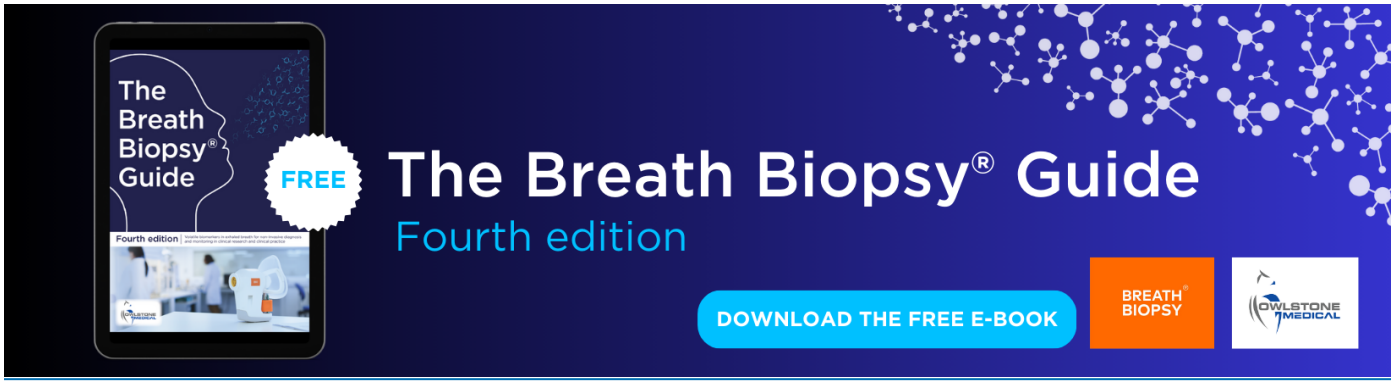
PAPER

Off-line breath acetone analysis in critical illness



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Off-line breath acetone analysis in critical illness

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Abstract
Analysis of breath acetone could be useful in the Intensive Care Unit (ICU) setting to monitor evidence of starvation and metabolic stress. The aims of this study were to examine the relationship between acetone concentrations in breath and blood in critical illness, to explore any changes in breath acetone concentration over time and correlate these with clinical features. Consecutive patients, ventilated on controlled modes in a mixed ICU, with stress hyperglycaemia requiring insulin therapy and/or new pulmonary infiltrates on chest radiograph were recruited. Once daily, triplicate end-tidal breath samples were collected and analysed off-line by selected ion flow tube mass spectrometry (SIFT-MS). Thirty-two patients were recruited (20 males), median age 61.5 years (range 26–85 years). The median breath acetone concentration of all samples was 853 ppb (range 162–11 375 ppb) collected over a median of 3 days (range 1–8). There was a trend towards a reduction in breath acetone concentration over time. Relationships were seen between breath acetone and arterial acetone ($r_s = 0.64$, $p < 0.0001$) and arterial beta-hydroxybutyrate ($r_s = 0.52$, $p < 0.0001$) concentrations. Changes in breath acetone concentration over time corresponded to changes in arterial acetone concentration. Some patients remained ketotic despite insulin therapy and normal arterial glucose concentrations. This is the first study to look at breath acetone concentration in ICU patients for up to 8 days. Breath acetone concentration may be used as a surrogate for arterial acetone concentration, which may in future have a role in the modulation of insulin and feeding in critical illness.

S Online supplementary data available from stacks.iop.org/JBR/7/037102/mmedia

(Some figures may appear in colour only in the online journal)

1. Introduction

Non-invasive breath tests for volatile organic compounds (VOCs) are useful in the Intensive Care Unit (ICU) as they provide rapid, frequent or continuous assessment of physiological changes occurring as a consequence of critical illness. For VOCs that are produced by metabolic pathways, it is important to understand the relationship between their concentrations in exhaled breath and the systemic circulation in order for them to be useful (Schubert *et al* 2005).

Acetone is produced by hepatic decarboxylation of acetoacetate through either an enzymatic or non-enzymatic pathway, mainly during lipolysis. The enzymatic catalyst, acetoacetate decarboxylase, is induced by starvation and inhibited by acetone. The acetone produced cannot be returned to acetoacetate and is excreted in breath (Kalapos 1999, 2003). Beta-hydroxybutyrate is also produced from acetoacetate in a reaction catalysed by beta-hyroxybutyrate dehydrogenase. The direction of this reaction is dependent upon the redox state of the cells; therefore, the relationship between the relative quantities of acetoacetate, acetone and beta-hydroxybutyrate is not constant (Carmant 2008).

Breath acetone has been investigated as a marker of ketosis (Kundu *et al* 1993, Musa-Veloso *et al* 2002) and metabolic stress in patients with heart failure (Kupai *et al* 1995) and during cardiac surgery (Pabst *et al* 2007). We have previously demonstrated that acetone can by accurately and reproducibly measured by selected ion flow tube mass spectrometry (SIFT-MS) in healthy volunteers (Dummer *et al* 2010), individuals with diabetes (Storer *et al* 2011) and intubated and ventilated patients in the ICU setting (Sturney *et al* 2012). The high variability seen in repeated measurements of acetone concentration in plasma compared to breath (O'Hara *et al* 2009) may be the result of blood sampling itself, movement of acetone from plasma into headspace gas or the spontaneous decarboxylation of acetoacetate to acetone within samples at room or body temperature (Crofford *et al* 1977). The monitoring of breath acetone concentration may, therefore, be more reliable than plasma measurements (O'Hara *et al* 2009).

Sepsis is a major cause of mortality in the ICU, due to haemodynamic compromise and multi-organ failure (Van den Berghe 2004, Vincent *et al* 2006, Sakr *et al* 2008, Harrois *et al* 2009). Activation of the inflammatory cascade is known to cause 'stress hyperglycaemia', which is common in patients with critical illness in the absence of pre-existing diabetes mellitus (Van den Berghe 2004). It is characterized by hyperinsulinaemia, peripheral insulin resistance and increased gluconeogenesis and glycogenolysis (Van den Berghe *et al* 2001, Van Cromphaut *et al* 2008). Glucose control appears to be important in the prevention of unnecessary morbidity and mortality in this situation (Van den Berghe *et al* 2001, 2006, Nice-Sugar Study Investigators *et al* 2009, Ichai and Preiser 2010), and in patients with hyperglycaemia after myocardial infarction (Malmberg *et al* 1995). Maintenance of glucose concentration below 10 mmol L^{-1} appears to reduce mortality whilst avoiding hypoglycaemia when compared to more aggressive glycaemic control (Nice-Sugar Study Investigators *et al* 2009, Ichai and Preiser 2010). The specialized relative insulin nutrition tables (SPRINT) protocol is a novel system for the control of glucose in patients in the ICU, Christchurch Hospital, implemented in 2005 (Chase *et al* 2008). The protocol uses blood glucose concentration and modelled insulin sensitivity to modulate hourly insulin dose and volume of enteral or parenteral feed administered.

The aims of this study were to investigate the measurement of acetone in intubated and ventilated patients in the ICU via SIFT-MS, to explore any changes in breath acetone concentration over time and correlate them with clinical parameters. We sought to examine the relationships between breath acetone and arterial acetone, beta-hydroxybutyrate and glucose concentrations in patients with stress hyperglycaemia and/or new pulmonary infiltrates on chest radiograph.

2. Methods

2.1. Subjects

Study procedures were approved by the Upper South A Regional Ethics Committee, New Zealand. Consecutive critically ill non-diabetic adult patients requiring mechanical ventilation on a controlled mode in the ICU, Christchurch Hospital, were enrolled if they had either or both of the following: stress hyperglycaemia requiring insulin therapy as per local protocol (Chase *et al* 2008), with baseline sampling prior to insulin administration, and/or new pulmonary infiltrates on chest radiograph, with baseline sampling within 24 h of intubation or development of infiltrates if already intubated. Patients or their next of kin provided written consent prior to sample collection. In addition, if the patient had not given their own consent prior to inclusion, and regained adequate cognitive functioning, they confirmed their consent to participate in retrospect.

Each patient was ventilated using a Nellcor Puritan Bennett™ ventilator system (USA). Some ventilator circuits included a humidifier (Fisher and Paykel MR 730 Respiratory Humidifier, NZ). Ventilator modes and settings were determined by ICU staff.

Breath and contemporaneous arterial blood samples were taken at enrolment (between 07:00 and 17:00) and daily thereafter. Subsequent samples were taken between 07:00 and 13:30 unless the patient was spontaneously breathing or not present on the ICU, for example, in the operating theatre or radiology department. Blood samples were sent to Canterbury Health Laboratory immediately for processing; the sample for acetone testing was sent on ice and the headspace analysed by gas chromatography. Sampling was discontinued if the patient no longer required controlled ventilation, was extubated, underwent tracheostomy or died. The following data were collected for each patient: diagnosis and reason for admission, number of days on the ICU, age, gender, ethnicity and smoking status. A note was made of physiological parameters at the time of breath collection, as well as feeding, current medication and disease severity scores.

2.2. Inspiratory air sampling

All methods utilized a T-piece (oxygen enrichment attachment, Respironics Inc, USA) inserted into the breathing circuit at the ventilator gas outlet, or humidifier outlet, for inspiratory gas sampling.

2.3. Exhaled breath collection

The experimental set-up can be seen in figure 1. A T-piece was inserted into the respiratory circuit on the ventilator side of the suction catheter mount (Fisher & Paykel, NZ). Two lengths of suction catheter tubing (4.7 mm × 53 cm, Ch14, Pennine Healthcare, UK) with a sampling filter (DISMIC®-25, Toyo Roshi Kaisha Ltd, Japan) between them were connected at one end to the T-piece and at the other to a handheld pump (Gilian® Personal Air Sampler, Sensidyne, USA). During tidal breathing, the expiratory pause button was held down for 4 s to allow the collection of approximately 150 ml of end-tidal breath into a 1 L transparent Tedlar® bag (SKC Inc, USA) via the pump. The volume of the tidal breath was noted. The ventilator delivered several standard breaths before this process was repeated in triplicate using separate Tedlar bags. At the time of breath sampling, the pressure sensitivity setting on the ventilator was temporarily altered so that the

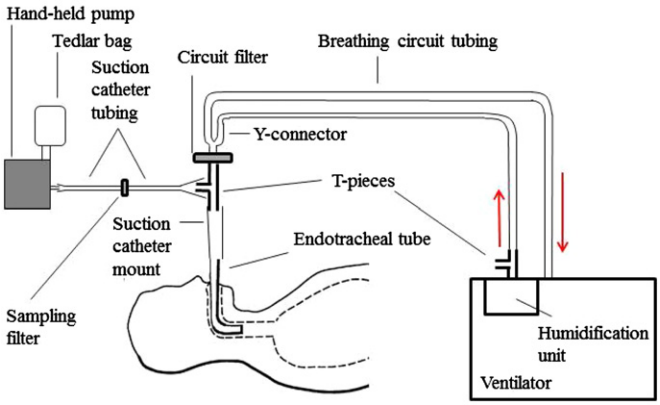

Figure 1. Schematic of the experimental set-up.

collection of exhaled breath did not trigger the ventilator to deliver another inspiratory breath.

A pneumotachometer (RSS 100, Hans Rudolph Inc, USA) was inserted into the respiratory circuit adjacent to the T-piece, on the ventilator side, to check the direction of flow during breath sample collection. All inspiratory and exhaled breath samples were incubated at 40 °C and analysed within 40 min of collection by SIFT-MS. The suction catheters and sampling filter were disposable and used for only one set of samples. The T-piece was decontaminated for re-use. Disinfectant wipes were used on the surfaces of the hand-held pump and Tedlar bags (Azo wipes, W. M. Bamford and Co. Ltd, NZ). None of the cleaning procedures changed the VOC composition of samples. The effects of storage of acetone in Tedlar bags were investigated in a laboratory-based experiment, revealing no appreciable reduction in acetone concentration over 120 min (unpublished data). The effects of the breath collection apparatus on breath acetone concentration have been examined (supplementary data available at stacks.iop.org/JBR/7/037102/mmedia). Inspired and exhaled acetone concentrations quoted in this paper are corrected for losses due to the sampling apparatus.

2.4. Selected ion flow tube-mass spectrometry

Breath acetone concentration was measured by SIFT-MS, Voice200® (Syft Technologies Ltd, NZ), using selected ion monitoring (SIM) mode. The technique has been described in detail previously (Prince *et al* 2010). Each Tedlar bag was attached to the sampling capillary end cap of the SIFT-MS and samples were analysed for 30 s. The NO^+ reagent ion was used to analyse breath acetone. The reaction is an ion–molecule collisional association with He atom stabilization (Spanel *et al* 1997):

$$NO^+ + CH_3COCH_3(+He) \rightarrow NO^+ \cdot CH_3COCH_3(+He).$$

The NO^+ reagent ion was monitored at a mass-to-charge ratio (m/z) of 30, and the $NO^+.H_2O$ hydrated reagent ion formed in humid breath mixtures at m/z 48. The $NO^+.CH_3COCH_3$ product ion was monitored at m/z 88.

2.5. Calculations and statistical analysis

Means and percentage coefficients of variation (CVs) of each set of three end-tidal breath samples were calculated. Comparisons between groups were made using Mann–Whitney U tests for unpaired and Wilcoxon signed rank tests for paired data. Correlations were determined by Spearman's rank correlation (r_s). In some cases, only data from days 1 and 2 were compared so that data from patients enrolled for many more days did not skew the results. As the units of measurement were different, a modified Bland–Altman plot was used to compare breath acetone and arterial acetone measurements as percentages of their maximum concentration (Takita *et al* 2007). A value of $p < 0.05$ was considered to be statistically significant.

Alveolar-arterial oxygen gradient (A-a gradient) was calculated by using the formula (FiO_2 × (atmospheric pressure − water pressure) − ($PaCO_2/0.8$)) − PaO_2, when atmospheric pressure and water pressure at sea level are 760 mmHg and 47 mmHg, respectively. The ratio of inspiratory acetone as a percentage of exhaled acetone concentration ((C_{insp}/C_{exp}) × 100) was calculated with a mean and standard error of the mean (SEM). Systemic inflammatory response syndrome (SIRS) when two or more of the following criteria were met: (1) temperature >38 °C or <36 °C, (2) heart rate >90 beats per minute, (3) respiratory rate >20 breaths per minute or $PaCO_2$ <32 mmHg, (4) white blood cell count >12000 per ml or <4000 per ml or >10% immature forms.

3. Results

Thirty-four consecutive patients admitted to the ICU, Christchurch Hospital, who fulfilled the inclusion criteria were enrolled; however, data from two patients were subsequently excluded from analysis as consent from their next of kin was revoked. Results from 32 patients were analysed; 20 males and 12 females, median age 61.5 years (range 26–85 years). Twenty-eight patients had new pulmonary infiltrates on chest radiograph and 19 patients were hyperglycaemic requiring insulin therapy. It was not possible to obtain breath and blood samples prior to administration of insulin in 8 of the 19 patients with hyperglycaemia. Demographic data can be seen in table 1.

At enrolment, 22 patients fulfilled the criteria for SIRS (Bone *et al* 1992), 17 of whom had microbiological evidence of infection and therefore fulfilled criteria for sepsis (Bone *et al* 1992). Twelve patients had pneumonia; two of these patients did not have sepsis. Positive microbiological results were obtained from 8 of the 12 patients with pneumonia; five patients had positive legionella DNA polymerase chain reaction (PCR) tests (*Legionella longbeachae* was cultured from one of these patients), two patients had tracheal aspirates with moderate to heavy growth of *Haemophilus influenza* and *Streptococcus pneumonia*, and one patient grew Group B Streptococcus on blood culture. At enrolment, 22 patients fulfilled PO_2/FiO_2 ratio criteria for acute respiratory distress syndrome (ARDS) (ARDS Definition Ranieri *et al* 2012) (9 mild, 11 moderate, 2 severe) and 6 patients had pulmonary oedema. Samples were collected for a median of 3 days (range 1–8 days). Sampling was most frequently discontinued due to development of spontaneous breathing (65.6%), which prevented breath sampling by the method described. The next

Table 1. Patient demographics, inflammatory status, physiology and ventilation mode at enrolment.

Subject	Gender	Age (years)	SIRS	Sepsis	Infiltrates	Insulin	Sampling days	Ventilator mode	Breath acetone (ppb)	PO_2/FiO_2	$P_{et}CO_2$ (mmHg)	Alveolar dead space
1	m	61	3	y	y*	#	3	Bi-Level	11375	54.7	35	0.54
2	m	66	3	y	y*	#	5	Bi-Level	1130	101.4	–	–
3	m	81	3	y		y	2	Bi-Level	1785	150.0	22	0.19
4	f	71	3	y	y*		2	SIMV	3054	167.5	31	0.30
5	f	60	4	y	y		1	SIMV	763	220.0	41	0.11
6	m	51	1	n	y		1	SIMV	1793	225.7	40	0.15
7	m	85	3	y	y*		3	Bi-Level	701	222.5	43	0.22
8	m	74	0	n	y	#	3	SIMV	2565	226.7	29	0.42
9	m	62	4	y	y*		2	Bi-Level	722	165.8	52	0.31
10	f	77	3	n	y		2	SIMV	853	363.3	33	0.27
11	f	78	0	y	y*	#	2	Bi-Level	2265	284.4	27	0.36
12	f	42	3	n	y		1	SIMV	839	168.3	53	0.17
13	f	40	2	y	y*		6	SIMV	1598	101.3	43	0.26
14	m	67	2	y	y	y	3	SIMV	515	72.0	40	0.07
15	m	46	1	n	y*	y	3	Bi-Level	1750	323.3	38	0.19
16	m	54	2	y	y*	#	8	SIMV	1332	118.3	33	0.38
17	m	60	2	n	y	y	2	Bi-Level	2379	168.3	34	0.29
18	m	81	2	y	y*	y	5	SIMV	1191	141.8	43	0.15
19	f	55	3	y	y*	#	5	Bi-Level	6696	245.7	32	0.24
20	f	53	1	n		y	2	SIMV	1201	260.0	27	0.27
21	f	59	3	y	y	y	3	SIMV	2590	217.5	31	0.52
22	m	46	2	y	y	y	8	Bi-Level	1266	146.0	43	0.17
23	m	38	2	n	y		3	SIMV	1569	336.7	26	0.41
24	m	37	1	n	y*		6	SIMV	259	116.0	57	0.23
25	m	74	0	n		y	4	SIMV	2227	240.0	37	0.14
26	m	64	3	n	y	#	6	Bi-Level	753	152.0	32	0.16
27	m	83	3	n	y	#	5	SIMV	260	202.0	42	0.29
28	f	83	2	y	y		2	SIMV	4236	385.7	37	0.14
29	f	26	3	y	y		2	Bi-Level	333	426.7	46	0.04
30	m	39	1	n		y	1	Bi-Level	1147	348.0	40	−0.14
31	m	67	2	n	y		2	Bi-Level	4366	108.3	50	0.43
32	f	85	1	n	y	y	2	SIMV	2354	358.3	43	0.19

SIRS (systemic inflammatory response syndrome) (American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference 1992): 0–1, no SIRS; 2–4, SIRS. Sepsis: n, criteria for sepsis (American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference 1992) not fulfilled; y, criteria for sepsis fulfilled. Infiltrates, new pulmonary infiltrates on chest radiograph; y, yes; n, no; *, pneumonia present; #, requiring insulin but no pre-insulin samples obtained. SIMV, synchronized intermittent mandatory ventilation. Breath acetone, mean breath acetone concentration. PO_2/FiO_2, arterial partial pressure of oxygen divided by the fraction of inspired oxygen; $P_{et}CO_2$, partial pressure of end-tidal CO_2; alveolar dead space calculated by (arterial PCO_2-$P_{et}CO_2$)/arterial PCO_2.

most common reasons for discontinuation of sampling were death (18.8%), extubation (12.5%) and tracheostomy (3.1%).

The pneumotachometer was used to visually confirm that samples were collected from the patient and there was no significant back flow from the ventilator circuit. The median inspired acetone concentration was 110 ppb (range 73–412 ppb) for all samples obtained. The median breath acetone concentration of all samples collected was 853 ppb (range 162–11 375 ppb). There was a high degree of inter-subject variation in breath acetone concentration. The median intra-subject CV for breath acetone concentration of all breath samples collected was 8.51% (range 0.7–36.2%). There was no relationship between increased variability in triplicate breath samples and alveolar dead space or lower PO_2/FiO_2. There were changes in alveolar dead space fraction and PO_2/FiO_2 over time; however, there was no relationship between changes in these measurements and changes in the mean breath acetone concentration. There was a relationship between inspired and exhaled acetone concentrations ($r_s = 0.39, p < 0.0001$). Mean $(C_{insp}/C_{exp}) \times 100$ was 11.8% (SEM ± 1.4).

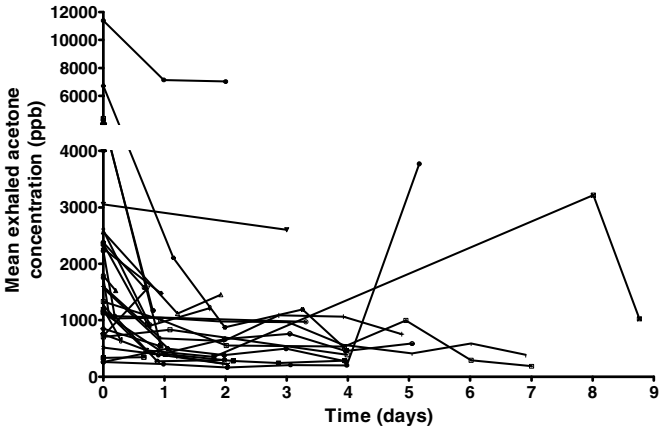

Figure 2. Variation in breath acetone concentration for each patient over time (lines join each sampling event for an individual patient).

There was no significant difference in breath acetone concentration between hyperglycaemic and euglycaemic patients. In general, there was a reduction in breath acetone concentration over time (figure 2). Increases in breath acetone

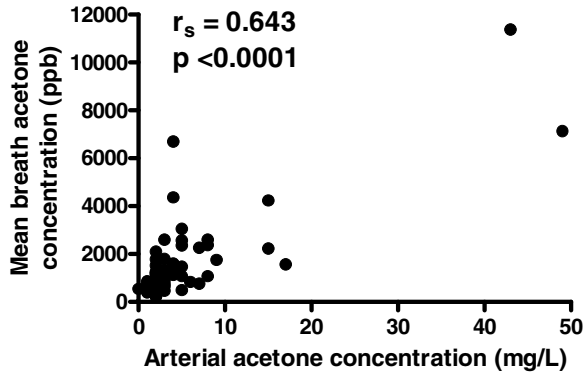

Figure 3. Individual data points of the relationship between breath and arterial acetone concentrations in the first two sets of samples collected from each patient.

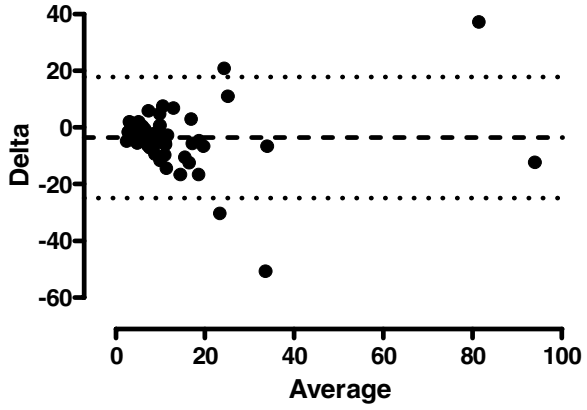

Figure 4. Modified Bland–Altman plot displaying the relationship between breath and arterial acetone concentrations as percentages of their maximal value. Average = average value of the normalised breath and arterial acetone concentrations; delta = difference between the normalised breath acetone concentration and the normalised arterial acetone concentration. Bias was −3.58 (dashed line) with 95% limits of agreement of −24.9 and 17.8 (dotted lines).

concentration were associated with surgery, reduction or discontinuation of feeding, increase in gastric aspirate volume (indicating a reduction in gut motility and/or malabsorption of enteral feed), general deterioration in condition (which may be an indicator of a reduction in gut motility and/or malabsorption of enteral feed) and imminent death. Changes in breath acetone concentration over time mirrored changes in arterial acetone concentration, although the relationship between breath and arterial concentrations was not constant.

There was a significant difference in PO_2/FiO_2 ratio between men and women ($p = 0.022$), men had poorer lung function, but this did not result in a difference in breath acetone concentration ($p = 0.32$).

There was a strong relationship between breath and arterial acetone concentrations in the first two sets of samples collected from each patient ($r_s = 0.64$, $p < 0.0001$) (figures 3 and 4). The modified Bland–Altman plot shows that as the normalized average concentration of acetone increases, the difference between the two measurements (breath and arterial) increases. There was a stronger correlation between breath and arterial acetone concentrations in patients with pneumonia than in all patients ($r_s = 0.78$, $p < 0.0001$). The relationship between breath acetone and arterial beta-hydroxybutyrate concentrations ($r_s = 0.52$, $p < 0.0001$) was similar to the relationship between arterial acetone and arterial beta-hydroxybutyrate concentrations ($r_s = 0.56$, $p < 0.0001$). Neither relationship was changed by subtracting inspired from exhaled acetone concentration. Removing the results from the patient with the highest breath and arterial ketone concentrations did not alter the relationships between breath acetone and arterial ketone concentrations. The Bland–Altman plot, however, showed a reduction in bias but an increase in the 95% limits of agreement (−37.4–42.2), i.e. the variability between the two measurements of acetone concentration appears to increase.

There was no relationship between breath acetone and arterial glucose concentrations in the first two sets of samples collected from each patient, either in all patients or in the group of hyperglycaemic patients. Figure 5 shows the relationship between exhaled acetone and arterial glucose concentrations at enrolment (a) and in the next set of samples taken (b). The graphs highlight that some patients remained ketotic, signified by high breath acetone concentration, despite normal, or near normal, arterial glucose concentration. There was a significant reduction in breath acetone concentration between pre-insulin and post-insulin samples in patients with hyperglycaemia ($p = 0.002$) (figure 6). There was no clear relationship between breath acetone concentration and modelled insulin sensitivity in the group of patients receiving exogenous insulin.

There was a reciprocal relationship between breath acetone concentration and the percentage of estimated feed requirement administered hourly in all patients ($r_s = -0.39$, $p < 0.0001$) and when subjects requiring insulin were analysed separately ($n = 19$, $r_s = -0.36$, $p = 0.0018$).

There was no significant difference in breath acetone concentration or acetone elimination rate between patients with pneumonia, SIRS, sepsis or ARDS and those without. There was no relationship between initial breath acetone concentration and mortality in the ICU or during hospital stay, acute physiology and Chronic Health Evaluation (APACHE II) score (Knaus *et al* 1985) or the requirement for renal replacement therapy. There was no relationship between breath acetone concentration and Sequential Organ Failure Assessment (SOFA) score (Vincent *et al* 1996), tidal breath volume, respiratory rate, heart rate or systolic blood pressure (SBP) on a daily basis. There was no significant difference in SBP between patients receiving ionotropic or vasopressor drugs, and no difference in breath acetone concentration was seen between patients in these two groups.

4. Discussion

Using a single breath end-tidal breath collection method, we have presented a reliable and repeatable method of breath collection for analysis of acetone concentration by SIFT-MS. The technique is simple to perform and uses equipment readily available in the ICU environment, thus making it attractive for use in routine clinical practice. Disruption to a patient's ventilation pattern was minimized as much as possible and arterial blood samples were collected from an arterial cannula

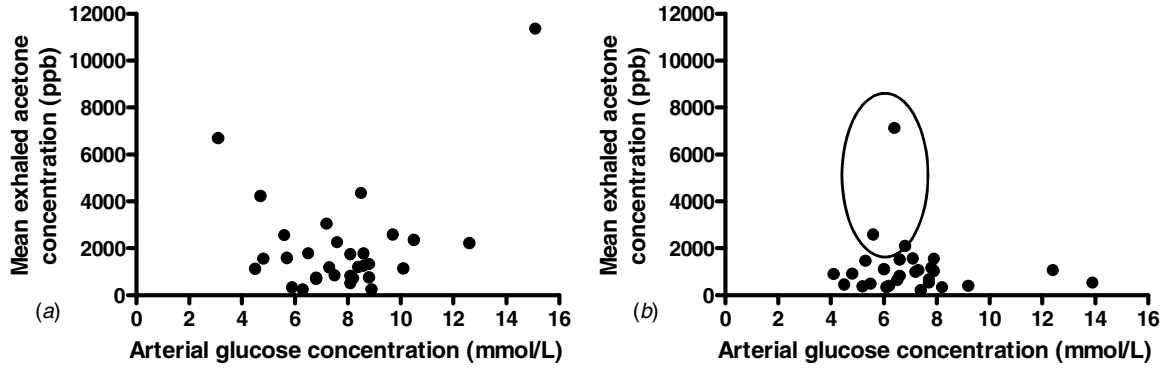

Figure 5. Individual data points of the relationship between breath acetone and arterial glucose concentrations at enrolment (*a*) and the next sample collection (*b*). The circle indicates patients with high breath acetone concentration with normal, or near normal, arterial glucose concentration.

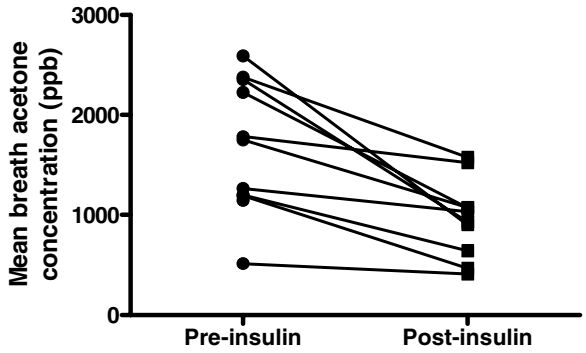

Figure 6. Individual data points show the change in mean breath acetone concentration after insulin administration in patients with hyperglycaemia, in whom pre-insulin and post-insulin samples were obtained ($n = 10$).

in situ for continuous blood pressure monitoring and routine blood sampling.

In this study, we have been able to show that the relationship between breath and arterial acetone concentrations, also seen in other studies (Schubert *et al* 2005, Reichard *et al* 1979, O'Hara *et al* 2009), may enable breath acetone to be used as a non-invasive marker of ketosis in intubated and ventilated patients in the ICU. Our Bland–Altman plot does, however, seem to indicate that the relationship changes as acetone concentration increases. In the study, by Reichard *et al* (1979) the ratio of plasma to breath acetone in fasted subjects was smaller when the plasma concentration was above 1 mg L^{-1} when compared to lower plasma concentrations. The relationship was not changed by using breath concentration after subtracting inspired acetone concentration; however, there was a correlation between inspired and exhaled acetone concentrations that is likely to be related to contamination of inspiratory samples by exhaled acetone in the inspiratory limb of the ventilator and by components of the ventilator or breathing circuit itself. Inspired gas samples were collected as close to the ventilator outlet as possible to minimize contamination and in all cases the ventilator, breathing circuit and breath collection apparatus were the same, so limiting the differences in acetone released from these components in different individuals.

The concentration of acetone in arterial samples at room temperature is not stable as it rapidly moves into the gas phase (Crofford *et al* 1977). Spontaneous decarboxylation of acetoacetate also occurs (Crofford *et al* 1977); therefore, blood samples must be kept cold until analysis. It has been suggested that the variability of repeated measurements of blood acetone concentration in single blood samples is higher than that of breath samples, indicating that breath acetone concentration may be a more reliable measurement (O'Hara *et al* 2009). Due to the problems with blood acetone measurement and with a reasonable correlation between breath and arterial acetone concentrations in this experiment, it can be concluded that breath acetone may be used as a surrogate for systemic acetone concentrations.

The relationship between breath acetone and arterial beta-hydroxybutyrate concentrations was not as strong but still significant. Our results were very similar to the relationship seen in subjects fed a ketogenic diet (Musa-Veloso *et al* 2002). The relationship between the different ketone bodies is dependent on the redox state of the cells; therefore, the relationship between breath and arterial acetone concentrations can be expected to be stronger than that between breath acetone and other arterial ketone concentrations (Carmant 2008).

This is the first study to collect daily breath and blood samples for up to 8 days from patients in the ICU. Changes in breath acetone concentration were seen over time, with a general trend towards reduction in acetone concentration with treatment and feeding. The reduction in breath acetone concentration over time was not constant, with a more rapid decrease in the first 48 hours, which may have reflected the fact that patients who had rapid clinical improvement started to breathe spontaneously earlier and therefore did not provide many breath samples. Patients who were enrolled for a greater length of time were likely to be more unwell and may have shown less rapid improvement in breath acetone concentration over time.

Unfortunately, because many factors appear to affect breath acetone concentration, including degree of lipolysis, breathing pattern (Cope *et al* 2004), dead space ventilation and pulmonary shunt (West and Wagner 1998), cardiac output (Schubert *et al* 2004) and airway perfusion, some of which

cannot be controlled for, interpretation of results remains difficult. In this study, we were not able to measure cardiac output or pulmonary shunt as patients did not have a pulmonary artery catheter in situ, and alveolar dead space ventilation was calculated instead of total dead space ventilation as we could not measure mixed expiratory CO_2. We saw no relationship between the change in alveolar dead space and breath acetone concentration over time, which indicates that factors other than alveolar dead space fraction were more important in determining breath acetone concentration. We were able to collect representative breath samples using a single breath collection technique as each patient was ventilated on a controlled mode without spontaneous breathing, so an almost identical tidal volume was delivered with each breath. A problem with this method compared to other breath collection methods was that breath sampling had to be discontinued when the patient's condition improved enough for spontaneous assisted ventilation. Each patient was ventilated using a mode, tidal volume and respiratory rate set by the ICU staff, so it was possible that different patients' breathing patterns may have led to differences in breath acetone concentration (Cope *et al* 2004); however, we did not see a relationship between respiratory rate or tidal breath volume and breath acetone concentration.

In a study by Schubert *et al* (2005), the degree of pulmonary shunt was greater in patients with sepsis but dead space ventilation was not different between groups. We found a difference in the degree of alveolar dead space but not A-a gradient in patients with ARDS versus no ARDS, and patients with pneumonia versus those without; however, our study population was different (data not shown). We enrolled patients with pulmonary infiltrates and/or insulin requirement, and could only collect samples from patients requiring mandatory ventilation, so spontaneously breathing ventilated patients who had less severe illness were excluded. The benefit was that we were able to take samples very early in a patient's ICU stay, before aggressive treatment had been instigated; therefore, patients should have had the severest metabolic derangement making any changes in breath concentration more obvious. It is possible that if a less unwell group of patients were included as a control, then differences between patients with sepsis, SIRS and ARDS versus no sepsis, SIRS or ARDS would have been greater. ARDS guidelines have recently been updated (ARDS Definition Ranieri *et al* 2012) so, in comparison to previous studies, our group of ARDS patients included those with less severe lung injury, previously termed acute lung injury (Bernard *et al* 1994). Moving this sub-group of patients to the non-ARDS group did not, however, change the results of our analysis.

Our study shows that it is not possible to use breath acetone concentration or acetone elimination rate to distinguish between pneumonia and other causes of pulmonary infiltrates, or ARDS and those with less severe lung injury. This is in agreement with a study by Schubert *et al* (2005), who found no difference in breath acetone concentration between septic and non-septic patients. They did, however, find a significant difference in mixed venous and arterial blood acetone concentrations between septic and non-septic patients, which we did not see in our study.

In the sub-group of patients with pneumonia, there appeared to be a stronger relationship between breath and arterial acetone concentrations. The explanation for this is unclear. Eight of the 12 patients had positive microbiological findings for *Legionella* species, *H. influenza* and *S. pneumonia*. Unlike *Pseudomonas aeruginosa*, which produces characteristic compounds, including hydrogen cyanide (Carroll *et al* 2005, Julak *et al* 2006, Ryall *et al* 2008) and 2-aminoacetophenone (Scott-Thomas *et al* 2010), there are no data on VOCs produced by *Legionella* species and little data for *H. influenza* and *S. pneumonia*. Studies of VOCs produced by bacteria in various culture media (Allardyce *et al* 2006, Julak *et al* 2006, Zhu *et al* 2010, Filipiak *et al* 2012) show individual patterns related to different species, and that most produce small quantities of acetone, although only one of these studies included samples containing the bacteria detected in our study (Filipiak *et al* 2012). Acetone was frequently detected in the headspace above various bacterial cultures on agar plates, although seldom at significantly higher concentrations than background levels produced by heated culture media (Thorn *et al* 2011). It is possible that acetone was produced in the lungs by pathogenic bacteria in this study; however, it is unlikely to have reached high enough concentrations to be detected in single breath samples without pre-concentration (Chambers *et al* 2012).

There was no relationship between breath acetone and blood glucose concentrations in these non-diabetic subjects. Starvation and ketogenic meals increase breath acetone concentration (Smith *et al* 1999, Musa-Veloso *et al* 2002, Spanel *et al* 2011), with a rise in breath acetone concentration seen in our patients when feed volume was reduced, feeding was stopped or the patient was not absorbing their feed adequately. There are no data to suggest that enteral feed itself produces acetone or that feed sitting in the stomach of patients with high gastric aspirates may interfere with breath acetone concentrations. In fact, the presence of a cuffed endotracheal tube is likely to reduce contamination of breath samples by gastric contents, for example, ethanol, which may be released by food itself or by the activity of gut bacteria (Turner *et al* 2006b). Increased feed volume appeared to reduce breath acetone concentration, which is in agreement with studies in healthy subjects who were fasted and then fed (Smith *et al* 1999, Turner *et al* 2008). Feeding in the ICU is administered continuously; therefore, any changes in breath acetone concentration are likely to occur gradually, and there should not be a diurnal variation as seen in normal subjects (Turner *et al* 2006a, Dummer *et al* 2010).

There was a significant reduction in breath acetone concentration between pre- and post-insulin samples in hyperglycaemic patients, which is likely to be due to a combination of treatment with insulin and feeding. There is no formal agreement around several areas of feeding in the ICU, mainly when feeding should be started and the carbohydrate content and composition of the feed. Overfeeding can be detrimental, not only due to the increased risk of aspiration if gastric emptying is delayed, but it may also contribute to hyperglycaemia if it is present. Despite high glucose requirements due to accelerated protein catabolism (Cerra *et al*

1997), lower calorie feeding may improve ICU and hospital outcomes (Arabi *et al* 2011, Krishnan *et al* 2003). As can be seen from our results, some patients remained ketotic despite normal, or near normal, glucose concentrations, which may be because insufficient time had elapsed following insulin treatment to see a reduction in breath acetone concentration, or may reflect a group of patients with a high degree of inflammation and catecholamine release driving the ketogenic pathway and promoting insulin resistance (Kupai *et al* 1995, Van den Berghe 2004). These patients may also be ketotic due to relative starvation and require more carbohydrate feeding. To explore further whether breath acetone concentration could track the degree of ketosis and aid the modulation of feeding and insulin regimes in this group of patients, the frequency of breath acetone sampling must be increased or a continuous sampling method should be employed.

In conclusion, we were able to accurately and reliably measure breath acetone concentrations in intubated and ventilated patients in the ICU via SIFT-MS. We saw a relationship between breath acetone and arterial acetone and beta-hydroxybutyrate concentrations that may enable breath acetone to be used as a surrogate for blood concentrations. The administration of insulin and feeding caused a significant reduction in breath acetone concentration in patients with stress hyperglycaemia, and the reduction in feed volume and difficulty absorbing feed resulted in increased breath acetone concentration. The relationship between breath acetone, feed volume and insulin administration needs further investigation before breath acetone can be used to aid the modulation of feeding regimes and insulin therapy.

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