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2013 J. Breath Res. 7 037109

(http://iopscience.iop.org/1752-7163/7/3/037109)

J. Breath Res. **7** (2013) 037109 (18pp) doi:10.1088/1752-7155/7/3/037109

Is breath acetone a biomarker of diabetes? A historical review on breath acetone measurements

Zhennan Wang and Chuji Wang[1]

Department of Physics and Astronomy, Mississippi State University, Starkville, MS 39759, USA
E-mail: cw175@msstate.edu

Received 1 March 2013
Accepted for publication 8 July 2013
Published 20 August 2013
Online at stacks.iop.org/JBR/7/037109

Abstract

Since the ancient discovery of the 'sweet odor' in human breath gas, pursuits of the breath analysis-based disease diagnostics have never stopped. Actually, the 'smell' of the breath, as one of three key disease diagnostic techniques, has been used in Eastern-Medicine for more than three thousand years. With advancement of measuring technologies in sensitivity and selectivity, more specific breath gas species have been identified and established as a biomarker of a particular disease. Acetone is one of the breath gases and its concentration in exhaled breath can now be determined with high accuracy using various techniques and methods. With the worldwide prevalence of diabetes that is typically diagnosed through blood testing, human desire to achieve non-blood based diabetic diagnostics and monitoring has never been quenched. Questions, such as is breath acetone a biomarker of diabetes and how is the breath acetone related to the blood glucose (BG) level (the golden criterion currently used in clinic for diabetes diagnostic, monitoring, and management), remain to be answered. A majority of current research efforts in breath acetone measurements and its technology developments focus on addressing the first question. The effort to tackle the second question has begun recently. The earliest breath acetone measurement in clearly defined diabetic patients was reported more than 60 years ago. For more than a half-century, as reviewed in this paper, there have been more than 41 independent studies of breath acetone using various techniques and methods, and more than 3211 human subjects, including 1581 healthy people, 242 Type 1 diabetic patients, 384 Type 2 diabetic patients, 174 unspecified diabetic patients, and 830 non-diabetic patients or healthy subjects who are under various physiological conditions, have been used in the studies. The results of the breath acetone measurements collected in this review support that many conditions might cause changes to breath acetone concentrations; however, the results from the six independent studies using clearly-defined Type 1 and Type 2 diabetic patients unanimously support that an elevated mean breath acetone concentration exists in Type 1 diabetes. Note that there is some overlap between the ranges of breath acetone concentration in individual T1D patients and healthy subjects; this reminds one to be careful when using an acetone breath test on T1D diagnostics. Comparatively, it is too early to draw a general conclusion on the relationship between a breath acetone level and a BG level from the very limited data in the literature.

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

1. Introduction

Approximately 346 million people worldwide have diabetes. Nearly 25.8 million people in the United States are diabetic [1]. Diabetes is directly responsible for 3.5% of deaths arising from non-communicable diseases (NCDs) [2]. The World Health Organization (WHO) assessed that diabetes deaths will double between 2005 and 2030. Therefore, the prevalence of diabetes mellitus is a worldwide problem and becoming

[1] Author to whom any correspondence should be addressed.

more and more serious. Diabetes mellitus is a heterogeneous group of metabolic diseases characterized by abnormally high blood glucose (BG) levels resulting from defects in insulin secretion, insulin action, or both. Due to the complicated pathphysiology of diabetes, the criteria of diagnosis are still open. Several suggested diagnostic criteria have been used to date [3–9]. Nevertheless, all of the criteria with rare exception need to conduct careful plasma glucose measurements [10]. Also, BG monitoring is necessary for patients and clinicians to evaluate diabetic control and management. At present, blood glucose meters (BGMs) are predominantly used to determine an approximate glucose concentration in the blood. Due to its low cost and compact size, a BGM has become a key element for home BG monitoring and diabetic self-management. However, the blood sampling in using BGMs is intrusive, painful and inconvenient. Especially for juvenile diabetes, typically Type 1 diabetes (T1D), they depend completely on injection of insulin for diabetic managements, i.e. insulin dose adjustments; therefore their blood must be sampled several times a day. After many years of using finger sticks, they may not only suffer the physical pains, but also bear a psychological burden from daily frequent blood sampling.

Breath analysis, an emerging new technique that is built on the advancement of modern measurement technologies, provides a non-invasive method for disease diagnosis, therapeutic monitoring, and metabolic status monitoring by testing exhaled breath components [11–13]. In 1971, Pauling *et al* discovered that more than 200 chemical components existed in human exhaled breath [14]. This finding marked the beginning of the modern breath analysis. It has now been found that there are more than 3500 chemical species presented in exhaled breath, and most of them are volatile organic compounds (VOCs) in low concentrations, i.e. from parts per million (ppm) to parts per billion (ppb) or parts per trillion (ppt) [15]. Abnormal concentrations or presence of specific breath gas species, which are specifically related to particular disease(s) or metabolic disorder(s), are known as a breath biomarker in the new field of breath analysis research. According to the definition proposed by the Biomarkers Definitions Working Group of the National Institutes of Health in 2001, more generally speaking, a biomarker or biological marker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Biomarkers can serve as a tool for disease detection and health status monitoring, such as abnormal condition diagnostics, disease staging, disease prognosis, prediction and monitoring of clinical response to an intervention [16]. Based on this definition and the research results from over the last 60 years' measurements of breath acetone in healthy and diabetic human subjects, as reviewed in this paper, breath acetone is arguably a biomarker of diabetes mellitus, and statistically, elevated breath acetone concentration is a good indicator of diabetes mellitus.

During the last ten years, exhaustive reviews have covered the technical aspects of breath analysis including sample collection, sample preparation, measurement techniques and instrumentation. The potential of breath analysis for clinical applications has been extensively discussed [17]. In the most recent review by Minh *et al* [18], general sources and roles of gases in a human body were introduced. The discussion mainly focused on breath testing for diabetes mellitus, including the diabetic features that could potentially influence testing. Breath analysis technologies and VOCs possibly relevant to diabetes mellitus were also summarized. In 2011, Španěl *et al* updated the progresses made in the non-invasive monitoring of breath VOCs. Turner discussed the possibility of inferring BG concentration by monitoring VOCs in exhaled breath and emitted from skin; meanwhile she presented a five-year's view on this application [19]. Smith *et al* reviewed the studies of breath analysis in diabetes, with an emphasis on the breath metabolites that might be related to diabetes [20]. Acetone, isoprene, methyl nitrate and some other factors which might confound their interpretation, were discussed in this perspective article. Special attention was paid to selected ion flow tube-mass spectrometry (SIFT-MS) and proton-transfer-reaction mass spectrometry (PTR-MS) that can potentially be used for the non-invasive monitoring of metabolic conditions including diabetes mellitus. Wang *et al* reviewed detection limits of 32 breath biomarkers including acetone using various laser-based techniques at the selected wavelengths [21]. Inspired by the heated discussion in the recent breath analysis research conference [22], the collective efforts on breath acetone measurements, and continuous pursuits of the goal of non-blood diabetes diagnostics using breath analysis, in this review, we try to look at the reports on the breath acetone measurements conducted over the last 60 years to help answer the question, if it has not been answered yet, whether or not breath acetone is a biomarker of diabetes.

In this paper, section 2 briefly discusses the sources and pathways of acetone. Section 3 presents the reported breath acetone concentrations measured under various situations to date. Section 4 reports on current measurements of acetone in clearly defined diabetic patients. Section 5 reviews on major methods and techniques used in the breath acetone measurements. The acetone breath analyzers that have been used in clinical studies to date are presented in section 6. Finally, an open question is raised along with some latest updates: what is the quantitative correlation of the breath acetone concentration with the BG level, which is the key element to bridge the current research efforts in breath acetone measurements and technology developments to the ultimate goal—diabetes diagnostics and monitoring using breath acetone.

2. Sources of acetone and pathways

Acetone (C_3H_6O) is one of the three ketone bodies that are produced in the liver, as illustrated in figure 1. The other two abundant ketone bodies are acetoacetate (AcAc, $C_4H_6O_3$) and 3-β-hydroxybutyrate (3HB). The acetone sources and pathways reported in the literature are summarized in table 1. There are two sources for acetone production: the

Table 1. Sources of acetone and acetone pathways reported from the literature.

References	Acetone sources and pathways
[30, 41]	Acetone passes into the urine through diffusion, and the concentrations in the blood and in the urine are usually the same. Acetone elimination through the lungs is a pure diffusion process.
[42]	Acetoacetic acid decarboxylase is responsible for the decarboxylation of acetoacetic acid to form acetone in Clostridium acetobutylicum.
[34]	The partition coefficient of acetone between air and plasma is 330.
[25]	Demonstration of the existence of acetoacetate decarboxylase of mammalian origin; acetoacetate decarboxylase accelerates the decomposition of acetoacetate into acetone.
[27]	Alcohol dehydrogenase, rather than catalase, plays an important role in the metabolic pathways involved in the oxidation of isopropanol into acetone.
[43]	Acetone blood/air partition coefficient at 37 °C is 341.
[26]	Acetone is generated by spontaneous decarboxylation and enzymatic conversion of acetoacetate.
[35]	Blood/air partition coefficient of acetone at 37 °C is 245 ± 32.
[23]	Breath and urinary excretion of acetone accounted for a 2–30% of the endogenous production rate. During maximum acetonemia, conversion of AcAc to acetone could account for ∼37% of the AcAc produced in fasting human; ∼4–11% of plasma glucose production could be theoretically derived from acetone.
[36]	The mean value of the individual blood/air partition coefficient for acetone determined *in vitro* blood sample is 275 ± 14 at 37 °C.
[24]	In moderate and severe ketoacidosis, acetone production could account for ∼52%. In diabetic patients, ∼20% and ∼80% of the production rate were accounted for by breath acetone at low and high plasma acetone concentrations, respectively.
[40]	The methylglyoxal and the propanediol pathways are the two pathways that are capable of converting acetone to glucose.
[37]	Partition coefficients of acetone in blood/gas, plasma/gas and lung/gas at 37 °C are 196 ± 31, 217 ± 14 and 160 ± 8, respectively.
[44]	Develop a physiologically based pharmacokinetic model for acetone and the model can predict the kinetic behavior of acetone in the human body.
[45]	Review acetone metabolism in mammals and summarize two pathways of acetone production, decarboxylation of acetoacetate and dehydrogenation of isopropanol.
[28]	Isopropanol is converted into acetone by oxidation with class I isoenzymes of hepatic alcohol dehydrogenase.
[31, 32]	Most exhaled acetone exchange occurs in the airways of the lung; 96% of acetone exchange occurs in the airways during a tidal breath, while 73% of that during a prolonged exhalation. Normalized end-exhaled acetone concentration is dependent on the exhalation flow rate.
[38, 39]	*In vivo* mean (range) arterial blood/breath ratios of acetone are 580 (280–1060).
[33]	Airway gas exchange contributes to the major part of short-term variability in breath acetone concentrations, with minimal changes in the underlying blood and tissue concentrations.

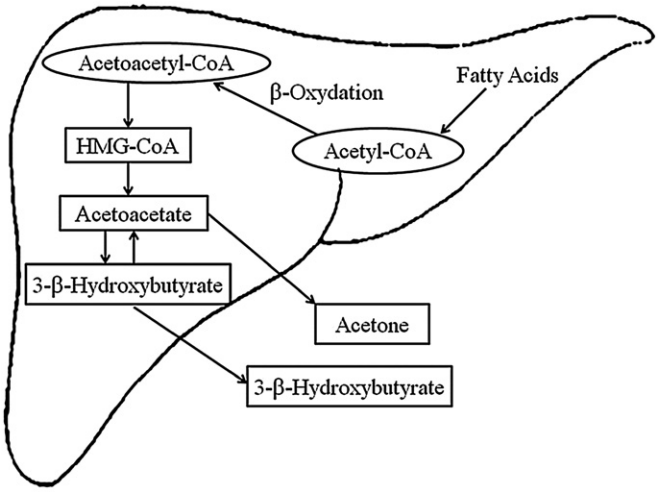

Figure 1. Generation of acetone in the liver via decarboxylation of acetoacetate.

decarboxylation of acetoacetate and the dehydrogenation of isopropanol. The former is a process of the conversion of acetoacetate through elimination of CO_2:

$$CH_3COCH_2COO^- + H^+ \rightarrow CH_3COCH_2COOH \rightarrow CH_3COCH_3 + CO_2.$$

This process accounts for ∼37% of the acetoacetate produced in fasting subjects [23] and ∼52% in diabetic patients in moderate to severe ketoacidosis [24]. Acetoacetate decarboxylase is a type of enzyme that can accelerate the decomposition of acetoacetate into acetone. In the 1970s, Steklenburg *et al* presented the evidence for the existence of acetoacetate decarboxylase in mammals and reported the presence of acetoacetate decarboxylase in rat liver, kidney and brain tissue [25, 26]. Nordmann *et al* have concluded that alcohol dehydrogenase, rather than catalase, plays an important role in the metabolic pathways involved in the oxidation of isopropanol into acetone after conducting experiments using intact rats [27]. Further, isopropanol is converted into acetone by an oxidation process with class I isoenzymes of hepatic alcohol dehydrogenase [28]. Lindinger *et al* monitored the breath acetone profile of the subject after consumption of 0.45 g isopropanol (diluted in water). A rapid increase in acetone concentration, from 1 to 80 ppm, was observed during the first 30 min after the start of the experiment, followed by a quite slow decline over the next 5 h [29].

Acetone is eliminated from a human body through the lungs, and the acetone passes into the urine through diffusion [30]. Reichard *et al* pointed out that breath and urinary excretion of acetone accounts for a 2–30% of its endogenous

production rate, and the *in vivo* metabolism accounts for the rest [23]. In the study of the acetone metabolism in nine diabetic patients who were under the moderate to severe ketoacidosis, Owen *et al* found a positive linear relationship between the percentage of the acetone production rate excreted as breath acetone and the plasma acetone concentration. At low plasma acetone concentrations, acetone excretion through exhalation accounted for approximately 20% of the acetone production rate; while at high plasma acetone concentrations, approximately 80% of the acetone production rate was attributed to exhaled breath [24]. It is reported that most exhaled acetone exchange occurs in the airways of the lungs [31, 32], 96% of acetone exchange occurs in the airways during a tidal breath, but during a prolonged exhalation maneuver 73% of acetone exchange occurs in the airways. Therefore, Anderson *et al* suggested that exhaled acetone measurements need to be reevaluated because they might underestimate blood acetone concentrations. King *et al* also reported that airway gas exchange contributes to the major part of the short-term variability in breath acetone concentrations, with minimal changes in the underlying blood and tissue concentrations [33]. The partition coefficient of acetone between air and plasma reported in the literature varies from 217 to 330, while the blood/air partition coefficient of acetone varies from 196 to 275 [34–37]. Surprisingly, O'Hara *et al* [38, 39] reported an *in vivo* mean arterial blood/breath ratio of acetone to be 580 by measuring blood and breath acetone concentrations in ten healthy subjects. This value is much higher than those *in vitro* partition coefficients, although they are on a comparable order of magnitude.

Two pathways contribute to the conversion of acetone to glucose: the methylglyoxal and the propanediol pathways [40]. Reichard calculated that ∼4–11% of plasma glucose production could be derived from acetone based on their study of acetone metabolism in lean and obese subjects during a starvation ketosis [23].

3. Breath acetone concentrations measured under various situations

Both cross-sectional and longitudinal approaches have been extensively applied to breath studies. In cross-sectional studies, acetone in exhaled breath has been investigated to see whether it could be used as a valid biomarker of diseases or metabolic disorders by comparing a group of controlled subjects with a group of patients. While in longitudinal studies, a temporal profile of exhaled acetone concentration has been investigated as an index of accessing the degree of control in diabetics, or for subjects under various situations, such as healthy subjects, under fasting, conducting oral glucose tolerance test, during an exercise, subjects on a surgery, etc.

According to the published work, the mean breath acetone concentration of healthy people varies from 0.39 to 1.09 ppm [38, 39, 46–56]. In a breath study of 30 healthy subjects over a six-month period, only a small difference in the mean exhaled acetone concentrations between males (558 ppb) and females (406 ppb) was observed [50]. Neither age nor body mass index (BMI) was a significant factor that was correlated to the acetone concentration in this group of volunteers, except that there might be a possible negative correlation between BMI and acetone for the male subjects. However, only 19 male subjects were tested in this study. Another investigation was carried out through analyzing variations of breath acetone levels with age, gender, and BMI in 243 healthy adults and 44 healthy children (5–11 years old) [52]. In the group of 215 adults with overnight fast, acetone concentrations ranged from 177 to 2441 ppb with an overall geometric mean (GM) of 628 ppb, while the group of 28 adults without dietary control had a corresponding range of 281–1246 ppb with an overall GM of 544 ppb. The difference in the acetone levels between the males and the females was not statistically significant in these two adult groups. Likewise no correlation was found between the acetone concentration and BMI in the adults. A slight positive correlation between age and acetone level was observed in the fasted females, but not in the males. The children group had a lower mean acetone concentration with a median of 263 ppb.

Španěl *et al* studied the dependence of breath acetone in healthy subjects on age [57, 58]. Breath acetone levels in each different age cohort were found close to a log normal distribution. The data showed that the young group (17–18 years) had a lower mean acetone concentration than the adult group (20–60 years). However, the age dependence variation was not very significant. In the study of 41 healthy children by Nelson *et al* [59], the result exhibited a tendency toward lower exhaled acetone concentrations with increase in age. With regard to newborn infants in this work, the infants in catabolic states exhaled much higher acetone concentrations than those in anabolic states did.

However, many other factors could influence breath acetone concentration, such as fasting, exercise, consuming ketogenic diet, etc. Smith *et al* were the first to report the true and absolute acetone concentrations in exhaled breath of healthy adults in the fasting and replete state [60]. After a 12 h fast, the subjects were fed by a liquid protein-calorie meal; their breath acetone concentrations fell from the maximum (∼200–600 ppb) during the fasting to their nadir (∼200 ppb) between the fourth and fifth h after the feeding, and then showed a tendency to increase. Elevated breath acetone concentrations were noticed in the subjects after they ingested disulfiram [61], ketogenic meals [62–64] and allicin [65].

The oral glucose tolerance test (OGTT) is a commonly performed medical test in screening of diabetes and insulin resistance. Galassetti *et al* analyzed the breath gases in ten healthy volunteers during an OGTT (ingestion of 75 g of glucose followed by 2 h of sampling) [47]. The mean glucose values showed a typical OGTT pattern—a rapid increase with peak values in 30–60 min, followed by a gradual return to the baseline in 120 min. Breath acetone decreased progressively after the start of the OGTT, from the baseline levels of 392 ± 85 ppb to 280 ± 64 ppb at 120 min, with the peak values of 364 ± 71 ppb at 30 min and 300 ± 64 ppb at 60 min.

King *et al* characterized the breath acetone behavior in six sleeping healthy subjects over two consecutive nights by monitoring end-tidal exhaled breath [66]. Breath acetone concentrations increased overnight in all of the measurements

with the maxima usually recorded 2–3 h before they woke up. Acetone concentrations varied from 234 to 580 ppb at the onset of sleep and ranged from 575 to 1460 ppb at the end of the sleep.

Diskin *et al* carried out an investigation into breath acetone concentrations in five subjects over a period of 30 days [67]. Their breath samples were collected in the early morning and the mean acetone concentrations ranged from 293 to 870 ppb.

In order to test the hypothesis that congestive heart failure (CHF) is a ketosis-prone state, Kupari *et al* studied 31 patients with chronic CHF, 19 cardiac patients without CHF and 24 healthy persons [68]. After 12 h of fasting, their breath acetone concentrations were measured. It was found that the mean breath acetone concentration in the CHF patients was approximately four times higher than that of the patients without CHF and the healthy persons, i.e. 1.81, 0.52 and 0.43 ppm for the three groups of subjects, respectively. Marcondes-Braga *et al* evaluated exhaled acetone as a new biomarker of heart failure by comparing exhaled breath acetone in the heart failure patients and in the healthy subjects [69]. The results showed that breath acetone concentrations in the acute decompensated heart failure group (median = 7.8 μg L^{-1} and the inter-quartile range (IQR) = 3.6–15.2 μg L^{-1}) were higher than those in the chronic heart failure group (median = 1.22 μg L^{-1} and IQR = 0.68–2.19 μg L^{-1}) and the control group (median = 0.39 μg L^{-1} and IQR = 0.30–0.79μg L^{-1}). A positive correlation was observed between the breath acetone and the B-type natriuretic peptide (a blood biomarker for heart failure). The results from this study suggested that exhaled breath acetone is a promising, non-invasive biomarker for diagnostics of heart failure and heart failure severity.

Pabst *et al* carried out a study of 24 patients undergoing a cardiac surgery with the extracorporeal circulation (ECC) [70]. Breath acetone profiles under the impact of the heart surgery with ECC were reported. In this observational study, the mean exhaled acetone concentration was 8.84 $\pm$ 6.85 nmol L^{-1} (equivalent to 0.20 ppm) after the induction of anesthesia, and then acetone concentration increased slightly to 13.8 $\pm$ 8.83 nmol L^{-1} after sternotomy. Exhaled acetone increased noticeably and kept at the same level (ranging from 28.4 to 32.9 nmol L^{-1}) during the first 2.5 h after the surgery. Also, exhaled acetone concentrations were found to have positive correlations to the serum C-reactive protein concentrations and to the serum troponine-T concentrations.

Ross reported changes in oral trace gas concentrations in an orthognathic surgery case [71]. Oral acetone concentration in a 42 years old male patient, who consumed a liquid diet after the orthognathic surgery, was measured. In this case, the oral acetone concentration was 423 ppb before the surgery. And it increased by approximately three-fold on the 13th day post-surgery. The acetone concentration kept at this high level until the 21st day, and then dropped to the baseline level. This patient, with daily consumption of a liquid diet, had lost 18 lbs in weight after 21 days post-surgery. This study indicated that the observed elevation in acetone concentration was likely due to the calorific restriction by the surgery.

Senthilmohan *et al* reported a breath acetone profile for the subjects who were doing exercise during the testing [72]. The expired breath acetone concentrations were in the range of 100–1400 ppb. The acetone concentration increased for most of the subjects during the exercise period. Schwoebel *et al* measured breath acetone concentrations in seven healthy volunteers when they were doing exercise on a stationary bicycle [73]. Their expired acetone concentrations ranged from 130 to 2600 ppb. Acetone concentrations at different respiratory phases (inspiratory, alveolar and mixed expiratory phases) were qualitatively determined at any time during the experiment.

Barker *et al* measured 12 VOCs, including acetone, in the exhaled breaths of 20 cystic fibrosis patients and 20 healthy subjects [49]. No significant difference in the exhaled acetone concentrations between the cystic fibrosis and the healthy subjects was observed, and the mean acetone concentrations were 400 ppb and 467 ppb for these two groups, respectively.

Table 2 tabulated the measured acetone concentrations under the various conditions as reviewed above. In the table, physiological conditions of the subjects, number of the subjects studied, breath acetone concentrations (converted to ppm), and measuring methods or techniques are tabulated. Due to the wide range of physiological conditions, large variations in the number of subjects used in different studies, we made no attempt to generate a global mean acetone concentration for the healthy subjects and for the rest.

The results reviewed above seem to support that change of breath acetone is related to various physiological conditions and diabetes is one of them that may cause a change in breath acetone concentration.

4. Current measurements of breath acetone in clearly specified (T1D and T2D) diabetic patients

According to the classification recommended by the National Diabetes Data Group of the National Institutes of Health and by the WHO Expert Committee on Diabetes, diabetes mellitus is classified into four categories: insulin-dependent diabetes mellitus (Type 1 diabetes (T1D)), non-insulin-dependent diabetes mellitus (Type 2 diabetes (T2D)), gestational diabetes mellitus, and other specific types. For example, in the United State, about 5% of people with diabetes have Type 1 diabetes, and approximately 95% of the diabetes is Type 2. Diabetes mellitus can occur at any age. Generally, Type 1 diabetes predominantly appears in juveniles. Some of Type 2 diabetes may convert to Type 1 later on.

The earliest studies of breath acetone in diabetic subjects date back to the early 1950s. In 1952, Henderson *et al* investigated breath acetone in diabetic and non-diabetic subjects using the breath sample pre-concentration technique followed by the mass spectrometry (MS) method [74]. Exhaled acetone concentrations in the non-fasting diabetic subjects were found to be higher than acetone concentrations in the non-fasting healthy subjects. In 1966, Rooth *et al* measured acetone concentrations in the alveolar air from 218 subjects using a gas chromatograph with a flame-ionization detector [75]. In this study, the mean acetone concentration in the 49 patients with juvenile diabetes, typically accorded with the T1D symptoms, was determined to be 4.42 $\pm$ 0.71 μg L^{-1}, which was much

Table 2. The measured acetone concentrations under various conditions. The references are listed in chronological order in the last column.

Subjects	No.	Measured concentrations	Converted to ppm	Technique	References
Healthy subjects	106	4.6 ± 2.4[a]		MS and microchemical method	[74]
diabetics	70	9.1 ± 8.2[a]			
Healthy subjects	67	1.10 ± 0.88 μg L^{-1}	0.42		
diabetics					
Borderline	40	0.80 ± 0.41 μg L^{-1}	0.31	GC with flame ionization detector	[75]
Chemical	42	1.17 ± 0.95 μg L^{-1}	0.45		
Late onset	20	1.70 ± 1.03 μg L^{-1}	0.65		
Juvenile	49	4.42 ± 4.94 μg L^{-1}	1.71		
Fasting diabetic patients	251	Varied with diabetic management and different blood glucose levels		GC	[76]
Fasting adults		Median (range) nmol L^{-1}			
With chronic CHF	31	81 (17–787)	1.18	GC with flame-ionization detector	[68]
Cardiac without CHF	19	23 (9–128)	0.52		
Healthy	24	19 (8–47)	0.43		
Newborn infants		μg L^{-1}			
Catabolic	8	5.99 (0.81–37.38)	2.31		
Anabolic	8	0.76 (0.30–11.60)	0.29		
Pre-school	23	0.51 (0.19–3.33)	0.19	GC combined with UV spectrophotometry	[59]
School	18	0.38 (0.16–0.64)	0.14		
Diabetic					
Nonfasting	7	1.09 (0.20–1.89)	0.42		
Fasting 0 h	8	1.97 (0.97–7.66)	0.76		
Postprandial 2 h	7	3.14 (1.10–9.16)	1.21		
Fasting healthy adults (24–61 years)	6	∼200–600 ppb, premeal;			
		∼200 ppb, 4–5 h after meal;		SIFT-MS	[60]
Healthy adult consumed ketogenic meals	12	Increase from 33 ± 13 to 116 ± 19 nmol L^{-1}	Increase from 0.74 to 2.59	GC	[62]
Children:		nmol L^{-1}		GC and hand-held breath acetone analyzer	[63]
Seizures on ketogenic diet	10	2530 ± 600;	56.67		
On antiepilepsy medication	10	19 ± 9;	0.43		
Healthy	13	21 ± 4;	0.47		
Healthy adults observed over 30 days	5	293–870 ppb		SIFT-MS	[67]
T2D	15	1.76–3.73 ppm;		GC-MS	[77]
Controls	15	0.22–0.80 ppm;			
Healthy adults (27.4 ± 3.1 y), OGTT	10	392 ± 85 ppb, baseline level; 280 ± 64 ppb, 120 min after OGTT;		GC-MS	[47]
Healthy adults (22–62 years)	11	176–518 ppb;		LED-based photometric method	[48]
Healthy adults after allicin consumption	7	Baseline: 0.68 ± 0.21 μg L^{-1}; Increase depends on dose;	0.26	GC-MS	[65]
Healthy adults (24–59 years)	30	Median 462 ppb; GM 477 ± 1.58 ppb;		SIFT-MS	[50]
Cystic fibrosis patients	20	400 ppb;		GC	[49]
Healthy controls	20	467 ppb;			
Health ingested disulfiram	1	Increase from 300 to over 4000 ppb;		SIFT-MS	[61]
Healthy control	1	∼300 ppb;			

Table 2. (Continued.)

Subjects	No.	Measured concentrations	Converted to ppm	Technique	References
Children with epilepsy on ketogenic diet	7	5063 ± 933 nmol L^{-1}	113.41	GC and hand-held breath acetone analyzer	[64]
Healthy men after ingest technical alcohol	35	Median 233 μg L^{-1},	89.99	FT-IR spectrometer	[78]
		Max. 800 μg L^{-1}	308.97		
Adults having cardiac surgery with ECC	24	After anesthesia: 8.84 ± 6.85 nmol L^{-1};	0.20	SPME-GC-MS	[70]
		2 h after surgery: 32.9 ± 22.3 nmol L^{-1};	0.74		
Healthy senior adults (60–83 y):	13	Median 440 ppb		SIFT-MS	[57]
Healthy young adults (17–18 years)	26	Median 263 ppb		SIFT-MS	[58]
T1D fasting 14 h overnight	4	0.80–3.97 ppm		CRDS	[79]
Healthy university students	451	0.53 ± 0.45 ppm		Biogas acetone analyzer, GC equipped with a semiconductor gas detector	[51]
Healthy subjects	441	Median 627.5 ppb		PTR-MS	[53]
Lung cancer patients	220	Median 458.7 ppb			
Healthy adults (20–50 years)	10	Mean 1090 (515–2335) ppb		PTR-MS	[39]
Healthy adults (20–50 years)	10	Mean 1090 (510–2900) ppb		PTR-MS	[38]
Healthy children (7–18 years)	200	Median 297 ppb;		SIFT-MS	[54]
		GM 308 ± 1.7 ppb;			
Healthy children (5–11 years)	44	Median 263 ppb;			
Healthy adult fast overnight	215	GM 628 (177–2441) ppb;		PTR-MS	[52]
Healthy adult without dietary control	28	GM 544 (281–1246) ppb;			
Healthy adults (25–30 years) during exercise	8	∼1 ppm		PTR-MS	[80]
T1D during insulin clamp	8	Baseline: 1–21 ppm;			
		Breath acetone decreased linearly with blood glucose concentration		SIFT-MS	[81]
Controlled T2D	21	0.19–0.66 ppm;		GC-MS	[82]
Medically untreated	1	0.92–1.20 ppm;			
Healthy adult under 24 h fasting	1	Increased to 5.66 ppm;			
Healthy adults (25–45 years)	15	Mean: 0.48 ppm;			
T1D (10–77 years)	34	2.19 ppm;		CRDS	[46]
T2D (34–72 years)	10	2.05 ppm			
Healthy adults (34 ± 5 years)	12	GM (log SD) ppb: 619 (1.83), flow rate 170 mL s^{-1}, $V_{70–85\%}$;		SIFT-MS	[55]
		618 (1.82), flow rate 330 mL s^{-1}, $V_{70–85\%}$;			
		636 (1.82), flow rate 170 mL s^{-1}, $V_{85–100\%}$;			
		631 (1.83), flow rate 330 mL s^{-1}, $V_{85–100\%}$;			
T2D	38	Median 337 (160–862) ppb		SIFT-MS	[83]

Table 2. (Continued.)

Subjects	No.	Measured concentrations	Converted to ppm	Technique	References
Healthy adults (23 ± 3 years) during exercise	7	130–2600 ppb		PTR-MS	[73]
Adult (42 years) following orthognathic surgery	1	Baseline before surgery: 423 ppb; During 43 days after surgery: 74–312%;		SIFT-MS	[71]
Healthy subjects	30	14.44–531.45 ppb		SPME-GC-TOF/MS	[56]
Lung cancer patients	23	34.57–390.60 ppb			
		Median (interquartile range) μg L^{-1}			
Acute decompensated HF patients	59	7.8 (3.6–15.2)	3.01	GC-MS and spectrophotometry	[69]
Chronic HF patients	30	1.22 (0.68–2.19)	0.47		
Healthy subjects	20	0.39 (0.30–0.79)	0.15		
Healthy adults (20–29 years) during sleep	6	Start 234–580 ppb; Max 685–1529 ppb; End 575–1460 ppb;		PTR-MS	[66]
T1D	<69[b]	Not displayed		Metal oxide semiconducting sensors	[84]
T2D	123				

[a] Mean ± Standard Deviation with unit to be micrograms/gram condensate/square meter of body surface; exhaled acetone, water vapor and carbon dioxide are collected as condensate in a series of liquid air traps.
[b] This number includes T1D and other types patients.
OGTT: oral glucose tolerance test; CHF: congestive heart failure; HF: heart failure; ECC: extracorporeal circulation; SIFT-MS: selected ion flow tube mass spectrometry; PTR-MS: proton transfer reaction mass spectrometry; T2D: Type 2 diabetes; T1D: Type 1 diabetes; $V_{70–85\%}$: fraction 70–85% by volume of exhaled vital capacity; GM: geometric mean; FT-IR: Fourier transformed infrared; SPME-GC-TOF/MS: solid phase microextraction-gas chromatography-time of flight-mass spectrometry.

higher than 1.10 ± 0.88 μg L^{-1} measured in the 67 controls, and even higher than 1.70 ± 0.23 μg L^{-1} measured in the 20 patients with late-onset diabetes after the age of 50 (typically T2D). In 1969, Tassopoulos *et al* conducted an even more specifically designed study of breath acetone in diabetic patients [76]. In this study, the 251 diabetic patients were grouped by the type of diabetic managements and the measured overnight fasting BG levels. Likewise, elevated breath acetone levels were observed in the diabetics with elevated BG values. Regardless of the types of diabetic managements, patients with higher BG tended to have higher breath acetone levels. This tendency seemed to be more significant in the 103 insulin-treated patients, their breath acetone increased from 1.90 ± 0.16 to 7.49 ± 4.90 μg L^{-1} monotonically with elevated BG when patients were grouped by BG levels (namely 51–120, 121–200, 201–300, and 301–450 mg per 100 mL). To the best of our knowledge, these three studies are the earliest reports on breath acetone in clearly-known diabetic patients and all of their results support that elevated breath acetone exists in diabetic patients.

After a time gap of about 30 years, in 1998, Nelson *et al* measured acetone and isoprene in exhaled breath collected from the newborn infants and the diabetic children [59]. There was no significant difference in breath isoprene between the healthy and the diabetic children. However, elevated exhaled acetone concentrations were observed in diabetic children, as compared with breath acetone concentrations in the healthy children. In the healthy children, the median (range) breath acetone concentrations in pre-school (0.1–6.1 years old) and school (8–16 years old) subjects were 0.51 (0.19–3.33) and 0.38 (0.16–0.64) μg L^{-1}, respectively. Non-fasting diabetic children (9–19 years old) had breath acetone concentrations of 1.09 (0.20–1.89) μg L^{-1}, while acetone concentrations in the fasting diabetic children were 1.97 (0.97–7.66) μg L^{-1}. A further increase was found in some of the diabetic children after breakfast consumption, and breath acetone concentrations were determined to be 3.14 (1.10–9.16) μg L^{-1}. This has been the fourth independent study showing that elevated exhaled acetone concentrations were observed in diabetic patients.

Turner *et al* monitored the breath acetone in eight T1D patients during the 'insulin clamp' studies in which insulin and glucose were infused into the subjects to lower their BG levels in steps from normal values into the low glucose (hypoglycaemic) range [81]. The baseline breath acetone concentrations (ranged from 1.0 to 21 ppm) in these eight T1D patients were not invariably higher than those in the healthy subjects, and the baseline BG did not correlate with the breath acetone baseline. Nevertheless, it was observed that the breath acetone level decreased linearly with the BG level in all of the subjects during the 'insulin clamp'.

In the more recent study by Wang *et al* breath acetone in 34 T1D outpatients and 15 apparently healthy individuals was measured [46]. Their diabetic statuses were justified by their BG levels and some of them even had records of A1C levels. A single exhaled breath gas was taken and measured

on-line. The mean breath acetone concentration in the 34 T1D patients was 2.19 ppm, higher than the mean breath acetone concentration of 0.48 ppm in the 15 healthy subjects.

The aforementioned independent studies of breath acetone in a total of 242 medically defined Type 1D patients, bridging over the last half century, unanimously support that the mean elevated breath acetone concentration in Type 1D patients is a common observation.

When it comes to T2D, however, the current investigations have not had a unanimous agreement that breath acetone increase abnormally in T2D patients. For example, Deng *et al* studied the exhaled breath in 15 T2D patients and 15 healthy controls [77]. They reported a considerable difference in acetone concentration between these two groups. The breath acetone value in the T2D patients was higher than 1.71 ppm, while that in the controls was lower than 0.76 ppm.

In the investigation conducted by Greiter *et al* [85], 21 T2D patients on an insulin therapy and 26 healthy controls participated in the breath analysis study. The T2D subjects had a mean age of 66.1 $\pm$ 8.3 years and diabetic onset time of 8.1 $\pm$ 8.9 years. Higher acetone levels in the diabetic patients were observed, but the difference between the T2D patients and the controlled subjects was not significant. Researchers also carried out sensitivity analyses. Based on acetone alone to identify diabetes, the discrimination sensitivity was 76%. The corresponding sensitivity was up to 90% when seven other VOCs were added to separate a patient from a control. An even higher sensitivity of 95% was obtained by using an alternative set of VOCs (acetone included). This work provides the evidence that differences exist in the exhaled breath acetone between the insulin-treated T2D patients and the healthy controls.

Differently, in the breath acetone analysis of the T2D patients carried out by Ueta *et al* [82], the exhaled acetone level in 21 controlled T2D patients was determined to be in the range of 0.19–0.66 ppm, while acetone concentration in the 10 healthy subjects was in the range of 0.18–0.59 ppm. No significant difference in breath acetone was observed between the healthy individuals and the controlled T2D patients. However, higher concentration, ranging from 0.92 to 1.20 ppm, was observed in one medically untreated T2D patient with 8-year duration. In another study [57], no significant difference in acetone concentration was observed between the four T2D patients (a range of 220–1024 ppb) and the nine healthy subjects (a range of 238–698 ppb), and all of them were senior people in the age of 60–83.

Similarly, Storer *et al* reported their study of 38 T2D patients [83]. They found that the median acetone concentration was 337 ppb and the breath acetone in all of the T2D subjects varied between 160 and 862 ppb. The mean breath acetone concentration in the 38 T2D was not obviously different from that in the healthy people.

Also in 2010, Wang *et al* tested exhaled breath acetone in 10 T2D outpatients and 15 apparently healthy individuals [46]. The results showed that the mean breath acetone concentration in the T2D subjects, i.e. 2.05 ppm, was higher than 0.48 ppm in the healthy people. However, the breath acetone concentration of those 10 T2D patients unevenly distributed from 0 to 5.99 ppm. No obvious trend was found.

As reviewed above (see table 3), unlike the cases in T1D, the six independent studies of breath acetone in T2D subjects do not support a commonly agreeable solution with regard to whether T2D patients have an elevated breath acetone.

5. The techniques used in the measurements of breath acetone

Quantification of breath acetone is challenging due to its trace concentration and interferences from other substances in the exhaled breath. Therefore, it is necessary for the measurement techniques to have high sensitivity and high selectivity. Furthermore, point-of-care (POC) requires the capability of real-time and on-line measurements. This section reviews the only techniques that have been used to date in the measurements of breath acetone. They are gas chromatography (GC) techniques; mass spectrometry (MS)-based techniques, electrochemical sensors and laser spectroscopy techniques, as listed in table 4.

GC. Gas chromatography has detection sensitivity at the ppb to ppt levels. GC has been accepted as the standard technique for the determination of exhaled VOCs. Basically, after sample collection and pre-concentration procedures, an analyte of interest is injected into a GC system, particular species are separated and then detected by various detectors. The commonly used detectors include flame ionization detectors [23, 49, 62, 63, 68, 75, 86, 87], electron capture detectors [86], mass spectrometers [31, 47, 49, 65, 77, 86], semiconductor gas detectors [51], spectrophotometers [59], etc. Solid phase micro-extraction (SPME) technique is a promising sampling method, which has been combined with GS-MS (thus, SPME-GC-MS) for breath acetone analyses [56, 73, 82, 88–90].

In order to achieve quantification, calibration using known compounds in the GC technique is necessary. The sample preparation procedure sometimes increases the quantification uncertainty. Furthermore, the chemical separation process in GC is time-consuming and not suitable for real-time, on-line measurements.

MS-based techniques. MS-based analytical techniques have been developed rapidly for on-line breath analysis. The two most popular MS-based techniques used in breath acetone analysis are (PTR-MS) [29, 38, 39, 52, 66, 73, 80, 91–93] and SIFT-MS [50, 54, 55, 61, 67, 71, 72, 81, 83, 94–99]. The main advantages of PTR-MS and SIFT-MS are their fast response (hundreds of milliseconds), low detection limit (ppb levels or lower) and capability of analyzing a single breath.

Schwoebel *et al* monitored the breath biomarkers' (acetone, isoprene, acetaldehyde and hexanal) profiles using PTR-MS in seven healthy volunteers when they were exercising using a stationary bicycle [73]. The schematic of the setup is shown in figure 2. With their PTR-MS data processing algorithm, the measurements could tell a distinct difference between alveolar phase and inspiratory phase even under high respiratory rates up to 60 min^{-1}. This study demonstrated that the PTR-MS technique can be used for fast on-line monitoring of exhaled VOCs.

Smith and his coworkers conducted extensive studies of breath acetone analysis using SIFT-MS [20, 50, 54, 57, 60,

Table 3. Measurements of breath acetone in T1D and T2D subjects.

Researchers	Type of diabetes	No.	Subjects information	Correlation	
				Blood glucose	HbA1c
[75]	T1D[a]	49		No	N/A
	T2D[a]	20			
[76]	T1D[b]	70	Overnight fast	The mean breath acetone levels rose progressively through groups arrayed in order of the simultaneous blood glucose.	N/A
	T2D[b]	156	Overnight fast	Subjects with higher blood glucose tended to have higher breath acetone values.	N/A
[81]	T1D	8	Mean age 28 ± 3 years, BMI 26.4 ± 1.0, mean HbA1c 8.8 ± 0.4%, had 'insulin clamp' test	Breath acetone declined linearly with blood glucose in all patients with R^2 ranging from 0.60 to 0.94.	N/A
[82]	T2D	21	12 males and 9 females; age 50–83 years	No	Correlation coefficient $r = 0.776$
[46]	T1D	34	14 males and 20 females; age 10–77 years;	When subjects were grouped by different BG levels, $R = 0.98$.	When subjects were grouped by different A1C levels, R = 0.99.
	T2D	10	Age 34–72 years	No	N/A
[83]	T2D	38	Age 32–76 years; BMI 23.8–49.1; 17 nonsmokers and 21 ex-smokers; all subjects were not asked to fast, but with other restrictions;	No	N/A
[86]	T1D	8	3 males and 5 females; age 25.8 ± 1.7 years; overnight fast	Plasma glucose estimates based on two groups of four gases each (both groups contain acetone) showed strong correlations with glucose levels, 0.883 and 0.869, respectively.	N/A
[84]	T1D	<69[c]	110 males, 82 females;	The accuracy to classify the diabetes samples using 12 metal oxide semiconducting sensors can be up to 68.66%.	N/A
	T2D	123	overnight fast		

[a] The type of diabetes is deduced based on onset age.
[b] The type of diabetes is deduced based on the treatments.
[c] This number includes T1D and other types of patients.

61, 67, 93, 95, 96, 100, 101]. The initial SIFT-MS study of five healthy subjects conducted over a 30-day period provided sufficient data to summarize breath acetone distributions [67]. And then, the longitudinal study of 30 healthy volunteers over a 6-month period was carried out to obtain a wider picture of the breath acetone [50]. The SIFT-MS technique shows the advantages of rapid response and a large dynamic range, as shown in figure 3.

Although MS is an established analytical technique, it has the issue with selectivity when it comes to discrimination of the gases with the same mass-to-charge ratios (m/z) [102, 103]. However, in SIFT-MS and PTR-MS techniques, the usage of different reagent ions, namely H_3O^+, NO^+ and O_2^+, endows them the capability of distinguishing isomeric compounds and different species on the same nominal mass [104, 105]. Ion mobility spectrometry-mass spectrometry (IMS-MS) is another MS-based technology that has been used for breath acetone analysis [106]. IMS-MS potentially overcomes the mass overlapping problem by providing further information (i.e. size and shape) on collision cross sections of compounds besides mass and charge [107–109]. IMS has the capability of separating analytes on the millisecond time scale. This feature makes this technique qualified for on-line analysis of breath gas.

Electrochemical sensors. During the past decade, electrochemical sensors, often referred to as electronic noses, have also been intensively studied and developed for breath acetone analysis [84, 110–115]. The major merit of electrochemical sensors is the cost-effectiveness and compact size. Electrochemical sensors can be fabricated as

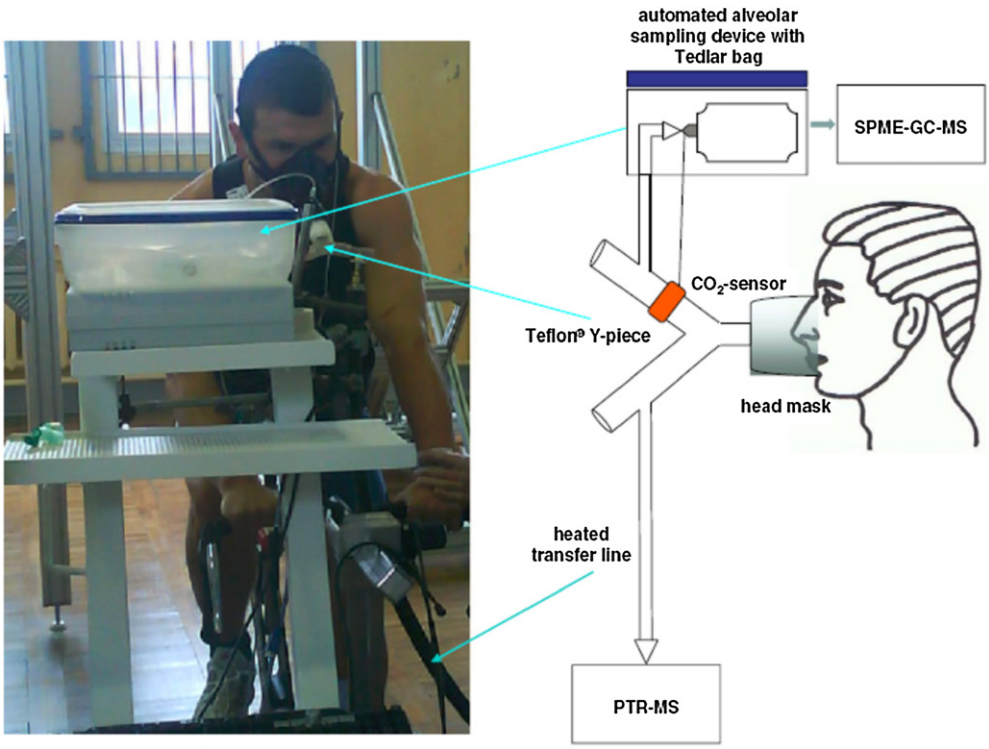

Figure 2. Experimental setup and schematic of the breath sampling procedures for real-time breath analysis during exercise using on-line PTR-MS and off-line SPME-GC-MS methods (Copyright permission from Schwoebel *et al* 2011 [73]).

Table 4. The techniques used in the measurements of breath acetone.

Techniques/methods	Features	References
GC	Detection limit down to ppb to ppt; pre-concentration methods, such as exhaled breath condensate (EBC), solid phase micro-extraction (SPME), breath collecting apparatus (BCA), and so on, have been applied for breath acetone analysis using GC.	[23, 31, 47, 49, 51, 56, 59, 62, 63, 65, 68, 73, 75, 77, 82, 86–90, 125–127]
MS-based (PTR-MS, SIFT-MS, IMS-MS)	Hundreds of milliseconds response time; down to the ppb level detection limit; use different reagent ions to distinguish isomeric compounds and different species on the same nominal mass, IMS-MS potentially overcomes the mass overlapping problem by providing further information on compound collision cross sections (i.e. size and shape) besides mass and charge.	[20, 29, 38, 39, 50, 52, 54, 55, 57, 60, 61, 66, 67, 71–73, 80, 81, 83, 91–105, 107–109]
Electrochemical sensors and nanosensors/electronic nose	Cost-effectiveness and compact size; Various sensors have been developed with the purpose of improving the sensitivity and selectivity.	[84, 110–120]
Laser spectroscopy-based techniques	Down to the ppb level detection limit; high selectivity, near real-time response, low instrument costs and POC function; so far, CRDS, Fourier transform broadband CEAS, and ICOS have been used.	[46, 79, 121–124]

a sensor array combined pattern recognition systems. To date, several electrochemical sensors such as ε-WO_3 nanoprobes [116], Si-doped WO_3 nanoparticles [117], WO_3 nanocrystal-based sensor [118], multiwall carbon nanotubes/SnO_2 gas sensor [119], chemiresistor sensor arrays with nanoparticle-structured thin films [120], etc have been reported for breath acetone measurements.

Besides the concern of inadequate sensitivity for trace acetone measurements, the main drawback of electrochemical sensors is low selectivity. Although much progress has been made in this regard, current electrochemical sensors do not have the desired sensitivity and selectivity for breath acetone analysis. Furthermore, the long response and recovery times, which are of tens of seconds or hundreds of seconds, restrict their applications in real-time, on-line breath acetone monitoring.

Laser spectroscopy-based techniques. Laser spectroscopy-based techniques are promising for breath analysis owing to their high sensitivity, high selectivity and real-time response. Various laser spectroscopic techniques have been used for detections of breath biomarkers so far [21]. These techniques include laser induced fluorescence spectroscopy, multi-pass laser absorption spectroscopy, integrated cavity output spectroscopy (ICOS), cavity leak-out spectroscopy, cavity ringdown spectroscopy (CRDS), cavity enhanced absorption spectroscopy (CEAS) and cavity-enhanced frequency comb spectroscopy. To date, only a few of them have been applied to breath acetone analysis.

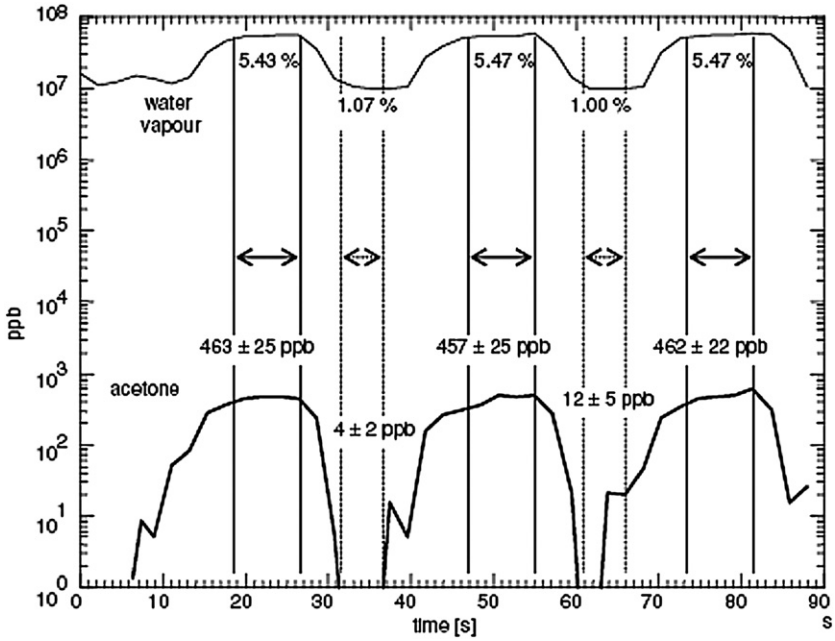

Figure 3. Quantitative SIFT-MS analysis of acetone levels in three sequential breath exhalations (Copyright permission from Turner 2006 [50]).

Wang *et al* developed a portable breath acetone analyzer using CRDS and evaluated its performance using both acetone sample solutions and human breath from subjects under various situations [79, 121, 122]. The laser wavelength was chosen to be 266 nm to make full use of the large absorption cross-section at this wavelength and avoid potential spectral interferences from other breath gases. This instrument was set in a clinic to measure breath acetone from diabetic outpatients and healthy subjects [46]. Denzer *et al* applied the Fourier transform broadband cavity enhanced absorption spectroscopy using both a supercontinuum source and superluminescent light emitting diodes in the spectral range of 1.5–1.7 μm to record absorption spectra of several breath analytes, including acetone [123]. Acetone was mixed with other gases and then buffered in air to simulated breath-like mixtures. The initial results showed that the system had detection sensitivity for acetone of ∼10 ppm due to the small absorption cross-sections in this wavelength range. Arslanov *et al* reported breath acetone measurements using ICOS at 3.4 μm with a detection limit of 100 ppb and a time resolution of 0.4 s. Part of the results is shown in figure 4 [124].

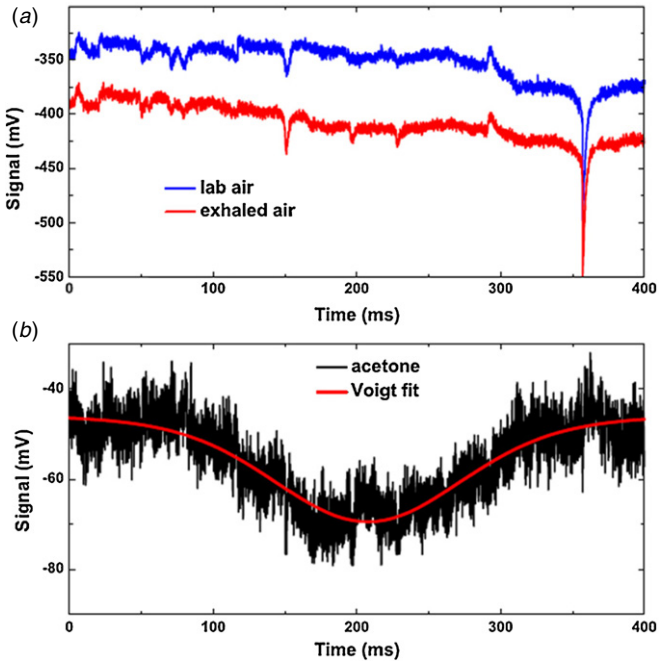

Figure 4. Real-time measurements of human breath acetone using ICOS. In panel (*a*), the upper line indicates the spectrum of the lab atmosphere and the lower line shows that of exhaled human breath. Panel (*b*) displays the result of subtraction of both lines from panel (*a*) and its fitting curve with a Voigt profile. (Copyright permission [124]).

As briefed above, different types of techniques have been used to measure breath acetone, using different breath gas maneuvers. One should be mindful when comparing their advantages and limitations in breath acetone measurements. First, from the technical specifications point of view, some techniques are pushed for sensitivity, selectivity, real-time response, or capability of on-line monitoring, while other methods are driven by potential instrumentation cost, size, or capability of POC. Secondly, from the measurement standardization point of view, currently there is no golden-standard method widely-accepted for real-time, on-line, high sensitivity, high-selectivity and high accuracy breath acetone measurements. Although the GC-MS technique has been considered a golden-standard method for lab-based trace gas analysis in most analytical applications, when it comes to breath acetone quantification, concerns resulting from the sophisticated breath sampling should be made aware. On the other hand, some techniques, i.e. laser spectroscopy-based techniques, can measure acetone in a single breath

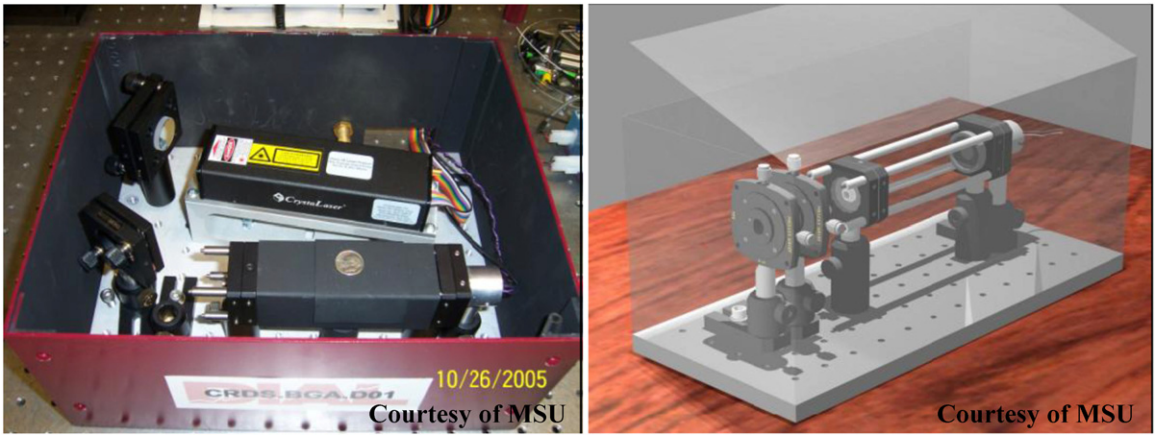

Figure 5. A prototype breath acetone analyzer using pulsed-CRDS at 266 nm. *Left*: the instrumental package; *right*: detailed pictorial view of the optical cavity configuration. (Copyright permission [21]).

on-line without using an additional sampling procedure. In this case, however, breath-by-breath fluctuations in chemical compositions and concentrations should be taken care of.

6. Portable acetone analyzers tested in a clinic or commercially available

In recent years, development of breath analysis instruments for POC clinical application is becoming more and more interesting. Non-invasive, POC breath gas analysis depends on the availability of a portable, fast response and highly sensitive device. Some breath biomarkers have commercially available analyzers now [128–134]. At present, most analyzers are available for research purpose or clinical investigation only. Mashir *et al* summarized the breath analysis devices that had been approved by the US Food and Drug Administration (FDA) [135].

On breath acetone analysis, several prototypes are still under trials or clinical tests toward commercial instruments, but two breath acetone analyzers are commercially available now. One utilizes GC for gas separation and a semiconductor gas sensor as the detector. The acetone analyzer has a detection range of 100 ppb–20 ppm. The instrument requires 5 to 60 min for warm-up and one measurement takes 8 min [136]. The other is a semiconductor sensor that uses tungsten oxide as the acetone detector. The sensor is compact and weighs only 125 g. The sensor has a detection limit for acetone of 0.2 ppm [137]. One prototype hand-held breath acetone analyzer (HHBAA) has been used in a clinical study to measure breath acetone in children with refractory seizures, children on antiepilepsy medication, and children without epilepsy history [63]. The results obtained from the GC and the HHBAA sensors were in a very good agreement, demonstrating the accuracy and reliability in breath acetone measurements. Kinoyama *et al* introduced a breath acetone analysis device with a detection limit for acetone of 0.1 ppm, namely Biogas Acetone Analyzer (BAS-2000) [51]. This device is a GC equipped semiconductor gas detector with a high-sensitivity and has been used to measure breath acetone and isoprene concentrations in 451 college students. Laakso *et al* employed a commercial pilot-case-sized FT-IR gas spectrometer as a POC gas analyzer to carry out breath screening in the clinical study of the patients attending emergency departments [138]. Acetone was one of the analytes that were studied in this clinical test. The results demonstrated the utility of this FT-IR analyzer. Wang *et al* employed prototype CRDS-based breath analyzer to measure the breath acetone concentrations in 32 human subjects under various situations, e.g. T1D patients, healthy subjects post-exercise, post-meal, post-alcohol-consumption and after overnight fast [79]. This pilot-scale analyzer was then used to conduct real-time, on-line breath tests with T1D and T2D outpatients in a clinic [46]. A photograph of a portable breath acetone analyzer is shown in figure 5.

7. Breath acetone concentration versus blood glucose level

Blood glucose (BG) level is the clinical parameter currently used in diabetes diagnostics, monitoring and management. After an extensive review of both the established and the emerging epidemiological evidences on glycated hemoglobin (A1C or HbA1c), which represents an average amount of glucose level in blood over the last two to three months, an International Expert Committee recommended the use of the A1C test to diagnose diabetes mellitus in 2009, with a threshold of $\geqslant$6.5% [139]. Soon afterwards, American Diabetes Association (ADA) and WHO endorsed this recommendation [5, 140]. Therefore, it is naturally curious: what is the relationship between breath acetone and blood glucose?

In 1969, Tassopoulos *et al* conducted breath acetone and blood sugar measurements in 251 diabetics after overnight fasting and then grouped the results by blood sugar levels [76]. It was found that breath acetone levels increased with elevated blood sugar values. They made no attempt to explore possible quantitative relations between the breath acetone concentration and the BG level. In the work by Ueta *et al* with 21 controlled T2D patients, the results showed a clear correlation between breath acetone concentration and HbA1c level with a correlation coefficient of 0.776 [82]. For the purpose of predicting plasma glucose, Minh *et al* used the standard least-squares regression on several

subsets of exhaled gases to generate multi-linear models [86]. Seventeen healthy subjects and eight T1D patients participated in this study, and the plasma glucose concentrations were estimated based on two groups of four gases (group A: acetone, methyl nitrate, ethanol and ethyl benzene; group B: 2-pentyl nitrate, propane, methanol and acetone). The predicted values displayed obvious correlations with measured BG concentration for both healthy and T1D subjects, and the mean correlation coefficients obtained with these two groups were 0.883 and 0.869, respectively.

Wang *et al* carried out a clinical test with well-defined diabetic patients using a pilot-scale breath acetone analyzer base on the CRDS technique [46]. They measured breath acetone concentrations in single exhaled breath and used a BGM to check the patient's BG levels within 3–5 min when the breath acetone was measured. HbA1C numbers of some of the patients were also provided by their doctors (the research processes followed the approved IRB procedures strictly!). These simultaneous measurements of the breath acetone and BG allowed them to explore possible correlation between breath acetone concentration and BG level in the diabetic patients. Among the 34 T1D patients studied in this work, a linear correlation was observed between the mean group acetone concentration and the mean group BG level with a correlation coefficient of 0.98 when all of the T1D patients were grouped by different BG levels, 40–100, 101–150, 151–200 and 201–419 mg dL^{-1}. A linear correlation between the mean group acetone concentration and the mean group HbA1C was also found with a correlation coefficient of 0.99 when the patients' A1C levels were grouped by <7, 7–9.9 and 10–13%. However, no correlations were found when the BG and HbA1C numbers were not grouped.

Very recently, Guo *et al* employed a chemical sensor to measure breath acetone of 192 diabetic patients (123 of them were T2D and the rest were T1D and other types) being classified into four groups according to their BG levels [84]. A regression technique was applied to analyze the obtained acetone concentrations to classify the diabetic patients into different groups. They reported a classification accuracy of 68.66%, which described the correlation between the breath acetone and the BG.

In an oral glucose tolerance test (OGTT) (ingestion of 75 g of glucose followed by 120 min of sampling) with ten healthy volunteers, breath acetone concentrations were observed to decrease continuously from baseline 392 ± 85 to 280 ± 64 ppb; however, they were not correlated with BG changes [47]. In a work done by Turner *et al*, they monitored the breath acetone of eight T1D patients during the 'insulin clamp'. There appeared to be no correlation between the baseline glucose level and breath acetone concentration. Nevertheless, they found that breath acetone levels decreased linearly with BG levels in all subjects during 'insulin clamp' [81].

The advancement in the effort of breath acetone analysis toward diabetes diagnostics and monitoring is ultimately determined by the bottle-neck question—what is the relationship between breath acetone concentration and blood glucose level? The answers to this question could be (1) there is no quantitative relation between breath acetone and BG or (2) there exists a quantitative relation, but it has not been obtained yet. The first answer would lead to termination of the pursuit of non-invasive diabetes diagnostics and monitoring using breath acetone analysis. The second answer would stimulate further research effort. To date, neither a single study nor the collective results over the last 50 years' study in breath acetone can give such an answer as (1) or (2). As reviewed above, such a strong argument stems from the fact that the reported research data in this regard are spotty and not sufficient to allow one to give either of the two answers.

8. Conclusions

To date, 41 independent studies have measured breath acetone using different techniques and methods under various situations. More than 3211 human subjects, including 242 T1D, 384 T2D, 1581 healthy subjects, 174 undefined and 830 non-diabetic patients or healthy subjects under particular treatments, have been tested. The studies covered in this review bridge over the last half century. Acetone concentration in human subjects (diabetic or non-diabetic) spans a wide range. However, all of the six independent studies with clearly-defined T1D patients unanimously support that breath acetone is a biomarker of T1D and that elevated mean breath acetone concentration is a statistically common observation in T1D patients. However, no such observation is available from the reported literature for T2D. It must be emphasized that the conclusions are drawn from the limited studies only and remain to be confirmed by a larger number of studies. Secondly, the mean acetone concentration has been widely used in the current studies of breath acetone; the inferred information from the mean acetone measurements could be misleading when it is used as a gauge to judge an individual. Moreover, one limitation of the current studies is that they invariably include controlled diabetics; more information might be gained from studies using uncontrolled diabetic patients. Finally, there has been no 'golden-standard method' for breath acetone measurements without concerns in sampling, time-lapse and potential interferences; this gap forces one to be careful when comparing results from different studies using different techniques and methods.

What is the quantitative correlation between the breath acetone concentration and BG level in T1D remains to be a challenging question. The research effort to tackle this problem has just begun. Although some results from the initial exploration are encouraging, a collective effort in simultaneous measurements of breath acetone and BG using a significantly large number of clearly-defined T1D patients is needed. Research findings in this regard will bridge the gap between the laboratory or clinic breath acetone measurements and the ultimate reality—breath analysis for diabetes diagnostics and monitoring.

Acknowledgments

This work was supported by National Science Foundation through grants CMMI-0927539 and CBET-1066486.

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