**Breath Ammonia and Ethanol Increase in Response To a High Protein Challenge**

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**Introduction**

Ammonia, a by-product of protein metabolism, and ethanol, a by-product of carbohydrate metabolism, are both relevant to human health. Ammonia is produced and metabolized by bacterial and mammalian cells, whereas ethanol is presumed to be produced only by bacteria. Hydrogen, which we used as a comparator, is a by-product of solely bacterial carbohydrate metabolism. Commercial hydrogen breath testing is recognized as a method to detect bacterial fermentation in the gastrointestinal tract (Saad & Chey, 2013). And, unabsorbed sugars, such as lactulose, serve to provoke an increase in gut bacteria hydrogen production (Simrén & Stotzer, 2006).

Human ammonia physiology is complex due to multiple sources and sinks (Adeva, Souto, Blanco, & Donapetry, 2012). And, ammonia balance is influenced by numerous physiologic and pathophysiologic events, including food intake and composition, bowel function, muscle physiology, and kidney disease. Given its role in several pathophysiologic conditions, accurate, rapid, and non-invasive quantification of ammonia levels has clinically important applications (Davies, Spanel, & Smith, 1997).

Ammonia physiology research is hindered by its volatility. It is a ‘sticky’ molecule. This makes reliable and reproducible ammonia measurement difficult by any method. In the past, most research utilized ammonia blood assays; these can be variable and vexed by technical errors. Moreover, phlebotomy generally occurs via limb venipuncture, and other body compartments are rarely evaluated. Perhaps more importantly, research relying on ammonia blood assays is inherently limited by episodic sampling and therefore cannot fluidly capture ammonia physiology.

Breath researchers have, for many years, attempted to advance ammonia research using a variety of monitors and measurement protocols. Numerous pilot studies have been published on a range of topics related to ammonia physiology in normal (Hibbard & Killard, 2011)(Turner, Spanel, & Smith, 2006) and disease states (DuBois et al., 2005a)(Adrover et al., 2012)(Davies et al., 1997). However, recent research suggests that exhaled breath ammonia may not reliably determine systemic ammonia; these investigators assert that its volatility and the multiple sources of contamination in oral-pharyngeal membranes cannot be overcome (Schmidt et al., 2013). And, others have preferred nose-exhaled ammonia over mouth exhaled ammonia (Wang, Pysanenko, Dryahina, Spaněl, & Smith, 2008). Papers reporting the difference in nose-exhaled versus mouth-exhaled ammonia have consistently found lower ammonia levels in nose-exhaled samples.

Ethanol is somewhat less volatile than ammonia and, since it is produced only by bacteria, has fewer potential sources. Interest in endogenous ethanol has been growing as it has been increasingly implicated in the pathophysiology of fatty liver and the metabolic syndrome (Bikov et al., 2013)(Cope, Watson, Foster, Sehnert, & Risby, 2004)(Wang, Pysanenko, Dryahina, Spaněl, & Smith, 2008b). Because the gut, specifically the distal small bowel and colon, hosts the largest bacterial community in humans, this compartment has generally been assumed to be the source of exhaled ethanol; however, this assertion has not been rigorously tested.

In this study, we have evaluated the capacity of our breath collection apparatus and procedure to determine systemic ammonia. And, we also hypothesize that exhaled breath ethanol is gut-derived. Our aim was to measure the ammonia and ethanol response to a high protein oral challenge versus a negative control oral challenge. Further, we used breath hydrogen as the gold standard for gut-derived breath products, and also compared the concordance between peaks in ammonia, ethanol, and hydrogen.

**METHODS**

**Study Participants**

Participants were recruited via flyers and advertisements. All eligible participants provided informed consent as required by the St. Luke’s University Hospital Institutional Review Board. Thirty healthy volunteers, without periodontal, liver, or kidney disease or report of tobacco use, fasted 12 hours prior to presentation. Volunteers abstained from exercise the morning of the study and brushed their teeth at least an hour before arrival.

**Data Collection**

We measured the alveolar portion of the exhaled breath for NH3, EtOH, and H2 with three devices serially over 6 hours on 2 days in each subject. Each day began with 30 minutes of baseline breath collection; three NH3 measurements were taken 10 minutes apart and two samples were measured for EtOH and H2 each taken 15 minutes apart. This baseline breath measurement phase was followed by an oral intervention. On Day #1 (control trial), we mapped trends of NH3, EtOH, and H2 in response to the oral ingestion of Gatorade (32 fl oz containing: 200 calories, 52 g sugar, 0 g fiber, 0 g protein, and 0 g fat). On Day #2 (intervention trial), we measured breath NH3, EtOH, and H2 in response to the consumption of lactulose (10g) as well as a high protein challenge (Rockin Refuel Muscle Builder shakes containing: 380 calories, 12g sugar, 6g fiber, 60g protein, and 9g fat). The oral intervention was followed by a 30 second water rinse to flush any residue from the mouth. Breath samples for NH3, EtOH, and H2 were taken every 30 minutes for 5 hours following the rinse.

*Breath collection:* The participants were required to exhale for at least 10 seconds 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 10 seconds. This mouth pressure corresponds to a flow rate of 50 ml/s. Latex gloves were worn when inserting the disposable mouthpiece into the breath sampler in order to prevent contamination with ammonia from the skin. The mouthpiece was not touched for the remainder of the study.

*Determination of breath ammonia:* NH3 was measured with a novel, sensitive, selective and fast quartz enhanced photoacoustic spectroscopy monitor (Lewicki et al., 2009) (Rice University, Houston, TX) as previously described (Steven F Solga et al., 2013) (Solga, et al, JoVE 2014, *in press*). Since each study subject was his/her own control, consistent breath sampling technique 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 (FeNO) (ATS/ERS 2005). 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 measured during the phase III portions of the exhalation profiles were reported in parts per billion (ppb).

*Determination of breath ethanol:* Ethanol was measured by a thermal desorption-capillary gas chromatograph (Fast GC)-differential mobility spectrometer (DMS) (microAnalyzer, Sionex Inc, Bedford, MA) capable of real-time measurement of ethanol, acetone, and isoprene. The sampled breath (10 mL) was adsorbed onto sequential adsorbent beds (Carbopack X (13 mm long, 60/80 mesh) and Carboxen 1003 (13 mm long, 80/100 mesh); Supelco, Bellefonte, PA) contained in a stainless steel tube (6.6 cm long, 1.59 mm od, 1.30 mm id) at 40°C. After breath had been sampled, the trap was purged for 15 seconds with dry air. After purging, the trap was switched to the head of the capillary column and the gas chromatographic separation was initiated. After a delay of 1 second, the adsorbent trap was heated to 300°C to thermally desorb the collected breath molecules. Separation was performed on a wall coated silicosteel capillary column (0.53mm od, 15 m MXT VMS crossbond diphenyldimethyl polysiloxane phase; Siltek, Restek, Bellefonte, PA). The column was maintained at 40°C for 150 seconds, temperature programmed from 40 to 140°C in 250 seconds and held isothermally at 140°C for 140 seconds.

The column effluent was passed into the source of the differential mobility spectrometer and ionized with thermalized electrons. For the first 90 seconds, the radiofrequency (RF) voltage was set to 1200 volts and then to 1000 volts. The compensation voltage was scanned from -30 to 4.99 volts and the ion current was monitored continuously. The complete analysis took 540 seconds. Data were recorded as a function of gas chromatographic retention time and compensation voltage. Calibration curves were obtained for ethanol, acetone, and isoprene. Breath concentrations for isoprene, acetone and ethanol were reported in parts per billion (ppb).

*Normalization by correction CO2 factor:* Both the ammonia and ethanol values were normalized by a CO2 correction factor in the same fashion as H2 is normalized in the MicroLyzer protocol as shown below. We used an approximated alveolar CO2 pressure (PACO2) of 40 mmHg: Corrected Breath Value = Raw Breath Value \* (40/Sample CO2 Pressure).

*Determination of breath hydrogen:* H2 was measured using a Quintron SC MicroLyzer (Milwaukee, WI). The protocol includes sample correction by normalizing each sample with a correction factor based on an alveolar CO2 pressure of approximately 40 mmHg (torr). Peak hydrogen > 20 ppm was deemed a positive test and provided evidence of gut activity. In addition to hydrogen, methane breath levels were quantified, as 5-10% of hydrogen testing may result in false-negative results due to methane rather than hydrogen production (de Lacy Costello, Ledochowski, & Ratcliffe, 2013a).

**Statistical Analysis**

We compared baseline values versus post-rinse maximum ammonia, hydrogen, and ethanol values for each subject in each study trial. As the raw data approximated log normal distributions, all data was log transformed (Limpert & Stahel, 2011)(Sorrentino, n.d.). Geometric means (µ\*) and multiplicative standard deviations (MSD) (Braithwaite, 2014) were calculated. Reported results were back-transformed to the original scale after analysis. Two-way repeated measures analysis of variance (ANOVA) for treatment type (control versus challenge), change from baseline to maximum, and the interaction of treatment and change from baseline to maximum was performed. All statistical analyses were performed with SAS v9.2 (SAS Institute, Inc., Cary, NC). For all tests, a p-value of < 0.05 was considered significant.

We also explored the potential diagnostic utility of exhaled breath ammonia. We identified thresholds by which diagnostically significant increases in ammonia levels could be recognized and interpreted as a positive result. Increase in breath ammonia (maximum ammonia minus baseline ammonia) for each participant was calculated and compared to the following thresholds: 300 ppb, 400 ppb, 500 ppb, 600 ppb, 1.5 x baseline, 1.8 x baseline, and 2.0 x baseline. We calculated the sensitivity, or true positive rate, by counting those participants with an increase in breath ammonia during the intervention trial that exceeded a pre-defined threshold (positive result) and by dividing that count by the total number of participants (N=30). And, we calculated the specificity, or true negative rate, by counting those participants with an increase in breath ammonia during the control trial that did not exceed a pre-defined threshold (negative result) and by dividing that count by the total number of participants (N=30).

**Results**

For 30 study participants, mean age was 24 years (SD, 7 yrs), 47% were men (14/30), and mean body mass index was 24.2 kg/m2 (SD, 4.0). Table 1 lists geometric means (µ\*) and multiplicative standard deviations (MSD) for NH3,EtOH, and H2. Figure 1 illustrates the baseline and maximum values for control and intervention treatment types for NH3 (1a), EtOH (1b), and H2 (1c). The interaction of treatment type and change from baseline to maximum was significant for NH3 (p<0.0001), EtOH (p=0.017), and H2 (p<0.0001).

Figure 2a illustrates the mean ammonia and mean hydrogen calculated at each time point without consideration of subject-specific data, with maximum mean ammonia occurring at 300 minutes and maximum mean hydrogen at 240 minutes. The mean time difference between peak ammonia to peak hydrogen calculated for each participant was 45 minutes. Figure 2b shows the trends for ethanol and hydrogen calculated for each time point without consideration of subject-specific data; ethanol peaked 30 minutes after the oral challenge. And, when calculated for each participant, the peak ethanol occurred on average within one hour of the mouth rinse.

We explored the utility of increased breath ammonia by comparing breath values for intervention and control trials (Table 3). Sensitivity to discern response to protein challenge was greatest (97%) when defined as an increase in breath NH3 of at least 300 ppb. When greater increases in breath NH3 were required, the sensitivity was lower. At a pre-defined threshold of 600 ppb increase, the specificity was 100%; however, sensitivity was 77%. We also evaluated multiplicative increases from baseline (x1.5, x1.8 and x2) to determine an “elevation” in breath ammonia after high protein challenge compared to control trials, but this did not generate sensitivity above 87%.

Four participants did not contribute to the EtOH analysis due to malfunction of data collection equipment and missing data for control trial EtOH values. Of these four, two were also missing data for the intervention trial. Furthermore, an additional two participants were excluded from the EtOH analysis due to exceptionally high EtOH levels attributable to alcohol consumption within 12 hours prior to testing. Twenty-eight participants contributed to H2 measurements because two participants did not produce measureable hydrogen (de Lacy Costello, Ledochowski, & Ratcliffe, 2013b).

**Conclusions**

Our results show that ammonia significantly increases over time in response to a high protein oral challenge compared to a negative control oral challenge, and we hypothesize that exhaled breath ammonia represents systemic ammonia. We also found that the ammonia peak following a high protein challenge is temporally related to the hydrogen peak (which identifies the time at which the food bolus encounters the major bacterial population of the distal small bowel and colon). This result is consistent with a gut-derived source of the systemic increase in ammonia. There are two putative mechanisms: increased activity of gut flora and/or activity of small bowel glutaminase. Notably, our interpretation of this temporally-associated increase in ammonia and hydrogen runs counter to the conclusions of others. However, we note that this temporal association does not prove the ammonia is gut-derived, and other mechanisms may contribute. For example, amino acid absorption in the proximal small bowel might result in ammonia release by inter-organ trafficking (e.g. from skeletal muscle) that could explain the temporal association. More research is needed to evaluate these possibilities.

Our median ammonia value is comparable to those measured by other groups (Schmidt et al., 2013; Smith, Spanel, & Davies, 1999; Turner et al., 2008) (Turner, Spanel, & Smith, 2006b). Some groups have concluded that exhaled breath ammonia measured via the mouth may be contaminated by oral bacterial products and exhaled breath ammonia does not reflect systemic or plasma ammonia (Schmidt et al., 2013)(Spaněl, Dryahina, & Smith, 2013). The differences between groups may be due to protocols followed during breath collection, mouth- versus nose-exhaled breath, or difference in devices used to collect samples.

Importantly, we measured the phase III portion of the breath and reported an immediate decrease in breath ammonia before a steady increase to above baseline seen in samples collected over 5 hours. We propose that a recovery period after rinsing is necessary. If measurements are recorded without adequate lag time or not followed for a long enough duration, recorded breath ammonia values may be relatively low as maximum levels have not yet been achieved. Some investigators may have recorded low breath ammonia levels due to this methodological difference. For example, Adrover *et al.* report that samples were collected 15 minutes after tooth brushing with results that are much lower than those reported in the literature (Adrover et al., 2012).

As an element to explore diurnal variation, we compared intervention to control trials. During control trials, we measured a modest increase in ammonia. Hibbard and Killard noted “a consistent decrease in oral breath ammonia concentrations by the early afternoon (post-prandial)...followed by gradual increase towards late afternoon” in two individuals (Hibbard & Killard, 2011a). As acknowledged by Hibbard and Killard, values they reported are lower on average than those reported here and in other studies (Enderby et al., 2009; Turner et al., 2006b). In our study, by comparing breath ammonia levels measured after oral intervention to levels after high protein intervention, each participant served as their own control. Breath ammonia was significantly increased after high protein intervention when compared to the control.

Some investigators have recommended measurement of nose-exhaled breath indicating that it represents systemic ammonia and avoids oral contamination that occurs with mouth-exhaled samples (Wang et al., 2008a). Wang *et al.* report geometric means (MSD) of ammonia: 1088 (1.3) ppb, 885 (1.3) ppb, and 855 (1.3) ppb for three volunteers using a protocol that sampled “direct exhaled breath via the mouth.” Additionally, they employed protocols for direct exhaled breath via the nose and static gas in the oral cavity during breath hold. Others incorporated a urea mouth wash to evaluate the potential for oral contamination and found supra-physiologic ammonia levels (4500 ppb) seen in mouth-exhaled breath of a single subject (Smith, D, Chippendale, TWE, Dryahina, K, Spanel, 2013). However, this maneuver does not rule out a gastrointestinal source of mouth-exhaled breath ammonia.

In reviewing the literature, results for breath ammonia collected via nose-exhaled versus mouth-exhaled protocols are different (Hibbard & Killard, 2011b; Wang, Pysanenko, Dryahina, Spaněl, & Smith, 2008c). Future research must clearly report the method of breath exhalation: nose- or mouth-exhaled, especially since the evidence indicates that nose-exhaled ammonia values are consistently lower than mouth-exhaled samples. This may be due to the greater surface area of the nasal cavity compared to the oral cavity to act as an ammonia sink. In that reproducible ammonia levels have been published in the literature, we propose that consistent use of either method will allow for the study of gut-derived ammonia.

Investigations have not definitively or reproducibly described the biological mechanisms that produce measurable ammonia in exhaled breath. Van de Poll *et al*. and Yang *et al*. have presented data supporting the thesis that gut-derived ammonia does not increase systemic ammonia in healthy persons without porto-systemic shunting (van de Poll et al., 2008) (Yang et al., 2000). The difference between our study results and these conclusions may be related to the methods used in the determination of ammonia levels. In our study, we collected exhaled breath, whereas van de Poll *et al*., for example, used phlebotomy from multiple body compartments during laparotomy. As well, the work of Olde Daminik *et al*. was exceptional, specifically because portal and hepatic vein phlebotomy occurred simultaneously (Mpabanzi et al., 2011).

While our study lacks data from blood assays, we do not believe this is an important impediment for the study of ammonia or ethanol. To illustrate with ammonia only, our breath assays measure exhaled NH3 presumably derived from the lungs while venipuncture measures NH4+ derived from a limb. As already noted, both approaches have the potential for significant variability and error. In the case of blood assays, this concern has been repeatedly reviewed. Thus, while NH3 and NH4+ may have a precise stoichiometric relationship at a given pH in a chemistry lab, the same relationship may not be observed when this most volatile metabolite is measured from different compartments of a whole organism. In fact, DuBois *et al*. used fiberoptic sensors to detect breath ammonium and compared it to arterial ammonium in 15 cirrhotic individuals and found no correlation (DuBois et al., 2005b). Nevertheless, our own preliminary work on this comparison using a separate cohort suggests a fair correlation between breath and blood ammonia (unpublished data). And, our current study provides an internal control by comparing repeated levels of breath ammonia in participants after an oral control intervention followed by a treatment intervention.

Our results also demonstrate significant increases in breath ethanol following a high protein challenge, compared to a negative control oral challenge that showed a similar yet less pronounced increase. This is consistent with the concept of gut-derived “endogenous ethanol”, which has previously been shown in several small studies using highly sensitive blood assays in response to food (Watanabe-Suzuki, Seno, Ishii, Kumazawa, & Suzuki, 1999) (Sarkola & Eriksson, 2001) as well as prior breath research using a murine model (K. Cope, Risby, & Diehl, 2000) and humans (Nair, Cope, Risby, Diehl, & Terence, 2001) (S F Solga et al., n.d.). Endogenous ethanol is postulated to contribute to the pathogenesis of non-alcoholic fatty liver and perhaps the metabolic syndrome (Aron-Wisnewsky, Gaborit, Dutour, & Clement, 2013) (Abu-Shanab & Quigley, 2010).

However, a unique aspect of this study, especially in regards to the interpretation of the ethanol data, is the use of breath hydrogen. In contrast to ammonia and ethanol, hydrogen is easy to measure, inert, and its source is relatively non-controversial: it is produced when the food bolus residue encounters the bulk of bacteria in the distal small bowel and, to a greater degree, colon. It therefore serves as a distinctive and essential timing marker. This is important to the consideration of serial data after an oral challenge because gut transit time is variable and unpredictable (Huizinga & Lammers, 2009). Since “endogenous ethanol” has been postulated to be derived from this same microbial community (Zhu et al., 2013), then these peaks should be approximate temporally.

Notably, though, our ethanol peak, on both days, was consistently and considerably earlier than the hydrogen peak, suggesting that the source of the ethanol peak is unrelated to the direct impact of the food bolus residue entering the distal small bowel or colon. Both the source of this early peak and the absence of a later peak coinciding with the hydrogen peak were unexpected and not easily explained. However, the presence of an early peak has been appreciated by other breath researchers (Cope, Watson, Foster, Sehnert, & Risby, 2004b)(Smith, Spanel, & Davies, 1999). It is possible, therefore, that “gut derived” endogenous ethanol is actually from the relatively sterile stomach or proximal small bowel.

Finally, we note more variability in ethanol response compared to ammonia. Since alcohol dehydrogenase is inducible and differentially expressed in various tissue beds including the gastrointestinal tract and liver (Engeland & Maret, 1993), increased variability may be expected. It is also possible, therefore, that endogenous ethanol produced in the distal small bowel and colon are not measurable in breath due to rapid clearance and first pass metabolism in the liver. Our ethanol results are generally consistent with recent work that has explored the use of breath ethanol (often coupled with acetone) to determine blood glucose amongst diabetes (Table 3)*.*

Our study also has various limitations, including small sample size and single-center experience. As our monitors are unique prototypes, our results may not be generalizable. Unfortunately, this is a common problem for trace breath analysis research. Furthermore, we are not able to verify the exact source of either ammonia or ethanol in our study. Perhaps the most important limitation, though, is that neither exhaled breath ammonia nor ethanol has yet been linked to a clinical outcome of interest.

However, our study also highlights a key strength of breath analysis: the ability to non-invasively evaluate the individual’s response to a physiologic challenge. Each individual may therefore serve as his or her own control, and multiple data points can be easily obtained over several hours and testing days.

Another important strength of both our work and breath analysis in general is the capability to evaluate gut flora activity in real time through a combination of biomarkers. Naturally, each metabolite offers distinct information, but when combined they offer insights into digestion and metabolism not matched by other methods. In this instance, ammonia and ethanol represent by-products of protein and carbohydrate metabolism, respectively, while hydrogen serves as a reliable marker to time the passage of bolus through the gut. To our knowledge, no previous human breath study has measured these metabolites together in response to a physiologic challenge compared to a negative control day. We believe this approach; however, holds great promise and is timely given the worldwide effort to evaluate the impact of the gut microbiome on health and metabolism (Owyang and Wu 2014). Ongoing work will evaluate the differential response of breath ammonia to various kinds of proteins.

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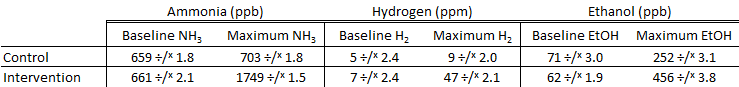


Table 1. Geometric mean (µ\*) times and divide (÷/x) multiplicative standard deviation (MSD). 95.5% confidence interval (95.5% CI) is calculated as µ\* ÷/x MSD. Ammonia (NH3) and ethanol (EtOH) are expressed in ppb. Hydrogen (H2) is expressed in ppm. Interaction of treatment and change from baseline to maximum was significant for NH3 (p<0.0001), H2 (P<0.0001), and EtOH (p=0.017). Calculation of hydrogen values excludes 2 participants (#1 and #2). Calculation of ethanol values excludes 6 participants (#4, #5, #21, #22, #28, #29).

Figure 1a. Breath ammonia (ppb) in control versus intervention groups. Interaction of treatment type and change from baseline to maximum was significant for NH3 (p<0.0001).

Figure 1b. Breath ethanol (ppb) in control versus intervention groups. Interaction of treatment type and change from baseline to maximum was significant for ethanol (p=0.017).

Figure 1c. Breath hydrogen (ppm) in control versus intervention groups. Interaction of treatment type and change from baseline to maximum was significant for hydrogen (p<0.0001).

Figure 2a. Breath ammonia (NH3) in ppb and hydrogen (H2) in ppm for control and intervention trials. N=30 for all NH3 measurements, N=28 for all H2 measurements. Open circle designates control trial NH3; closed circle, intervention trial NH3; open square, control trial H2; closed square, intervention trial H2. Arrow designates the time of intervention.

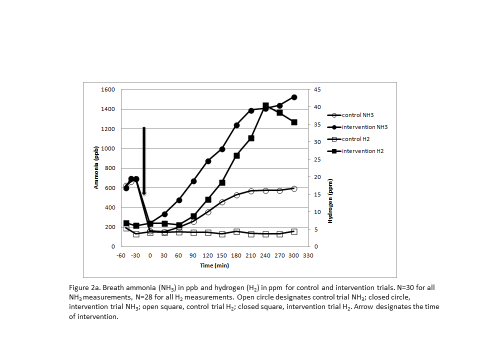
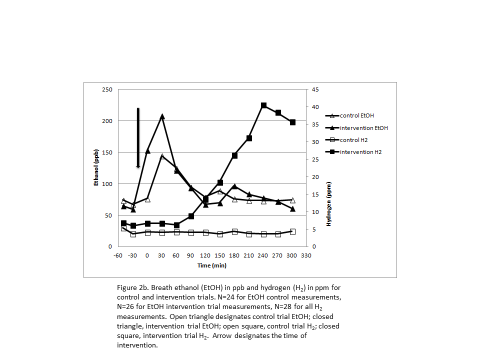


Figure 2b. Breath ethanol (EtOH) in ppb and hydrogen (H2) in ppm for control and intervention trials. N=24 for EtOH control measurements, N=26 for EtOH intervention trial measurements, N=28 for all H2 measurements. Open triangle designates control trial EtOH; closed triangle, intervention trial EtOH; open square, control trial H2; closed square, intervention trial H2. Arrow designates the time of intervention.



|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Intervention Trial Data | | | | | | Control Trial Data | | | | | |
| Sensitivity | | | | | | Specificity | | | | | |
| True positive rate | | | False negative rate | | | True negative rate | | | False positive rate | | |
|  | n | % | 95%CI | n | % | 95%CI | n | % | 95%CI | n | % | 95%CI |
| 300 ppb | 29 | 97 | 83-100 | 1 | 3 | 0.1-17 | 25 | 83 | 65-94 | 5 | 17 | 6-35% |
| 400 ppb | 27 | 90 | 73-98 | 3 | 10 | 2-27% | 28 | 93 | 78-99 | 2 | 7 | 1-22% |
| 500 ppb | 26 | 87 | 69-96 | 4 | 13 | 4-31% | 28 | 93 | 78-99 | 2 | 7 | 1-22% |
| 600 ppb | 23 | 77 | 58-90 | 7 | 23 | 10-42% | 30 | 100 | 88-100 | 0 | 0 | 0-12% |
| 1.5 x baseline | 26 | 87 | 69-96 | 4 | 13 | 4-31% | 26 | 87 | 69-96 | 4 | 13 | 4-31% |
| 1.8 x baseline | 23 | 77 | 58-90 | 7 | 23 | 10-42% | 29 | 97 | 83-100 | 1 | 3 | 0.1-17% |
| 2.0 x baseline | 22 | 73 | 54-88 | 8 | 27 | 12-36% | 30 | 100 | 83-100 | 0 | 0 | 0-12% |

Table 2. Table 2. Exploratory diagnostic application of breath ammonia levels (ppb). True positive (sensitivity) and false negative rates were calculated based on the intervention trial data, by counting those participants with increase in breath ammonia (maximum – baseline ammonia) that exceeded a pre-set threshold (positive result) and dividing by total number of participants (N=30). True negative (specificity) and false positive rates were calculated based on the control trial data by counting those participants with increase in breath ammonia (maximum – baseline ammonia) that did not exceed a pre-set threshold (negative result) and dividing by the total number of participants (N=30).95% CI, 95% confidence interval.

