

An exploratory comparative study of volatile compounds in exhaled breath and emitted by skin using selected ion flow tube mass spectrometry (SIFT-MS)

Short title: Measuring compounds from skin and breath

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Abstract.

Selected ion flow tube mass spectrometry, SIFT-MS, has been used to carry out a pilot parallel study on five volunteers to determine changes occurring in several trace compounds present in exhaled breath and emitted from skin into a collection bag surrounding part of the arm, before and after ingesting 75g of glucose in the fasting state. SIFT-MS enabled real time quantification of ammonia, methanol, ethanol, propanol, formaldehyde, acetaldehyde, isoprene and acetone. Following glucose ingestion, blood glucose and trace compound levels were measured every 30 minutes for two hours. All the above compounds, except formaldehyde, were detected at the expected levels in exhaled breath of all volunteers; all the above compounds, except isoprene, were detected in the collection bag. Ammonia, methanol and ethanol were present at lower levels in the bag than in the breath. The aldehydes were present at higher levels in the bag than in breath. The blood glucose increased to a peak about one hour post ingestion, but this change was not obviously correlated with temporal changes in any of the compounds in breath or emitted by skin, except for acetone. The decrease in breath acetone was closely mirrored by skin-emitted acetone in three volunteers. Breath and skin acetone also clearly change with blood glucose and further work may ultimately enable inferences to be drawn of the blood glucose concentration from skin or breath measurements in type 1 diabetes.

Keywords: volatile monitoring, breath, skin, SIFT-MS

Introduction.

The rationale for this study

The new science of breath analysis is now receiving much attention in several laboratories worldwide because of its growing potential as a non-invasive clinical diagnostic, as reported in a recent book [1]. The inception of appropriate analytical techniques, especially those that allow real time analyses of breath metabolites, obviating sample collection into bags or onto traps, can now provide immediate results to the clinician and reveal the short time influences of therapy such as drug administration and dialysis. Breath analysis is patient friendly and especially suitable for children and frail patients who can usually provide breath samples without difficulty. However, there are circumstances where breath sampling is more difficult, for example, when a patient is comatose or under intensive care. Although monitoring such patients has been demonstrated [1], it is attractive to consider if the release of endogenous volatile compounds through the skin could be measured and be clinically meaningful. The skin is a complex organ a major function of which is to act as a barrier to the passage of bioactive species into the body. Yet it is clear that endogenous volatile compounds pass through the skin, as any real time trace gas analytical technique can readily indicate; the strong ammoniacal odour in dialysis units is a manifestation of this. Some trace compounds are generated only on the skin surface and in the pores by bacteria resulting in “body odour”; the detection of these compounds has little clinical value. The challenge is to establish which of the truly endogenous (blood stream) volatile components readily cross the skin barrier into the air and be useful as a diagnostic or monitor of the physiological status of the individual. The potential value of this was recognised by [2] who have carried out experiments in which both skin emission and breath content of acetone and hydrogen were correlated, with interesting results, about which more will be said later in this paper. VOC emissions at skin surfaces have also been used as a potential exposure index to toxic substances [3]. In another study [4], skin volatiles have been measured using a skin cuvette to study ultraviolet (UV) light-induced lipid peroxidation in human skin.

Endogenous volatile compounds can now be measured in breath to a clinically acceptable accuracy using the recently developed selected ion flow tube mass spectrometry, SIFT-MS, technique, which was developed in our laboratories [1, 5]. Using SIFT-MS, on line real time analyses of single breath exhalations can be achieved down to the parts-per-billion, ppb, level [5]. Thus, using SIFT-MS, the concentration distributions of the common breath

metabolites, viz. ammonia, acetone, methanol, ethanol, propanol, acetaldehyde and isoprene, have been established and reported in a series of papers published recently [6, 7, 8, 9]. Importantly, using this technique the concentrations in the alveolar portion of the breath can be obtained, since the concentration time profile through the exhalation can be defined because of the fast response of the SIFT-MS instrument, and it is known that this alveolar concentration can be related to the blood concentrations of some metabolites [6] using the appropriate gas phase/blood phase partition coefficients (for example [10]). With this information as a reference, emissions through/from the skin can now be investigated. This is the subject of the current report.

However, skin emissions cannot be so readily quantified, since factors such as skin area and temperature are difficult to assess and control, and bacterial action can chemically modify some of the truly endogenous compounds that pass through the skin and also generate other compounds. But if the blood concentrations of certain endogenous blood compounds can be deliberately modified and monitored by breath analysis and their skin emissions monitored, then correlations between breath/blood levels and skin release can be investigated. If such connections can be established then skin emission monitoring can have practical value, as mentioned in the previous paragraph. People with diabetes, for example, monitor their blood glucose status through frequent finger pricking to produce a drop of capillary blood in which they measure the glucose concentration. An alternative non-invasive technique could be to monitor the concentration of an appropriate volatile marker in exhaled breath or from the skin, if such a marker can be recognised. In this paper we show that changes in blood glucose and breath and skin related acetone occur during the course of an oral glucose tolerance test (OGTT) and very tentatively suggest that breath/skin analyses may provide the desired non-invasive monitor of this important parameter.

A brief description of SIFT-MS.

The SIFT-MS analytical technique, including its application to breath analysis, has been described in detail elsewhere [1, 5, 11, 12]; hence we need only provide a brief summary here. A chosen precursor ion species (either H_3O^+ , NO^+ or O_2^+) is selected from the mixture of ion species formed in a microwave discharge through moist air by a quadrupole mass filter and injected into fast-flowing helium carrier gas. Air containing trace gases, exhaled breath or liquid headspace can be presented to the entry port of the SIFT-MS instrument via a disposable cardboard mouthpiece and a sample enters the carrier gas at a known flow rate via a heated calibrated capillary. Characteristic product ions are produced from the reactions of each trace

gas compound with each particular precursor ion species. It is the ratio of the count rates of the product ions to the precursor ions that is the essential parameter in the quantification of particular trace gas compounds. More details relating to the modes of data acquisition in SIFT-MS are given in [5] and [13].

There are two distinct analytical modes of operation of SIFT-MS. Firstly, the *full scan mode*, where a conventional mass spectrum is obtained over a chosen range of ion mass-to-charge ratio, m/z , in order to identify the precursor and product ions and to determine their respective count rates. From these, the on-line computer immediately calculates the partial pressures of those trace gas compounds present in the breath sample exploiting the in-built kinetics database, which comprises the rate coefficients and the product ions of the particular precursor ion/trace gas compound reactions. This database has been constructed from numerous detailed selected ion flow tube (SIFT) studies of the reactions of various classes of compounds (including alcohols, aldehydes, ketones and hydrocarbons) with the three SIFT-MS precursor ions given above [5, 11, 12]. Secondly, the *multiple ion monitoring mode* in which the downstream analytical mass spectrometer is rapidly switched between selected m/z values for both the precursor ions and the product ions, in order to quantify both water vapour and the targeted trace compounds. This mode of operation provides more accurate quantification of the chosen trace compounds than does the broad sweep *full scan mode*. An example of the breath data obtained using the *multiple ion monitoring mode* is presented in figure 1a, and in skin, figure 1b. Note, also, the important point that during the breath inhalation cycles the levels of the targeted compounds in the ambient (laboratory) air are determined, which allows an assessment to be made as to whether or not the ambient levels influence the derived breath levels. The water vapour concentration in the air/breath samples are also calculated from the levels of the hydrated precursor H_3O^+ ions and such measurements act as an internal calibration of the sampling system [5]. With the skin measurements (Fig 1b), note that the water concentration is lower: this is due to the fact that the skin has a lower temperature than the blood/lung interface and also that the air is not fully saturated with water, as is the case in human breath.

Experimental protocol

Monitoring breath and skin volatiles and blood glucose (OGTT).

Five healthy male volunteers (ages: three in their mid twenties, one of 42 and one of 49 years) were asked to fast overnight (for at least 12 hours) and on arriving in the laboratory were

asked to wash their hand and arm thoroughly with tap water. The use of soap and other cleansing agents was avoided, because exploratory tests had shown that these resulted in contamination by extraneous volatile compounds. Each volunteer's blood sugar was measured with a Therasense Blood Glucose sensor (Abbott Diabetes Care, 1360 South Loop Road Alameda, CA 94502, USA) and then a "bag" was clamped around the hand and forearm, secured with a plastic tie and filled with dry clean cylinder air. The bag was made from Nalophan tubing, which is manufactured from polyethyleneterephthalate and available in a range of sizes from Kalle UK Limited (Perry Road, Industrial Estate East, Witham, Essex); in this experiment, it had a "filled" diameter of 135mm. Nalophan is used here due to the fact that it does not emit contaminating compounds detectable by SIFT-MS. One end of the Nalophan tube was attached to a polypropylene tube fitted with a Swagelok connector for attachment to the SIFT-MS instrument; the other open end could be slid over the forearm and secured with a plastic tie. The bag was secured over the arm and while the skin volatiles were equilibrating within the bag, the volunteer was asked to provide direct breath samples into the SIFT-MS instrument for analysis. The air from the bag attached over the skin of the arm was then analysed using SIFT-MS, having accumulated the volatiles for approximately 7 minutes during which time we assume that equilibrium between skin emission and absorption was reached (as in a separate unpublished experiment, it was found that skin volatiles are emitted quickly and an equilibrium reached within a few minutes). The approximate volume of air in each bag was about 4 litres. No attempt was made to optimize the time and proportion of gas to skin surface; rather the analysis took a snapshot of what was present in the bag 7 minutes after "bagging the arm". Figure 2 shows the setup for arm sampling. No attempt was made to stir the trapped gas in the bag; rather natural convection was relied upon to mix the volatiles emitted from the skin with the air. The volunteer then consumed 75g of glucose dissolved in water, and at 30 minute intervals for two hours, blood sugar and the breath and skin volatiles ammonia, acetone, methanol, ethanol, propanol, formaldehyde, acetaldehyde and isoprene were measured.

SIFT-MS analysis of each component

Analysis of ammonia, methanol, ethanol, propanol, formaldehyde and acetaldehyde was carried out using the H_3O^+ precursor; isoprene and acetone was analysed with NO^+ . The rate coefficients for the reactions of each of the compounds with H_3O^+ , included in the kinetic library for quantitative SIFT-MS analysis were: ammonia: $2.6 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ (product ions at mass-to-charge ratios, m/z, at 18, 36 & 54); methanol: $2.4 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ (product ions at m/z 33, 51, 69); ethanol: $2.7 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ (product ions at m/z 47, 65, 83); propanol: $2.7 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$

(product ion at m/z 43); formaldehyde: $2.7 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ (product ion at m/z 31); acetaldehyde: $3.7 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ (product ions at m/z 45, 63, 81). Similarly, for NO^+ , acetone: $1.8 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ (product ion at m/z 88); isoprene: $1.7 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ (product ion at m/z 68). The absolute concentrations were calculated according to the method described in [13]. Ionic diffusion was accounted for in the SIFT-MS instrument used in this study according to equation (14) in [13] with the D_e values for ions approximated as a function of their m/z values as:

$$D_e = 1 + 0.0167(m/z - 19) \quad (1)$$

The precursor ion count rate for NO^+ (m/z 30) was approximately 130,000 counts per second, c/s, and for H_3O^+ (m/z 19) was 40 000 c/s, although the sum total c/s of m/z 19, m/z 37, m/z 55 and m/z 73 was approximately 100,000 c/s. At these count rates the limit of detection for a 5 seconds measurement is better than 5 ppb in the SIFT-MS Mk.2 instrument used. The precision and accuracy of measurement for values above 100 ppb is better than +/- 10% [14].

Comparison of skin and breath – full scan spectra

In order to compare not just the quantitative analysis of breath and skin compounds, but also the qualitative assessment of the differences between breath and skin, full spectra (using full scan mode described above) using three SIFT-MS precursor ions (H_3O^+ , NO^+ and O_2^+) were taken for two minutes each of skin and breath. For the skin spectra the SIFT-MS instrument analysed the bag directly for two minutes in the m/z range of 10 – 130 as described earlier after incubation of seven minutes. In order to obtain a direct comparison and because a volunteer cannot be expected to exhale into the instrument directly for 2 minutes, the same volunteer was asked to breathe into a bag made from identical material (Nalophan) and this bag was analysed for two minutes at the same m/z range (10 – 130) immediately after being produced. These analyses were made after breakfast i.e. without fasting.

Results and discussion.

Prior to the glucose ingestion the blood glucose levels in all five volunteers were within the range 4-6 mmol/litre and following the glucose ingestion the levels typically increased by about a factor of two after one hour, after which they declined. This blood glucose modification and the obvious maxima reached in the glucose levels allow comparisons with breath levels and skin emissions of the various volatile compounds.

It must be recognised immediately that direct comparisons of the magnitudes of the concentrations of the various volatile compounds measured in alveolar breath and in the collecting bag around the arm must be treated circumspectly, because the equilibrium concentrations of the various volatiles between blood and breath relate to the alveolar interface temperature (close to 36°C) whereas the skin temperature is lower than this. Thus, even if the skin release rates are in direct proportion to the blood concentrations and equilibrium is reached, the data interpretation is difficult. In part, this is due to the lack of skin temperature control, the arbitrary time for which the volatiles were allowed to accumulate into the bag volume and the likelihood of some condensation onto the cooler bag surface of some compounds, notably water, and very water soluble compounds, especially ammonia. It is for these reasons that in these initial experiments we only consider comparing trends in breath and skin data. However, having made these points, it is worth noting that the actual equilibrium values of bag volume concentrations are only a factor of three or so lower than the corresponding alveolar concentrations, as can be seen in the graphical data presented below. We now discuss the results obtained for each of the targeted volatiles, this being the simplest way to show the confounding factors that confuse the skin data obtained for most volatiles. Our results suggest that in the majority of cases, factors such as skin bacterial action confound the data. However, acetone appears to be largely uninfluenced by such effects and the results are seemingly more readily interpreted against blood glucose concentration.

Isoprene:

This hydrocarbon is present in the exhaled breath of all persons at levels typically within the range 40 to 300 ppb [8]. The mean value for the present 5 volunteers is 212 ppb with a standard deviation (s.d.) of 60 ppb, the value remaining relatively constant in the breath of each volunteer during the 2 hours of the experiments; so there is no detectable variation of the breath isoprene with blood glucose variation. However, isoprene could not be detected in the gas volume surround the skin, which is somewhat surprising. Isoprene is a stable hydrocarbon that is present in the global troposphere at a level of 300 parts-per-trillion, ppt, mostly emitted by trees and plants [15]. It is not obvious why it was not present in the skin/air sample above the detection limit (5 ppb) of the SIFT-MS instrument used for these studies, so this requires further investigation. However, in a separate study (data not shown here), breath isoprene analysed from a bag (even after several hours) is representative of direct breath samples into the SIFT-MS instrument, indicating that this inability to detect isoprene from skin is not down to adsorption onto the bag or losses from it.

Ammonia:

The results obtained for breath ammonia show similar concentration time profiles for all five volunteers. The mean value for the breath samples given by all five volunteers here is 854 ppb, and the median value is 830 ppb. The actual levels measured for these volunteers fall within those expected, as determined from the study involving a cohort of 30 healthy volunteers (spanning the age range 20-60 years), as reported in the recent paper [9] that indicated a geometric mean breath ammonia concentration (in this log-normal distribution) of 833 ppb. However, in all cases there is an obvious decrease in breath ammonia following the ingestion of the glucose solution, as is exemplified in Figure 3. This “ammonia dip” is due to two combining factors that are transitory (operative for about 30 minutes following liquid ingestion): the washing of the mouth by the glucose solution and the increase in portal blood flow stimulated by the entry of the solution into the stomach, as has been reported in a previous paper [16]. Also, it has recently been observed that the majority of ammonia seen in mouth exhaled breath has its origin in the oral cavity, being formed by bacterial and/or enzymatic activity on salivary urea (D. Smith, private communication). Unfortunately, these phenomena mask any change in breath ammonia that might occur as the blood glucose increases, but changes in blood equilibrium levels are not expected due to the ingestion of a carbohydrate. No pattern in the comparisons of breath and skin ammonia emerges from the data for these volunteers. The measured bag (skin) levels are generally somewhat lower than the breath levels but quite scattered. It is not surprising that the bag levels are so variable in view of the high solubility of ammonia in water and water vapour condensation and temperature gradients within the arm/bag system. The ammonia emission will also strongly depend on the pH of the blood and water near the skin surface. Indeed, it is somewhat surprising that the bag levels differ so little from the exhaled breath levels.

Methanol, ethanol and propanol.

These three alcohols are always present in exhaled breath; their distributions have been determined for a representative cohort of healthy individuals previously [6, 7, 9]. The mean values in ppb (and their standard deviations in parentheses) of methanol, ethanol and propanol for that cohort are reported as 502 (239), 196 (244) and 22 (17) respectively. All three alcohols are also emitted from the skin, but there are only weak correlations between the breath and skin data for these. However, it consistently emerges that the apparent breath ethanol levels increase shortly after the intake of the glucose solution, as can be seen in Figure 4. For clarity, we show

the results of just one volunteer, but a similar pattern is seen for all volunteers in this experiment. This is due to the production of ethanol by the action of mouth flora on the glucose, as has similarly been shown previously following the intake of sucrose solution [7, 17]. Actually, there is generally a slow decrease in both the breath and skin levels of these alcohols with time, indicating that the glucose does not significantly increase their endogenous production, even though sugar ingestion has often been seen to increase breath ethanol, again most probably due to mouth flora although some gut flora activity may play a role [7]. This trend can be seen in Figure 5, which shows values for the same single volunteer. These measurements also indicate that the levels of propanol in the bag are consistently higher than those in breath, whereas for methanol and ethanol the reverse is true. The median concentrations of methanol, ethanol and propanol for all volunteers are: 444, 153 and 94 ppb respectively. Background levels of volatiles are known to affect measured blood/breath levels of volatiles; typical background concentrations measured during this study are 150 ppb for methanol, 100 ppb for ethanol and 150 ppb for propanol. In comparison to the typical breath levels this is particularly high in the case of propanol, which may be why the propanol levels measured in breath in this study are significantly higher than those previously reported [9]. Mean (and standard deviation) values in ppb for each volunteer are as follows:

- methanol - V1: 1525 (296); V2 : 228 (117); V3: 443 (114); V4: 733 (172); V5: 138 (91);
- ethanol - V1: 186 (171); V2: 143 (104); V3: 110 (91); V4: 207 (231); V5: 215 (115);
- propanol - V1: 105 (62); V2: 124 (87); V3: 122 (95); V4: 98 (35); V5: 61 (36).

That the bag levels of propanol are higher than the breath levels may be due, in part, to diffusion of this alcohol across the Nalophan bag material because of its relatively higher concentration in the laboratory air.

Formaldehyde and acetaldehyde.

Both of these aldehydes have been detected in exhaled breath at relatively low levels, formaldehyde at less than 10 ppb (A. Amman; priv.com) and acetaldehyde at a typical level of 20 ppb [7]. In the present experiments, formaldehyde was not detected in the exhaled breath, presumably because the level was below the detection limit of the current SIFT-MS instrument. Acetaldehyde was detected in breath at the expected levels, as indicated above. The interesting observation is that both aldehydes were present in the bag samples at higher levels than in the breath. Why should this be? It is most probably due to the partial conversion of skin released methanol and ethanol to the aldehydes by bacterial action and /or by isoenzymes such as

alcohol dehydrogenase. Formaldehyde and acetaldehyde were immeasurable in the laboratory air. These observations are worthy of further investigation.

Acetone.

It is known from several previous studies, including an early SIFT-MS study [16], that breath acetone levels are relatively high in the morning following overnight fasting. An increase in breath acetone has also been observed following 24 hour fasting periods [2]. The concentration distribution for breath acetone has been established for a cohort of healthy volunteers under normal dietary status [9] and the geometric mean level (and multiplicative standard deviation) is seen to be 477 ppb (1.58). As can be seen in Figure 6, the breath acetone level for one of the five volunteers prior to the glucose ingestion is about 1400 ppb, which is higher than average probably due to the overnight fast. Following the glucose ingestion and the subsequent increase in the blood glucose, the breath acetone levels fall in all five volunteers; in 3 volunteers this fall is obvious; less so for the other 2 volunteers. This is to be expected when the body is fed, as reported in previous publications, for example [16]. What is more, the acetone levels in the bags also fall and closely mirror the breath levels and there are indications in all cases that these acetone levels begin to increase again as the blood glucose levels decrease. After the initial fall, the levels start to rise or stay the same, until the last time point where 2 volunteers had a slight fall in breath acetone; a similar trend is mostly seen in the skin acetone of the same volunteers. Hence, there is a clear relationship between the levels of breath acetone and acetone release from the skin, unlike the other volatile compounds, and is probably explained by the relative chemical inertness of this ketone. This relationship is shown in Figure 7, where the skin acetone relative to pre-dose level is plotted against the breath concentration relative to the pre-dose level, yielding a correlation with a R^2 of 0.7. The significance for the 24 observations involved being $p < 0.0001$. The correlations for each of the volunteers for breath acetone versus skin acetone yielded R^2 values as follows: V1: 0.27; V2: 0.3; V3: 0.71; V4: 0.92; V5: 0.85. These values are very variable but overall quite significant. A similar close correlation has been shown previously by [2] using GC-MS analyses of breath and skin acetone. Their measurements of the absolute emission rates of acetone from the skin resulted in a correlation factor R^2 of 0.8 between these parameters. This offers an opportunity to estimate the blood levels of acetone from the skin emission rates, but such comparisons must involve a consideration of the variation of partition coefficient with temperature of the dermal fluids and the alveolar interface, as mentioned in the Introduction.

Qualitative differences between breath and skin

The easiest way to visualise a comparison between breath and skin studies is to compare the mass spectra taken from breath and skin samples. Figure 8a shows the spectrum obtained from the bag which had been fitted to the arm for seven minutes. By comparison, a spectrum from breath analysed for 2 minutes from a breath bag, also using the H_3O^+ precursor, is shown in Figure 8b. Apart from the quantitative differences as described for various compounds above, there are differences in the compounds present. Firstly, note that for some compounds, the different ions visible are due to the higher amount of water present. For instance, acetone, which forms MH^+ with H_3O^+ gives a product ion at m/z 59, but in conditions of high humidity, it also has the addition of water cluster ions, with 1 H_2O at m/z 77 and 2 H_2O at m/z 95. In figure 9b (skin), note that the m/z 95 ion is absent. A similar situation occurs for acetaldehyde, where MH^+ would be at m/z 45. This is absent from both skin and breath spectra but in the skin sample, a 1 H_2O cluster is visible at m/z 63 but not in breath, although a 2 H_2O cluster is visible in breath (9a) at m/z 81. Another difference is the clear presence of formaldehyde in skin (9b) at m/z 31 which is not detectable on breath, as described earlier in the text.

Concluding remarks.

These pilot experiments have shown the promise and the pitfalls in comparing the skin emission rates of volatile compounds with their breath/blood levels. One problem appears to be that some compounds, notably alcohols, may be partially chemically modified by the action of skin bacteria or isoenzymes as they pass through the skin. The concentration of water in the bags surrounding the skin was between 3.5 to 4.25 %, and this is clearly lower than the concentration in breath (approximately 6%). This difference is mostly due to the lower skin temperature compared to the alveolar interface temperature and the lower temperature of the bag surface compared to the skin surface, which can result in condensation of water vapour and some VOCs onto the bag surface, thus reducing the concentration of some compounds in the air in the bag. Notwithstanding these problems, it is clear that most of the volatile compounds that appear in exhaled breath also appear in the collection bag around the skin and so skin-emission levels may be a useful monitor of changes in the blood levels. This could be particularly useful for blood ammonia monitoring, because this metabolite builds up to very high levels in the blood stream and in the breath of patients with kidney failure, which reduces dramatically during haemodialysis [18]. Thus, skin emission rates of ammonia could be used to monitor the efficacy of haemodialysis treatment.

But the most impressive data obtained in the present study relate to the correlation of breath and skin-released acetone. As the blood glucose level is increased by glucose ingestion the breath/skin acetone levels decrease and as the glucose is metabolised these acetone levels start to increase. In principle, therefore, a breath and/or a skin level measurement of acetone may indicate an approximate blood glucose status in people with type 1 diabetes, as emphasized in [2] following their sample collection/GC-MS studies. These non-invasive analyses are more patient friendly than skin pricking for blood samples and might thus be the monitor of choice if suitably convenient detectors of acetone can be produced. Although the relationship between blood sugar and breath acetone is not direct, as early as 1969, [19] found that “measurement of the breath acetone can detect inadequacies of control not revealed by measurement of the blood-sugar alone”, indicating that monitoring acetone is likely to have clinical value in diabetes control. In [20] the usefulness of analysis of breath compounds, including acetone, was shown in monitoring serum glucose levels. However, we emphasize that this is far from a proven technique and its usefulness may be limited in determining gross changes in blood glucose. Clinical studies are required to assess the potential for this in people with type 1 diabetes.

The difference between skin and breath values for the other compounds is open to speculation. One possibility is that the skin values better reflect values in blood, as some component of breath volatiles may originate from the action of mouth flora. Further study is required in which these compounds are analysed in blood, breath and skin.

One great advantage of the SIFT-MS analytical technique and other direct monitoring techniques is that they can be used at the bedside to perform on line, real time analyses of both exhaled breath and skin-released acetone and other trace gases. Thus, it could be used to monitor blood glucose status in the hospital ward. However, as SIFT-MS is unlikely to become small and inexpensive enough for personal use, smaller, handheld devices specifically for acetone quantification should be developed for personal use in the home.

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Captions to Figures

Figure 1. SIFT-MS traces showing the real time monitoring mode of the instrument analysing breath and skin. Figure 1a: Analysis of breath, showing the inhalations (where background air is analysed) and exhalations, showing alveolar breath into the instrument. Note the higher water concentration in the exhalations. Figure 1b: Analysis of skin, made by connecting the arm bag directly to the SIFT-MS instrument and analysing the contents in real time.

Figure 2. Photograph of the sampling bag attached to the arm and the setup for SIFT-MS analysis of skin volatiles.

Figure 3. Ammonia concentrations measured from breath and skin for 5 volunteers before and after glucose ingestion.

Figure 4. Change of breath and skin-emitted ethanol concentrations plus blood glucose concentrations associated with an oral glucose tolerance test for a single volunteer. Results for all 5 volunteers are similar and show an increase in breath ethanol about 30 minutes after glucose consumption, mostly due to production by mouth flora.

Figure 5. Concentrations of methanol, ethanol and propanol in exhaled breath and in the bag surrounding the skin from a single volunteer as an example before and at 30 minute intervals after glucose ingestion.

Figure 6. The results from all 5 volunteers for breath and skin-released acetone and blood glucose before glucose ingestion and at 30 minutes intervals after glucose ingestion.

Figure 7. Correlation of skin acetone levels and breath acetone levels, each taken relative to the initial levels before glucose ingestion.

Figure 8. Mass spectra, in full scan mode, from m/z 10 to 130 for analyses of breath from a breath bag (8a) and from skin (8b).