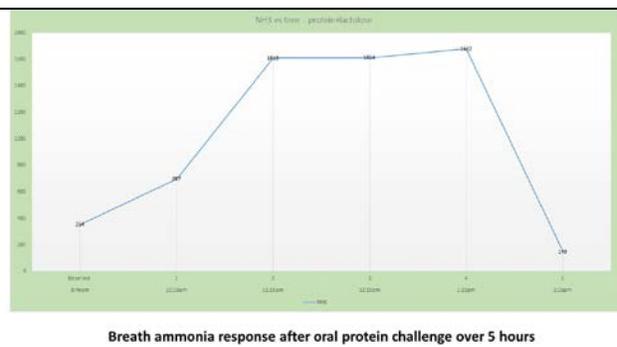


QCLs for Breath Analysis

Breath ammonia measurement is being used to evaluate protein metabolism in cirrhotic subjects. By assessing the response to an oral protein challenge and comparing results both to healthy subjects and blood amino acid profiles, we are demonstrating novel biology highly relevant to the care of cirrhotic patients.



Key project goals for supported project period from inception

Our Overall Hypothesis remains “minute variation in the level of breath ammonia will be an effective and robust biomarker for ammonia physiology”.

Breath ammonia has been measured in exhaled breath since the 1970’s. However, despite clear rationale for a better ammonia measurement method than blood assays and the promise of exhaled breath to fulfil this unmet need, breath research to date had little impact. By testing a fast and accurate QCL based Rice ammonia monitor in human subjects, our goal has been to establish that breath ammonia measurement can indeed become practical and meaningful.

Using the ammonia monitor provided by our colleagues at Rice University, we believe that we have convincingly demonstrated for the first time that breath ammonia reflects systemic ammonia. We made this demonstration by combining breath ammonia measurement with other breath measurements (hydrogen, acetone) in order to create novel and compelling breath metabolic profile. While used an oral high protein challenge in healthy subjects to make this initial demonstration, we are now using a more moderate protein challenge in order to include cirrhotic subjects. We are also evaluating complete amino acid profiles to better determine the source of the breath ammonia.

We believe ammonia physiology remains highly relevant and that that breath analysis is uniquely suited to the evaluation of nutrition physiology and metabolism. A key strength of this approach is the ability to evaluate an individual’s response to a physiologic challenge non-invasively. 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.

Project role in strategic plan

Our chief connection to the 10-year strategic plan of MIRTHE to provide a compelling rationale for the use of a QCL based laser in breath measurement. As ammonia is a highly reactive and difficult molecule to measure, the successful demonstration of a breath ammonia monitor would not only propel breath analysis and ammonia physiology research, but it would provide justification for the use of QCLs for other breath projects.

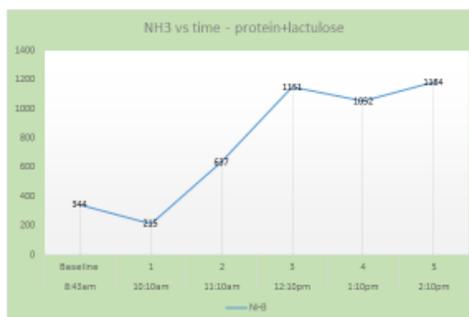
Summary of fundamental research barriers and methodologies to address them

Since inception, we have noted many engineering barriers to the accurate measurement of this volatile metabolite. Prior reports detail our successes overcoming these barriers. More recently,

we have found it challenging to prove that breath ammonia indeed reflects systemic ammonia because presently available ammonia measurement methods are poor and there are many unknowns. For example, little is known about the systemic ammonia response to a food challenge in healthy subjects, and even less is known about the ammonia response amongst various body compartments (e.g. intestinal, kidney, etc). Therefore, in the absence of a reference standard, it has been challenging to demonstrate that our human breath measurement is at once superior to existing measurement techniques (i.e. blood assays) and also physiologically meaningful.

Summary of achievements

Using the ammonia monitor provided by our colleagues at Rice University, we believe that we have convincingly demonstrated for the first time that breath ammonia reflects systemic ammonia. We made this demonstration by combining breath ammonia measurement with other breath measurements (hydrogen, acetone) in order to create novel and compelling breath metabolic profile. While used an oral high protein challenge in healthy subjects to make this initial demonstration, we are now using a more moderate protein challenge in order to include cirrhotic subjects. We are also measuring complete amino acid profiles to better determine the source of the breath ammonia.



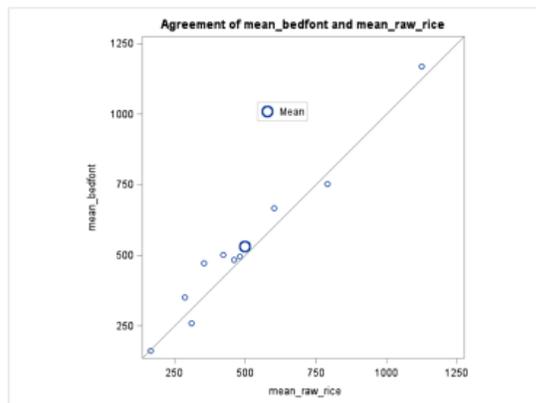
Breath ammonia response to an oral moderate protein challenge: Healthy Subject (left panel) vs Cirrhotic Subject (right panel). By demonstrating a difference between the response of healthy vs cirrhotic subjects to an oral protein meal, this research further supports our belief that breath ammonia reflects systemic ammonia. Furthermore, comparison of the breath results to blood amino acid profiles will provide a biological context and yield novel insights into ammonia physiology.

Goals abandoned or not achieved

Because it has been challenging to prove that breath ammonia reflects systemic ammonia (and is not, for example, merely derived from oral bacterial artefact), we have not rigorously pursued the evaluation of breath ammonia for hepatic encephalopathy. Since encephalopathy endpoints (e.g. psychometric tests, EEG, etc) are also subject to considerable error, such a clinical study would need to involve a large number of subjects in order to be adequately powered. We are, however, including one quantitative measurement of encephalopathy (the critical flicker frequency) in our moderate protein study above.

Description of related projects

In addition to continuing to measuring breath metabolic profiles, we are also comparing results from the Rice ammonia monitor to a non-QCL based ammonia monitor provided by a commercial partner (Bedfont Scientific). Because the Bedfont monitor is smaller, more portable, and easier to use, a successful demonstrate will enable greater clinical research opportunities.



Breath ammonia measurement comparing the QCL-based Rice monitor vs a non-QCL based monitor.

Because multi-monitor studies are rare in trace breath research, we believe this work is significant as it builds confidence in the output of both monitors. Further, because the Bedfont monitor is small, more portable, and easier to use, it enables greater clinical research opportunities.

Plans for the next year

We will finish the oral protein study in cirrhotics. Will also complete our analysis of the Rice vs Bedfont monitors. We also anticipate delivery of prototypes from Thor Labs for testing and additional clinical study.

Member company and practitioner benefits

We are collaborating with Thor Labs (Newton, NJ) on the design and testing of additional QCL based monitors. Our collaboration involves routine onsite visits as well as phone conferences. Thor Labs has provided support to keep our breath infrastructure operational.

Commercialization activities and efforts

Bedfont Scientific (Maidstone, Kent, UK) remains interested in the potential to commercialize breath ammonia measurement. to compare their breath ammonia monitor with the Rice ammonia monitor. The Bedfont monitor is much smaller and more portable than the Rice monitor, but appears to be equally capable in terms of accuracy and speed. We are presently comparing this monitor to the Rice standard.

Names of MIRTHE team members involved with the project in Year 10

MIRTHE faculty:

Steven Solga, PI, St. Luke's University Hospital
Terence Risby, Co-PI, Johns Hopkins University
Lisa Spacek, MD, PhD, Co-PI St. Luke's University Hospital

Other staff (post-docs, visitors, etc.):

Arthur Strzepka, Research Assistant. St. Luke's University Hospital

Industry and practitioner participants:

Eric Geoffrion, General Manager, Thor Labs, Member
Marshall Scott, Engineer, Thor Labs, Member

Trevor Smith, Managing Director: Bedfont Scientific: Portable Ammonia and Hydrogen Monitor

References:

Solga Steven F, Mudalel M, Spacek LA, Lewicki R, Tittel F, Loccioni C, Russo A, Risby TH. *Factors influencing breath ammonia determination.* J Breath Res. 2013 Jun 18;7(3):037101

Solga, Steven F, Mudalel M, Spacek LA, Risby TH. *Fast and Accurate Exhaled Breath Ammonia Measurement.* J Vis Exp. 2014 Jun 11; (88).

Solga Steven F, Mudalel M, Spacek LA, Lewicki R, Tittel FK, Loccioni C, Russo A, Ragnoni A, Risby TH. *Changes in the Concentration of Breath Ammonia in Response to Exercise: A Preliminary Investigation* J Breath Res. 2014 Sep;8(3):037103

Lisa Spacek, Matthew Mudalel, Frank Tittel, Rafal Lewicki, Terence Risby, Jill Stolfus, Solga Steven F. *A High Protein Challenge Increases Gut-Derived Exhaled Breath Ammonia.* Biomarkers (2015) Mar;20(2):149-56.

Lisa A. Spacek, Matthew Mudalel, Frank Tittel, Terence H. Risby, Steven F. Solga. *Clinical utility of breath ammonia for evaluation of ammonia physiology in healthy and cirrhotic adults.* J Breath Res. 2015 Dec 14;9 (4):047109