

Breath ammonia levels in a normal human population study as determined by photoacoustic laser spectroscopy

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Abstract

Photoacoustic laser spectroscopy was used as a technique to measure real-time levels of ammonia in exhaled human breath in a small, locally recruited, normal healthy population (n=30). This yielded an average level of breath ammonia of 265 ppb, ranging from 29 to 688 ppb. Although average levels were marginally higher in male volunteers, this was not statistically significant. In addition, no correlation could be found between age, body mass index, or breath carbon dioxide levels. Monitoring of the daily routine of two individuals showed a consistent decrease in oral breath ammonia concentrations by the early afternoon (post-prandial), but was followed by a gradual increase towards late afternoon. However, in a comparison of oral and nasal breath in two volunteers, nasal breath ammonia levels were found to be significantly lower than oral levels. In addition, the daily variation was only seen in oral rather than nasal measurements which may indicate that significant background levels are predominantly of oral origin and that nasal sampling is the preferred route to eradicate this background in future studies. These results provide a healthy human breath ammonia baseline upon which other studies may be compared.

Key Words

Human breath, diagnostics, volatile compound, ammonia, photoacoustic spectroscopy

1. Introduction

There are at least 1,000 trace volatile compounds (VCs) in human breath [1]. Furthermore, VCs found in human breath have been linked to various physiological conditions. For example, nitric oxide is indicative of pulmonary dysfunction, acetone has been linked to diabetes, and pentane is suggestive of lipid peroxidation [2]. Hence, understanding the correlation between breath compounds and the condition of the human body has attracted increasing interest in clinical diagnostics.

Among breath compounds, ammonia has proven to be an important trace gas. It is a key component of the nitrogen cycle which results from protein metabolism and arises naturally from metabolic processes in the stomach and intestines [3]. When the liver and/or kidneys are not functioning correctly, ammonia found in blood cannot be filtered out of the body properly. The increased levels due to liver dysfunction make it possible for hyperammonaemia to develop causing neurological disorders such as hepatic encephalopathy [3]. Where the kidneys are not operating efficiently, conditions such as uraemia [4], acidosis, and edema [5] may also develop. Hence, focus on measurement of ammonia levels in the blood is of great value to those with potential liver or kidney disorders. Any excess ammonia that has not been filtered out of the body is capable of diffusing into the lungs and can be exhaled in breath [6]. Breath ammonia levels have been correlated with blood urea ($R^2=0.95$) and creatinine levels ($R^2=0.83$) using optoacoustic spectroscopy [7]. However, development of technologies that are capable of measuring ammonia in breath has proven to be a challenging task.

Breath is a complex matrix where flow rate, temperature, humidity, and gas concentration are multifarious, time-dependent variables. The goal is to isolate the particular VC signal from all other molecular interferences that can arise in the stream of breath. In an attempt to gain a deeper understanding of how conditions in the human body correlate with the constituents of breath, techniques such as chemical ionisation, gas chromatography, laser spectroscopy, and chemical sensing have been used. However, there is still a lack of data in this area due to the limited number of instruments available which are further deterred by their individual limitations. Instruments based on chemical ionisation can detect breath ammonia as low as 10 ppb, but due to complexity are still in the development of a more portable version [8]. Gas chromatography is

capable of quantifying breath ammonia at levels of 14 ppt, but the need to pre-collect the samples removes the possibility for real-time analysis [9]. Laser spectroscopy can reach 10 ppb, although the differentiation of molecules that fall into categories of similar frequency ranges and spectral regions can prove difficult [10]. Chemical sensing methods are capable of detecting 18 ppb, but condensation from breath can decrease the accuracy of readings [11]. Due to such drawbacks, a need for additional research is necessary for more conclusive comparisons to be made. Recently, a form of photoacoustic laser spectroscopy has been developed specifically for the real-time monitoring of trace gases in breath. Using this technique eliminates the need for pre-collection which removes the potential of contamination or loss of sample. Furthermore, by using high laser strength, increasing the intensity of photoacoustic signals received from chosen gas molecules, and reducing noise signals from the adjoining interfering gases, the sensitivity of a photoacoustic instrument is usually orders of magnitude higher than conventional measurement technologies [12]. However, it must be acknowledged that specificity errors can arise where impeding molecules of similar gas species are absorbed from the background. When attempting to detect gaseous ammonia, photoacoustic systems can typically use a wavelength of 1531.68 nm [13].

The work here outlines the application of photoacoustic spectroscopy to the analysis of a small cohort of healthy human volunteers to determine baseline ranges of ammonia concentration. This was also correlated against other biometric data including gender, age, body mass index (BMI), and breath carbon dioxide levels since previous studies have suggested that a relationship may exist among healthy people. The effect of sampling route (nasal vs. oral) and daily routine (post-prandial) was also investigated. Oral was examined more extensively in this preliminary work since it has shown a strong correlation with blood ammonia levels in previous literature. However, a quick nasal study was performed to see if it may bear more potential for future works.

2. Experimental Procedures

A breath sample was defined as a relaxed full exhalation from the volunteer after a relaxed full inhalation. Over a period of ten minutes, breath measurements were made using the NephroluxTM photoacoustic laser ammonia analyser (Pranalytica Inc., CA, USA) (Fig. 2.1). The NephroluxTM



Figure 2.1. Breath ammonia measurement using photoacoustic spectroscopy. The typical procedure for volunteer breath collection using the NephroluxTM.

underwent a multipoint ammonia calibration during development at Pranalytica which generated a strong R^2 of 0.9986. Photoacoustic spectroscopy (PAS) is a well-known technique that has the advantage of being relatively simple while still capable of high sensitivity detection. Previous literature has explained the process of PAS in depth [12][14]. To summarise, every 41 seconds, the system pulled a breath sample into the photoacoustic chamber by way of a vacuum flow. As ammonia (NH_3) and carbon dioxide (CO_2) breath gases entered the photoacoustic chamber of the NephroluxTM, the tunable CO_2 -laser [15] excited the gas molecules. The light energy used for absorption was then converted to heat [7]. Pressure from this temperature change was detected by the chamber microphone during the de-excitation process [16]. The photoacoustic signal, S , provided a magnitude in arbitrary units displayed as:

$$S = S_m P C \alpha \quad (1)$$

where S_m is the sensitivity of the microphone (in units volts per pascal), P is the power of incident laser radiation (in units of watts), C is a constant for the cell-geometry, measurement conditions, and modulation frequency (in units of pascal centimetres per watt), and α is the

absorption cross-section of the transition being interrogated (in units of inverse centimetres) [13]. The CO₂-laser used by the NephroluxTM is tuneable for gas selectivity allowing for ammonia detection as low as 1 ppb \pm 10% and carbon dioxide down to 0.1% \pm 5% (of reading) to generate results within 120 seconds. Once the breath was analysed by the signal processor, the data was transmitted through the single-board computer system of the NephroluxTM and saved to an external lap-top (Fig. 2.2).

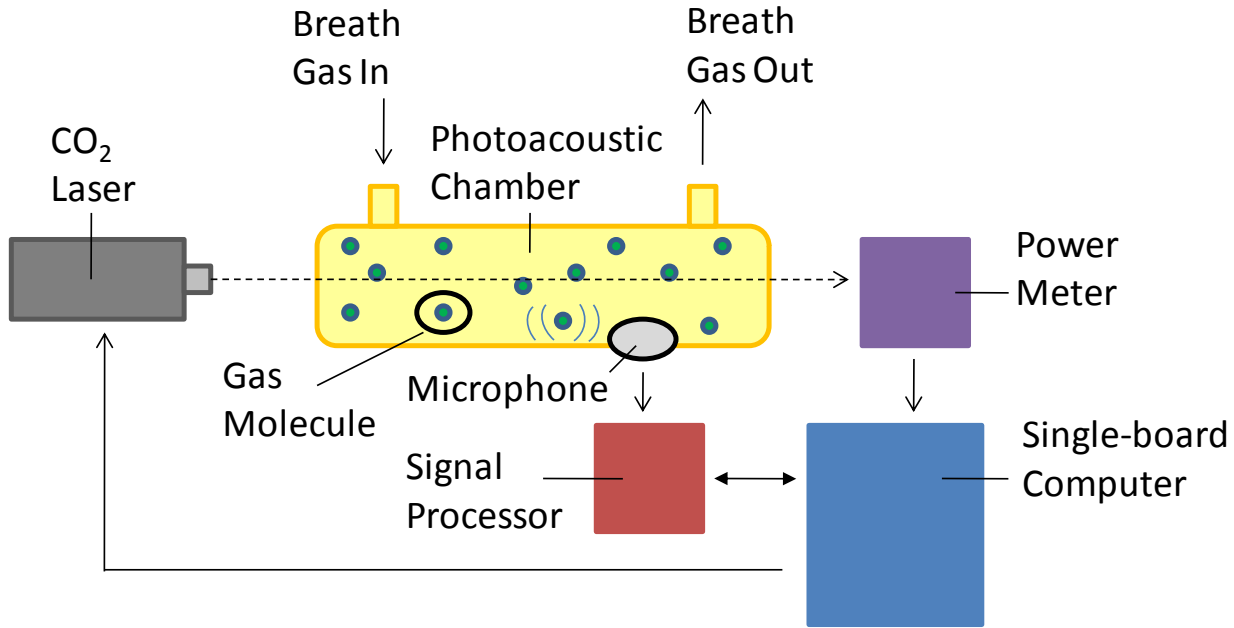


Figure 2.2. Ammonia gas molecule measurement using photoacoustic spectroscopy. Schematic of the NephroluxTM system. The CO₂ laser enters the photoacoustic chamber and excites the gas molecules. As de-excitation occurs, the pressure is released and detected by the microphone where it is transmitted to the signal processor [16]. Figure reproduced from reference [17].

A cohort of 30 normal healthy human volunteers was locally recruited following institutional ethical approval. Testing was carried out over a period of approximately six months. Following a questionnaire, only those with no breathing abnormalities or related medical conditions were entered into the study population. Gender, ethnicity, age and BMI were recorded. For breath sampling, volunteers were asked to sit in a relaxed position while breathing into a face mask which was connected to a breath delivery tube. All volunteers were asked to breathe using either oral or nasal respiration. Furthermore, each breath sample was from a relaxed full exhalation where breath concentrations were averaged. For the purpose of clinical analysis, it is useful to define whether whole breath averaged concentrations or peak alveolar concentrations are being

observed. When focusing specifically on the alveolar concentrations, the compounds exclusively from within the lungs are monitored. With this study, however, whole breath examination took place which provided samples consisting of both the alveolar region and dead space (e.g. trachea) within the respiratory system [18]. Previous literature has described ammonia as being a sticky substance which could potentially adhere to the walls of the system [13]. To prevent this adsorption, the tubing of the NephroluxTM was constantly heated while breath consistently flowed into the system. Each sample was an average of five breath measurements.

A single male and female volunteer were studied for five consecutive days to observe daily variation in their oral ammonia breath concentrations. The volunteers fasted from midnight to 10:00 am at which point they were sampled. They were again sampled at 13:00 pm (post-prandial), and at 16:00 pm following a normal daily routine. In addition, a single volunteer provided three repetitions of nasal and oral breath on two separate occasions.

3. Results and Discussion

Oral Breath Ammonia and Statistical Confidence

The survey population had a mean age of 34. There were 19 female and 11 male volunteers, and the average body mass index was 25.18 kg/m². Of these volunteers, 20 identified themselves as ethnically Irish, with several Europeans, two Americans and one Asian. The biometric data, mean oral breath ammonia, and mean oral breath carbon dioxide concentrations from the 30 volunteers are compiled in Table 3.1. The mean breath ammonia range was calculated by averaging five breath samples per individual. The mean oral breath ammonia of the 30 volunteers was 264.9 ppb with a standard deviation of ± 182.8 ppb while the inter-individual (i.e. averaged five breath samples) deviation was ± 43 ppb. Mean oral breath ammonia concentrations recorded from this test population ranged from 28.8 ppb to 687.8 ppb.

Previous literature has shown that oral breath ammonia ranges for healthy populations of different sample sizes can vary. Research using selected ion flow mass spectrometry (SIFT-MS) observed oral breath ammonia concentrations of 220 ppb to 550 ppb from a population of five healthy volunteers [19], and 200 ppb to 1800 ppb from a separate cohort of six volunteers [20]

whereas, a larger group of 30 volunteers produced an oral breath ammonia range of 248 ppb to 2935 ppb [21], and another of 200 volunteers gave 112 ppb to 2865 ppb [22]. Understanding the significance of the sample size is helpful in relating the resulting breath ammonia ranges to the

Table 3.1. Compilation of biometrics, mean oral breath ammonia, and mean oral breath carbon dioxide concentrations for 30 volunteers (mean = average of five breath samples per person).

Volunteer (number)	Gender (M/F)	Age (years)	Body Mass Index (kg/m ²)	Mean NH ₃ (ppb)	NH ₃ Standard Deviation (ppb)	Mean CO ₂ (%)	CO ₂ Standard Deviation (%)
1	Female	23	24.08	157	11	3.1	0.2
2	Male	29	23.10	458	84	3.1	0.5
3	Female	27	17.51	397	24	2.8	0.3
4	Male	33	25.83	308	35	3.2	0.9
5	Female	30	20.81	59	4	3.2	0.2
6	Female	58	28.38	194	9	3.7	0.3
7	Female	54	23.83	283	43	3.2	0.4
8	Male	41	28.96	264	44	2.1	0.2
9	Female	33	N/A	688	42	2.9	0.5
10	Male	25	29.35	237	27	2.9	0.5
11	Female	46	35.02	503	111	2.4	0.1
12	Male	33	31.08	356	68	3.5	0.9
13	Female	26	35.15	326	28	3.2	0.3
14	Female	27	24.38	89	10	3.3	0.4
15	Female	43	36.23	442	99	2.7	1.2
16	Female	51	28.47	244	61	2.4	0.5
17	Female	N/A	21.97	57	6	2.5	0.4
18	Male	36	20.33	327	29	2.1	0.5
19	Female	26	20.81	118	22	2.5	0.8
20	Female	30	21.22	421	47	2.3	0.5
21	Female	38	26.49	99	16	3.5	0.5
22	Female	44	18.28	104	13	2.9	1.2
23	Female	45	20.38	467	52	2.8	1.0
24	Male	38	20.76	84	24	3.4	0.6
25	Male	25	22.86	81	41	2.2	0.9
26	Male	23	22.86	448	238	2.6	1.1
27	Male	24	24.17	598	58	2.3	0.5
28	Male	27	30.93	29	21	3.8	0.2
29	Female	30	24.42	45	6	2.8	0.2
30	Female	28	22.68	67	8	2.6	0.5

rest of the human population. In other words, larger sample sizes are more likely to generate results that are closely related to the target population as well as produce a smaller margin of error [23]. In population statistics in which a sample is used, a measure of confidence is needed in order to know if the sample results can be related to the larger population. Assuming that the sample studied here was not statistically abnormal, it is safe to claim that the results of a confidence interval calculation for the sample will be true 95% of the time [24]. In calculation of the confidence interval for the given sample size and oral breath ammonia concentrations, it can be claimed that there is 95% confidence that the interval of 196.7 ppb to 333.2 ppb contains the true mean of the local population. There was a standard error of 33.3 ppb as well as a skewness of 0.48 and a kurtosis of -0.70. It should be recognised that the population sample ($n=30$) examined in this study was of a small group where metabolic ammonia concentrations could be related to local factors such as ethnicity, diet and localised environmental factors, and may result in a mean and range distinct from those of other research.

Oral Breath Ammonia and Gender

The 19 female volunteers ranged from a mean minimum of 45 ppb to a mean maximum of 688 ppb, with an average of 250 ± 189 ppb. The 11 male volunteers had a mean minimum of 29 ppb and a mean maximum of 598 ppb, with an average of 290 ± 177 ppb showing that the female standard deviation was slightly higher than that of the males. To compare the breath ammonia levels of females against males, a box plot analysis was used (Fig. 3.1). From the box plot, the oral breath ammonia of females and males combined gave a median of 254 ppb where the upper quartile ($Q3$) was 415 ppb and the lower quartile ($Q1$) was 91 ppb. The upper quartile represents the median of the top half of the data set. Hence, the upper 25% of the total ammonia concentrations exist between $Q3$ and the Max Limit. The lower quartile is indicated by the median of the bottom half of the box data. This area between $Q1$ and the Min Limit contains the lower 25% of the total concentrations. The breath ammonia of the females was skewed towards a lower median of 194 ppb ($Q1 = 94$ ppb, $Q3 = 409$ ppb) while the males leaned more towards a higher median concentration of 308 ppb ($Q1 = 160$ ppb, $Q3 = 402$ ppb). Further comparison of the ammonia levels between females and males via a t -test indicated a p -value of 0.57. For the t -test, a null hypothesis was used stating that the two populations will produce similar breath ammonia, and any differences were due to chance. With no predictions or expectations of a

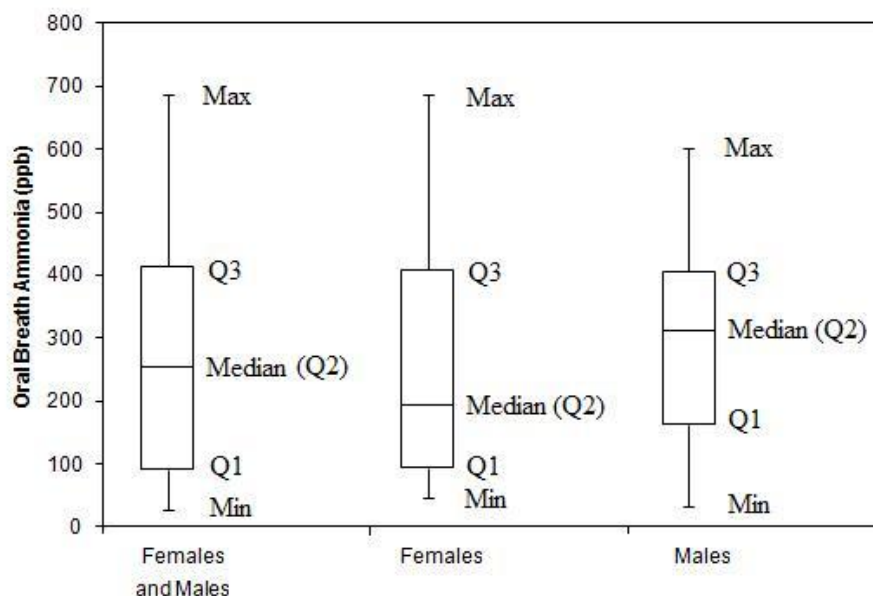


Figure 3.1. Box plot comparison of mean oral breath ammonia levels from female and male populations. Centre line shows median, box shows upper and lower quartiles, and whiskers give maximum and minimum range. Total sample population, n=30 total; n=11 male, n=19 female.

difference being made, a two-tailed p -value was sought. Furthermore, since the two variables were independent of each other and a slightly different standard deviation was observed, an unequal type-2 measurement took place. Since the resulting p -value was greater than 0.05, there was no significant difference between the female and male breath ammonia concentrations of this cohort. Two other studies from literature using selected ion flow tube mass spectrometry (SIFT-MS) also provided comparisons between oral breath ammonia and gender. In one, 95 females were compared against 105 males. The concentrations of breath ammonia were found to be significantly different ($p = 0.004$) between the two genders [22]. In contrast, the other study involving 11 female and 19 male subjects between showed no significant difference ($p = 0.98$) among the genders [21]. The fact that the cohort sizes of these studies were quite different from each other must be acknowledged. Such differences make comparisons difficult and imply that more research is necessary in this area.

Oral Breath Ammonia and Oral Breath Carbon Dioxide

The relationship between oral breath ammonia and oral breath carbon dioxide was also studied (Fig. 3.2). Mean CO_2 levels were recorded as being between 2.1% and 3.8% among the 30

volunteers with an average of $2.9 \pm 0.5\%$. Literature has shown that the approximate amount of CO_2 found in exhaled oral breath can reach as high as 4.5% [25], but this concentration can differ from individual to individual resulting in a high level of variation [26]. A correlation coefficient of 0.08 indicated an absence of a correlation ($p=0.12$). With a lack of literature comparing human oral breath ammonia against oral breath carbon dioxide, comparisons with previous breath ammonia work could not be made. However, similar comparisons based on the blood work of 17

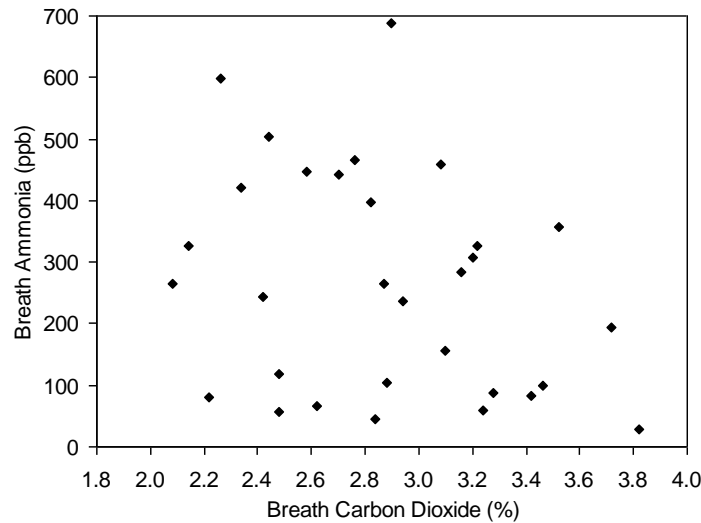


Figure 3.2. Relationship between mean oral breath carbon dioxide (n=5) and mean oral breath ammonia (n=5) in a population of 30 normal healthy human volunteers.

volunteers with hepatic cirrhosis have shown that inhalation of CO_2 up to five percent led to a decrease in blood NH_3 [27]. When CO_2 concentrations in blood decrease, the blood's pH increases possibly indicating that metabolic alkalosis has taken place. In such instances of lower CO_2 , renal venous blood NH_3 concentrations are expected to increase as well [28]. Since it has been stated in previous works that high concentrations of blood NH_3 are capable of diffusing into the lungs [6], perhaps future work could be performed comparing the link between exhaled NH_3 and CO_2 respiration.

Oral Breath Ammonia and Age

The correlation between mean oral breath ammonia and age was examined (Fig. 3.3). The sample age range was between 23 and 58 years with an average of 34 ± 10 years. It is believed that liver volume and portal blood flow decrease in function with increasing age [29]. The

correlation was found to be insignificant given an R^2 of 0.004 ($p=0.74$), suggesting that no link between breath ammonia and age could be determined from this population. Several volunteers with ages between the 20's and 40's appear to have above average ammonia levels while those over 50 years all had ammonia levels close to or below the mean level. Previous literature based on SIFT-MS have shown the coefficient of determination (R^2) between oral breath ammonia and age to be 0.18 over an age range of 7 to 18 years ($n=200$) [22], approximately 0.25 for an age range of 24 to 59 years ($n=30$) [21], and 0.50 among an age range between 4 and 83 years ($n=17$)

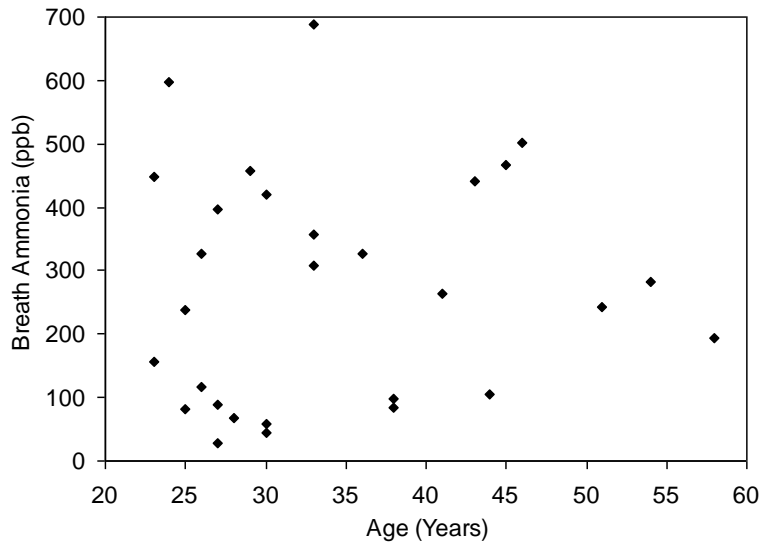


Figure 3.3. Correlation between age and mean oral breath ammonia ($n=5$) among a population of 29 volunteers.

[30] indicating that a small relationship may exist for those populations. Other techniques focusing on spectrophotometric examination of exhaled breath condensate (ages 23 to 79 years) gave results similar to ours which indicated that age has no effect on ammonia levels [31].

Oral Breath Ammonia and Body Mass Index

Mean oral breath ammonia levels were compared against body mass index (Fig. 3.4). Volunteer BMIs ranged between 17.51 kg/m^2 and 36.23 kg/m^2 with an average of $25.18 \pm 5 \text{ kg/m}^2$. The correlation was insignificant given an R^2 of 0.04 ($p=0.28$), suggesting that no link between breath ammonia and body mass index could be determined from this population. Previous publications using SIFT-MS technology have shown the coefficient of determination (R^2) between oral breath ammonia and BMI to be 0.004 for a range of 18.4 Kg/m^2 to 30.6 Kg/m^2 ($n=30$) [21], and 0.08 for

a range from approximately 13 Kg/m² to 33 Kg/m² (n=200) [22] further emphasising the lack of significance between the two variables.

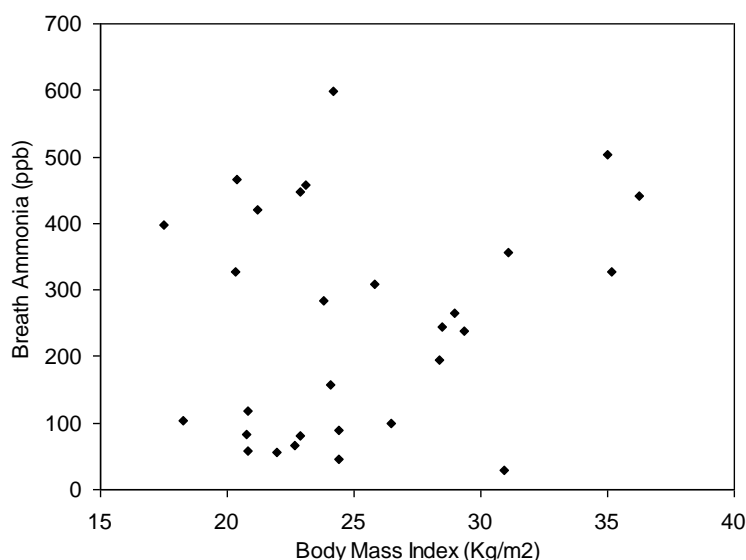


Figure 3.4. Relationship between body mass index and mean oral breath ammonia (n=5) from a population of 29 volunteers.

Daily Variation in Oral Breath Ammonia Levels

The variation in oral breath ammonia levels were monitored over an eight hour working day for five consecutive days in both a male and female volunteer. Ammonia levels were measured at 10:00 h, 13:00 h (post-prandial) and 16:00 h. In both studies, there was a drop in breath ammonia from the morning measurement to the mid-day measurement and then a progressive increase as the day continued (Fig. 3.5). Over the period of five days, the female volunteer expressed a mean breath ammonia of 205 ±12 ppb in the morning, 74 ±41 ppb at 13:00 h, and 202 ±152 ppb by the afternoon. The male volunteer generated a mean breath ammonia of 414 ±35 ppb in the morning, 275 ±95 ppb at 13:00 h, and exhaled 702 ±110 ppb by the afternoon. At 13:00 h, the ammonia level of the male decreased by 119 ppb while the female breath ammonia dropped by 111 ppb. Previous literature states that this decrease in ammonia may be a result of ingestion of food since the liver has a tendency to increase portal blood flow when eating [20]. As for the increase in ammonia afterwards, the male showed a difference of 439 ppb and the female changed by 140 ppb. This shows a significant increase, but the absolute value is still low. Literature has also proposed that such an increase may have been a result of purine nucleotide deamination and

amino acid catabolism that occur during the physical processes that take place over the day [32]. Furthermore, protein consumption is a major source of ammonia within the body due to the fact

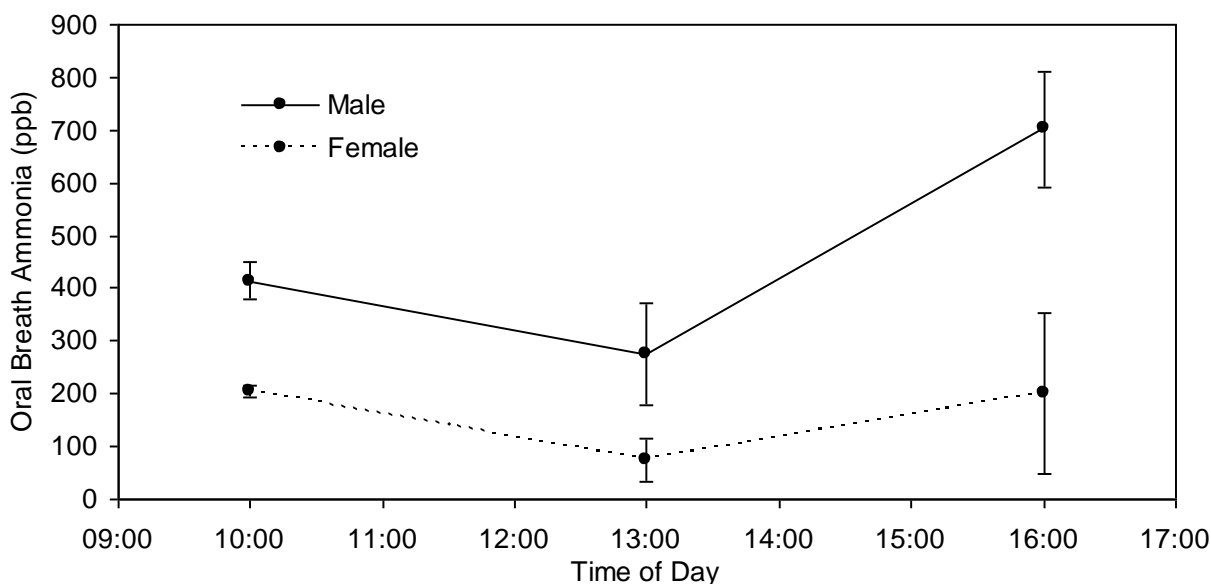


Figure 3.5. Comparison of the daily variation in oral breath ammonia for a male and female volunteer averaged over a period of 5 days. Volunteers fasted for 12 h until 10:00 h, and were tested again at 13:00 h, post-prandial. At 16:00 h, the volunteers gave further breath samples following their regular daily routine.

that it is broken down into its nitrogenous structural components via proteolysis [3]. Hence, the larger amount of proteins ingested by the male could be one factor related to the greater increase of breath ammonia he displayed.

Oral Breath Ammonia and Nasal Breath Ammonia

Three comparisons were made between the mean oral and mean nasal breath ammonia concentrations in two volunteers. The comparisons were made following a similar timeline to the previous daily variation section. Oral breath ammonia concentrations were consistently higher than those of nasal from both volunteers. For volunteer one, morning nasal exhalations (Fig. 3.6) gave a mean ammonia concentration of 38 ± 9 ppb while oral exhalations were 210 ± 24 ppb. Towards mid-day (post-prandial), the mean nasal breath ammonia level decreased to 26 ± 2 ppb and the mean oral breath ammonia dropped to 64 ± 4 ppb. Measurements taken in the afternoon displayed an increased mean nasal ammonia concentration of 57 ± 6 ppb and mean oral ammonia at 553 ± 75 ppb.

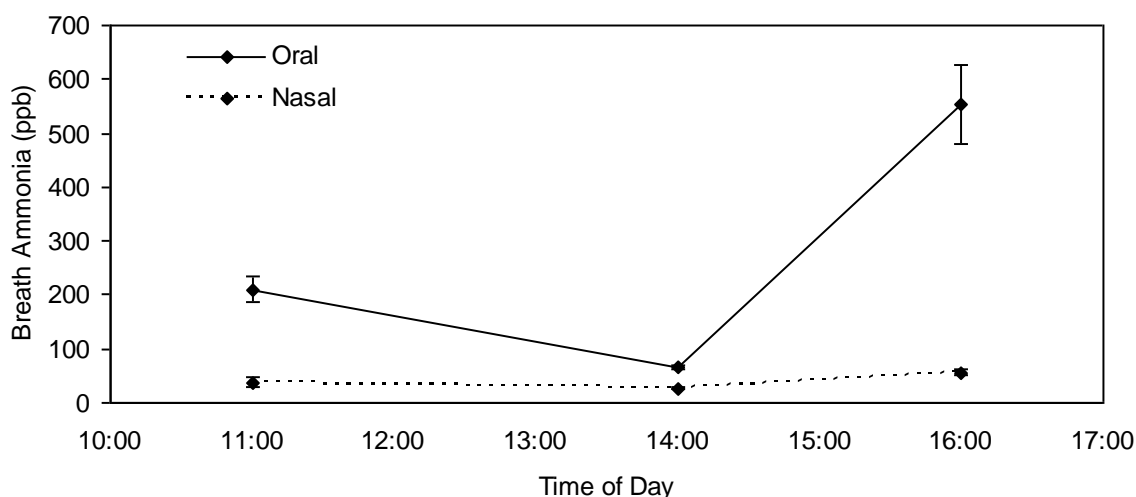


Figure 3.6. Comparison between the mean oral and mean nasal breath ammonia concentrations from volunteer one (n=5). Breath samples were taken in the morning (11:00 h), post-prandial (14:00 h), and in the afternoon (16:00 h).

From volunteer two (Fig. 3.7), the mean nasal breath ammonia concentration in the morning was 47 ± 17 ppb along with an oral concentration of 246 ± 25 ppb. By mid-day (post-prandial), the ammonia concentrations did not vary as much as those of volunteer one. The mean nasal breath ammonia recorded was 68 ± 9 ppb while the mean oral ammonia was 253 ± 29 ppb. Similar to volunteer one, measurements from the latter part of the day were higher.

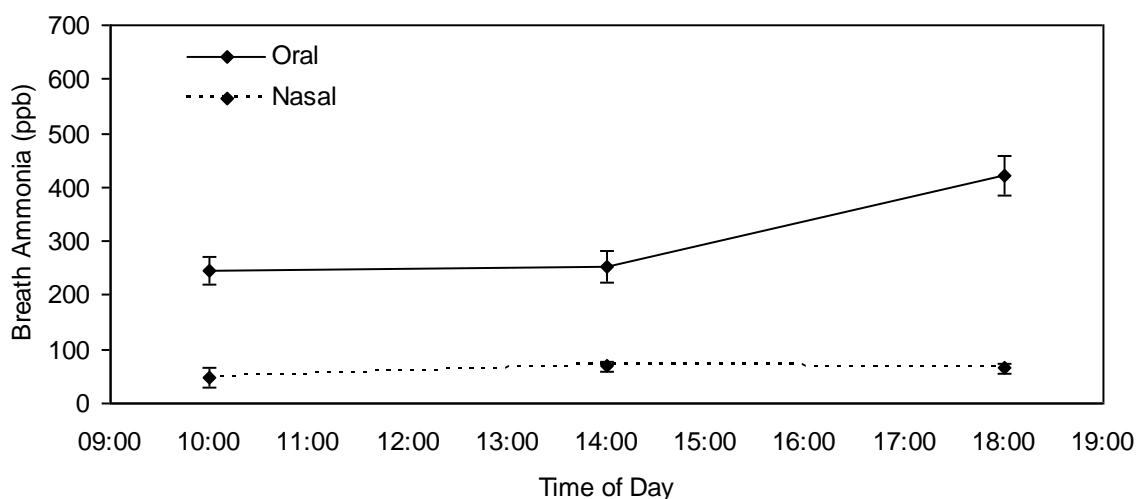


Figure 3.7. Comparison between the mean oral and mean nasal breath ammonia concentrations from volunteer two (n=5). Breath samples were taken in the morning (10:00 h), post-prandial (14:00 h), and in the evening (18:00 h).

By the evening, volunteer two emitted a nasal ammonia concentration of 65 ± 9 ppb and an oral of 420 ± 36 ppb. This display of nasal breath ammonia concentrations being lower than oral is similar to the results in other studies where measurement from nasal exhalations was shown to be preferable over oral. It was stated that a possible reason for the higher oral ammonia levels was due to bacteria and other compounds that reside there since bacteria are known to produce ammonia and are readily found in the saliva [33]. Furthermore, this may provide some reasoning as to why the standard deviation of the oral ammonia is so much larger than that of the nasal.

4. Conclusions

Photoacoustic laser spectroscopy has the potential to be a viable tool for monitoring real-time concentrations of ammonia in human breath. Being able to extract an ammonia signal from flowing breath samples provided the advantage of collecting large amounts of data in a short period of time. Furthermore, the sensitivity and accuracy were highly supportive of the results found in previous literature. In a small cohort of 30 healthy human volunteers, breath ammonia concentrations were found to range from 29 ppb to 688 ppb which was broadly in line with other studies. However, no correlation between oral breath ammonia and oral breath carbon dioxide, gender, age or BMI was established. While monitoring daily routines, breath ammonia levels were shown to decrease until mid-day (post-prandial), but were followed by a large increase into the afternoon. The large variation in baseline levels was originally believed to be a result of a number of confounding factors such as diurnal variation, inter-individual variation, food intake and metabolic activity, and the presence of significant levels of ammonia in oral breath from bacterial sources which would serve to obscure more subtle relationships. However, a comparison between nasal and oral breath ammonia appeared to imply that confounding factors may mostly exist in oral breath. Oral ammonia was consistently and significantly higher than nasal which may be pointing to the significance of oral bacterial contributions of ammonia as mentioned by *Smith, et al.* [33] in previous literature. If so, this suggests that more appropriate ways of sampling are needed to avoid this unwanted contribution. Nasal breath sampling may assist with this. However, if monitoring of patients is the goal, issues of nasal congestion and comfort will need to be explored as well. More extensive studies with larger patient cohorts using nasal monitoring may add clarity to this preliminary study.

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