The origin of mouth-exhaled ammonia

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Abstract. It is known that the oral cavity is a production site for mouth-exhaled NH_3. However, the mechanism of NH_3 production in the oral cavity has been unclear. Since bacterial urease in the oral cavity has been found to produce ammonia from oral fluid urea, we hypothesize that oral fluid urea is the origin of mouth-exhaled NH_3. Our results show that under certain conditions a strong correlation exists between oral fluid urea and oral fluid ammonia (NH_4^++NH_3) (r_s=0.77, p<0.001). We also observe a strong correlation between oral fluid NH_3 and mouth-exhaled NH_3 (r_s=0.81, p<0.001). We conclude that three main factors affect the mouth-exhaled NH_3 concentration: urea concentration, urease activity and oral fluid pH. Bacterial urease catalyses the hydrolysis of oral fluid urea to ammonia (NH_4^++NH_3). Oral fluid ammonia (NH_4^++NH_3) and pH determine the concentration of oral fluid NH_3, which evaporates from oral fluid into gas phase and turns to mouth-exhaled NH_3.

1. Introduction

Ammonia in the human body originates mainly from the metabolism of diet protein and is converted to urea in the liver [1]. The average pK_a value of ammonia in blood and water is 8.95 at 37 °C [2], which is higher than the pH value of physiological fluids, ranging from 4.5 to 8.0 [3]. Hence, ammonia is present mostly in the ammonium ion (NH_4^+) form, and only a small fraction is in the ammonia molecule (NH_3) form. In this text, we use ammonia as a general term to represent both forms (NH_4^++NH_3), except for further notification. The normal blood ammonia concentration is 11–50 μmol/L [4]. Since ammonia passively diffuses from blood to both salivary and sweat glands, it can be detected in oral fluid and sweat [5,6]. In addition to body fluids, NH_3 has been detected in exhaled breath using various methods, including selected ion flow tube mass spectrometry [7], ion mobility spectrometry [8], cavity ring-down spectroscopy [9], and photoacoustic spectroscopy [10].

In some previous studies, a positive correlation was observed between plasma NH_4^+ and mouth-exhaled NH_3 in patients with hepatic diseases [11,12]. Given such results, a logical conclusion would be that mouth-exhaled NH_3 originates from blood ammonia, based on the gas exchange between blood and the air in the alveoli [13,14]. However, recent studies found no correlation between plasma NH_4^+ and mouth-exhaled NH_3 in either hepatic disease patients or healthy people [9,15]. Therefore, it is unclear whether mouth-exhaled NH_3 reflects the systemic ammonia level directly. It has been proven that the mouth-exhaled NH_3 concentration is higher than the nose-exhaled NH_3 concentration [9,16-18], indicating that in addition from the alveoli, mouth-exhaled NH_3 also originates from the oral cavity. Smith *et al* further demonstrated that the NH_3 concentration in the oral cavity during breath

holding is correlated to the mouth-exhaled NH_3 concentration [17], implying that mouth-exhaled NH_3 is dominantly generated in the oral cavity.

Exhaled breath NH_3 has been proposed as a non-invasive biomarker in several different clinical applications. It has been found that mouth-exhaled NH_3 is statistically significantly correlated to blood urea in chronic kidney disease patients during haemodialysis [7,8,19-21]. Additionally, elevated mouth-exhaled NH_3 concentrations were detected in *Helicobacter pylori* infected patients after urea ingestion [22]. Furthermore, ammonia gas in the oral cavity has been proposed as a useful tool to assess halitosis [23]. In light of these potential clinical applications, we feel that it is essential to understand the mechanism of mouth-exhaled NH_3 production in detail. To investigate the mouth-exhaled NH_3 production, we decided to measure ammonia simultaneously in oral fluid and mouth-exhaled breath. Recently, we applied a similar methodology to investigate the oral production of hydrogen cyanide (HCN) [24]. We believe that by combining the breath measurements with the simultaneous oral fluid analysis, we can gain important insight into the production mechanisms of these orally generated volatile species.

It is known that urease can hydrolyse oral fluid urea into ammonia [25,26]. Urease is produced by oral bacteria, such as *Streptococcus salivarius* and *Actinomyces naeslundii* [25,26]. In addition, it has been shown that oral fluid pH affects the mouth-exhaled NH_3 levels. Smith *et al* showed that rinsing the mouth with vinegar lowers the oral fluid pH value and the mouth-exhaled NH_3 concentration [17]. On the other hand, a mouth wash with bicarbonate solution, which increases the oral fluid pH value, can increase the mouth-exhaled NH_3 concentration [17]. Schmidt *et al* [9] and Solga *et al* [27] obtained similar results by showing that an acidic mouth rinse reduces the concentration of mouth-exhaled NH_3.

Based on the description above, we assume that there are three main factors affecting the mouth-exhaled NH_3 concentration: urea concentration, urease activity and oral fluid pH. We hypothesize that the mechanism of the mouth-exhaled NH_3 production in the oral cavity is as follows. Oral fluid urea is first hydrolysed to ammonia (NH_4^++NH_3) by oral bacterial urease. The oral fluid ammonia and hydronium ion concentration determine the concentration of oral fluid NH_3, which evaporates into gas phase and becomes mouth-exhaled NH_3. To test this hypothesis, we measured the oral fluid pH value, the concentrations of oral fluid ammonia, urea and mouth-exhaled NH_3 of one healthy subject under both fasting and normal conditions (an intra-subject test) as well as of 30 healthy subjects (an inter-subject test) and investigated the respective correlations. *In vitro* and *in vivo* tests with an oral disinfectant were also employed to confirm the hypothesis.

2. Material and Methods

2.1. Human subjects and sampling

Thirty one volunteers participated in the study. A written consent was obtained from all participating individuals. Coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa approved the research. In the intra-subject, inter-subject and oral disinfectant *in vivo* tests, we measured mouth-exhaled NH_3 levels on-line and took stimulated oral fluid samples from volunteers to measure the pH value, as well as ammonia and urea concentrations. A healthy female volunteer, aged 27, took part in the intra-subject test, including the fasting and diurnal tests. In the fasting test, the volunteer had breakfast two hours before the test. Afterwards eating and drinking was forbidden from 9:00 to 16:00. We measured 38 samples during two days. In the diurnal test, the diet of the volunteer was not controlled. We measured 24 samples in two days. Thirty healthy volunteers participated in the inter-subject test, each giving one sample. Sampling time was at least two hours after the last meal. Altogether 22 males and 8 females aged between 19 and 60 participated in this test. Samples were taken between 9:00 and 12:20.

Sampling time was at least two hours after the last meal in the comparison test of urea and ammonia concentrations in sublingual saliva and oral fluid. Three volunteers participated in this study:

one male and two females aged between 21 and 37. The volunteers gave sublingual saliva and oral fluid samples at the same time. Samples were taken every 15 min from each volunteer. We obtained altogether 23 samples from three volunteers.

In the oral disinfectant *in vitro* test, we used Corsodyl as the disinfectant. It contains 0.2% of chlorhexidine digluconate, which destroys most of the oral bacteria and inhibits enzymatic activity [28]. In addition to the active ingredient, Corsodyl also contains ethanol, macrogolglycerol hydroxystearate, sorbitol, peppermint oil and purified water. We used two different spiked urea concentrations: 0.6 mol/L and 2.5 mol/L. We prepared six tubes for this test (table 1). Solutions were mixed and kept at about 37 °C. After 45 min, ammonia concentrations of the solutions were measured. The same protocol was repeated three times on two volunteers.

Table 1. The compositions of six test tubes in the disinfectant *in vitro* test.

Tube	Oral fluid (μL)	Distilled H_2O (μL)	Corsodyl (μL)	0.6 mol/L Urea (μL)	2.5 mol/L Urea (μL)
1	300	300	--	--	--
2	300	300	--	20	--
3	300	--	300	20	--
4	300	300	--	--	--
5	300	300	--	--	20
6	300	--	300	--	20

In the oral disinfectant *in vivo* test, three volunteers participated: two males and one female aged between 28 and 52. Each volunteer gave two samples before the oral disinfectant mouth rinse. Then the volunteers rinsed their mouths with 10 mL of Corsodyl for one minute and gave samples every 15 min. Each volunteer gave 10 samples.

2.2. Measurement of mouth-exhaled NH_3

Mouth-exhaled NH_3 was measured on-line by a commercial ammonia analyser (Picarro, G2103), based on cavity ring-down spectroscopy. The setup, performance of the analyser and breath gas sampling have been described in detail [9]. A metronome to control the breathing rate was not used in this study. Volunteers were asked to inhale normally through the nose, exhale through the mouth, and breathe to a mouth piece, which is connected to the analyser inlet tube. The mouth-exhaled NH_3 concentration was recorded after three minutes of breathing.

2.3. Stimulated oral fluid and sublingual saliva sampling

Volunteers chewed a piece of a plastic paraffin film (30 mm × 30 mm, Parafilm) for one minute. During chewing, components in the oral cavity are mixed within the oral fluid. Volunteers were asked to keep the oral fluid in the oral cavity without swallowing it. After one minute, all of the fluid was collected onto a plate. To sample the sublingual saliva, the volunteer touched the back of the upper front teeth with the apex of her tongue, tilted her head forward and let the freshly secreted saliva flow out directly from the sublingual area onto a plate.

2.4. Measurement of oral fluid pH

Oral fluid pH was measured with a Horiba D-51 pH-meter using a flat tip ISFET electrode (Horiba, 0014-D00). The pH-meter was calibrated on every measurement day. After an oral fluid sample was collected onto a plate, the electrode was immediately dipped into the oral fluid sample and the pH value was measured.

2.5. Determination of oral fluid and sublingual saliva ammonia

The indophenol reaction was used to measure the oral fluid ammonia (NH_4^+ + NH_3) concentration [29,30]. First, we prepared reagents A and B. Reagent A: 0.005 g of sodium nitroprusside ($Na_2[Fe(CN)_5NO]\bullet 2H_2O$), 1.25 g of phenol and 1.0 g of NaOH were added into a 100 mL volumetric flask, and filled with distilled water to the mark. Reagent B: 1 mL of 14% sodium hypochlorite

(NaOCl) was added into a 100 mL volumetric flask, and filled with distilled water to the mark. NH_4^+ standard solutions (25 μmol/L, 50 μmol/L, 100 μmol/L, 200 μmol/L) were prepared from $(NH_4)_2SO_4$. To measure the oral fluid ammonia concentration, 20 μL of oral fluid and 980 μL of distilled water were added into a 15 mL glass tube. Then 2 mL of reagent A and 1 mL of reagent B were added into the tube and mixed well. The tube was incubated in a dark water bath at around 37 °C for 20 min. After the indophenol reaction, the solution was transferred from the tube to a cuvette. The absorption of the solution was measured (Ocean Optics, USB4000 and USB-ISS-UV/VIS) at 623 nm. The same procedure was used to measure the ammonia concentration of sublingual saliva. For a standard curve, 1 mL of distilled water (blank solution) and 1 mL of each standard solution were added into separate tubes. Reagents A and B were added and the measurement steps were the same as just described.

Based on the oral fluid pH value and oral fluid ammonia (NH_4^++NH_3) concentration, we calculated the oral fluid NH_3 concentration with the Henderson-Hasselbalch equation [31]:

$$\mathrm{pH} = \mathrm{pK_a} + \log_{10}\left(\frac{\mathrm{cNH_3}}{\mathrm{cNH_4^+}}\right)$$

where cNH_3 and cNH_4^+ are the concentrations of NH_3 and NH_4^+, respectively, in oral fluid, and pK_a is the acid dissociation coefficient of NH_4^+. At 37 °C, the pK_a value of NH_4^+ in water is 8.890 [2]. We assume the same pK_a value applies for saliva.

2.6. Determination of oral fluid and sublingual saliva urea

Ehrlich's reagent was used to measure the oral fluid urea concentration [32]. It was prepared by adding 1 g of *p*-dimethylaminobenzaldehyde and 1 mL of concentrated H_2SO_4 (98%) into a 25 mL volumetric flask, and filled with ethanol to the mark. Urea standard solutions (0.5 mmol/L, 1.0 mmol/L, 1.5 mmol/L, 2.0 mmol/L) were prepared for a urea standard curve. To determine the urea concentration in oral fluid, we added 100 μL of oral fluid, 900 μL of distilled water and 250 μL of Ehrlich's reagent into a 1.5 mL microcentrifuge tube. The solution was centrifuged for 10 min at 6000 rpm (Hettich, EBA 3S). The clear solution was transferred into a cuvette. The absorption of the solution was measured at 422 nm. Same procedure was used to measure the sublingual salivary urea concentration. For the urea standard curve, 1 mL of distilled water (blank solution) and 1 mL of each standard solution were added into tubes. Then, 250 μL of Ehrlich's reagent was added. After 10 min, the absorption of the mixed solution was measured.

2.7. Repeatability test of oral fluid urea, ammonia, pH and mouth-exhaled NH_3 measurement

For the repeatability test of oral fluid urea and ammonia (NH_4^++NH_3), both the urea and ammonia concentrations were measured from the same oral fluid sample ten times. For the pH measurement repeatability test, ten oral fluid samples were obtained five minutes apart. For the mouth-exhaled NH_3, 12 breath samples were measured on-line five minutes apart.

2.8. Statistical analysis

Spearman's rank correlation test was used to analyze the correlations between mouth-exhaled NH_3, the various oral fluid components (urea, ammonia and pH), and volunteers' information (age and body mass index). In this test, the *p* value refers to the probability of obtaining the observation results assuming the correlation coefficient r_s is zero (null hypothesis).

The Mann-Whitney U test was used to analyze differences in urea and ammonia concentration between sublingual saliva and oral fluid, as well as differences in mouth-exhaled NH_3 between the male and female groups.

3. Results

3.1. Repeatability test of oral fluid urea, ammonia, pH and mouth-exhaled NH_3 measurement

Table 1 shows the mean value, standard deviation (SD) and coefficient of variation (CV) of oral fluid urea, ammonia, pH and mouth-exhaled NH_3 in the repeatability test. The CV value represents the error from measurement itself. The error in the urea measurement is higher than in the other experiments. After repeated tests, we have come to a conclusion that this variation is due to an interaction between the plastic pipette tip material and the Ehrlich's reagent. This leads to a variation in the amount of added reagent and subsequently results in a higher CV in the urea measurement.

Table 1. The repeatability test.

	Urea	Ammonia (NH_4^++NH_3)	pH	Mouth-exhaled NH_3
N[a]	10	10	10	12
Mean	8.3 mmol/L	2.2 mmol/L	7.06	820 ppb
SD	1.2 mmol/L	0.2 mmol/L	0.06	30 ppb
CV[b]	0.14	0.07	0.009	0.04

[a] The quantity N is the number of samples.

[b] The coefficient of variation (CV) is defined as the standard deviation divided by the mean value.

3.2. The correlations in intra-subject and inter-subject tests

Both in the intra-subject and inter-subject tests, we observed statistically significant correlations between oral fluid urea and ammonia (NH_4^++NH_3) (table 2). This implies that oral fluid urea plays an important role in the oral fluid ammonia production. The strongest correlation was observed in the fasting test (r_s=0.77, p<0.001) (figure 1) of a single individual. There is a strong correlation between oral fluid NH_3 and mouth-exhaled NH_3 (r_s=0.81, p<0.001) in the inter-subject test (figure 2). The correlation in the intra-subject test is weaker (table 3). In addition, we observed a moderate correlation between oral fluid urea and mouth-exhaled NH_3 (r_s=0.49, p=0.002) in the fasting test.

Table 2. Correlations between oral fluid urea and ammonia (NH_4^++NH_3) in the intra-subject and inter-subject tests.

Tests		Urea ↔ NH_4^++NH_3		Urea (mmol/L)		NH_4^++NH_3 (mmol/L)	
		r_s	p	Mean	CV[a]	Mean	CV[a]
Intra-subject	Fasting (n=38)	0.77	<0.001	5.3	0.64	3.3	0.21
	Diurnal (n=24)	0.51	0.01	5.6	0.29	2.3	0.26
Inter-subject (n=30)		0.46	0.01	6.9	0.43	4.7	0.41

[a] The coefficient of variation (CV) is defined as the standard deviation divided by the mean value.

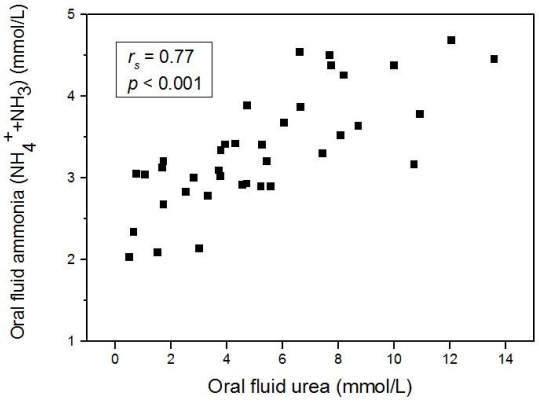

Figure 1. The correlation between oral fluid urea and ammonia (NH_4^++NH_3) in the fasting test of a single individual.

Table 3. Correlations between oral fluid NH_3 and mouth-exhaled NH_3 in the intra-subject and inter-subject tests.

Tests		Oral fluid NH_3↔Mouth-exhaled NH_3		Oral fluid NH_3 (μmol/L)		Mouth-exhaled NH_3 (ppb)		pH	
		r_s	p	Mean	CV	Mean	CV	Mean	CV
Intra-subject	Fasting (n=38)	*0.19*[a]	*0.26*[a]	33	0.28	630	0.16	6.89	0.014
	Diurnal (n=24)	0.53	0.008	33	0.24	360	0.24	6.98	0.018
Inter-subject (n=30)		0.81	<0.001	42	0.68	630	0.49	6.79	0.038

[a] The one without statistically significant correlation is written in *italics*.

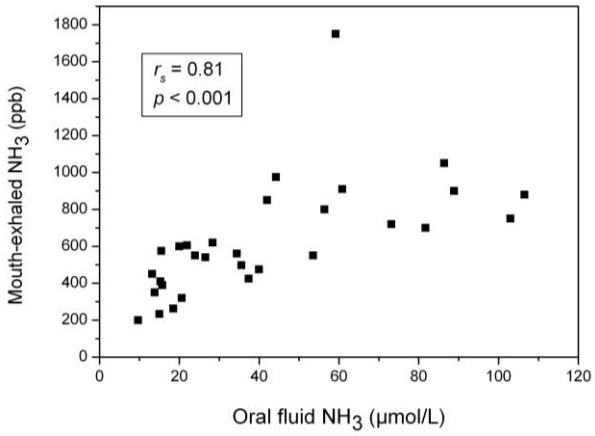

Figure 2. The correlation between oral fluid NH_3 and mouth-exhaled NH_3 in the inter-subject test.

Additionally, we found that volunteers' age was statistically significantly correlated to oral fluid pH (r_s =0.54, p =0.002) and negatively correlated to oral fluid urea (r_s=-0.45, p=0.014). The mouth-exhaled NH_3 concentration of males (690±310 ppb) was statistically significantly higher than that of females (460±240 ppb) (p=0.04). The mean value of oral fluid pH and mouth-exhaled NH_3 were 6.79 and 630 ppb, respectively, in the inter-subject test. These are close to our previous study, where the mean values of oral fluid pH and mouth-exhaled NH_3 were 6.84 and 780 ppb, respectively [9].

3.3. The urea and ammonia concentration in sublingual saliva and oral fluid

There was no statistically significant difference of the urea concentration between sublingual saliva (6.7±2.3 mmol/L) and oral fluid (6.5±1.0 mmol/L) (p=0.68) (figure 3a). However, the ammonia concentration in oral fluid (2.9±1.9 mmol/L) was significantly higher than in sublingual saliva (0.4±0.2 mmol/L) (p<0.001) (figure 3b).

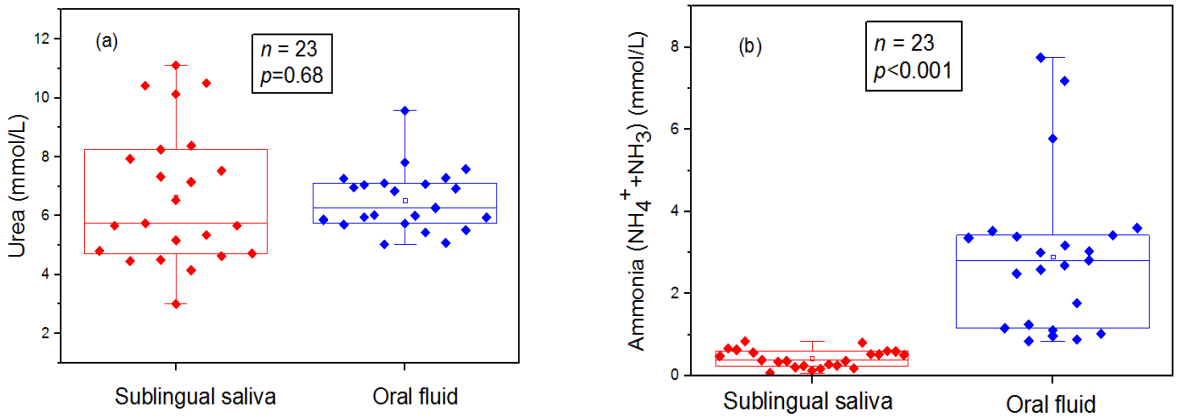

Figure 3. The urea concentration in sublingual saliva and oral fluid (a), the ammonia concentration in sublingual saliva and oral fluid (b) from three volunteers. The bottom and top of the box are the first and third quartiles, and the band inside the box is the median. The ends of the whiskers represent the minimum and maximum of all the data.

3.4. Oral disinfectant in vitro and in vivo test

In the oral disinfectant *in vitro* test, we added 20 µL of 0.6 mol/L and 2.5 mol/L spiked urea into oral fluid samples. The final concentrations of the spiked urea in mixed solutions were 20 mmol/L and 80 mmol/L respectively. The mean value of oral fluid urea in this study was 6 mmol/L. An increase in the ammonia concentration was observed after spiking (figure 4). However, if an oral disinfectant was added at the same time, no increase in the ammonia concentration was observed. This result implies that without bacterial and enzymatic activity, urea cannot be hydrolysed into ammonia in oral fluid.

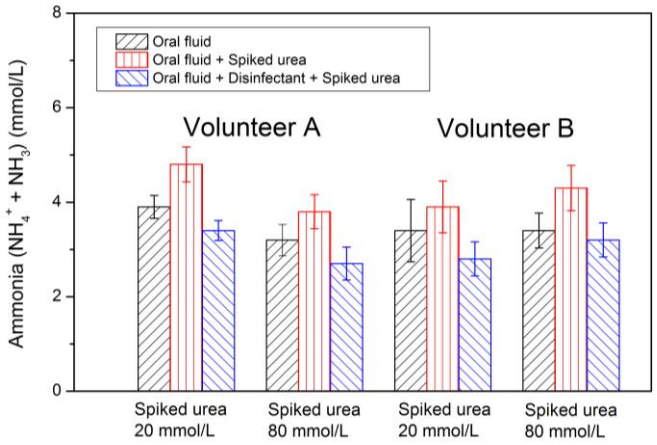

Figure 4. The mean values of ammonia (NH_4^++NH_3) concentration from three repeat *in vitro* experiments. Error bars represent one standard deviation.

In the oral disinfectant *in vivo* test, a statistically significant correlation between oral fluid ammonia (NH_4^++NH_3) and mouth-exhaled NH_3 was observed only in one of the volunteers (A). However, there was a strong correlation between oral fluid NH_3 and mouth-exhaled NH_3 in the case of volunteer A (r_s=0.90, p<0.001), and a moderate correlation in the case of volunteer C (r_s=0.64, p=0.048). Statistically significant correlation was not found for volunteer B (r_s=-0.061, p=0.87). Results of the test are given in table 4.

Table 4. Correlations between oral fluid ammonia and mouth-exhaled NH_3, and between oral fluid NH_3 and mouth-exhaled NH_3 in the oral disinfectant *in vivo* test.

	Oral fluid ammonia (NH_4^++NH_3)↔Mouth -exhaled NH_3		Oral fluid NH_3↔Mouth-exhaled NH_3		Oral fluid NH_3 (µmol/L)		Mouth-exhaled NH_3 (ppb)		pH	
	r_s	p	r_s	p	Mean	CV	Mean	CV	Mean	CV
Volunteer A	0.81	0.004	0.90	<0.001	22	0.45	330	0.38	7.14	0.020
Volunteer B	*-0.097*[a]	*0.79*[a]	*-0.061*[a]	*0.87*[a]	53	0.28	259	0.17	7.09	0.006
Volunteer C	*0.16*[a]	*0.65*[a]	0.64	0.048	36	0.31	379	0.40	7.06	0.024

[a] The one without statistically significant correlation is written in *italics*.

4. Discussion

We observed statistically significant correlations between oral fluid urea and ammonia (NH_4^++NH_3) in the intra-subject and inter-subject tests. Because only one subject participated in the intra-subject test, this might be considered as a potential weakness of our study. However, we also observed statistically significant correlation in the inter-subject test. Therefore, we believe that our results indicate that oral fluid urea is a dominant contributor to the oral fluid ammonia production. Furthermore, we find a moderate correlation between oral fluid urea and mouth-exhaled NH_3 (r_s=0.49, p=0.002) in the fasting test. This implies that oral fluid urea is a significant source of mouth-exhaled NH_3. The correlation between oral fluid urea and ammonia (NH_4^++NH_3) in the fasting test (r_s=0.77, p<0.001) is stronger than in the diurnal test (r_s=0.51, p=0.01). The coefficient of variation (CV) of urea concentration in the diurnal test (CV=0.29) is smaller than in the fasting test (CV=0.64), and is closer to the CV in the repeatability test (CV= 0.14). This implies that the variation of urea concentration in the diurnal test is largely affected by the measurement error. Hence, the rank order of the urea concentrations in the diurnal test is more random, resulting in a weaker correlation obtained from Spearman's rank correlation test. In the fasting test, the higher CV of urea concentration implies a larger data range and less effect from the measurement error. Therefore, the rank order is less random, leading to a stronger correlation. The low CV of urea concentration in the diurnal test is probably due to the urea regulation system in the human body. However, during fasting, dehydration of the human body elevates the urea concentration [33], leading to a larger data range and higher CV. In the inter-subject test, the correlation is the weakest. This occurs because the variation in the oral conditions of 30 volunteers is larger than that of one volunteer in the intra-subject test. The ureolytic process varies among volunteers, leading to a weaker correlation between oral fluid urea and ammonia in the inter-subject test. In addition to ureolysis, arginolysis also produces ammonia in the oral cavity by catabolizing arginine [25]. However, the concentration of free form arginine in oral fluid is around 50 µmol/L [25], which is much less than the urea concentration in oral fluid measured in the inter-subject test (mean=6.9 mmol/L).

There was no significant difference in the urea concentration between oral fluid and sublingual saliva (p=0.07), but the ammonia concentration in oral fluid was significantly higher than in sublingual saliva (p<0.001). These results confirm that the ureolytic process takes place in the oral cavity. Since sublingual saliva is secreted freshly from sublingual glands, it contains less bacteria and enzymes than oral fluid. This results in a lower ammonia concentration in sublingual saliva. Previous studies have shown that oral fluid ammonia is generated through the hydrolysis of urea by urease [34,35], which is produced by oral bacteria [25,26]. Hence, urease activity affects the oral fluid ammonia concentrations. Similar conclusion can be drawn from the oral disinfectant *in vitro* test. We observed that spiking an oral fluid sample with urea increased the ammonia concentration. However, after adding a disinfectant, spiking with urea did not increase the ammonia concentration. This is because the bacterial and enzymatic activity is inhibited by the oral disinfectant. Without the bacterial and enzymatic activity, the spiked urea cannot be hydrolysed into ammonia.

Oral fluid ammonia further transfers to mouth-exhaled NH_3. Depending on the oral fluid pH value, certain amount of ammonium ion (NH_4^+) turns to ammonia molecule (NH_3) in oral fluid and further evaporates into the gas phase. Based on the oral fluid ammonia concentrations and oral fluid pH, we calculated the oral fluid NH_3 concentrations. We found a strong correlation between oral fluid NH_3 and mouth-exhaled NH_3 in the inter-subject test (r_s=0.81, p<0.001) and a moderate correlation in the diurnal test (r_s=0.53, p=0.008). However, in the fasting test, there is no statistically significant correlation between them. Oral fluid NH_3 is calculated from the Henderson-Hasselbalch equation using the value of oral fluid pH. The CV of pH in the fasting test (CV=0.014) is close to that in the repeatability test (CV=0.009). This implies that the variation of oral fluid pH in the fasting test is affected to a large extent by the measurement error. The accuracy of pH measurement in oral fluid is influenced by the sampling and measurement techniques [36].

In the oral disinfectant *in vivo* test, Corsodyl was applied as the mouth rinse. As possible side effects, the manufacturer mentions swelling of the parotid glands, among other things. Although we cannot rule out the possibility that such side effects might affect the retrieved ammonia concentrations,

we assume that the main effect of the mouth rinse is to destroy most of the oral bacteria and inhibit enzymatic activity. After the application of the mouth rinse, the influence of urease is thus minimized and oral fluid pH becomes the main factor affecting the mouth-exhaled NH_3 concentration. In volunteer A and volunteer C, we observed a stronger correlation between oral fluid NH_3 and mouth-exhaled NH_3 than between oral fluid ammonia (NH_4^++NH_3) and mouth-exhaled NH_3. This implies that oral fluid pH has important effect on mouth-exhaled NH_3 production. In addition, we notice that there is a stronger correlation in volunteer A (r_s=0.9, p<0.001) than in volunteer C (r_s=0.64, p=0.048), probably because CV of oral fluid NH_3 in volunteer A (CV=0.45) is higher than in volunteer C (CV=0.31). However, no statistically significant correlation was found in volunteer B. This is most likely due to the low CV in oral fluid pH (CV=0.006) and in mouth-exhaled NH_3 (CV=0.17).

Overall, the mouth-exhaled NH_3 concentration in this study was affected by three primary factors: oral fluid urea concentration, bacterial urease activity and oral fluid pH. Oral fluid urea is first hydrolysed to ammonia by bacterial urease. Oral fluid NH_4^+ then transfers to NH_3, depending on the oral fluid pH value. Finally, oral fluid NH_3 evaporates into gas phase and turns to mouth-exhaled NH_3.

In healthy people, since the urea concentration in body fluids is regulated to a certain level, the change of urea concentration is small. As a result, it is difficult to observe the correlation between body fluid urea and mouth-exhaled NH_3. However, the situation is different in chronic kidney disease patients, because their body fluid urea concentration is abnormally high. Previous studies have shown that mouth-exhaled NH_3 could be a potential marker to monitor the haemodialysis progress in chronic kidney disease patients [7,8,19-21]. Mouth-exhaled NH_3 levels decrease during haemodialysis and there is a statistically significant correlation with blood urea. It was first shown by Kopstein *et al* that blood urea positively correlates to oral fluid ammonia [34]. Španěl *et al* showed that oral exposure to urea elevates the mouth-exhaled NH_3 concentration, indicating that exogenous urea can influence the mouth-exhaled NH_3 levels [37]. In our study, we demonstrated that endogenous urea (oral fluid urea) also influences the mouth-exhaled NH_3 levels. Since blood urea is strongly correlated to oral fluid urea [38,39], a decrease in blood urea leads to a decrease in oral fluid urea. The decrease in oral fluid urea results in a lowering of ammonia in oral fluid, followed by a subsequent decrease in mouth-exhaled NH_3. In addition, Bots *et al* have shown that oral fluid pH decreases at the end of haemodialysis [40]. This will also have an effect of lowering the retrieved mouth exhaled NH_3 levels by shifting the acid-base equilibrium in oral fluid. Endre *et al* have also shown that the decay of mouth-exhaled NH_3 during dialysis does not necessarily follow a simple exponential behaviour and that there are distinct differences between individuals [20]. It is possible that some of their observations can be explained by individual differences in bacterial urease activity or changes in pH during dialysis. Simultaneous oral fluid and breath measurements should be conducted on haemodialysis patients to find out whether the changes in mouth-exhaled NH_3 during haemodialysis can be explained by oral NH_3 production from urea.

5. Conclusion

To our knowledge, this is the first study to explore the mechanism of the mouth-exhaled NH_3 production by measuring ammonia simultaneously in oral fluid and mouth-exhaled breath. We show that mouth-exhaled NH_3 is significantly affected by hydrolysis of urea in the oral cavity. We demonstrate that there are three main factors influencing mouth-exhaled NH_3 levels: oral fluid urea concentration, bacterial urease activity and the oral fluid pH value. We conclude that oral fluid urea is hydrolysed to ammonia by oral bacterial urease. Depending on the oral fluid pH, oral fluid total ammonia (NH_4^+/NH_3) converts to a certain amount of oral fluid NH_3, which further evaporates into gas phase and turns to mouth-exhaled NH_3.

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