Potential of breath and skin analysis for monitoring blood glucose concentration in diabetes

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Abstract

The ability to monitor blood glucose non-invasively has long been a goal of those with diabetes due to the pain and inconvenience of current blood glucose monitoring devices. This review investigates the potential for monitoring compounds in breath and emitted through skin for inferring blood glucose concentration. Potential markers and an assessment of their suitability for non-invasive monitoring are discussed. The varying technologies developed for monitoring volatile organic compounds (VOCs) in breath and from skin of diabetics and their suitability for development as a hand held device is reviewed. The potential exists for the use of breath and skin monitoring as an alternative to blood glucose but it may take years to collect sufficient clinical data for robust correlations to be possible.

Keywords:

Diabetes mellitus; non-invasive monitoring; breath monitoring; skin monitoring; VOCs; blood glucose concentration; gas chromatography; mass spectrometry; gas sensing.

1. Introduction

One of the biggest problems in trying to keep diabetics healthy is in maintaining blood glucose concentrations within safe limits. Technology is available to monitor and maintain blood glucose concentration, yet many people with diabetes do not use the blood glucose monitor and fail to monitor their glycaemic state. Although devices are getting progressively easier to use, are less painful and invasive, they are still largely ignored by many diabetics, especially those with type 2 diabetes. One study in the USA in 1993 showed that more than two thirds of people with diabetes failed to monitor their blood glucose levels at all [1]. A more recent study has indicated that 43% of adolescents and 30% of children do not regularly monitor blood glucose and the same patients were not very good at recognising when they were hypo- or hyperglycaemic [2].

It is the combination of pain, inconvenience (especially where lancets, test strips and device all need to be carried around) and difficulty in interpreting the results that make people reluctant to monitor their blood glucose concentrations.

For this reason, there is a growing desire to develop methods for monitoring blood glucose that are non-invasive, continuous, unobtrusive and portable in order to alert the diabetic or their carer to glycaemic status. This may potentially be done by either monitoring one or more volatile compounds present in breath or those emitted from skin. This review discusses these two possibilities.

2. Breath analysis

The main advantages of breath analysis to monitor a medical condition or diagnose disease are that it is non-invasive, that breathing is very frequent, samples can be readily taken at any time even if a patient is unconscious, and people do not mind giving breath samples. If it is possible to infer blood glucose concentration from sampling breath and analysing one or more compounds, it is likely that this would be acceptable to patients and better glycaemic monitoring would ensue.

There are three main issues:

- what needs to be monitored
- how it should be monitored
- what affects the measurement or its relationship with blood glucose

2.1 Biomarkers for diabetes and blood glucose

The earliest recorded medical histories of diabetes from ancient Greece at the time of Hippocrates note that sufferers had the smell of rotten apples on their breath. We now know that this is acetone, present in very high concentrations on the breath of diabetics. For this reason, modern breath analysis has concentrated on acetone as marker of diabetes.

Acetone is one of three ketone bodies which are produced in the mammalian liver as an alternative energy source when glucose is not readily available. People who are fasting and/or performing prolonged exercise will tend to have low blood glucose levels and will, therefore, have elevated levels of ketone bodies in their blood and the fruity acetone smell on their breath. Diabetics, either lacking insulin or having insulin resistance, will not be able to use the glucose that is present, so it builds up in the blood (hyperglycaemia). At the same time, the ketone bodies will increase too as the body responds to an energy shortage, and the liver will break down fat. Acetoacetate and 3-β-hydroxybutyrate are the two main ketone bodies, being much more abundant than acetone, which is produced by the spontaneous decarboxylation of acetoacetate. However, they are less volatile than acetone so are much harder to detect on breath.

2.1.1 Acetone in non-diabetics

Levels of breath acetone in healthy volunteers have been determined in a number of studies [3, 4, 5]. [3] took weekly breath samples from 30 healthy volunteers (age range 24-59 years) for 6 months and found that the geometric mean acetone concentration in breath was 477 parts-per-billion, (ppb), with the range being 148 to 2744 ppb. Spanel et al (2007), in his study showed that children had slightly lower acetone concentrations, but the mean value was within the range described by [3]. Thus we have typical breath acetone concentrations for healthy (non-diabetic) people of a range of ages, body mass indices and of both genders in longitudinal studies. These studies were single breath samples and they do not relate breath acetone to blood glucose concentration. [6] looked at the relationship between breath acetone and blood glucose after fasting and for two hours following the consumption of 75g of glucose. They found that after fasting, when blood glucose was low, then acetone

tended to be relatively high. Following the consumption of glucose, blood glucose increased and breath acetone declined. The relationship was not a direct linear correlation but the general trend in healthy volunteers was that when blood glucose was low, breath acetone was relatively high and vice versa. Other studies have stated that the normal breath acetone concentration in non-fasting individuals is usually below 900ppb [7, 8, 9].

How does this differ for people with diabetes?

2.1.2 Acetone in diabetics

There have been many studies where acetone concentrations have been measured in diabetics, in breath and in blood. [7] back in 1969 reported that breath acetone was elevated in diabetics on insulin, hypoglycaemic tablets or reducing diets, even if their blood glucose levels were near normal. Another early study [10] measured plasma acetone concentrations in nine diabetic patients with moderate ketoacidosis and found that the plasma acetone concentrations were 1.55 to 8.91 mM. They also measured the rate of acetone production and found that there was a wide variation between individuals. Thus conventional wisdom is that acetone is markedly elevated in hyperglycaemia. However, this is a long way from a method to replace blood glucose testing. What is needed is an attempt to determine the correlation, if it exists, between blood glucose and breath acetone for a number of different individuals at different blood glucose concentrations and under different conditions, e.g. insulin levels etc. It is also very likely that the relationship between blood glucose and breath acetone is different in type 1 and type 2 diabetes, with levels of breath acetone being reported as being between 0.92 and 1.2 ppmv [11]. Much higher concentrations of acetone have been reported in type 1 diabetics (up to 20 ppm) [12] than in type 2, however there have been very few studies comparing the two with respect to breath acetone and other potential breath biomarkers.

Some aspects of this have been carried out. [13], reported that breath acetone was measured in 34 Type 1 diabetics, type 2 diabetics and15 healthy individuals. Blood glucose (BG) concentrations were measured at the time the breath samples were taken. This study found a correlation between mean group acetone and mean group BG for the blood glucose levels when the BG levels are grouped as follows: 40 to 100; 101 to 150, 151 to 200 and 200 to 419 mg/dL. The same study also found a mean acetone concentration in the T1DM at all the BG levels measured of 2.19 ppm as opposed to 0.48 ppm for the healthy volunteers.

One study has been reported in which patients at lower BG concentrations (hypoglycaemia) were monitored in a glucose clamp experiment, in which dextrose and insulin were fed intravenously. [12] recruited 8 type 1 diabetics to participate in the study. The 8 volunteers had diabetes for a mean of 28 +/- 3 years and had sub-optimal glycaemic control (Hb1A level of 8.8 +/- 0.4%). The volunteers were admitted to a clinical research facility overnight to stabilise BG concentrations using intravenous insulin. The following morning, the insulin clamp technique enabled a controlled reduction in BG concentration in steps of 40 minutes duration by varying intravenous dextrose while keeping insulin infusion rates constant. The nominal BG concentrations for which data were obtained were 5, 3.8, 3.3, 2.8 and 2.2 mM/L. BG levels were measured every 5 minutes and when stable, breath samples were taken for determination of acetone concentrations. Results from this study showed very clearly

that there was a linear relationship between breath acetone and BG concentration for all 8 volunteers. However, what was also apparent in this study was that the range of breath acetone values for baseline levels of BG (i.e. at the start of the experiment – around 5mM/l) varied very widely from 1.0 to 21 ppm. All declined throughout the clamp. This demonstrates that the absolute breath acetone concentration is not on its own an indicator of blood glucose level. What is also not clear is whether this would be reproduced in the absence of a constant insulin infusion, and whether the linear correlation would be extended in the euglycaemic and hyperglycaemic range.

Although these studies do not definitively prove a linear and reliable correlation between breath acetone and blood glucose concentrations, they do indicate a strong link, especially for type 1 diabetes. However, it is clear that as the levels of acetone vary so widely for each blood glucose concentration in each individual that a direct and absolute relationship between blood glucose and acetone does not exist. One other major advantage of using acetone as a potential marker of blood glucose level in diabetes is its abundance- after methane, it is probably the most abundant volatile organic compound in breath even in healthy volunteers. In diabetes, as we have seen, it generally becomes much more abundant, which makes analysis much easier.

2.1.3 Other potential volatile biomarkers for diabetes monitoring

Acetone seems the most promising breath marker for BG monitoring as it is very abundant in breath, even of healthy volunteers. However, some studies have found other potential markers which, tantalisingly, offer the possibility of monitoring multiple markers to provide a more robust correlation. There has been a report of high levels of isopropanol being present in the blood (and presumably breath) of a patient in a state of diabetic ketoacidosis [14]. This is a plausible finding given the finding that acetone may be metabolised to isopropanol in some diseases by the enzyme alcohol dehydrogenase. High levels of isopropanol are only expected in such diseases; nevertheless a correlation has been found between isopropanol and acetone even in healthy volunteers [3]. Iso-propanol is present at much lower levels than acetone, with a typical value of around 20 ppb in healthy volunteers [3] with insufficient reported data to show a typical level in diabetics.

The levels of methyl nitrate in breath has been correlated with blood glucose concentration [15] and the correlation has been explained by the fact that methyl nitrate is a marker of oxidative stress. This correlation is a potentially significant finding, however its very low concentration on breath (parts-per-trillion levels) makes its detection in a hand held device rather challenging. Other markers of oxidative stress (C4 – C20 straight chain and mono-methylated alkanes) have also been found in diabetes, however no strong correlation was found between these markers and blood glucose concentration [16].

Another single compound which has been postulated as a breath marker for blood glucose in diabetes is ethanol [15, 17, 18]. Ethanol may be a marker for blood glucose, however, the consumption of glucose in an oral glucose tolerance test which was used in these studies contaminates the mouth with glucose. Some studies [19, 20] have shown that mouth rinsing with glucose will increase the concentration of ethanol to as much as 1 ppm on breath due to metabolic activity by bacteria in the mouth. In this case, the breath ethanol is not systemic and for this reason, it may not be possible to isolate the two effects.

At the current time, although these studies have shown a potential correlation between these other metabolites and blood glucose, there are insufficient data to indicate that levels can directly mirror blood glucose concentration.

2.1.4 Multiple biomarkers

The advantage of using a single biomarker present in high concentrations (such as acetone) is obvious, however it may, on its own, not directly correlate with blood glucose concentrations for all diabetics under all conditions. We have already seen that type 1 diabetics with the same blood glucose levels have widely varying breath acetone concentration [12]. Another approach is to look at a number of biomarkers and correlate the biomarker patterns (i.e. a combination of biomarkers and their concentrations, or instrument responses) with blood glucose concentration. This may ultimately be a more robust approach, however at the time of writing, such research is not very advanced. PTR-MS (proton transfer mass spectrometry) was demonstrated analysing the breath of type 2 diabetics and healthy controls and using a number of ions, found a clear difference between the two groups [21]. Another group [16] developed a single parameter: the oxidative age, to compare oxidative stress in diabetics. Oxidative age was determined from measurements of multiple markers in breath plus chronological age. An alternative approach was developing models using multi-linear regression analysis of exhaled compounds in breath to predict blood glucose concentration [17]. These approaches all reported some potential although it was acknowledged that further work was required.

2.2 Monitoring breath biomarkers

Analysing breath is not straightforward, and in spite of an early study [22], when gas chromatography-mass spectrometry (GC-MS) was used, there are still very few validated, FDA approved breath tests for medical diagnosis or disease monitoring. Of course, analysis of ethanol in breath for enforcement of the drink driving regulations is well known, however in this case, the concentration of ethanol will be very much higher than the concentration of volatile compounds in the breath of healthy, non-intoxicated individuals. In the United Kingdom, the drink drive limit for ethanol is 180 parts-per-million, which is more than 1000 times higher than the mean concentration of ethanol in the breath of healthy volunteers who had not consumed alcohol.

Apart from the ethanol breathalyser for drink-driving, there are few other hand held devices for monitoring breath. Such devices as there are may only monitor total VOCs (or volatile sulphur compounds, VSCs, for the halitosis industry) non-selectively, or contain specific sensors that can only monitor specific compounds. For this reason, most research on breath testing in diabetes has used large, complex and expensive equipment such as mass spectrometers, infra red analysers and gas chromatographs.

Although there has been a lot of research carried out on instrumentation, methods and techniques for measuring breath, the methods for taking a breath sample have not been standardised, which makes it difficult to compare the results of studies. Issues with the methods for taking breath samples, e.g. alveolar or end tidal breath, or whether taken after the individual has been resting or active for a period of time need

to be addressed as they all may affect the result. Some attempt has been made to model the exhalation kinetics of breath markers including acetone to try and optimise this and to understand the physical and physiological factors affecting VOCs in breath [23], but standard methods have not yet been specified.

2.2.1 Chromatography and mass spectrometry

One of the most widely used techniques for monitoring breath of diabetics is gas chromatography, which is usually coupled to mass spectrometry (GC-MS), especially where the identity of compounds is being sought. GC-MS is so useful because the chromatography separates all the components of the breath sample. The mass spectrometer detector will then analyse and identify each of the components in turn as they come off the chromatography column. Quantitative data are also possible, allowing the potential for determining correlations between blood glucose and breath VOCs [24].

Chromatography can typically resolve several hundred compounds. GC of the headspace of a breath sample will enable the analysis of the major organic compounds present in breath, for example acetone, methanol, methanol etc. This technique has been used without MS to determine acetone concentration and how it related to blood glucose concentration at single time points in the breath of diabetics [25, 26]. This method can only be used for compounds at known retention times present at high enough concentrations.

In order to identify unknown compounds, more sensitive techniques are needed, and MS is used for identification. If breath is trapped and concentrated, for example using a tube containing sorbent packing material, then very low concentrations down to parts per trillion may be resolved and identified using the technique thermal desorption GC-MS (TD-GC-MS). Solid phase microextraction (SPME) fibres may also be used to concentrate VOCs. These concentration methods have been demonstrated in a number of studies [15, 16, 27] and a similar method using a particle packed separation needle has also been reported for concentrating acetone from breath and urine headspace [11]. Membrane extraction using a sorbent interface was also used [28].

Although GC and GC-MS are powerful techniques, the need to concentrate, adsorb and desorb compounds off a column and sorbent tube (if used) means that absolute quantification is difficult. The presence of other compounds will affect the chemical interaction, so there is always some doubt about absolute concentrations. For this reason, mass spectrometers are sometimes used, without the sample separation step. This enables real time, direct quantification on compounds such as acetone, however the sample will be complex and identification of all compounds is not usually possible. However, such methods are unsurpassed at obtaining rapid, quantitative data. Examples of the use of real time mass spectrometry to determine acetone and other VOC concentrations are with selective ion flow tube-mass spectrometry (SIFT-MS) [3, 12, 29, 30]. A similar mass spectrometric technique, proton transfer reaction mass spectrometry (PTR-MS) has also been used in quantifying key breath metabolites in diabetics [21].

2.2.2 Electronic olfaction/gas sensing

Chromatography and/or mass spectrometry may be extremely useful research tools in identifying and quantifying potential markers and their relationship with blood glucose, however their use is not very helpful to the diabetic who wants to monitor his or her blood glucose levels non-invasively. The best option in this instance is to use gas sensors which can be made to be semi-selective. Increasing selectivity is usually possible, but generally at increased cost. How selective the sensors need to be is debatable. If a close correlation can be shown between blood glucose and breath acetone, then it does not matter if the sensor is not very selective as the levels of acetone in breath will usually be much higher than the levels of other breath compounds. However, if a marker VOC is present at a much lower concentration, then it has to compete with the other compounds in breath, so the sensor needs to be more specific. This can in part be circumvented by using an array of gas sensors in a so-called electronic nose or artificial olfaction.

There are different types of gas sensors, but most do not specifically detect just one compound, most respond to many, to a greater or lesser extent. The use of an array of sensors means that complex samples (such as breath) will yield a series of signals, one for each sensor. Each signal may itself contain complex information. A computer must be used to analyse the signals, and there are many ways of doing so, using multivariate statistics or pattern recognition tools. In order to be useful as a tool for monitoring blood glucose, many different diabetics will need to be monitored with a big variation in blood glucose; this will enable a model to be built relating blood glucose to signal from the sensors. It seems unlikely at the present time that a breath signal will be universally applicable; it is probable that it will need to be calibrated for each individual.

Of course, because acetone is so usually abundant in the breath of diabetics, it is conceivable that a single sensor sensitive acetone may be sufficient to monitor blood glucose, subject to calibration for each individual. Technology is readily available to build a portable breath sensor which can detect acetone, and realistically, the use of such sensors is the only way to make a breath testing device for monitoring blood glucose sufficiently cheap and portable. One example is the use of LAPS (light addressable potentiometric sensors) which are promising sensors for use in breath testing devices [31]. In another study [32], a metal oxide sensor array was used to detect acetone with data processing using support vector machine (SVM) method to demonstrate fluctuating blood glucose of diabetics. Another type of chemi-resistive sensor made from Si doped WO_3 was found to effectively distinguish between low and high acetone levels [33]. This offers the opportunity of developing a single sensor portable breath test for acetone. A different type of sensor array, conducting polymer, was shown to be effective in monitoring acetone in the breath of diabetics, but they did not relate the signal to blood sugar levels [34]. A different type of sensor array - a QCM (quartz crystal microbalance) was also used to monitor breath acetone and relate that to blood glucose, with some success demonstrating the potential of this approach [35]

Of course, this all depends on future work determining the exact relationship between acetone and blood glucose for each individual, so calibration for each individual user will probably be necessary.

Sensors in an electronic nose array have also been used to diagnose type 2 diabetes from the headspace of urine. Artificial neural networks (ANNs) plus principal component analysis (PCA) and logistic regression (LR) were used to process data

from sensors and were effective in diagnosing diabetes [36]. However, diagnosis is not as big a problem as monitoring blood sugar.

One major issue in the use of sensors is the impact that large concentrations of other compounds may have under certain circumstances. One example of this is ethanol, which when consumed in quantities, will induce very high levels on the breath. This is likely to affect many of the sensor types that respond to acetone so any sensor on the market would need to be able to distinguish between the two. Other compounds from diet or other metabolic conditions could also conceivably have an effect.

2.2.3 Other methods

While mass spectrometric techniques are the best methods for identifying marker compounds, and portable sensors the most likely contenders for production of a portable breath testing device, other techniques have been used in establishing breath markers for blood sugar. Cavity ringdown spectroscopy (CRDS) was used to determine breath acetone concentration, which was then shown to be correlated with grouped blood glucose concentration of type 1 diabetics [11]. Acetone in breath was also quantified using a light emitting diode based photometric method [37].

Breath testing has been used for another application: determining the levels of insulin resistance in those at risk of pre-diabetes. This is often not easy to determine, but one method makes use of the modified oral glucose tolerance test (OGTT). In this method, subjects are given 75g glucose plus 150mg ^{13}C labelled glucose. Breath samples were collected and the $^{13}CO_2/^{12}CO_2$ ratio was monitored using infrared spectrophotometry to identify how quickly the glucose was broken down. Those at greatest risk had a much slower excretion of $^{13}CO_2$ [38, 39].

3 Skin Analysis

Breath volatile organic compounds (VOCs) originate at various sites around the body, are transported in the blood and then cross the alveolar membranes, which collectively have a large surface area to promote gas exchange, to get out into breath. One would expect that a similar thing would happen to skin, which has a good blood supply and a large surface area.

The analysis of the VOCs being excreted by skin is at a much earlier stage than breath analysis, nevertheless it has shown some promise. As is the case in breath, acetone is the most abundant compound excreted via skin in diabetics.

One group [40] devised a method for trapping skin acetone by placing a finger into an FEP sample bag and after a period of time, removing air from the bag which was then analysed by gas chromatography. They also measured blood β- hydroxybutyrate (βHB) and found a good correlation between skin acetone and βHB and also between skin acetone and blood glucose.

In another study on healthy volunteers [6] VOCs from the skin of the forearm were trapped in a Nalophan sampling bag which was then monitored using selected ion flow tube mass spectrometry (SIFT-MS) during an OGTT, while also taking breath samples. This showed a clear correlation between skin acetone and breath acetone, opening up the possibility of also using skin VOCs as a method of monitoring blood sugar.

Further work needs to be done on monitoring VOCs from skin, but these early studies show potential.

4 Commentary

Breath and skin analysis offer a non-invasive method for monitoring blood sugar. As alternatives to the traditional blood testing method, they may prove to be more acceptable to patients and thus increase compliance in the use of blood testing with the concomitant decrease in morbidity and mortality that arises from the failure of maintaining glycaemic control.

It has been shown that acetone may be monitored using a variety of methods, including those with the potential for producing low cost and portable devices. Acetone is present in abundance in diabetics, which is its main advantage. Other compounds may also be markers of blood glucose, however the fact that they are present at much lower concentrations makes their analysis by non-specific sensors or portable devices much more difficult, even when using sophisticated pattern recognition techniques. For this reason, it seems likely that if breath and skin VOCs offer potential for non-invasive blood glucose monitoring, acetone is currently the only potential marker, although there are still too many unanswered questions regarding its exact relationship with blood glucose.

The most important next step is to establish the relationship between blood glucose and acetone or other VOCs in diabetics at different blood glucose concentrations and where insulin levels differ. Other diseases or conditions, or even diet may also affect the relationship between VOCs and blood glucose and these things need to be taken into account too. It will almost certainly be the case that this relationship is not identical for all subjects, as it is already certain that acetone levels vary greatly between individuals, even at the same blood glucose concentration. However, even if a non-invasive breath or skin sensor needs to be clinically calibrated against blood glucose, the availability of such a device may well increase compliance in blood glucose testing and improve glycaemic control across the population of diabetics.

5 Five year view

There are numerous devices with the ability to measure VOCs from breath and skin; including those which have been shown to be markers of diabetes. Acetone is currently the only potential biomarker as it is present in abundance in diabetics and is easily monitored using portable devices, for example gas sensors, however, it still does not fulfil all requirements. If non-invasive monitoring of VOCs to infer blood glucose concentration is possible, the key step is to investigate the relationship between blood glucose and acetone under a variety of conditions and in many individuals. This can be achieved through studying many individuals on many occasions with varying blood glucose concentrations at different times of the day and night. It is also important to determine the exact relationship between acetone and blood glucose for each individual, and may require routine calibrating of acetone with blood glucose for each individual. Other health factors and the influence of diet need to be investigated. The relationship between acetone and insulin levels may also be important and should be taken into account. In short, a comprehensive clinical study of breath and skin acetone compared with blood glucose levels in a large number of

type 1 and type 2 diabetics is required. This will establish whether the plethora of devices already available will be of any use in trying to infer blood glucose concentration.

6 Key issues

- Are volatile organic compounds (VOCs) from breath or skin markers for blood glucose concentration?
- Devices capable of measuring volatile markers of blood glucose in breath or from skin must be portable.
- The most promising VOC is acetone because it is abundant in breath, but its relationship with blood glucose is not straightforward. Other potential markers are present at too low a concentration for small portable devices to be able to accurately monitor
- More studies have been carried out on monitoring breath than skin
- Developments in this area should concentrate on clinical studies not technological developments.
- Clinical studies should investigate the relationship between acetone and blood glucose in many patients under a variety of conditions.
- The relationship between insulin levels and acetone should be investigated.

References

1. Harris MI, Cowie CC, Howie LJ, Self-monitoring of blood glucose by adults with diabetes in the United States population, *Diabetes Care*, 16, 1116-1123 (1993).

2. Dalton P, Gelperin A, Preti G, Volatile metabolic monitoring of glycaemic status in diabetes using electronic olfaction, *Diabetes Technol. & Ther*. 6, 534-544 (2004).

3. Turner C, Španěl P, Smith D, A longitudinal study of ammonia, acetone and propanol in the exhaled breath of 30 subjects using selected ion flow tube mass spectrometry, SIFT-MS. *Physiol. Meas*. 27, 321-337 (2006).

4. Diskin AM, Španěl P, Smith D, Time variation of ammonia, acetone, isoprene and ethanol in breath: a quantitative SIFT-MS study over 30 days. *Physiological Measurement* 24, 107-120 (2003).

5. Španěl, P, Dryahina K, Smith D, Acetone, ammonia and hydrogen cyanide in exhaled breath of several volunteers aged 4–83 years, *J. Breath Research* 1 011001 (2007).

6. Turner C, Parekh B, Walton C, Španěl P, Smith D, Evans M, An exploratory comparative study of volatile compounds in exhaled breath and emitted by skin using selected ion flow tube mass spectrometry, *Rapid Commun. Mass Spectrom*. 22, 526–532 (2008).

7. Tassopoulos CN, Barnett D, Fraser RT, Breath acetone and blood sugar measurements in diabetes, *Lancet*, 293, 1282-1286 (1969).

8. Veloso KM, Likhodi SS, Cunnane SC, Breath acetone is a reliable indicator of ketosis in adults consuming ketogenic meals, *Am. J. Clin. Nutr*., 76, 65-70 (2002).

9. Anderson JC, Lamm WJE, Hlastala MP, Measuring airway exchangeof endogenous acetone using a single-exhalation breathing maneuver, *J. Appl. Physiol*., 100, 88-89 (2006).

10. Owen OE, Trapp VE, Skutches CL, Acetone metabolism during diabetic ketoacidosis, *Diabetes*, 31, 242-248 (1982).

**11. Ueta I, Saito Y, Hosoe M *et al.* Breath acetone analysis with miniaturized sample preparation device: in-needle pre-concentration and subsequent determination by gas chromatography-mass spectroscopy, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 877, 2551–2556 (2009).

This paper investigates the relationship between blood glucose and breath acetone in type 1 and type 2 diabetes

**12. Turner C, Walton C, Hoashi S & Evans M, Breath acetone concentration decreases with blood glucose concentration in type 1 diabetes mellitus patients during hypoglycaemic clamps, *J. Breath Res*., 3, 046004 (2009).

This paper investigates the quantitative relationship between breath acetone and blood glucose under controlled conditions.

13. Wang C, Mbi A, Shepherd M, A study on breath acetone in diabetic patients using a cavity ringdown breath analyser: Exploring correlations of breath acetone with blood glucose and glycohemoglobin A1C, *IEEE Sensors Journal*, 10, 54-63 (2010).

14. Jones AE & Summers RL, Detection of isopropyl alcohol in a patient with diabetic ketoacidosis, *J. Em. Med*., 19, 165-168 (2000).

15. Novak BJ, Blake DR, Meinardi S *et al*., Exhaled methyl nitrate as a noninvasive marker of hyperglycaemia in type 1 diabetes, *PNAS*, 104, 15613-15618 (2007).

*16. Phillips M, Cataneo RN, Cheema T , Greenberg J, Increased breath biomarkers of oxidative stress in diabetes mellitus, *Clin. Chim. Acta*, 344, 189-194 (2004).

Other breath biomarkers of oxidative stress in diabetes are investigated

*17. Lee J, Ngo J, Blake D *et al.,* Improved predictive models for plasma glucose estimation from multi-linear regression analysis of exhaled volatile organic compounds, *J. Appl. Physiol*., 107, 155-160 (2009).

This paper considers the possibility of using multiple biomarkers in a model to infer blood glucose levels.

18. Hu W, Galassetti P, Breath ethanol and acetone as indicators of serum glucose levels: An initial report, *Ethnicity and Disease*, 15, S4-32-S4-34 (2005).

19. Španěl P, Turner C, Wang T, Bloor R, Smith D, Generation of volatile compounds on mouth exposure to urea and sucrose: implications for exhaled breath analysis. *Physiol. Meas*., 27, N7-N17 (2006).

20. Turner C, Španěl P, Smith D, A longitudinal study of ethanol and acetaldehyde in the exhaled breath of healthy volunteers using selected ion flow tube mass spectrometry, *Rapid Commun. Mass Spectrom*., 20 61-68 (2006).

21. Greiter MB, Keck L, Siegmund T, Hoeschen C, Oeh U, Paretzke HG, Differences in exhaled gas profiles between patients with type 2 diabetes and healthy controls, *Diab. Technol., & Therap.*, 12, 455-463 (2010).

22. Teranishsi R, Mon T R, Robinson A B, Cary P, Pauling L Gas chromatography of volatiles from breath and urine *Anal. Chem.* 44 18–20 (1972).

23. King J, Unterkofler K, Teschl G *et al*., A mathematical model for breath gas analysis of volatile organic compounds with special emphasis on acetone. *J. Math. Biol*., In press. DOI 10.1007/s00285-101-0398-9 (2011).

24. Chakraborty S, Banerjee D, Ray I, Sen A, Detection of biomarker in breath: A step towards noninvasive diabetes monitoring, *Current Sci*., 94, 237-242 (2008).

25. Likhodii SS, Musa K, Cunnane SC, Breath acetone as a measure of systemic ketosis assessed in a rat model of the ketogenic diet, *Clin. Chem*., 48, 115-120 (2002).

26. Jones AW, Sagarduy A, Ericsson E, Arnqvist HJ, Concentrations of acetone in venous blood samples from drunk drivers, type 1 diabetic outpatients and healthy blood donors, *J. Anal. Toxicol*., 17, 182-185 (1993).

27. Deng C, Zhang J, Yu X, Zhang W, Zhang X, Determination of acetone in human breath by gas chromatography-mass spectrometry and solid phase microextraction with on-fiber derivatization, *J. Chromat. B*, 810, 269-275 (2004).

28. Ma V, Lord H, Morley M, Pawliszyn J, Application of membrane extraction with sorbent interface for breath analysis, *Methods Mol. Biol.* 610, 451–468 (2010).

29. Smith D, Španěl P, Davies S, Trace gases on the breath of healthy volunteers when fasting and following a protein calorie, meal: a preliminary study, *J. Appl. Physiol*. 87, 1584-1588 (1999).

30. Dummer JF, Storer MK, Hu W-P *et al.* Accurate, reproducible measurement of acetone concentration in breath using selected ion flow tube-mass spectrometry, *J. Breath Res.* 4, 046001 (2010).

31. Zhang Q, Wang P, Li J, Gao X, Diagnosis of diabetes by image detection of breath using gas-sensitive LAPS, *Biosensors & Bioelectronics*, 15, 249-256 (2000).

32. Guo D, Zhang D, Li N, Zhang L, Yang J, Diabetes identification and classification by menas of a breath analysis system, ICMB 2010, LNCS, 6165, 52-63 (2010).

33. Righettoni M, Tricoli A, Prtsinis E, Si:WO3 sensors for highly selective detection of acetone for easy diagnosis of diabetes by breath analysis, *Anal. Chem.* 82, 3581–3587 (2010).

34. Yu JB, Byun HG, So MS, Huh JS, Analysis of diabetic patient's breath with conducting polymer sensor array, *Sensors & Actuators B*, 108, 305-308 (2005).

35. Saraoğlu HM, Koçan M, Determination of diabetic blood glucose value from breath odor using QCM sensor based electronic nose, Paper no. 5479766 from 2010 15th National Biomedical Engineering Meeting, Antalya, 21-24 April (2010).

36. Mohamed EI, Linder R, Perriello G, Di Daniele N, Poppl SJ, De Lorenzo A, Predicting type 2 diabetes using an electronic nose-based artificial neural network analysis, Diabetes, *Nutr. & Metab.-Clin & Exp*., 15, 215-221 (2002).

37. Teshima N, Li J, Toda K, Dasgupta PK, Determination of acetone in breath, *Anal. Chim. Acta*, 535, 189-199 (2005).

38. Dillon EL, Janghorbani M, Angel JA *et al.,* Novel non-invasive breath test method for screening individuals at risk for diabetes, *Diabetes Care*, 32, 430-435 (2009).

39. Mizrahi M, Lalazar G, Adar T, Raz I, Ilan Y, Assessment of insulin resisitance by a ^{13}C glucose breath test: a new tool for early diagnosis and follow-up of high-risk patients, *Nutritional Journal* 9, 25-33 (2010).

40. Yamane N, Tsuda T, Nose K, Yamamoto A, Ishiguro H, Kondo T, Relationship between skin acetone and blood β-hydroxybutyrate concentrations in diabetes, *Clin. Chim. Acta* 365, 325-329 (2006).