A Study on Breath Acetone in Diabetic Patients Using a Cavity Ringdown Breath Analyzer: Exploring Correlations of Breath Acetone With Blood Glucose and Glycohemoglobin A1C

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***Abstract*—Acetone is qualitatively known as a biomarker of diabetes; however, the quantitative information on acetone concentration in diabetic breath is incomplete, and the knowledge of correlations of breath acetone with diabetic diagnostic parameters, namely, blood glucose (BG) and glycohemoglobin A1C (A1C), are unknown. We utilized a pilot-scale breath acetone analyzer based on the cavity ringdown spectroscopy (CRDS) technique to conduct breath tests with 34 Type 1 diabetic (T1D), ten Type 2 diabetic (T2D) patients, and 15 apparently healthy individuals. Relations between breath acetone and BG, A1C, and several other bio indices, such as the type of diabetes, onset-time, gender, age, and weight were investigated. Our observations show that a linear correlation between the mean group acetone and the mean group BG level does exist ($\mathrm{R} = 0.98, \mathrm{P} < 0.02$) when all the T1D subjects tested are grouped by different BG levels, 40–100, 101–150, 151–200, and 201–419 mg/dL. Similarly, among the T1D subjects studied, when their A1C's are grouped by <7, 7–9.9, and 10–13, a linear correlation between the mean group A1C and the mean group acetone concentration is observed ($\mathrm{R} = 0.99, \mathrm{P} < 0.02$). No strong correlations are observed when the BG and A1C numbers are not grouped. The mean breath acetone concentration in the T1D subjects studied in this work is determined to be 2.19 ppmv (parts per million by volume), which is higher than the mean breath acetone concentration, 0.48 ppmv, in the 15 healthy people tested.**

***Index Terms*—Acetone, blood glucose, breath analysis, cavity ringdown spectroscopy, correlations, diabetes, glycohemoglobin A1C.**

I. Introduction

DIABETES comprises a heterogeneous group of disorders characterized by high blood glucose (BG) levels, which is diagnosed by the presence of the classical signs and symptoms of diabetes, by fasting plasma glucose (FPG) ≥ 140 mg/dL (venous whole blood glucose ≥ 120 mg/dl), or by venous plasma glucose ≥ 200 mg/dl at 2 h after a 75-g oral glucose challenge [1]. Ninety-five percent of diabetic persons are classified as either insulin-dependent diabetes mellitus (IDDM), also widely referred to as T1D, or non-insulin-dependent diabetes mellitus (NIDDM) (T2D) [1]. Besides BG levels, another monitoring parameter of diabetic status is A1C that reflects the mean blood glucose level over the last three months. An A1C number (percentage) helps evaluate the adequacy of diabetic treatment and control. For instance, A1C is normally managed to be slightly less than or equal to 7% when a diabetic case is under good control [2]. The measurement of GB is normally achieved by using a standard self-management BG meter while the measurement of A1C is typically obtained through analyzing a blood sample in a certified medical facility. Both measurements require blood samples and intrusive.

Breath gas analysis may offer an ultimate solution for noninvasive monitoring and diagnostics of diabetes. The usefulness of breath acetone ($(CH_3)_2CO$) as a biomarker of Type 1 diabetes (T1D) has long been known although the mechanism has not been completely understood [2]. The common understanding of acetone in diabetic breath is supported by a combination of established metabolic science and specific research. Acetone, which is produced normally in the body, primarily results from the spontaneous decarboxylation of the ketone body acetoacetate and to a lesser extent from enzymatic conversion of acetoacetate to acetone by acetoacetate decarboxylase [3]. The "sweet odor" of the breath of ketotic individuals is due to the elevated presence of acetone. The elimination of free-acetone from lungs follows the diffusion law, and the acetone in expired air is approximately 1/330 of the acetone in plasma [4]. Breath acetone in expired air has also been known to be correlated with ketone bodies in plasma [5], blood acetone levels [6], [7], and β-hydroxybutyrate in venous blood [8].

Although acetone concentrations in normal human breath have been extensively studied, publications on acetone concentrations in actual diabetic breath are very limited and to date no study has reported the correlation of breath acetone with the standard diabetic monitoring parameters, blood glucose (BG) and glycohemoglobin A1C (A1C) levels. Numerous studies have reported on breath acetone measurements in healthy human subjects. The mean acetone concentrations in healthy breath measured in different studies range from 0.35 ppmv (parts per million by volume) to 0.85 ppmv [7]–[22]. Averaging

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over all of the acetone concentrations reported in the different studies yields a mean acetone concentration in healthy breath to be 0.49 ppmv.

Compared with the breath acetone analysis in healthy human subjects, studies on breath acetone in diabetic subjects are very limited [9], [10], [23], [24], [25] Abnormal acetone concentrations (typically higher than acetone concentrations in normal breath) in exhaled air of diabetic subjects have been reported [9], [10], [22], [23], [24]; however, the quantitative information on the correlation of breath acetone with BG level, A1C level, and other bio indices, such as age, gender, years of diabetes, and type of diabetes, has not been available yet. To the best of our knowledge, there is only one publication that reports the measurement of breath acetone and simultaneous BG level in diabetic subjects [9]. In that study, the diabetic subjects were insulin dependent (T1D) and investigated after an overnight fast; the progressive increase in breath acetone concentration with the increase in simultaneous BG level were observed. Interestingly, over the last four decades, no further studies on the similar research topic have been reported. In a recent study [18], Galassetti *et al.* investigated the correlation of breath acetone with BG, yet the subjects studied were healthy individuals treated by a glucose load, rather than diabetic patients. The current knowledge of the breath acetone versus the simultaneous BG in diabetic patients is based exclusively on that early study in which the breath gas analysis was conducted by GC-flame ionization detection. It is interesting and important to explore possible correlations of breath acetone with BG and/or A1C in diabetic subjects.

We have proposed a laser-based detection method of measuring breath acetone using high sensitivity and fast-response cavity ringdown spectroscopy (CRDS) technique. The fundamental spectroscopic study of acetone using the CRDS technique and the characterization of a pilot-scale acetone detection device using acetone sample solutions were conducted previously [26], [27]. A proof-of-principle, portable ringdown breath acetone analyzer has been recently tested with human subjects to demonstrate the technical feasibility and its detection specificity has been justified in detail [28]. The goal of this study was to test a hypothesis that breath acetone correlates with BG and A1C in T1D patients. We conducted clinical tests with diabetic patients using the breath analyzer. In this paper we present our preliminary findings.

II. Experimental

1) Ringdown Breath Acetone Analyzer: The instrument's principle is based on cavity ringdown spectroscopy (CRDS) [29], which has been described in detail in several excellent reviews (just to list a few) [30]–[33]. The basic design of the breath analyzer and the details of the instrument components can be seen elsewhere [28], [29]. Briefly, the instrument contained a laser source, a gas cell, a detector, a sample introduction part, and a data processing portion. The laser source, gas cell, detector, and associated parts were assembled on a 60 cm $\times$ 25 cm aluminum plate of 1 cm thickness. This optical-electrical platform served as the central part of the instrument. The light source (CrystaLaser) was a single-mode Q-switch Nd:YAG laser operating at 266 nm with a repetition rate of up to 1 kHz and a single pulse energy of $1-3$ μJ. Except for using two steering mirrors to direct the laser beam, no mode-matching optical parts were used to image the laser beam into the cavity. The gas cell was made of a section of stainless pipe that was 43 cm in length with an inner diameter of 2 cm (Tiger Optics). The gas cell was constructed with one gas inlet sitting in the middle of the cell and two gas outlets located at each end of the cell. One pair of mirror mounts (Los Gatos Research) was installed at each end of the gas cell through an O-ring seal. A high reflectivity ultraviolet (UV) mirror ($\mathrm{R} = 99.87\%$, Los Gatos Research) was housed in each mount. The spacing between the two mirror surfaces was 45 cm. The gas cell was sealed and operational at different pressures, which were monitored by a micro pressure transducer (MKS 872B) with a measuring range of 0.5–1000 Torr. The ringdown decay waveforms were digitalized by an A/D converter interfaced to a laptop computer [34]. The whole package, excluding the computer and a compact vacuum pump, is readily carried by a single person and placed on an office table for measurements.

A breath collection bag (Quintron Instrument) was equipped with a mouthpiece for single directional gas flow and a gas outlet valve, which was readily plugged into a gas cell inlet tubing with 5-mm inner diameter. The breath analyzer can operate in either an online breath mode or an offline breath mode. In the online breath mode, subjects directly breathed into the bag which was connected to the gas cell; in the offline mode subjects breathed into the bag which was separated from the gas cell and the breath gas was sealed for later measurement. In this study, the online mode was used. The volume of the breath bag was 750 mL. In the test, the subjects were instructed to take a deep breath and then inflate the bag with a single exhaled air up to slightly above atmospheric pressure. Except for a couple of senior and female outpatients, the volunteers readily inflated the bag by a single exhalation. It was tested in the experiments that the single breath flow rate had no effect on the measurement of breath acetone. The breath mouthpiece was used by only one volunteer and then disposed. Each breath bag was used 2–3 times. Before the bag was reused, the residual breath gas was pumped out and then flushed by compressed air.

2) Measuring Method: The background subtraction method, which was introduced in the previous study [29], was used to obtain breath acetone concentration. In the background subtraction method, the absolute concentration of acetone (the upper limit) in the breath gas is obtained by [29]

$$\Delta A = A_{\mathrm{breath}} - \bar{A}_{\mathrm{atm}} = \sigma_{266} n d \tag{1}$$

where ΔA is the absorbance difference, A_{breath} is absorbance of the breath gas (healthy breath gas, diabetic breath, etc.), $\bar{A}_{\mathrm{atm}}$ is the mean effective absorbance of the atmosphere, d is the mirror spacing, and n is the absolute acetone concentration. σ_{266} is the absorption cross-section of acetone at 266 nm, which is 4.5×10^{-20} cm^2/mol at atmospheric pressure and room temperature [35]. The applicability, justification of the method, and detection specificity were discussed in the previous work [29]. Absorbances at 266 nm of more than 16 major high concentration breath gases and high abundance breath VOCs were estimated; the measured absorbance of each of those gases or VOCs was 2–3 orders of magnitude lower than that of breath acetone [29]. Therefore, the method has high detection specificity of

acetone and minimal interference effects on determination of the breath acetone concentration.

In this study, the breath analyzer was further validated by measuring absorbances of laboratory atmospheres and standard acetone samples. The measurement uncertainty was evaluated by measuring gas mixtures with known acetone concentrations. In the experiments, the certified gas mixture of 1.18% acetone in nitrogen (Scott Specialty Gas) was introduced to the gas cell of the breath analyzer at 10 Torr; then the gas sample was diluted step-by-step by ambient atmosphere to different concentrations ranging from 1 ppmv to 10 ppmv. The measured acetone concentrations were then compared with the known concentrations. The same procedures were used for the determination of acetone concentration in healthy breath gases. The detection limit of the breath analyzer was 0.13 ppmv based on the 1σ criterion. The measurement uncertainty was $\pm 15\%$. Note that acetone concentration in the normal indoor air is lower than 0.04 ppmv, therefore, it is not detectable for this instrument.

3) Breath Test Volunteers and Patients: All participants were volunteers. The Institutional Review Board (IRB) of human subject research at Mississippi State University approved recruiting scripts, consent forms, and research protocol. The tests were conducted in two locations, our research laboratory and a clinic (the Endocrinology Consultants Center in Tupelo, MS). For the tests conducted in the laboratory, T1D volunteers were scheduled to visit the laboratory. For each volunteer, the participation activities included being briefed on the instrument operation, filling in a consent form and a datasheet form (age, gender, T1D/T2D, years of diabetes, the latest A1C level, body weight, type of diabetic management), exhaling into the breath bag, and measuring BG levels. The whole process took about 10–15 min, of which the actual time spent on measuring the breath gas was less than 1 min. The simultaneous BG levels were measured with a standard self-management BG meter owned by each individual subject. The BG measurements were performed by the subjects themselves in the same laboratory within 5 min before or after their breath measurements. A small portion of volunteers did not bring a BG meter with themselves and their concurrent BG levels were not recorded. Similarly, some diabetic subjects did not have their latest A1C numbers available. Fifteen apparently healthy, nondiabetic volunteers were also recruited to visit the laboratory for breath gas measurements. No BG levels were recorded for these healthy subjects.

For the tests performed in the clinic, the instrument was placed on a conference table of the facility. Each T1D volunteer, and their guardian if the volunteer was a minor, was asked to do the same things as previously described for the laboratory test. During the four-day testing period, 24 T1D outpatients were tested through the first three days and 10 T2D outpatients were tested on the fourth day. Volunteers were tested right after his/her doctor's office visit but before leaving the facility.

III. Results and Discussion

A. Breath Tests in 15 Nondiabetic Healthy Subjects

Fifteen apparently healthy subjects in the ages of 25–45 were tested. No pregnant or lactating female participants were recruited. No chronic alcoholics and individuals who were on diet or intensive exercise programs were included in the volunteers. The absorbance of the healthy breath at 266 nm had a mean value of 2.32×10^{-4} with 1σ of 0.51×10^{-4}. It should be clearly noted that this standard deviation is an indicator of the interindividual variation of the measurements. The intraindividual variation between samples of a single individual is significantly smaller, approximately 10%, which is smaller than our targeted clinically-acceptable sensitivity of the measurements, 15%. Further, the measurement uncertainty of the duplicated measurements of a single sample was smaller than 4%. Since the absorbance was attributed to the absorption of all species in the normal human exhaled air as well as the Rayleigh scattering loss, the absolute concentration of acetone derived from this mean absorbance only represents an upper limit of acetone in the healthy breath gases, which was 0.48 ppmv with a 1σ standard deviation of 0.20 ppmv.

B. Acetone in Expired Air of T1D Patients

1) Abnormal Breath Acetone Levels in T1D Subjects: Table I shows the testing results collected from 34 T1D subjects. The T1D subjects listed in Table I include juvenile-onset and adult-onset diabetes. The juvenile-onset is defined as diabetes onset at 15-year-old or younger and adult-onset is defined as diabetes onset at 16-year-old or older. Note that the classification of volunteers into T1D, T2D, or T2D-to-T1D groups was entirely based on the volunteers' medical records. Of the 34 T1D subjects, 10 of them were tested in the research laboratory. The remaining 24 subjects were outpatients and were tested at the clinic between 9:30 A.M. and 4:15 P.M. during the first three days of the clinical study. In Table I, the volunteers' gender, age, simultaneous BG level, body weight, the latest A1C, years of diabetes, and the measured acetone concentration (ppmv) are also listed.

The mean acetone concentration of the 14 T1D patients who have diabetic onset at 15-year-old or younger is 2.46 ppmv with a 1σ of 1.63 ppmv. The mean acetone concentration of the 15 T1D patients who have diabetic onset at 16-year-old or older is 1.75 ppmv with a 1σ of 1.07 ppmv. The results show that even with the same type of diabetes, juvenile-onset T1D, in general, has a significantly higher acetone concentration in expired air than adult-onset T1D. Five T1D patients were not included into this calculation since their onset years were not available in this study. A small difference of the mean acetone concentrations was found between male and female T1D patients. The mean value of the 20 female T1D subjects was 1.87 ppmv with a 1σ of 1.47 ppmv, which was slightly lower than that of the 12 male T1D subjects, 2.30 ppmv with a 1σ of 1.39 ppmv. Tassopoulos *et al.* [9] measured breath acetone of diabetic patients after an overnight fast. The acetone concentrations of the subjects under the five different diabetic treatments were reported in the range of $1.90-7.49\ \mu g/L$, equivalently, 0.74–2.92 ppmv. The two converted T1D patients were defined based on the fact that their diabetic statuses were changed from NIDDM (T2D) to IDDM (T1D) and the information was provided by their medical records.

All of the T1D subjects tested were under insulin treatment. They were wearing an insulin pump and carried a BG meter for

TABLE I
BREATH ACETONE IN T1D SUBJECTS INCLUDING THE 24 T1D OUTPATIENTS WHO WERE TESTED IN THE CLINIC

Subject No.	Gender	Age	BG (mg/dL)	Weight (lb)	A1C (%)	Yrs on T1D	ppmv
1	M	47	40	n/a	n/a	40	1.55
2	F	51	63	175	6.5	43	0.26
3	F	28	78	147	6.5	n/a	0.47
4	F	29	83	102	6.9	29	3.88
5	F	37	85	n/a	6.9	29	2.69
6	F	15	87	98	6.4	15	0.92
7	F	53	95	151	7.2	37	0
8	F	56	97	138	n/a	39	0.27
9	M	59	108	150	6.7	n/a	n/a
10	M	77	110	n/a	7.9	23	0.84
11	F	56	112	185	5.9	n/a	0
12	F	27	120	152	11	10	2.66
13	F	22	124	110	13	2	1.52
14	F	69	126	162	n/a	30	2.08
15	F	77	127	185	n/a	12	0.49
16	M	61	150	117	n/a	60	6.05
17	M	42	154	174	7.5	34	1.38
18	F	25	165	260	n/a	20	1.53
19	F	21	180	140	8	12	4.53
20	F	40	183	172	7.4	25	2.6
21	F	40	185	127	n/a	12	1.62
22	F	53	192	110	6.8	15	2.05
23	M	32	198	193	6.4	30	1.55
24	F	51	200	169	11	17	4.19
25	F	19	217	n/a	n/a	4	4.15
26	M	51	219	196	n/a	12	2.02
27	F	32	240	233	10.3	28	1.45
28	M	77	256	n/a	n/a	n/a	3.79
29♦	M	73	323	165	7.8	8	2.64
30♦	M	55	419	162	n/a	11	2.19
31	M	10	n/a	77	n/a	2	1.83
32	M	63	n/a	195	n/a	n/a	n/a
33	M	69	n/a	192	6.1	53	1.59
34	M	69	n/a	n/a	n/a	30	2.14

♦Onset with T2D and converted to T1D later.

self-management of their BG levels. For all of the diabetic subjects tested, except for the self-insulin-treatment, no additional control, such as fasting, load of glucose, or feeding with special meals, was managed for the test. It can be seen from Table I that even though the BG levels of the T1D patients (subject # 1–12 in Table I) were well controlled to be within or under the normal BG level, e.g., $< 100 - 120$ mg/dl, 50% of the 12 T1D patients still have abnormal acetone breath; and their mean breath acetone was 2.1 ppmv. This interesting observation indicated that the mild ketosis of diabetes was undiagnosed by simply monitoring the BG levels. Furthermore, this mild ketosis might also suggest an inadequacy of the insulin treatment while the BG levels showed normal levels. Another possible cause for those who had abnormal breath acetone but normal or low BG levels was that those patients might have hypoglycemia. For example, subject #1's BG level was only 40 mg/dL; yet the breath acetone was 1.55 ppmv. This phenomenon was also observed in the breath test of overnight fasting T1D patients in the early study [9]. To understand the cause of some T1D cases, in which the diabetic patients have abnormal breath acetone concentration but normal or low BG levels, is beyond the scope of the current study.

2) Correlation of Breath Acetone to BG Levels: Fig. 1 shows the graph of acetone concentration versus the simultaneous BG level of the 30 T1D subjects whose BG levels were recorded. The data in Fig. 1 shows a weak correlation between acetone concentration and BG level up to 217 mg/dL. A linear fitting yielded $\mathrm{R} = 0.49$ and $\mathrm{P} < 0.022$. However, when the fitting included all measured BG levels, no correlation was found. Galassetti *et al.* [18] reported a weak correlation between breath ace-

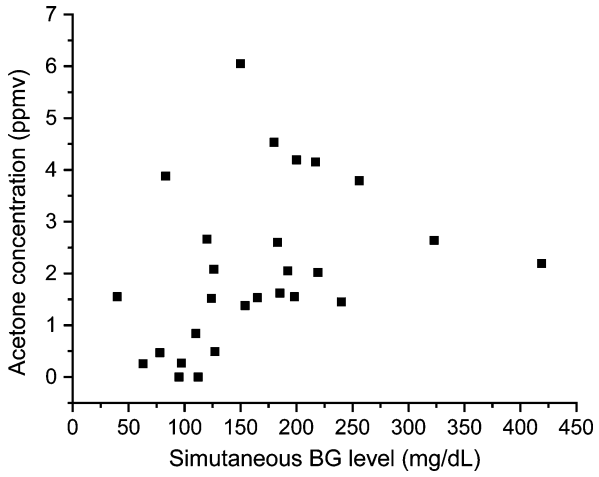

Fig. 1. Breath acetone concentration (ppmv) versus simultaneous blood glucose level (mg/dL) in 34 T1D subjects.

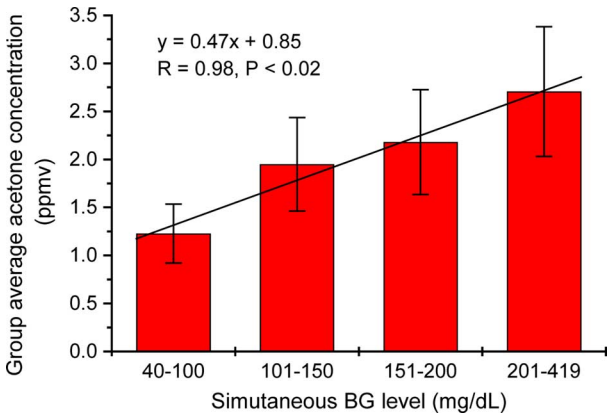

Fig. 2. Observed correlation of the breath acetone concentration (ppmv) with the simultaneous blood glucose level in the T1D subjects grouped by the different blood glocuse levels. The error bar shows 1σ standard deviation. The method of grouping the blood glucose level for data analysis was adopted from [7].

tone concentration and the serum glucose level in healthy subjects with a glucose load. However, this trend was not obvious in the test data presented in Fig. 1.

However, when all of the 30 T1D subjects were grouped by different BG levels, 40–100 (low BG), 101–150 (borderline), 151–200 (high BG), 201–419 (very high BG) mg/dL, a linear correlation between the mean group acetone concentration and the mean group simultaneous BG levels was observed. The fitting results are $\mathrm{y} = 0.47\mathrm{x} + 0.85(\mathrm{R} = 0.98, \mathrm{P} < 0.02)$, as shown in Fig. 2. The error bars show the 1σ standard deviation in each group. The classification of the four different BG groups was based on the consideration of two factors. First, the overall BG levels can be classified in general as low BG (or healthy BG), borderline BG, high BG, and very high BG. The average value of each group BG is well defined although different data sources give slightly different numbers [1]. Second, the group BG numbers (or ranges) used in this work were constructed to be approximately the same as the ones used in the early study [9], so that the results from these two separate studies can be more conveniently compared and discussed here. In that work, Tassopoulos *et al.* measured breath acetone in overnight

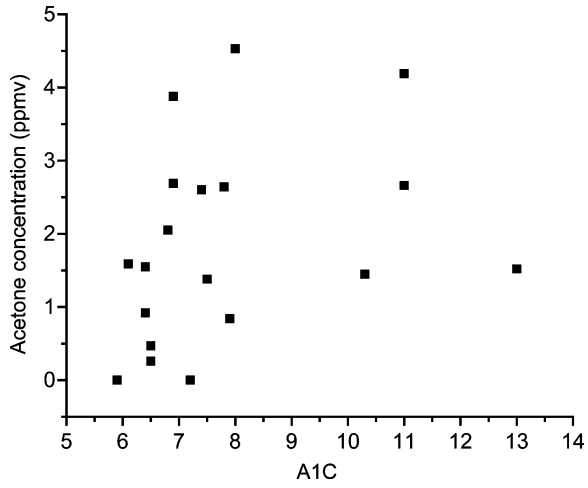

Fig. 3. Breath acetone concentration (ppmv) versus blood A1C level in 20 T1D subjects.

fasting T1D patients and grouped the subjects based on several different BG levels. Although no quantitative correlation of the breath acetone with the simultaneous BG levels was given in that study, the data did show a progressive increase in breath acetone concentration with the increase of the group BG level for the subjects in all of the different diabetic treatments. Interestingly, these two studies separated by four decades and using different measuring techniques observed a similar phenomenon while in that early study the diabetic subjects were tested after an overnight fast yet in the present work the subjects were nonfasting.

3) Correlation of Breath Acetone With A1C: In this study, A1C levels of the T1D subjects were provided by the medical records of the subjects. Column 6 in Table I lists the A1C for the subjects who had the A1C tests within the last 90 days. Of the 34 T1D subjects tested, 20 T1D subjects had A1C values. Their A1C numbers ranged from 5.9 to 13. Fig. 3 shows the graph of breath acetone concentration versus A1C value. No strong correlation was observed. A linear fitting yielded $\mathrm{R} = 0.32$ and $\mathrm{P} < 0.18$. It seems that a relatively weak correlation exists at the lower A1C values and this correlation trend reduces at the higher A1C levels. Similar trends exist in the graph of breath acetone concentration versus simultaneous blood glucose level, as shown in Fig. 1. Whether there is a general trend of the reduced correlation of the breath acetone concentration to the BG and A1C levels at the higher BG and A1C levels needs further study.

When the A1C levels were grouped into three subgroups: 5.9–6.9 (normal), 7.0–9.9 (high), and 10–13 (very high), a linear correlation between the mean group A1C and the corresponding mean group acetone concentration was found. The correlation is described by the linear fitting results, $\mathrm{y} = 1.0\mathrm{x} + 0.49(\mathrm{R} = 0.99, \mathrm{P} < 0.02)$, as shown in Fig. 4. To the best of our knowledge, no one has reported a study on the correlation of breath acetone with A1C; no experimental data are available for a comparison. In theory, it is well documented that BG level is correlated with A1C level and the correlation coefficient can be up to $\mathrm{R} = 0.99$ [36], [37]. Therefore, theoretically, if the breath acetone is correlated with the BG, then the breath acetone should

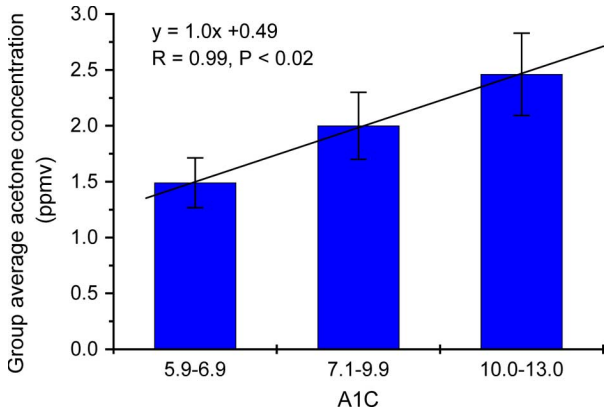

Fig. 4. Observed correlation of the acetone concentration (ppmv) with A1C in the T1D subjects grouped by the different A1C numbers. The error bar shows 1σ standard deviation.

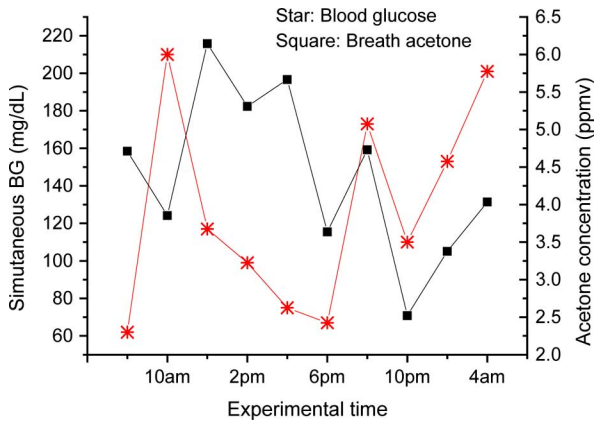

Fig. 5. Observed variations of breath acetone (ppmv) and the simultaneous blood glucose level in a 51-year-old, juvenile-onset, female T1D subject during a 24-h monitoring.

be correlated with the A1C as well. In this study, we also examined the correlation coefficient between the mean A1C and the mean group BG level and a correlation coefficient was found to be $\mathrm{R} = 0.87$. Note that the BG level measured by a BG meter indicates the present metabolic state while the A1C reflects a time-averaged steady-state. In Fig. 4, the y-axis represents the group average breath acetone and the x-axis denotes the group average A1C. Therefore, the correlation shown in Fig. 4 implies that the average breath acetone concentration reflects average BG level, as indicated by the results of Fig. 2. A physiological justification on whether acetone concentrations in the breath reflect the present metabolic state or a steady metabolic state of the T1D patient is the subject of future study and the related discussion is beyond the knowledge of the authors of this work.

4) Variation of Breath Acetone in T1D During a 24-h Period: T1D includes juvenile-onset T1D and adult-onset T1D. The category of the adult-onset T1D also includes T2D-converted T1D. In this preliminary study, we investigated the time-dependent variation of breath acetone in T1D subjects during a 24-h period, and the tests exclusively focused on the juvenile-onset T1D subjects in this study. Three female juvenile-onset diabetic subjects were selected for the 24-h monitoring on their breath acetone. The selection was only based on availability of the volunteers. The experiment was designed to explore the possibility of high temporal resolution correlation between breath acetone and simultaneous BG level during a 24-h period. In the experiment, each volunteer was invited to be in the laboratory and given instruction on how to handle the breath gas sample. Twelve pre-prepared breath gas collection bags were labeled with designated sampling time, e.g., at 8:00 A.M., 10:00 A.M., etc., and the bags were taken home by the volunteer. For each breath gas collection, the BG level was concurrently (within a few min) measured by a glucose meter. The BG levels and the brief information on the amount of calories, food gradients, and ingestion time were recorded in a predesigned sheet form. During the daytime of the 24-h period, the sampled breath bags were picked up about 3 h from their offices or at their homes and the breath gases were measured in the laboratory. For the breath collections conducted in the nighttime from 10:00 P.M. to 6:00 A.M., the breath bags were picked up and tested early in the morning of the second day. All of the sampled breath bags were kept under ambient conditions. According to the specification, the breath bags can store fresh breath gas for 24 h. No further analysis was conducted to confirm the freshness of the breath gas in this study. However, whether this specification was held for breath acetone or not was validated in the experiment by measuring the breath gas stored in a bag several times within a 24-h period and no noticeable difference was observed in terms of the change in the measured absorbance of acetone.

The first patient selected for this test was a 51-year-old female T1D patient, who had diabetic onset at 8-year-old. This subject weighed 175 lb and was wearing an insulin pump. Her latest A1C was 6.5. Fig. 5 shows the variations of her breath acetone and BG levels. As is evident in the figure, the highest BG level of 209 mg/dL occurred during the early morning and receded to the lowest point at the level of 68 mg/dL in the late afternoon before dinner time. The breath acetone concentrations ranging from 2.5 to 6.2 ppmv show a similar and correlated pattern except for a large difference between 8:00–10:00 A.M. when the BG was high (159 mg/dL), yet the breath acetone was low (2.2 ppmv). The record shows that the volunteer drank one can (12 oz) of Diet Dr. Pepper at 8:10 A.M. To determine if this BG peak was due to the drink, the change of BG levels after drinking one can (12 oz) of classical coke was observed and monitored. The test was conducted with another female T1D subject in a separated experiment. In the experiment, 15 min after drinking one can (12 oz) of Coca-Cola Classic, her BG levels were measured and the results showed a progressive increase within the next 0.5–1 h. However, no increase of breath acetone was observed during the same period. In Fig. 5, the second BG peak of 170 mg/dL came with the acetone peak of 4.8 ppmv at 8:00 P.M., right after dinner time when 65 grams of carbohydrates were consumed.

The second subject selected for the 24-h monitoring was a 29-year-old female who was born with diabetes. She weighed 110 lb and her latest A1C was 6.9. Her BG variation showed a different peak-valley pattern from the first subject while the correlation between the breath acetone and the BG level were strong, in general, as shown in Fig. 6. The record did not show that a normal breakfast was eaten; instead, a cup of coffee was

TABLE II
BREATH ACETONE IN T2D OUTPATIENTS WHO WERE TESTED IN THE CLINIC

Subject No.	Gender	Age	BG (mg/dL)	Weight (lb)	A1C (%)	Yrs in T2D	ppmv
1	M	34	103	305	n/a	n/a	5.99
2	F	40	121	300	n/a	6	0.49
3	F	48	n/a	244	8.3	25	0.49
4	F	51	101	228	n/a	4	0
5	M	51	n/a	239	n/a	12	0.74
6	M	58	151	226	7.1	32	2.19
7	F	59	128	174	n/a	13	0.75
8	n/a	61	101	265	n/a	15	3.76
9	F	71	246	320	11.1	5	0.27
10	F	72	137	223	n/a	7	5.8

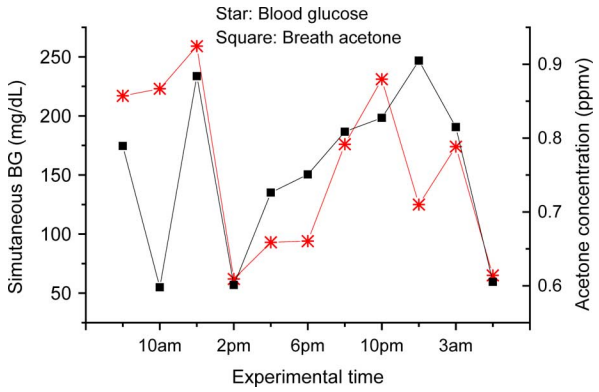

Fig. 6. Observed variations of breath acetone (ppmv) and the simultaneous blood glucose level in a 29-year-old, birth-onset, female T1D subject during a 24-h monitoring.

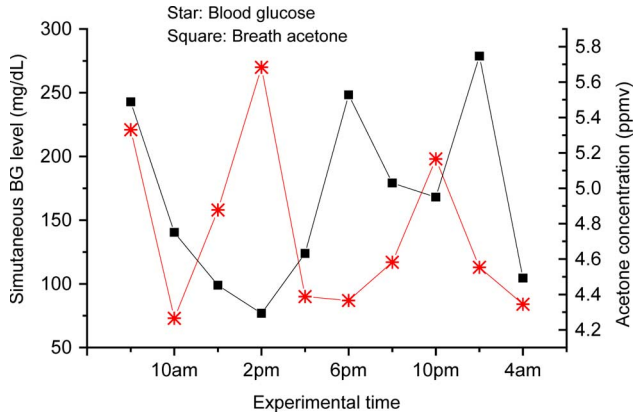

Fig. 7. Observed variations of breath acetone (ppmv) and the simultaneous blood glucose level in a 15-year-old, birth-onset, female T1D subject during a 24-h monitoring.

consumed at 9:00 A.M. The first BG peak of 260 mg/dL shown in the graph came with intake of the lunch at 11:00 A.M. The second BG peak of 226 mg/dL was after dinner at 7:00 P.M., but no food details were provided. It will be interesting to investigate how the time variation of breath acetone levels is affected by different meal time-tables. Although two studies reported previously on a decrease in breath acetone after food intake [19], [38], no consistent trend was observed in this study.

The third subject was a 15-year-old female who was born with T1D. Both the acetone concentration and the BG level show a cyclic pattern within the ranges of 4.3–5.8 ppmv and 74–269 mg/dL, respectively; however, there is a more than 4-h delay of acetone peaks to the BG peaks, as shown in Fig. 7. Whether or not a high-resolution time correlation between breath acetone and the BG exists only in some T1D patients or if the correlation depends on each individual metabolic mechanism needs further study. These preliminary observations showed a cyclic pattern of the variation of breath acetone during a 24-h period in all of the three cases. Tassopoulos *et al.* [9] reported a cyclic pattern of breath acetone in the overnight fasting diabetic patients. The time resolution of the data collections in that study was in days and their observations showed one peak each day and a strongly correlated variation pattern between the breath acetone and the BG level. For instance, in that study, both breath acetone and the concurrent BG were high in the morning and low in the late afternoon during the seven-day observation period.

Although the T1D subjects' weights and years of diabetes were also recorded, no obvious correlations were observed between each of these parameters and the breath acetone.

C. Acetone in Expired Air of T2D Outpatients

10 T2D outpatients were tested in the clinic in the fourth day of the clinical testing. The data is listed in Table II. Of the 10 T2D outpatients whose BG levels were in the range of 101–246 mg per 100 mL, some of them had high acetone breath and some of them had normal or even lower acetone breath than healthy people. One 72-year-old subject had a BG level of 137 mg/dL. His acetone concentration was 5.8 ppmv.

The mean acetone concentration of the rest of the 10 T2D subjects was 2.05 ppmv. From the BG levels, all of the T2D outpatients could not be considered as having severe diabetes or be in serious diabetic complications, e.g., ketoacidosis. However, 60% of the tested T2D outpatients had elevated acetone breath. This observation is contrary to a prevalent misconception: T2D patients do not have abnormal acetone breath unless they have diabetic complications or severe diabetes. In a recent study on breath acetone in 15 T2D subjects using a GC-MS method, abnormal acetone concentrations were observed in all of the 15

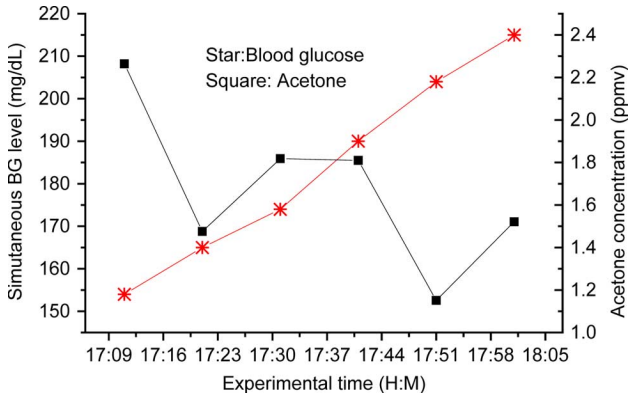

Fig. 8. 1-h monitoring on breath acetone and simultaneous blood glucose level on one female T1D subject, who temporarily stopped using the insulin pump for the 1-h testing period.

T2D subjects [15]. A common conclusion that can be drawn from these two independent studies is that at least some of T2D diabetes have abnormal acetone breath.

D. Investigation Into the Time-Delay Between Breath Acetone and Simultaneous BG

One 47-year-old female juvenile-onset T1D subject was selected for testing on her breath acetone when the insulin pump was temporarily stopped. Before the test, the volunteer had consulted with her doctor to determine the maximum feasible time she was allowed to go without using the insulin pump and which would not place her health at high risk. For this subject, a 1-h test without using the insulin pump was allowed. The test started at 5:00 P.M., and breath tests were performed approximately every 10 min. The concurrent BG levels were also measured using a standard glucose meter. The results are shown in Fig. 8. During this 1-h period, the breath acetone ranged from 1.1 to 2.3 ppmv. Interestingly, the BG level linearly increased from 154 mg/dL to 214 mg/dL after the insulin pump was turned off, but the breath acetone levels did not follow the same trend of the concurrent BG levels during this 1-h period. This testing result provides a piece of suggestive information that a delay-time between breath acetone and BG may exist under high temporal resolution. Limited by the tolerance threshold of the body under no-insulin treatment, experiments of longer duration on this subject could be performed. In order to ensure the least action of interference or potentially enhanced risk due to prolonged insulin-free duration, no repeat experiment was attempted. Similar experiments to explore the high-resolution temporal correlation are better to be conducted with T1D inpatients in a clinic in the future.

IV. Summary and Concluding Remarks

In this study, we utilized a prototype cavity ringdown breath acetone analyzer for clinical testing with diabetic patients. We explored relations between breath acetone and diabetic monitoring parameters, including BG level, A1C value, and several bioinformatic parameters, such as the type of diabetes, onset time, gender, age, weight, etc. The breath tests were conducted in a laboratory as well as a clinic. 34 outpatients (24 T1D and 10 T2D) were tested during the four-day period in the clinic. 10 T1D subjects and 15 nondiabetic volunteers were tested in the laboratory. Three juvenile-onset T1D subjects were selected for monitoring the variations of breath acetone versus the simultaneous BG level at 2–4 hour time intervals during a 24-h period. This is the first study of breath acetone in clearly classified (T1D, T2D, onset times, etc.) diabetic subjects without a load of glucose or an overnight fast.

The research findings are summarized below. 1) The mean breath acetone concentration in expired air of the T1D subjects studied is 2.19 ppmv. 2) Among the T1D subjects investigated, the mean breath acetone concentration in juvenile-onset diabetes is significantly higher than that from adult-onset diabetes. 3) When all the T1D subjects are grouped by different blood glucose levels, 40–100, 101–150, 151–200, and 201–419 mg/dL, a linear correlation between the mean group acetone concentration and the mean group BG level is observed. 4) Among the T1D subjects studied, when their A1C's are grouped by $<$7, 7–9.9, and 10–13, a linear correlation between the mean group A1C and the mean group acetone concentration is found. 5) Simultaneous monitoring of BG and breath acetone on three juvenile-onset T1D subjects over a 24-h period shows a weak correlation on the variations of the breath acetone and the BG levels. 6) Of the ten T2D subjects tested, six patients have elevated breath acetone and the mean breath concentration of the 10 T2D subjects is 2.05 ppmv. 7) The observation of the abnormal acetone concentrations in 4 T1D subjects, whose BG levels are well controlled (lower than 100 mg/dL), indicates that mild ketosis is undiagnosed by measuring BG. 8) A 1-h monitoring of the breath acetone and the simultaneous BG in one T1D subject, who temporarily stops using the insulin pump for the test, suggests that a time-delay between the breath acetone and the simultaneous BG level may exit under high temporal resolution.

It should be emphasized that this work is a preliminary clinical study and reports the first attempt to explore the correlations of breath acetone with BG, A1C, etc., through clinical testing on real diabetic patients using a ringdown breath acetone analyzer. Many related questions remain to be answered in future work. For instance, some T2D subjects investigated in this work have elevated breath acetone; is there any correlation between breath acetone with BG in T2D patients? In this study, we observed time variations of breath acetone and time delays between breath acetone and BG during a 24-h period, what factors are physiologically response for the temporal behaviors of breath acetone? Except for breath acetone, other species in human breath may be also biomarkers of diabetes. For example, a very recent study reports that exhaled methyl nitrate (CH_3ONO_2) is a biomarker of hyperglycemia in T1D [39]. Additionally, it is known that acetone with an elevated concentration may be also present in exhaled air of the persons after alcohol consumption or after/during intensive exercise [12], [29]. People who are on a ketogenic diet for a treatment to intractable epilepsy disease also have elevated breath acetone [11]. Finally, in this paper, the breath acetone was studied as a monitoring means for the people who have been diagnosed to be diabetic. How breath acetone can be used for diabetes diagnostics (measuring acetone concentrations to determine whether a subject is diabetic or not) is a challenging question. All of these are interesting topics of future study.

It should be also noted that this work is a study of the breath biomarker acetone towards noninvasive diabetic monitoring and diagnostics, instead of directly measuring blood glucose levels using the CRDS technique. During the last decade, other optical methods/techniques, such as Raman spectroscopy, optical coherence tomography, laser induced fluorescence, and reflectance spectroscopy, have been extensively studied and employed to directly measure/sense blood glucose [40]–[46]. Discussion of those techniques and their applications for diabetic monitoring and diagnostics is beyond the scope of this work.

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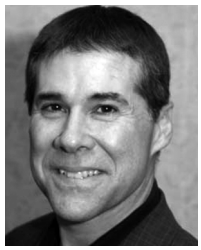

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