NO} [7]). This breath sampler monitors, displays, prompts and archives real-time measurements of mouth pressure and the concentration of carbon dioxide. Real-time ammonia concentrations determined by the ammonia sensor are also displayed on the breath sampler and archived. For all breath sampling a disposable one-way in-line valve was used on the mouth port of the breath sampler. Single breaths were sampled continuously into the ammonia monitor via a 50 cm long inlet line (Teflon) heated to 55 °C. Plateau breath ammonia concentrations during the phase III portions of the exhalation profiles were reported in picomoles normalized to respiration (carbon dioxide production, pmol/ml CO_2 [15–19]).

Statistical methods: we examined differences between pre-exercise and post-exercise ammonia and between pre-exercise and maximum post-exercise ammonia within participants. Further, we evaluated between group differences of gender, body mass index, and fitness across time by mixed randomized repeated analysis of variance (ANOVAs) for means and by the Mann–Whitney–Wilcoxon test for medians.

Table 1. Demographics of the study population, $n = 13$.

	Mean
Age (years)	21.3 ± 1.3
Men (n, %)	8, 62%
Height (cm)	179.9 ± 9.9
Weight (kg)	80.4 ± 14.6
BMI (kg/m^2)	24.7 ± 2.8
4k time (min)	17:38 ± 1:46
Pre-exercise heart rate (bpm)	66 ± 11
Post-exercise heart rate (bpm)	149 ± 23
Pre-exercise CO_2 (torr)	32.6 ± 3.3
Post-exercise CO_2 (torr)	31.8 ± 2.7
Post-exercise minimum CO_2 (torr)	28.8 ± 2.7
Pre-exercise mouth pressure (cm H_2O)	9.8 ± 0.4
Post-exercise mouth pressure (cm H_2O)	9.7 ± 0.3

Table 2. Changes in the mean concentrations of breath ammonia (pmol ml^{-1} CO_2) as a function of exercise.

	Breath ammonia (pmol NH_3 ml^{-1}CO_2)		
	Mean of pre-exercise ± SD	Maximum post-exercise achieved	Mean post-exercise ± SD
erg01	114 ± 53	1901	1273 ± 541
erg02	509 ± 85	1080	812 ± 188
erg03	1296 ± 156	1848	1466 ± 240
erg04	724 ± 82	1135	808 ± 159
erg05	556 ± 80	1180	893 ± 202
erg06	1009 ± 137	1827	1543 ± 195
erg07	218 ± 12	1503	1036 ± 272
erg08	34 ± 3	154	103 ± 34
erg09	792 ± 150	1024	871 ± 132
erg10	193 ± 48	905	714 ± 160
erg11	1366 ± 310	3139	1875 ± 584
erg12	830 ± 107	1571	880 ± 584
erg13	1070 ± 126	2216	1553 ± 405
Group mean	670 ± 446	1499	1064 ± 535

Parametric and non-parametric analysis compared pre-exercise mean and post-exercise minimum CO_2 levels. All statistical analyses were performed using the statistical package SAS 9.2 (SAS Institute, Inc Cary, NC, USA). For all tests, a p-value < 0.05 was considered significant.

3. Results

Table 1 summarizes the demographics of the 13 study participants. Body mass index for seven of the participants ranged from 18.5 to 24.9 (normal or 'LOW') and for the remaining six, ranged from 25 to 29.9 ('HIGH'). Four of the study subjects completed 4000 m row in less than 16.5 min, and for study purposes, these subjects were designated 'conditioned' rowers.

Of 13 subjects, nine subjects' CO_2 concentrations decreased from pre-exercise mean within the first four post-exercise breaths. Overall, pre-exercise mean CO_2 was 32.6 torr and post-exercise minimum CO_2 was 28.8 Torr, p=0.00007. These results suggest that subjects had hyperventilated as a result of exercise.

The exercise intervention increased the concentration of breath ammonia to varying degrees in all subjects. Table 2

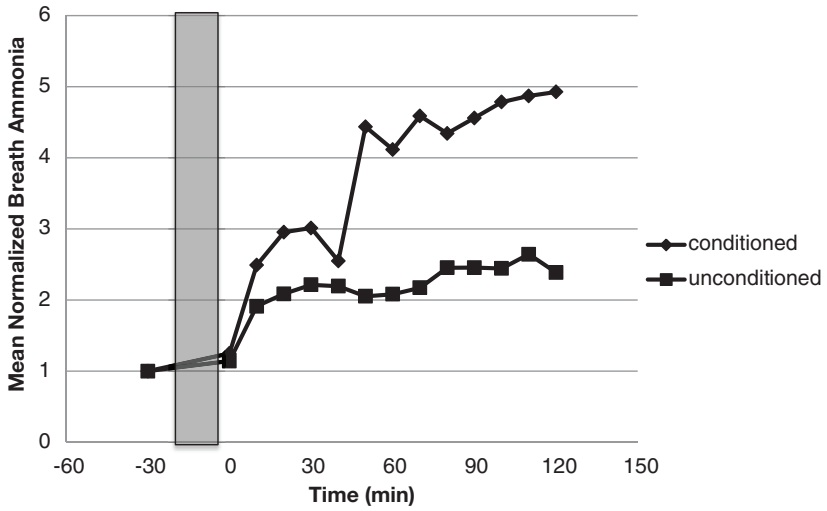

Figure 1. Normalized concentrations of breath ammonia (ppb) as a function of exercise on the basis of fitness status. Shaded area represents the time of exercise.

summarizes breath ammonia concentration as a function of controlled exercise and includes: mean pre-exercise ammonia, maximum post-exercise ammonia and mean post-exercise ammonia for each study subject. There is a wide variation of the concentrations of breath ammonia pre-exercise, data ranged from 34 to 1366 pmol ml^{-1} CO_2. For each study subject, the difference between mean pre-exercise versus maximum ammonia achieved was statistically different ($p = 0.0003$), and the difference between mean pre-exercise versus mean post-exercise ammonia was statistically different ($p = 0.0016$).

Since the baseline breath ammonia was study subject dependent the breath ammonia concentration at each sampling point was normalized to baseline. Each study subject acted as his/her own control. Figure 1 shows how the exercise intervention increased the breath ammonia in the study subjects stratified by normal versus conditioned rowers. Data are expressed as means of the normalized breath ammonia. The difference between the normal versus conditioned rowers was found to be significantly different ($p = 0.029$).

The data contained in table 2 were reexamined on the basis of gender (male, $n = 8$; females, $n = 5$). These comparisons are shown in figure 2(*a*). Differences between the mean breath ammonia concentrations pre-exercise versus post-exercise maximum, pre-exercise versus mean post-exercise for men and women were not statistically significantly different ($p = 0.33$).

The data contained in table 2 were reexamined on the basis of body mass index (Low < 25 BMI, $n = 7$; High ≥ 25 BMI, $n = 6$). These comparisons are shown in figure 2(*b*). Differences between the mean breath ammonia concentrations pre-exercise versus post-exercise maximum, pre-exercise versus mean post-exercise for study subjects with low and high BMI were not statistically significantly different ($p = 0.187$).

Figure 3 shows that the exercise intervention raised the heart rate for a sub-group of the subjects ($n = 3$). The baseline pulses were different for the study subjects so the measurements of pulse were normalized to baseline. The pulses for each subject reached maximum values shortly after exercise was completed and returned to pre-exercise rate after approximately 150 min. Pulse is used as a surrogate for the cardiac output, since exercise is not likely to change cardiac stroke volumes. Based upon comparisons of the pulse measurements before and after exercise, cardiac output increases more than two fold during this exercise protocol and the changes in the concentrations of carbon dioxide suggests that minute ventilation had increased even more. In future studies, a metabolic cart will be used to measure minute ventilation as a function of exercise.

4. Discussion

The results of this study are in agreement with our hypothesis that the concentration of breath ammonia increases with exercise, with a slight increase immediately after completion of the intervention followed by a steady rise for 2 h. Increases in breath ammonia with exercise were reported in three of the published studies [8, 12, 13] and in some subjects in the remaining exercise study [6]. The major difference between the study reported herein and these published studies is that no measurements of breath were taken during exercise and breath ammonia was measured for 2 h post exercise. Another important difference was in our study, subjects were not allowed to drink or eat food post-exercise. Drinking fluids has been shown to decrease breath ammonia for at least 15 min [20]. Ament *et al* were the only group to report significant data on the recovery period and they showed that breath returned to normal within minutes after recovery although no information was presented on the ingestion of fluids. However, this same group showed that blood ammonia remained elevated for greater than 30 min of recovery. These results are consistent with the data reported herein. Upon completion of our exercise intervention, a mean 19% and 25% ammonia increase for unconditioned and conditioned rowers, respectively, was found. These numbers are slightly less than the increase previously found, which may be due to a less rigorous workout. Subjects were not pushed to complete exhaustion. Further,

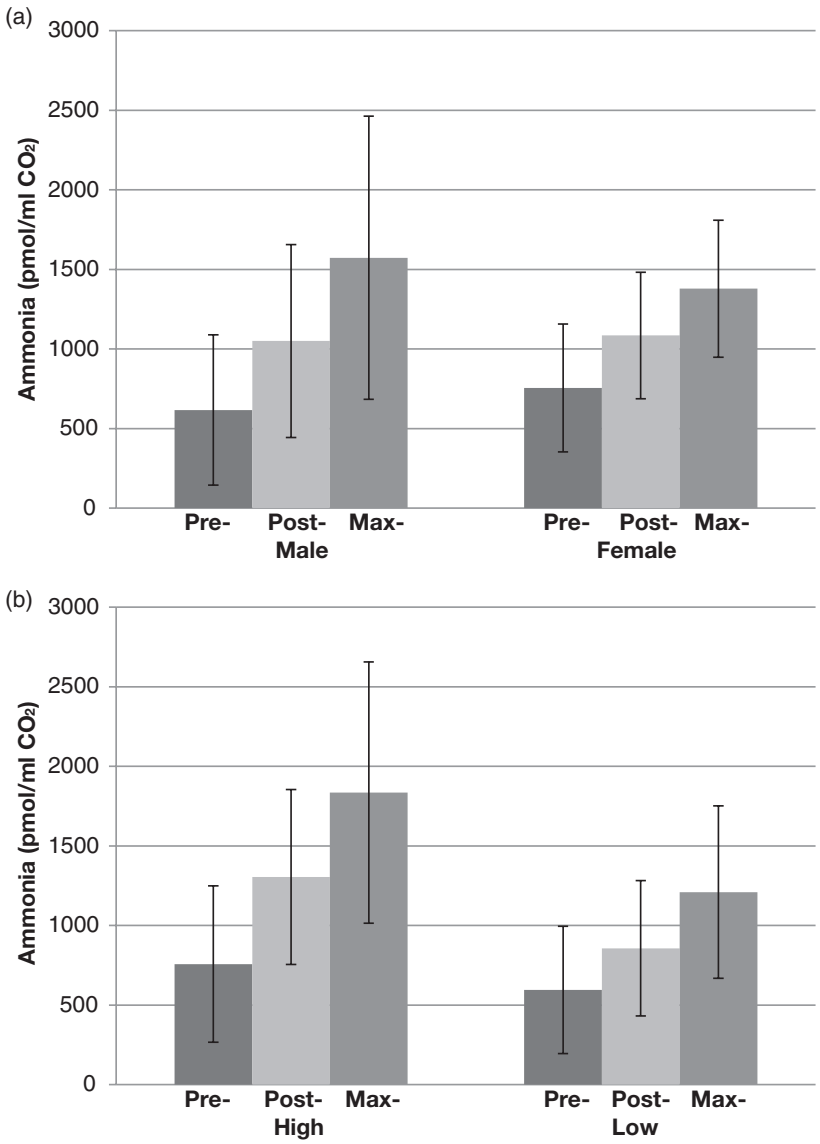

Figure 2. (a) Concentrations of breath ammonia (pmol ml^{-1} CO_2) as a function of exercise on the basis of gender. (b) Concentrations of breath ammonia (pmol ml^{-1} CO_2) as a function of exercise on the basis of body mass index.

'conditioned' rowers showed the greatest increases. While we cannot be certain as to the reason why we observed these differences in the rate of increase of breath ammonia, the conditioned rowers were all larger men, and therefore we propose that skeletal muscle might be the primary factor. Differences in blood ammonia based on athletic performance have been previously described [21]. This difference could also be explained by smaller increases in minute ventilation versus the rates of increase in cardiac output for the conditioned rowers. The steady rise in ammonia after exercise may be attributed to the increased use of amino acids as a fuel source to assist in muscle recovery [22]. Reidy *et al* have also demonstrated the increase in amino acid trafficking in human skeletal muscle for up to four hours after exercise [23]. Even though this group provided a protein blend to the subjects after exercise, it is still worth observing the need for human skeletal muscle to utilize amino acids, thus generating ammonia, for several hours after exercise. We did not find statistical differences based on gender or BMI, but our study sample size was small.

An important aspect of our study is the capability of real-time data collection including the use of parameters that define a repeatable breath sample, specifically carbon dioxide concentrations and mouth pressure. We believe our system is capable of accurately measuring end-alveolar ammonia, and thereby systemic ammonia levels. Measuring the concentration of carbon dioxide, in particular, allows physiological changes that occur during exercise to be observed post-exercise. Notably, the rate of respiration increases to a greater extent than the heart rate during exercise, thereby leading to a dilution of the amount of compounds in each breath (assuming a relatively consistent stroke volume). Indeed, we found decreases in carbon dioxide immediately following exercise for around 30–40 min in most subjects. If this trend is valid, all the concentrations of post-exercise ammonia will have been diluted. This dilution effect may explain our observation that, although all subjects exhibited increased ammonia concentration levels after exercise, six of the thirteen subjects showed initial decreases in ammonia after exercise.

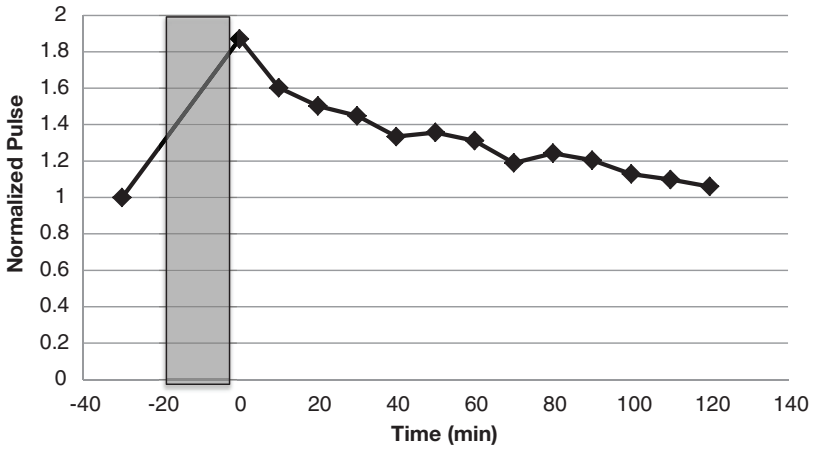

Figure 3. Mean of the normalized pulse as a function of exercise. Shaded area represents when exercise occurred.

Our study has significant limitations including limited data on exercise parameters (e.g. cardiac output, among other possible exercise variables, was not measured [8]). Furthermore, although we used a fast and accurate breath collection technique, sampling *during* exercise was not feasible since our method of breath ammonia measurement requires a breath maneuver. Moreover, our monitor is both expensive and sizable. As a result, study subjects come to our research lab and are studied one a time. This limits samples size for all of our ammonia studies, including the present work. Another important parameter for breath ammonia, namely oral pH change during exercise, was not measured. Oral pH has been reported to be a significant factor breath ammonia determination by our group and others, with reduced oral pH causing decreases in measured breath ammonia [5]. Thus, if vigorous exercise reduced the oral pH of the study subject, measured ammonia levels would be artificially lower. If this were true, our findings may underestimate the ammonia increase. However, while the exact effect of exercise on salivary pH is still debated, some have reported only negligible change [24].

As noted above, multiple other interventions are also plausible. However, incomplete knowledge of ammonia biology needs to be acknowledged, and where possible, addressed. Over the last decade, ammonia research has revealed ever more complexity. The once primary roles of the gut flora and liver are diminishing, while multiple other sites of ammonia regulation (e.g. kidney, muscle, small bowel) are being elucidated and emphasized as key factors. For example, while gut derived ammonia from oral intake, especially high protein meals, has long been considered an important ammonia source, recent work by Van de Poll *et al* using surgical blood collected from various venous compartments (portal, hepatic, renal) strongly contradicts this dogma [25].

Ideally, any measurement of ammonia, via blood, breath, or another method, should account for the key factors in its regulation, including these various sites of ammonia regulation and their influences (e.g. fed versus fasted state, hydration status, medications affecting hepatic enzyme pathways and so on). Furthermore, especially in the breath literature, most ammonia physiology studies are plagued by small sample size and are often designed by research groups most interested in putative applications of ammonia (e.g. hepatic encephalopathy or dialysis monitoring [26, 27] rather than the measurement process itself. As a result, recent comprehensive reviews of general ammonia physiology [2, 28], including those with a hepatic [29] and non-hepatic focus [30], still neglect to mention breath measurement, even for research purposes. Thus, despite the fact that multiple prior studies have used breath ammonia to evaluate exercise [6, 8, 12, 13], they have not had a major or lasting impact on the study of either exercise or ammonia physiology. Finally, we are in agreement Miekisch *et al* [31] that there are numerous important pitfalls in data interpretation for breath analysis. Because we share these concerns, we believe that comparisons between studies (each of which are small, single center studies with varying study designs) are challenging and risk over-interpretation and false conclusions.

Therefore, notwithstanding the fact that breath ammonia has been performed for many years [32], the most recent work by multiple highly experienced breath research groups is, paradoxically, pioneering [5, 33, 34]. These detailed studies re-focused on the breath measurement process, and suggest that exhaled breath ammonia measurement through the oral cavity is inherently flawed: either oral bacteria and/or saliva will contaminate or otherwise obscure significant measureable signal from the lung, and such measurements do not accurately reproduce blood ammonia levels. These groups have used fast and highly accurate collection techniques and their findings simultaneously cast doubt on the validity of prior breath ammonia studies even while they propel the field forward. Schmidt *et al*, for example, report detailed measurements of sweat, blood, salivary, nasal, and breath ammonia, and comes closest to replicating the strategy and effort of Van de Poll.

Though we believe that our system enables the measurement of end-alveolar ammonia and therefore systemic levels [20], we may be in error. Definitive proof would seemingly require simultaneous deep lung measurement via bronchoscopy and oral measurement. Using bronchoscopy to monitor breath ammonia in healthy subjects is impossible due to IRB policies; therefore, we selected the present strategy of studying the whole person via a short term intervention on healthy subjects. The strength of this strategy is that each subject

serves as his/her own control, a standard breath maneuver was used to perform each breath ammonia measurement, and confounders are minimized. But if even our results do reflect, for example, salivary ammonia in part or in whole, they may still have utility if salivary ammonia correlates to systemic levels. We doubt oral bacteria were influenced by exercise.

In summary, we found that vigorous exercise increases exhaled breath ammonia in all subjects and our findings are in agreement with our overall hypothesis. This suggests that exhaled breath ammonia measurement indeed reflects systemic levels. It is again emphasized that exercise was used as an intervention to evaluate our breath ammonia measurement protocol; therefore, these results were not intended to generate insights into exercise per se. Additional experiments are needed to further evaluate our hypothesis and we are presently evaluating the effect of oral challenge in healthy subjects.

Finally we note that this kind of experiment (i.e. multiple repeated measures before and after a physiologic intervention) would be difficult to achieve with blood ammonia measurement, further highlighting the potential of the breath approach. Thus, if (1) further engineering refinements result in fast, accurate monitors that are also inexpensive and portable and (2) if exhaled breath can be proven to reflect systemic ammonia, then perhaps exercise could become the experimental focus. Conceivably then exercise 'breath ammonia thresholds' could be defined and meaningfully impact the study exercise *and* ammonia physiology.

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