Published in final edited form as:
Curr Opin Clin Nutr Metab Care. 2013 July ; 16(4): 466–472. doi:10.1097/MCO.0b013e328361f91f.

Monitoring states of altered carbohydrate metabolism via breath analysis: are times ripe from transition from potential to reality?

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Abstract

Purpose of review—To introduce the potential of breath analysis as a potential diagnostic or monitoring tool in diabetes. .

Recent findings—Blood testing for plasma glucose and other metabolic variables is the base for the diagnosis and management of diabetes, whose two main types (type 1 and type 2, T1DM, T2DM) are projected to affect 450 million by 2030. As blood testing is often uncomfortable, painful, costly, and in some situations unreliable, the quest for alternative, non invasive methods has been ongoing for decades. Breath analysis has emerged as an ideal alternative as sample collection is easy, painless, flexible, non-invasive, practical, and inexpensive. No single exhaled gas can reflect systemic glucose concentrations. Multiple gases, however, have been linked to various aspects of glucose metabolism, and integrated analysis of their simultaneous profiles during prolonged glycemic fluctuations has yielded accurate predictions of plasma values, building expectation that clinically usable breath-based glucometer may be developed within a few years.

Summary—While prototypes of hand-held breath testing glucometers may still be several years away, current research shows the imminent promise of this methodology, and the widening support for its development.

Keywords

Breath Testing; Diabetes; Exhaled gases

INTRODUCTION

The Potential of Using Breath Analysis as a Diagnostic or Monitoring Tool in Health and Medicine

A large portion of diagnostic and monitoring procedures in clinical practice involve blood testing or other complex and costly methodologies (MRI, PET scanning, etc). The quest for alternative, non-invasive, practical, and ideally low-cost alternatives is the focus of constant efforts by researchers and manufacturers of medical devices. In this context, tests based on

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the analysis of exhaled gases represent ideal candidates, due to the ease and flexibility of breath collection. Once collected, exhaled gases can be quantified in a variety of ways, each entailing specific advantages and technical challenges. A concise synopsis of available techniques is provided in Table I, while more comprehensive evaluations of these methodologies can be found in several recent excellent reviews [1–4].

Unfortunately, few clinically approved breath-based tests currently exist. A large number of studies, however, in recent years have demonstrated the potential applicability of this methodology to a variety of clinical conditions. Several of such studies published in the last 12 months are summarized in Table II.

Diabetes and Blood Glucose Testing

Type 1 and type 2 diabetes (T1DM, T2DM) are an ever-growing problem, with a combined affected population potentially reaching, by some estimates, 450 million in the next twenty years [14]. An increased risk of developing cardiovascular complications is the main cause for long-term morbidity and mortality in diabetic patients. In these subjects, a subclinical state of inflammation is constantly present, whether they are aware of it or not; this is now believed to the main pathogenic mechanism leading to the onset and progression of cardiovascular complications. Dysregulated inflammation (activation of circulating leukocytes and systemic release of cytokines and chemokines) causes systematic, widespread damage to the vascular wall via build-up of atherosclerotic plaques, which gradually reduce and eventually may completely the vascular lumen in virtually all tissues of the body.

The main stimulus causing this exacerbation of inflammation in diabetes is the hallmark of the disease, i.e. hyperglycemia (excess amounts of glucose in the bloodstream); how high, how often and how long hyperglycemia is experienced, has in fact been clearly shown to correlate with the occurrence and severity of diabetic cardiovascular complications [18]. Prevention of hyperglycemic episodes is therefore the very base of diabetes management. The key piece of information required to achieve this goal is the patient's blood glucose level ideally measured at least at several critical time points during the day. Devices able to accurately monitor plasma glucose have therefore become integral parts of the life of diabetic patients.

Why Use Breath Analysis for Diabetes?

Currently, glucose meters requiring a finger prick to obtain a sample of the patient's blood are the main method used to monitor glucose levels. Although this methodology is relatively efficient, it is rather expensive (a new test-strip must be used each time, costing about 1 US dollar), and the constant need of piercing the skin to reach the capillary bed required an average of three to five times daily by most patients, is bothersome, painful, and may cause skin hardening and difficulty in obtaining good samples. In many cases, this results in poor patient compliance [16]. These concepts are well exemplified in the word of a patient who wrote to our laboratory upon learning that we were trying to develop alternative, non-invasive and painless techniques to measure blood sugar:

> "…. the pain of taking blood from my fingers has made it to where I just don't do it hardly anymore…"

This deterrent of patients from monitoring their blood glucose levels, therefore, almost inevitably results in worse glycemic control and higher likelihood of developing serious cardiovascular complications.

Any methodology allowing blood glucose measurement in a painless, non invasive way is therefore likely to be very well received by diabetic patients, and significantly improve compliance and glycemic control. Among several possible techniques being developed for this purpose, the use of exhaled volatile organic compounds appears especially promising. The noninvasive nature of this method and flexibility of its application may provide a number of key advantages. In addition to the obvious possible improvements in diabetic patients' management of the disease and quality of life (reduced pain and calluses from constant finger pricking), the application of breath analysis can be useful in a series of additional settings, such as real-time glucose monitoring during surgical procedures or other situations when subjects are unconscious (samples can be collected taken from patients without their collaboration) [17]. Further, the use of breath analysis can be beneficial in large-scale screening programs, where obtaining blood samples may be limited by a number of factors, such as difficult vein access, young age of screened populations, or simple refusal to undergo a needle stick.

Despite these many possible advantages, and considerable interest in the field, a clinically applicable breath-based glucose test has not yet been fully developed. With improved abilities to measure breath compounds present at extremely low concentrations with increasing accuracy, the creation and use of such a breath testing system however appears now to rapidly approach reality.

EXHALED COMPOUNDS IN HEALTHY AND DYSMETABOLIC CONDITIONS

Origin of Gases Found in Human Breath Mixture

The composition of human breath is complex. Exhaled gas concentrations may be the result of several diverse processes, such as re-exhalation of gases inhaled from ambient air (which may be inert, or partly metabolized endogenously); exhalation of gases produced by the lungs, or produced in peripheral tissues and transported to the lungs through the blood stream; and any combination of the above. A synopsis of these mechanisms is provided in Table III.

Functional aspects of exhaled gases

While at least some gases may be present in exhaled mixtures as mere "by-standers", i.e. with no apparent biological function (and these may either by be inhaled from ambient air, or be the by-product of endogenous biochemical reactions), others may exert a range of effects of cells and tissues. Some of these mechanisms are listed in Table IV.

Issues Associated With Breath-Based Testing in Diabetes

While the noninvasive component of breath analysis is attractive to people with diabetes, some intrinsic features of the condition may complicate the accuracy of breath-based-testing. The effects of diabetes are far-reaching into the body, and any alteration in physiological processes that may affect exhaled breath composition (and thereby affect the accuracy of breath testing) should be identified and taken into account for proper adjustments.

Lung function can be directly or indirectly affected by diabetes. It has been shown that T1DM and T2DM patients are likely to develop restrictive lung disease and that the occurrence of glycation in diabetics can decrease the lung's ability to efficiently transfer or diffuse gases [38]. Advanced glycation end products bind to RAGE receptors in greater concentrations in diabetics as opposed to healthy individuals. This increased binding has pro-inflammatory effects, increases pro-sclerotic and pro-fibrotic growth factors, and decreases bioavailability of nitric oxide leading to dysfunction in pulmonary arteries [23]. In addition, high levels of myeloperoxidase (MPO) and α-defensins, derived from circulating PMN's, have been reported in diabetic patients [23;12]. MPO exerts strong oxidative effects, and its excessive levels have been associated with chronic arterial disease; α-defensins also facilitate the development of atherosclerosis, and disrupt the barrier between capillaries and epithelial tissue in the lungs, thereby increasing the chances of lung injury [39;40].

In diabetic patients, several key endogenous metabolic processes may also be altered in a way that induces changes in exhaled gas profiles. For instance, during hyperglycemia, specific VOCs may increase in breath due to direct fermentation (or other chemical degradation) of glucose [19]. Other changes in exhaled gas profiles may be induced by hyperglycemia indirectly, such as a drop in exhaled ketones due to the anti-lipolytic effect of insulin, which increases in response to rising blood sugar [43]. Another factor affecting breath composition is diet, which has been show to differ considerably across groups of patients. T1DM patients, for instance, tend to reduce carbohydrate intake, in favor of a balance mix of nutrients rich in both protein and lipid, while the proportion of lipids appears much greater in T2DM. Fat ingestion is associated with greater exhaled levels of methyl nitrate and other ketones, as well as other gases [41].

Altered Gas Profiles in Diabetes

The initial approach to the use of breath analysis in the field of diabetes has traditionally been the identification of individual exhaled compounds whose concentration was either altered by the presence of diabetes per se, or associated with specific metabolic aspects or co-morbidities of diabetes. Glucose itself, for instance, in aerosolized form, was found to be increased in exhaled breath condensates (EBC) during induced hyperglycemia [42]. Exhaled levels, however, correlated rather poorly with the magnitude of hyperglycemia. Acetone, on the other hand, has been by far the compound receiving the greatest attention. Massively elevated acetone exhalation is typical of diabetic ketoacidosis, and smaller increases have been reported in diabetic populations; in fact, a linear relationship between average breath acetone levels and blood glucose concentration was reported in T1DM [43;44], but not in T2DM subjects [45;46]. Measurement of acetone alone, however, has proven insufficient to

estimate blood glucose levels, due to considerable variability across subjects, even in the presence of similar glucose levels.

A number of other exhaled compounds are linked with various aspects of diabetes. Methyl nitrate, for instance, was highly correlated with the magnitude of hyperglycemia in a cohort of T1DM children [47]. Isoprene was reported to reflect the degree of ongoing cholesterol synthesis [48]. Carbon monoxide, propane, ethane and pentane, appear to reflect the systemic level of oxidative stress. Propionic and butanoic acids' levels were greater in EBC after the ingestions of sucrose [49]. Aromatic compounds like ethylbenzene and toluene are actively metabolized by the liver, in a fashion dependent on hepatic glucose load [19]. Carbon dioxide is a direct oxidative by-product of energy substrates, including glucose. Finally, ethanol and methanol appear to derive from enteric bacterial fermentation of circulating glucose [50;51].

While all of the above studies revealed interesting correlations, none allowed the accurate prediction through the measurement of a specific exhaled gas of any diabetes-related variable, and in particular of plasma glucose. It is therefore apparent that if any such predictions were to be obtained, they would rather entail the mathematical integration of the simultaneous profiles of multiple gases, ideally measured during broad fluctuations of the variable of interest. Several studies have indeed been performed attempting to estimate plasma glucose via multilinear regression analysis of exhaled gas profiles obtained during an oral glucose tolerance test (10 healthy adults, model including exhaled acetone and ethanol) [50]; an I.V. glucose tolerance test (10 healthy subjects, model including ethanol, acetone, methyl nitrate, and ethylbenzene) [19]; and during a 4 hour hyperglycemic clamp study (16 healthy and 13 T1DM adults, same 4-VOC model) [52]. In these studies, a high level of concordance was reported between measured and breath-determined plasma glucose levels (in the latter two studies, the mean correlation coefficients were 0.91 and 0.86, respectively). In addition to plasma glucose, parallel studies with similar experimental design also allowed to estimate with considerable accuracy plasma levels of insulin [43;53], free-fatty acids, and triglycerides [54;55] both in healthy, obese, and T2DM subjects.

CONCLUSION

As the need for alternate modalities of diabetes management grows, large resources have been invested worldwide in developing non-invasive devices. Of these, breath analysis methods appear to be on the verge of major breakthroughs that can hopefully accelerate the transition of past theoretical concepts into practical clinical devices that will allow diagnosis and, more importantly, daily management of diabetes. A number of analytical challenges that impeded accurate measurement of very low gas concentrations have been now virtually eliminated, and several controlled, complex studies in children and adults with and without diabetes have demonstrated the feasibility of accurate breath-based tests for not only glucose, but other relevant variables such as insulin, FFAs, and triglycerides. The transition towards development of actual prototypes of testing devices that can be used on large scale clinical trials, however, still awaits the resolution of a number of issues. It appears for example, that the whole range of possible of glycemic levels relevant to diabetes, especially concerning hypoglycemia (whose accurate detection is crucial in type 1 diabetes) has not yet

been thoroughly explored. Further, current studies reporting breath-based predictions of blood glucose, have not yet documented the stability of their measurements over time, or the reproducibility of data obtained in highly controlled experimental conditions translated to real-life settings. Additional confounding variables that need to be addressed also include the effects of prior food ingestion, exposure to different air mixtures, prior glycemic control, duration of diabetes, concomitant presence of tissue complications. The logical next steps in the field therefore appear to address these complex issues, while developing viable partnership between clinical researchers and private sector entities, leading to the development, testing, and marketing of testing devices. Finally, it should be noted that, for the purposes of this review, exhaled gas profiles have so far been considered as simple "mathematical components" of predictive equations leading to the estimations of metabolic variables relevant to diabetes. Considerable intrinsic values, however, is definitely also retained by the study of the biochemical significance of the changes in exhaled gases per se, especially for gases present at very low concentrations or previously not associated with altered metabolism. These findings can lead to novel insights in the complex field of energy substrate metabolism.

Acknowledgments

Thank you to M. Martinez for editing this manuscript. The project described was supported by the National Center for Research Resources, the National Center for Advancing Translational Sciences, and the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, through Grants UL1 TR000153 and 5K24DK085223. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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- Breath analysis is an appealing, painless and non invasive alternative to current blood testing methodologies used to diagnose and monitor diabetes, measuring plasma glucose and other related variables.
- Technical and analytical difficulties that in the past have slowed the development of the technique have now largely been solved, raising the hope that practically usable testing devices will be developed within a few years.
- While no single exhaled compound has proved sufficient to predict plasma glucose or any other related variable, numerous compounds have been shown to be consistently affected by changes in glucose metabolism.
- These compounds have various origins, being produced endogenously by peripheral cells or tissues, or being inhaled with ambient air and partly metabolized.
- Integrated analysis of the simultaneous profiles of multiple exhaled gases collected during prolonged metabolic fluctuations in healthy, obese and diabetic subjects, has allowed the accurate prediction of corresponding plasma variables in controlled experimental conditions.

Table I

Current Breath Analysis Technology

Type	Description
Classic tracer studies	Infusion of molecules tagged with either stable/radioactive isotopes followed by isotope quantification in exhaled breath to study β-oxidation and other metabolic pathways (5)
Gas chromatography and mass spectroscopy of VOCs	Improved to such extent that VOCs are now routinely identified and quantified accurately in the part-per trillion range Sample are pre-concentrated by - Sorbent Trap Absorption - Solid Phase Micro Extraction - Cryofocussation Analysis is then performed by gas chromatography coupled with flame ionization or flame photometric detectors, or by mass selective detectors (1;4)
Direct measurement sin EBC of compounds of interest (glucose, lipids)	Exhaled breath trapped in collection tube, cooled to aqueous form, and analyzed by various chromatographic techniques (6;7)
Frequent gas sampling or real-time analysis	- Atmospheric pressure ionization mass spectrometry + electrospray charging (used for on-line measurements of volatilized fatty acids (33) - Proton transfer reaction mass spectrometry (PTR-MS) for measurement of breath VOCs
	Used for on-line measurements of volatilized fatty acids (30) and proton transfer reaction mass spectrometry for measurement of breath VOC

Table II

Recent studies applying breath analysis to various diseases

Condition	Breath Analysis Method	Results
Alzheimer's (AD) and Parkinson's (PD) Disease	Analysis of alveolar breath using nano-material based sensors and use of gas chromatography and mass spectrometry	Sensors able to distinguish between healthy and AD subjects, healthy and PD subjects, AD and PD subjects with respective accuracies 85%, 78%, 84% (8)
Cancer		
Breast Cancer	Gas Chromatography, Mass Spectrometry used to identify VOCs in subject's breath	Identification of 5 VOCs as distinguishing markers between healthy subjects and subjects with breast cancer (9)
Lung Cancer	Solid Phase Micro Extraction-Gas Chromatography Mass Spectrometry (SPME-GCMS) used for exhaled breath analysis; Linear Discriminatant Analysis (LDA) used to create diagnosis models	Lung cancer subjects distinguishable from healthy subjects based on 23 VOCs and 3 diagnosis models. 96.47% sensitivity, 97.47% specificity. Distinction between early and late stage cancer not evident (10)
Colorectal Cancer	Thermal-desorber gas chromatography-mass spectroscopy used to analyze the subject's exhaled breath.	VOC pattern differed between subjects with colorectal cancer and healthy subjects; subjects with colorectal cancer could be identified with greater than 75% accuracy (11)
Non-alcoholic Steatohepatitis (NASH)	Gas Chromatography, Mass Spectrometry used to analyze VOCs in subject's breath	3 specific exhaled VOCs necessary to between subjects with NASH and without (12)
Pulmonary Nodules	Gas Chromatography, Mass Spectrometry, Solid Phase Micro Extraction and chemical nanoarray used to analyze VOCs in subject's breath	Breath analysis able to distinguish between benign and malignant pulmonary nodules (13)

Table III

Origin of Gases Found in Human Breath Mixture

Atmospheric Air	- VOCs present in ambient air are inhaled and then exhaled at varying concentrations; if inert, inhaled and exhaled concentrations are equal (18) - If VOCs are also produced endogenously, exhaled concentrations will exceed inhaled concentrations - Some VOCs undergo partial or total enzymatic metabolisms, resulting in lower exhaled than inhaled concentrations. In this situation, acute metabolic changes can modify the inhaled/exhaled ratio, linking exhaled levels to specific metabolic events (i.e. hyperglycemia suppressing enzymatic catabolism of ethylbenzene and xylenes, reducing the net uptake of these gases) (19)
Lungs	- The internal surfaces of the lungs may directly contribute to exhaled mixture of gases - Pulmonary surface liquid can become aerosolized into the breath, resulting in measurable concentrations of arachidonic acid metabolites (eicosanoids), isoprostanes, leukotrienes, proteins, and cytokines in exhaled breath condensate (20) - Unique combinations of VOCs are generated specifically in diseased states (i.e. the interaction of 22 VOCs in lung cancer) suggesting the direct transmission of these VOCs from the lungs to exhaled breath
Peripheral Tissues	- Peripheral human cells and tissues contribute to exhaled VOC through their release into the bloodstream and subsequent transfer into the airways (i.e. ethane, isoprene, pentane, and other hydrocarbons are proposed biomarkers for metabolic processes such as fat oxidation, cholesterol/LDL concentrations and oxidative stress levels) - The exact mechanisms of transferring systemically produced VOCs from tissues into the lungs are still unclear
Blood	- Blood components themselves can produce VOCs - Acetone, dimethyl sulfide, isoflurane, isoprene, pentane and may more gases are commonly found in whole blood; as many as 74 compounds have been simultaneously identified in blood and exhaled breath (21) - Polymorphonuclear leukocytes (PMNs) have been shown to produce acetaldehyide, hexanaldehyde and other gases (22;23); as these cells are often found sequestered in the lungs at high concentrations disease states, VOC transfer into exhaled breath is possible (24); altered VOC production by PMNs may be present in dysmetabolic states, including diabetes, becoming a potential marker of onset/progression of disease (25;26)
Micro-organisms	- If identified with exhaled gas mixtures, unique gas profiles of bacteria and other microorganisms can be used for diagnostic or monitoring purposes (i.e. radiolabeled urea detects H. pylori, a urease-positive bacterium; hydrogen cyanide detects pseudomonas aeruginosa (27); a group of VOCs were found to be secreted in vitro by mycobacterium tuberculosis) (28)

Table IV

Functional aspects of representative exhaled gase

Endogenous Origin	Known signal transmission in humans	Known signal transmission in other organisms (possible, but not proven in humans)
	- NO→vasodilation of endothelial cells, neuronal synaptic plasticity, and antibacterial defense (29) - CO→platelet aggregation and inhibition of smooth muscle proliferation (29) - Hydrogen Sulfide→affect Katp channels and vasodilation (30)	- Ethylene→increases regulation of defensins and antagonize glucose homeostasis in Arabidopsis plants
Exogenous Origin	**Known molecular mechanisms**	**Known effect but unclear molecular mechanisms**
	- Trichloroethylene→upregulate PPARα (31) - D-limonene→antitumorigenic activity (32) - Carbon-dioxide→ regulates respiratory function; regulates gene expression, attenuate pro-inflammatory gene expression and enhance anti-inflammatory IL-10 production (33)	- Ethyl nitrate→vasodilatory activity and suppressed methemoglobin formation (34) - Methyl iodide→increase serum cholesterol and decrease triglycerides (35;36) - Dichloromethane, ethylbenzene, and trichloroethylene→shown to make 1217 identifiable changes in rate gene expression with unidentifiable metabolically active exogenous VOCs (37)