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Determination of Breath VOC Levels for Glucose Metabolism: A Study Using the GERSTEL TD 3.5+ Thermal Desorption System

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Keywords

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Abstract

Breath sampling is a technique used to aid in disease diagnosis and monitoring. Specific compounds found in exhaled breath are attributed to abnormal functional pathways due to various diseases. For instance, acetone and isoprene are indicators of poor glucose metabolism in diabetic patients. This application note investigates the use of GERSTEL TD 3.5+ Tenax TA® thermal desorption tubes for the non-invasive collection of volatile organic compounds (VOCs) from breath to determine biomarkers for glucose metabolism. Distinct changes were seen in acetone and isoprene levels corresponding to blood glucose spikes after a participant ate meals throughout the day.

Introduction

Sampling VOCs in the breath of diseased individuals is an increasingly popular diagnostic technique as it is simple, non-invasive, and unlike urine or blood, samples are available in unlimited supply. Doctors and researchers can use the data collected from an individual's breath to identify important biomarkers produced during metabolic processes that can ultimately aid in diagnosing and treating certain diseases. Studies have linked exhaled VOCs as biomarkers for diseases such as asthma, lung cancer, and renal failure [1]. In this study, diabetes, and more specifically, glucose metabolism processes, will be investigated.

The glucose molecule serves as a central component for energy whereby the body breaks it down using insulin. Insulin and glucose work in tandem to maintain healthy glucose levels, but a lack of insulin production and/or insulin resistance in type I and type II diabetes patients, respectively, prohibits this glucose metabolism process from occurring normally [2]. As a result, the body uses fat for energy, which produces excess ketones. Figure 1 shows the glucose metabolism process for healthy individuals versus what occurs in diabetic individuals without insulin. Exhaled VOCs in diabetic patients provide insight into glucose metabolism. Notably, compounds like acetone, isoprene, and dimethyl sulfide have proven relevance to metabolic imbalance and altered biochemical pathways [3], and acetone collected via breath showed a linear relationship with blood glucose and hemoglobin A1c [3,4].

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GERSTEL AppNote 253

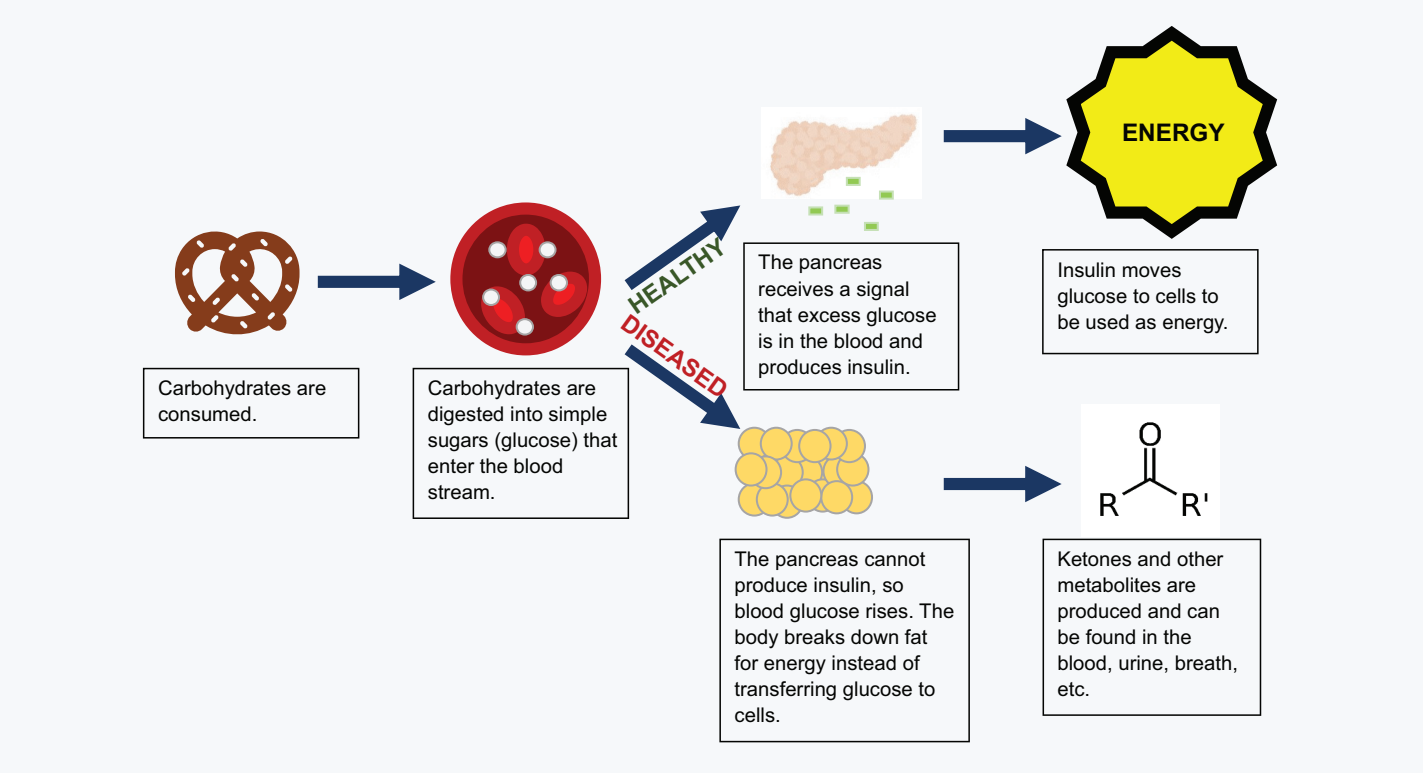

Figure 1: Summary of glucose metabolism process in healthy and diseased individuals.

For this study, exhaled VOCs were monitored over 6.5 hours, during which a diabetic patient consumed two meals. The patient was asked to provide an initial breath sample and two additional samples at 3-hour intervals afterward. GERSTEL's TD 3.5+ Tenax TA® thermal desorption tubes serve as an effective tool for collecting breath samples as they ensure optimal sorption of a wide range of VOCs and have a low affinity for water. Sample collection can be performed in the field where tubes can be stored and shipped to the lab for subsequent automated analysis using the GERSTEL TD 3.5+ thermal desorption system.

Experimental

Instrumentation

GERSTEL TD Core System on Agilent 8890 GC and 5977B Inert Plus MSD.

Analysis Conditions TD Core System

CIS

Split (10:1)
Vent flow: 50 mL/min until 0 min
Purge flow to split vent: 10 mL/min at 0.01 min
-120 °C; 12 °C/s; 275 °C (3 min)

TD 3.5+

Solvent Vent/Dry Purge, Vent time 3.33 min
40 °C (0 min); 60 °C/min; 60 °C (3 min)
400 °C/min; 280 °C (5 min)
Tenax TA®

Analysis Conditions Agilent 8890 GC

Column	30 m HP-5MS UI
	d_i= 0.25 mm d_f = 0.25 µm
Pneumatics	He; P_i = 7.0699
	Constant flow = 1 mL/min
Oven	40 °C (3 min); 10 °C/min; 300 °C (5 min)

Analysis Conditions Agilent 5977 Inert Plus MSD

Full scan	40-350 amu

Sample Preparation

The participant was required to fast from after dinner the night before until 10:00 AM the following morning, which was approximately 15 hours. At 10:00 AM, the participant consumed a piece of toast with peanut butter and another with hummus. At 1:30 PM, the participant consumed a gluten-free snack bar and a coffee with creamer. Breath samples were collected onto Tenax® TA ther-

GERSTEL AppNote 253

mal desorption tubes at their place of work. A 100-150 mL breath sample was collected from the participant at 9:00 AM, 12:00 PM, and 3:30 PM. The three-hour time intervals were selected to ensure that at least two meals were accounted for throughout the day. The participant's blood glucose was measured when the first and last breath samples were collected.

Sample introduction

Samples were desorbed in solvent vent/dry purge mode with a 50 mL/min helium flow at 280 °C for 5 minutes. The solvent vent/dry purge mode was used to purge any water that may have collected on the tubes during breath sampling before desorbing the analytes of interest. Analytes were cold trapped in the CIS 4 inlet at -120 °C on a glass bead-filled liner. When desorption was complete, analytes were transferred to the column in split mode (10:1) by rapidly heating the inlet to 275 °C.

Results and Discussion

Figure 2 shows the stacked view of total ion chromatograms of each breath sampling time point. At 9:00 AM, the participant's breath was monitored for pre-existing analytes of interest prior to eating and, subsequently, the glucose metabolism process. Acetone was identified in addition to salicylates, fatty acids, and squalene which are all present due to contact with the participant. Acetone levels were lowest at 9:00 AM because the participant fasted the night before. The participant's blood glucose was also at its lowest, at 6.8 mmol/L. Squalene is a common oily substance found on human skin to keep it hydrated, and fatty acids and salicylates can be found in various food and topical lotion products. These non-metabolites were likely introduced due to topical creams and skin oils that encountered the sorbent tube during sampling. For the purposes of this study, these compounds were not considered major contributors to glucose metabolism biomarkers, as they were detected before meal consumption and are not labeled in the chromatograms of Figure 2.

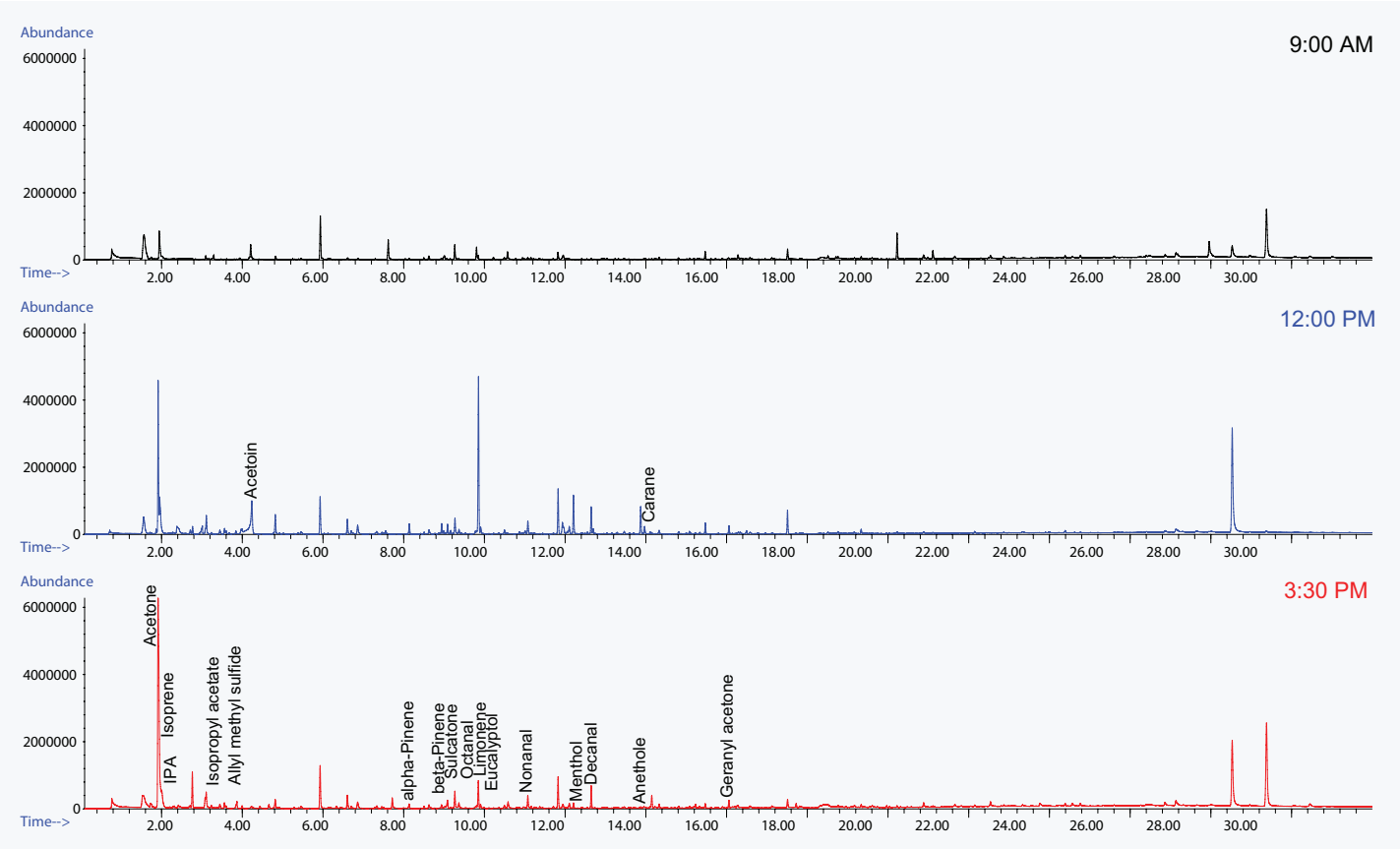

Figure 2: Stacked view of breath samples collected at 9:00 am (top), 12:00 pm (middle), and 3:30 pm (bottom).

GERSTEL AppNote 253

After eating the two meals, the remaining chromatograms in Figure 2 show a notable increase in signal intensity for allyl methyl sulfide and several aldehydes, ketones, and terpenes. These compounds are often classified as food components, but they could also increase in the breath due to metabolic processes.

At 12:00 PM, the participant's breath was monitored after consuming two pieces of toast. At this time, the acetone levels increased, and several new compounds were identified. These compounds included isoprene, isopropyl alcohol, isopropyl acetate, allyl methyl sulfide, and the aldehydes, ketones, and terpenes mentioned previously. Although there is literature indicating that acetone and isoprene play a role in glucose metabolism [3-4], there are limited studies that explain the role of the other metabolites. All of these may have been components of the food that lingered on the participant's breath after the first meal. Acetoin and carane were also newly identified in this breath sample, but not at other times.

At 3:30 PM, the participant's breath was monitored after consuming coffee and a snack bar. Acetone and isoprene levels were highest compared to the other sampling times. The participant's blood glucose was also higher at 8.0 mmol/L, indicating that acetone and isoprene share a direct relationship with blood glucose levels. All other compounds identified in the sample at 3:30 PM were the same as those found in the sample at 12:00 PM. All other compounds identified from the sample taken at 3:30 PM were like those found from the sample taken at 12:00 PM.

Table 1 shows the relative peak areas of the compounds collected in the participant's breath at all three time points. The peak area counts were normalized to the breath sample obtained at 3:30 PM. Acetoin and carane were normalized to the breath sample obtained at 12:00 PM because these two compounds were not detected at any other time point. The acetone and isoprene levels increased throughout the day because of the glucose metabolism process. In diabetic patients, insulin resistance and/or deficiency causes the body to take up glucose ineffectively. Therefore, the body resorts to breaking down fats as an alternative source of energy. During this process, ketones, like acetone, are produced and noted in the breath, and isoprene stored in the fat tissue is released [5].

Table 1: Percent relative area counts of compounds detected at the three breath sampling time points.

No.	Compound	9:00 AM	12:00 PM	3:30 PM
1	Acetone m/z 43	15.1%	51.5%	100.0%
2	Isoprene m/z 67	0.5%	29.1%	100.0%
3	Isopropyl alcohol m/z 45	0.0%	374.5%	100.0%
4	Isopropyl acetate m/z 43	0.0%	131.6%	100.0%
5	Allyl methyl sulfide m/z 88	0.0%	98.8%	100.0%
6	Acetoin m/z 45	0.0%	100.0%	0.0%
7	alpha-Pinene m/z 93	26.8%	265.7%	100.0%
8	beta-Pinene m/z 93	29.5%	350.9%	100.0%
9	Sulcatone m/z 43	0.0%	140.1%	100.0%
10	Octanal m/z 43	10.6%	93.4%	100.0%
11	Limonene m/z 68	12.8%	581.2%	100.0%
12	Eucalyptol m/z 43	0.0%	166.2%	100.0%
13	Nonanal m/z 57	11.2%	108.1%	100.0%
14	Menthol m/z 71	0.0%	752.9%	100.0%
15	Decanal m/z 57	6.1%	119.1%	100.0%
16	Anethole m/z 148	0.0%	2406.7%	100.0%
17	Carane m/z 95	0.0%	100.0%	0.0%
18	Geranyl acetone m/z 43	0.0%	120.1%	100.0%

Since the use of breath sampling for diabetes diagnostics is a relatively new field of research, further investigation is necessary for the additional compounds detected in the breath. The majority of the additional 16 compounds increased following meals. However, these compounds did not follow the same linear trend as acetone and isoprene. The area counts for compound numbers 3, 6, 7, 8, 11, 14, and 16 increased after the first meal but decreased significantly after the second meal. Compound numbers 4, 5, 9, 10, 12, 13, 15, and 17-18 increased after the first meal and remained relatively unchanged after the second meal. Although these compounds may be related to persisting food VOCs after meal consumption, they should be explored further to determine if each serves a specific role in diabetic biochemical metabolic pathways.

GERSTEL AppNote 253

Conclusion

Utilizing the GERSTEL TD 3.5+ thermal desorption system along with Tenax TA® sorbent-filled tubes offers a straightforward and non-intrusive breath collection technique for exploring glucose metabolism biomarkers in individuals with diabetes. This approach holds potential for broader applications where breath VOCs play a significant role as disease markers. These findings revealed a notable correlation between acetone, isoprene, and blood glucose levels, unveiling valuable insights into the processes of glucose metabolism. An additional 16 compounds of interest were effectively captured and identified using this breath analysis method. Although there is less information on these compounds, this technique can be used for supplemental biomarker exploration. Additionally, this system's ability to support field sample collection further establishes its suitability for similar investigative purposes with other major diseases.

References

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