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REVIEW ARTICLE

Noninvasive Monitoring of Glucose Levels: Is Exhaled Breath the Answer?

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Abstract

Monitoring of blood glucose levels is clinically important in the management of diseases affecting insulin secretion and resistance, most notably diabetes mellitus and cystic fibrosis. Typically, blood glucose monitoring is an invasive technique that may cause distress and discomfort, particularly in the pediatric population. Development of noninvasive methods of monitoring blood glucose is therefore indicated, particularly for use in children. Using respiratory fluids (the liquid present in the lumen of the airways and alveoli) to estimate blood glucose levels indirectly is one potential method. Glucose concentrations in respiratory fluids are typically low, maintained by the equilibrium between paracellular leakage of glucose from the lung interstitium and active cotransport of glucose by epithelial cells. Measurement of glucose in respiratory fluid by collection of exhaled breath condensate is therefore a potentially clinically useful method of estimating blood glucose levels if it can be shown that there is good agreement between these values. This article reviews the research in this area.

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Introduction

Monitoring of blood glucose levels is clinically important in the management of diabetes mellitus. However, blood glucose monitoring is typically an invasive technique that can cause pain and distress, particularly in the pediatric population. Development of noninvasive methods of monitoring glucose levels may therefore be particularly beneficial for children and is a growing area of research. Exhaled breath condensate (EBC) is one potential method of indirectly estimating blood glucose levels, however, research in this area is still at an early stage.

Diabetes Mellitus and Cystic Fibrosis

Diabetes mellitus (DM) is characterized by chronic hyperglycemia, with hypoglycemic episodes also a clinical concern. Frequent monitoring of blood glucose

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Abbreviations: (CF) cystic fibrosis, (CFRD) cystic fibrosis-related diabetes, (DM) diabetes mellitus, (EBC) exhaled breath condensate, (ISF) interstitial fluid, (PTR-MS) proton-transfer-reaction mass spectrometry, (SIFT-MS) selected-ion flow-tube mass spectrometry, (VOCs) volatile organic compounds

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levels is therefore clinically important. While type 1 DM accounts for >90% of all cases of diabetes in the pediatric population,[1] type 2 DM in children is a growing problem due to the increasing prevalence of childhood obesity.[2,3] Type 2 DM in adults is a significant cause of morbidity and mortality, with rapidly increasing rates worldwide leading to considerable economic and social burden.[4] Diabetes is also a significant complication of cystic fibrosis (CF). Many patients with CF subsequently develop cystic fibrosis-related diabetes (CFRD) with clinical impacts including decreased pulmonary function and higher mortality than those with CF but without diabetes.[5–7] Evidence of a decline in health before the clinical onset of CFRD suggests benefits of regular monitoring of blood glucose levels in CF patients.[8]

Blood Glucose Monitoring

Self-monitoring of blood glucose levels is recommended by the American Diabetes Association for patients with type 1 DM at least three times daily, and may also be helpful in achieving glycemic control for patients with type 2 DM.[6] Compliance, however, is often a major issue with self-monitoring, with a majority (61%) of type 1 DM patients not testing their blood glucose levels every day.[9] Self-monitoring of blood glucose levels is typically achieved using a portable glucose meter but glucose levels may also be monitored using a continuous glucose monitoring system, which provides frequent, real-time glucose measurements determined from interstitial fluid (ISF) glucose concentrations. The system is invasive as it requires the insertion of a sensor under the skin and several initial blood samples for calibration, with most systems also requiring later calibrations because of the time lag between changes in ISF and blood glucose concentrations.[10]

The development of noninvasive methods of monitoring blood glucose levels for patients has considerable clinical benefit, particularly in a pediatric setting, due to the painful and uncomfortable nature of blood sampling.[11] One questionnaire survey of children aged 3–18 years old found that they described venipuncture as extremely distressing, worse than they could imagine, and "horrible."[12] Although finger-prick tests are typically less painful than venipuncture due to a smaller needle and less blood being required, they still may be a cause of stress and discomfort for many children. Development of noninvasive methods is also relevant for adults; a Dutch study of adult patients with diabetes found that 10% of participants reported extreme fear of self-injecting or self-testing, which was associated with high levels of distress and poor general well-being.[13]

Alternative Techniques

Most methods of noninvasive monitoring of blood glucose levels use easily accessible sites such as the skin of the forearm as the measurement site; however, other sites including the eye and the mucosa of the mouth have been explored.[10,14] Although these noninvasive technologies have been demonstrated to be promising methods of estimating blood glucose levels, each method has limitations that restrict their usefulness and as such, few commercial systems are available. Many of these technologies monitor glucose levels in the ISF. Glucose diffuses readily down the concentration gradient from blood to ISF and as such, ISF glucose concentrations vary in relation to blood flow and rate of glucose uptake into cells. One issue with ISF glucose monitoring is the time lag between changes in blood glucose and ISF glucose concentrations. This varies between different sensor types and may range from 2 to 45 minutes.[15,16] Boyne and colleagues (2005)[17] compared blood glucose levels obtained by a subcutaneous ISF continuous glucose monitoring system (Medtronic MiniMed CGMS®, Sylmar, CA) with those obtained by venous blood sampling and found a mean time lag of 6.7 minutes between the two methods.

Infrared absorption spectroscopy uses measurements of relative light intensity after interaction with glucose molecules.[14,18] This technology has been used to measure blood glucose levels across the oral mucosa due to its high vascularity. Research using different wavelengths of light has been promising, however, the accuracy of the results obtained using this method is impeded by the interference of other molecules and scattering of light.[15] Transdermal sensors utilize reverse iontophoresis, where a current is applied between two electrodes on the skin, causing the flow of glucose from the anode to the cathode. This method has been demonstrated to cause erythema in some cases and has low sensitivity in detecting hyperglycemia.[15] A commercial monitor (GlucoWatch®, Cygnus, Redwood City, CA) was approved by the United States Food and Drug Administration (FDA), however, it was withdrawn in 2008 because of unsatisfactory performance. Other limitations with transdermal sensors include lengthy calibration time and limited accuracy.[15,19]

Biosensors such as an electronic nose system are being investigated.[20–22] In DM patients, breath acetone levels may be elevated because of increased lipolysis. Studies have suggested a linear relationship between blood and breath acetone levels, indicating that measurement of breath acetone may play a role in monitoring glycemic

control.[23] It is likely, however, that a profile of exhaled volatile organic compounds (VOCs) would be needed to monitor glycemic control rather than acetone alone. Breath isoprene has been suggested as another potential metabolite of interest.[24] Selected-ion flow-tube mass spectrometry (SIFT-MS) and proton-transfer-reaction mass spectrometry (PTR-MS) have been used to detect VOCs in exhaled breath. In SIFT-MS, precursor ions are injected into fast-flowing helium carrier gas, and the breath sample is introduced at a known rate of flow. The reaction of trace gas compounds in the exhaled breath with the precursor ion species produces characteristic product ions, which can then be used to quantify compound levels.[23] PTR-MS uses a similar technique, however, the most frequently used precursor ion is H_30^+ generated from water vapor in a hollow cathode discharge, without the need for a carrier gas. Both techniques allow real-time monitoring of VOCs in exhaled breath, however, further research in this area is necessary because of conflicting data and the wide range of error values.[24]

Exhaled Breath Condensate

The limitations of these alternative techniques indicate that there is a need to develop other noninvasive methods for estimating blood glucose levels. Measurement of glucose levels in respiratory fluids via collection of exhaled breath condensate (EBC) is one potential method. Collection of EBC is a useful technique for both adult and pediatric subjects as it is noninvasive, comfortable, and safe and allows for the measurement of various substances present in respiratory fluid.[25,26] The procedure for collection of exhaled breath involves the subject breathing tidally into a mouthpiece, which is attached by glass tubing to a cooling system that condenses the exhaled air as it passes through. Breathing for 10 to 20 minutes generally yields a sufficient amount of fluid for analysis (1–2 ml).[26] Studies of both healthy children and those with asthma report no adverse events, in particular, no clinically significant reduction in airway caliber, after EBC collection.[27,28] The largest component of EBC is condensed water vapor (which makes up greater than 99% of the fluid collected). Other volatile and nonvolatile substances present in the respiratory fluid can be detected, with the concentrations of these substances affected by lung disease.[26] Analysis of leukotriene B4, for example, has been used to assess airway inflammation in subjects with CF,[29,30] while levels of oxides of nitrogen and hydrogen peroxide have been shown to be elevated in asthmatic children and lung cancer patients, respectively.[31,32]

Glucose in Respiratory Fluids

In normoglycemic individuals, the glucose concentration in respiratory fluid (the liquid present in the lumen of the airways and alveoli) is typically maintained at low levels (<1 mmol/liter). Various animal studies have found glucose levels in respiratory fluids to be 0.3- to 0.05-fold that of blood glucose.[33] This low concentration of glucose in the respiratory fluid is maintained by the equilibrium between active cotransport of glucose with Na^+ by epithelial cells, and paracellular leakage of glucose from the lung interstitium and plasma (which is normally low unless disrupted).[34]

Cellular uptake of glucose from plasma into the airway epithelial cells occurs via the action of glucose transporters (carrier-mediated facilitated diffusion), with the concentration gradient across the cellular membrane maintained by cellular consumption of glucose. The presence of a Na^+-glucose cotransporter in the apical cell membrane of airway epithelial cells facilitates secondary cotransport of glucose.[35] Once in the cell, glucose is either metabolized or released at the basolateral membrane into the lumen of the airway. This occurs via facilitated diffusion, resulting in the presence of glucose in the epithelial lining fluid of the lungs. The equilibrium between these pathways (paracellular leakage, active cotransport, and facilitated diffusion) maintains a low concentration of glucose within the respiratory fluid under normal circumstances.[35,36]

Concentrations of glucose in respiratory fluid may be increased by certain pathological or physiological processes. Patients with lung disease such as CF have been observed to have elevated levels of glucose in the respiratory fluid, estimated by analysis of exhaled breath.[33] Hyperglycemia also causes elevated respiratory fluid glucose concentrations, with the increase in glucose levels in the respiratory fluid correlating with the increased in blood glucose.[33,34] Higher levels of glucose in respiratory fluids are clinically significant as they may predispose to infection.[37,38]

Glucose levels in nasal secretions have been examined in various studies. Philips and colleagues (2003)[39] looked at 19 healthy subjects and 20 subjects with DM to determine the glucose concentration of human nasal secretions. Glucose oxidase reagent strips were used to measure glucose levels, which were determined using color change of the reagent strips against a visual color scale. Glucose was not detectable in the nasal secretions of healthy subjects but was detected in 90% of subjects

with DM (18 out of 20 subjects), with the 2 individuals without glucose in their nasal secretions having plasma glucose <8 mmol/liter at the time of measurement. Wood and colleagues (2004)[40] demonstrated that as blood glucose levels increase and decrease, nasal glucose concentrations also increase and decrease. As with the earlier study, glucose oxidase reagent strips were used to measure glucose levels in nasal secretions, with the blood glucose levels of healthy subjects altered by hyperglycemic clamping. Glucose was not detected in nasal secretions until blood glucose reached a concentration of 6.7–9.7 mmol/liter, after which nasal glucose concentrations increased in correlation with blood glucose concentrations. Due to the inability to detect glucose in healthy normoglycemic subjects, the researchers suggested that there may be a blood glucose threshold above which glucose appears in respiratory fluid. Later studies, however, have used EBC to measure glucose levels and have demonstrated that glucose is present in low concentrations in the respiratory fluid of healthy subjects,[33] indicating that the sensitivity of glucose oxidase reagent strips (1 mmol/liter) used in the earlier studies was not sufficient to detect normal glucose levels in nasal secretions. Baker and colleagues (2007)[33] used EBC to measure breath glucose levels in four populations: healthy control subjects, patients with DM, patients with CFRD, and patients with CF without diabetes. Blood glucose and salivary glucose levels were also measured. Glucose was found to be present in the breath of healthy subjects at a mean level of 0.40 mmol/liter, and was elevated to a mean of 1.20 mmol/liter in DM patients without lung disease, 2.04 mmol/liter in patients with CF, and 4.00 mmol/liter for patients with CFRD after correction for dilution using conductivity measurements to determine the cation concentration of the condensate in relation to serum. The breath-blood glucose ratio was determined to be 0.08 in healthy subjects and 0.09 in patients with DM. It was markedly higher in patients with CF alone (0.29) and CFRD (0.54).

Similar results were observed by Srivastava and colleagues (2008).[41] Exhaled breath condensate was collected from 17 adult CF patients three times within 60 minutes and analyzed for glucose, with blood glucose levels also measured. The mean breath glucose level for the first measurement was 0.61 mmol/liter; for the second measurement, 0.19 mmol/liter; and for the third measurement, 0.22 mmol/liter. The mean blood glucose level was 4.8 mmol/liter and did not change between measurements. The authors speculated that the change in breath glucose concentrations may have been due to differences in lung secretions.

However, these results were not replicated in the later study by researchers Srivastava and colleagues (2010),[42] which analyzed the EBC of 227 adult patients (over 16 years old) with CF for glucose. Blood glucose levels were concurrently measured, and 82% of participants were found to have breath glucose levels of less than 1 μmol/liter, with a mean EBC glucose level of 0.72 μmol/liter. No association was found between breath glucose levels and blood glucose levels or other clinical parameters such as lung function. The researchers suggested that factors such as dilution and concurrent metabolic activity may be responsible for the lack of association by causing difficulty in quantifying breath glucose levels.

The methods of this study were similar to Baker and colleagues'[33] 2007 study; both used high-performance anion-exchange chromatography to measure glucose levels, and estimated the dilution of respiratory fluid in the EBC by using conductivity to calculate total cation concentration of the sample. It is unclear why the breath glucose levels found by Srivastava and colleagues (2010)[42] were considerably lower than those reported by Baker and colleagues[33] and Srivastava and colleagues (2008).[41] The larger sample size of the 2010 study (n = 227) compared to the two earlier studies (n = 56 and n =17, respectively) may be significant. Issues such as time lag seen with other noninvasive glucose monitoring technologies may also be relevant, however, little data is available on the subject.

Conclusion

Development of noninvasive methods of blood glucose monitoring is a growing area of research. It is particularly relevant for children with diabetes mellitus and cystic fibrosis. Measuring glucose levels in respiratory fluid via collection of exhaled breath condensate to indirectly estimate blood glucose levels is a viable method that may improve compliance with regular self-monitoring and therefore have the potential benefit of reducing disease progression. Exhaled breath condensate is a safe and noninvasive method of sampling lower respiratory tract fluid. If, with further development it, can be shown to reflect blood glucose levels accurately, it will have the potential to play a role in the clinical management of these diseases, particularly in children.

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