



Could Oral Ammonia Adversely Affect the EBC pH Assay?

There is one prolific letter writer who keeps pushing that alteration of oral ammonia is the cause of EBC acidification. What he completely lacks in original data is made up for by his strongly worded, albeit unfounded and incorrect notions. In our minds, data trump theory any day of the week. And the data are clear that oral ammonia, or absence of it, is not the cause of low EBC pH. (1)

Ammonia (NH_3) is a volatile base that is exhaled in reasonably large amounts. Although some is derived from the lower airway (on average 15% in healthy subjects), much of the exhaled NH_3 originates in the upper airway. Sources of oral NH_3 clearly include the activity of bacterial urease enzymes on salivary urea, although other, eukaryotic pathways including glutaminase likely also play an important role in oral NH_3 levels. It is to be noted that in a small percentage of subjects, over 90% of the EBC NH_3 is derived from the lower airway. Not all people are the same. There are always exceptions.

Oral NH_3 is strongly suspected of influencing the outcomes of acid inhalation challenges, as NH_3 can neutralize low concentration inhaled acids. When oral NH_3 is suppressed (with acidic rinses of the mouth which traps NH_3 as NH_4^+), acid inhalation challenges are more consistently effective at prompting airway responses.

So, what is the effect of upper airway NH_3 on EBC pH? Well, it turns out there is very little effect. But the situation is readily misinterpretable, and is therefore discussed below. We will use the term $\text{NH}_3/\text{NH}_4^+$ to signify total ammonia plus ammonium, which is what is measured with most (but not all assays) of EBC levels.

The $\text{NH}_3/\text{NH}_4^+$ levels found in EBC are in the range of 0-500 μM in health-when the pH is normal, but are in the range of 0-50 μM when EBC pH is low. This has suggested that a low EBC $\text{NH}_3/\text{NH}_4^+$ level is necessary, but not sufficient for the EBC pH to be low. Indeed, removal of the oral contribution to EBC $\text{NH}_3/\text{NH}_4^+$ by a variety of methods (oral rinsing, endotracheal collections, sample lyophilization and resuspension, extremely cold condenser temperatures) has resulted in no change in EBC pH whatsoever. Thus, although there are correlations between EBC pH and $\text{NH}_3/\text{NH}_4^+$ when looking at large numbers of samples, there is clearly not a cause and effect relationship. Low EBC NH_3 is NOT the cause of low EBC pH. Of course this makes complete sense when one realizes that the absence of a base does not make an acid.

It is helpful to consider what happens when the airway becomes acidic. When the airway becomes acidic, volatile NH_3 becomes trapped as NH_4^+ (which later may be transferred to the blood and dealt with in the liver). So, NH_3 that gets inhaled into the lungs from the mouth is not able to egress the lungs and is not available for capture in EBC. In turn, airway acidity makes acids, such as acetic acid, volatile-encouraging escape of acid from the airway for subsequent capture in EBC. Thus a low EBC pH (as determined by captured acids) and a low EBC $\text{NH}_3/\text{NH}_4^+$ are independently caused by a low airway pH. And this is the reason why there is a correlation when looking at the two measures in a large group of subjects.

Extensive data has been collected to determine what, if any, role oral NH_3 has in determining EBC pH. As a volatile base, it assuredly plays a role. However, the role is so small as to be unidentifiable in reasonably sized studies. Oral ammonia, or absence of it, is unequivocally NOT the cause of the profound EBC acidification seen in respiratory diseases.

Ammonia in EBC may be relevant to measure however, despite it being derived in most people (not all) predominantly from the upper airway. This is because of the correlation with EBC pH. EBC $\text{NH}_3/\text{NH}_4^+$ is even easier to measure than pH. Deaeration is not necessary or useful for $\text{NH}_3/\text{NH}_4^+$ assay. The assay can be done with a simple colorimetric scale. There are even dipsticks available that are perfectly suited for EBC and are perfect for the range found in EBC. Check your local aquarium supply store for these dipstick ammonia assays. They are used for assaying $\text{NH}_3/\text{NH}_4^+$ in fresh and salt water aquariums. Although very easy to measure, it must be kept in mind that $\text{NH}_3/\text{NH}_4^+$ can be low without the pH being low, and therefore there will be substantial false positivity when using this assay to identify airway acidification.

References

- 1 Wells, K., J. Vaughan, T. N. Pajewski, S. Hom, L. Ngamtrakulpanit, A. Smith, A. Nguyen, R. Turner, and J. Hunt. 2005. Exhaled breath condensate pH assays are not influenced by oral ammonia. *Thorax* 60(1):27-31.