Hyperglycemia and cystic fibrosis alter respiratory fluid glucose concentrations estimated by breath condensate analysis

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Baker EH, Clark N, Brennan AL, Fisher DA, Gyi KM, Hodson ME, Philips BJ, Baines DL, Wood DM. Hyperglycemia and cystic fibrosis alter respiratory fluid glucose concentrations estimated by breath condensate analysis. J Appl Physiol 102: 1969–1975, 2007. First published February 15, 2007; doi:10.1152/japplphysiol.01425.2006.—In animals, glucose concentrations are 3–20 times lower in lung lining fluid than in plasma. In humans, glucose concentrations are normally low (<1 mmol/l) in nasal and bronchial fluid, but they are elevated by inflammation or hyperglycemia. Furthermore, elevated bronchial glucose is associated with increased respiratory infection in intensive care patients. Our aims were to estimate normal glucose concentrations in fluid from distal human lung sampled noninvasively and to determine effects of hyperglycemia and lung disease on lung glucose concentrations. Respiratory fluid was sampled as exhaled breath condensate, and glucose was measured by chromatography with pulsed amperometric detection. Dilution corrections, based on conductivity, were applied to estimate respiratory fluid glucose concentrations (breath glucose). We found that breath glucose in healthy volunteers was 0.40 mmol/l (SD 0.24), reproducible, and unaffected by changes in salivary glucose. Breath-to-blood glucose ratio (BBGR) was 0.08 (SD 0.05). Breath glucose increased during experimental hyperglycemia (P < 0.05) and was elevated in diabetic patients without lung disease [1.20 mmol/l (SD 0.69)] in proportion to hyperglycemia [BBGR 0.09 (SD 0.06)]. Breath glucose was elevated more than expected for blood glucose in cystic fibrosis patients [breath 2.04 mmol/l (SD 1.14), BBGR 0.29 (SD 0.17)] and in cystic fibrosis-related diabetes [breath 4.00 mmol/l (SD 2.07), BBGR 0.54 (0.28); P < 0.0001]. These data indicate that 1) this method makes a biologically plausible estimate of respiratory fluid glucose concentration, 2) respiratory fluid glucose concentrations are elevated by hyperglycemia and lung disease, and 3) effects of hyperglycemia and lung disease can be distinguished using the BBGR. This method will support future in vivo investigation of the cause and effect of elevated respiratory fluid glucose in human lung disease.

exhaled breath condensate; diabetes mellitus; hyperglycemic clamp

The respiratory tract lumen from nose to alveoli is lined by a thin layer of fluid. The volume and composition of this fluid are tightly regulated to maintain normal physiological function. In animals, the normal glucose concentration of respiratory tract lining fluid is 3–20 times lower than plasma glucose concentration (1, 22). Glucose diffuses from plasma into respiratory fluid down its concentration gradient, probably through paracellular pathways and at a rate dependent on epithelial permeability (22). Low respiratory fluid glucose concentrations are maintained by active removal of this glucose from respiratory fluid by apical glucose transporters in respiratory epithelial cells (8, 22).

We have investigated factors that determine the glucose concentration of human respiratory fluid. Glucose was not detected in nasal secretions from healthy volunteers using glucose oxidase sticks with a sensitivity of 1 mmol/l (18). However, nasal glucose concentrations were elevated by hyperglycemia, which increases the plasma-lumen glucose gradient, (18, 26) and by nasal epithelial inflammation (colds), which may increase epithelial permeability or impair epithelial transport (18). Glucose concentrations were also elevated in lower airway secretions aspirated via endotracheal tubes from patients with hyperglycemia undergoing mechanical ventilation on intensive care (19). Patients with elevated lower airway glucose concentrations on admission to intensive care were more likely to develop respiratory infection, particularly with methicillin-resistant Staphylococcus aureus, than patients with normal lower airway glucose concentrations (19).

In this paper we describe development and validation of a noninvasive method of measuring glucose concentrations in fluid from the lower respiratory tract of human volunteers. Exhaled breath can be collected easily, safely, and repeatedly by condensation from human volunteers of all ages with and without lung disease (13). Exhaled breath condensate mainly comprises water vapor, but also it contains very low concentrations of solute, thought to derive from aerosolized respiratory tract lining fluid (10). Major challenges in condensate research are measurement of solute at these very low concentrations and estimation of variable dilution of respiratory fluid in condensate samples (13). In this study, we used anion-exchange chromatography with pulsed amperometric detection to measure glucose concentrations as low as 0.01 μmol/l and estimated respiratory fluid dilution using conductivity measurements (9). We designated condensate glucose values corrected for dilution as “breath glucose” values, which are estimates of respiratory fluid glucose concentrations. Having validated this method, we used it to determine the effect of hyperglycemia and lung disease on respiratory glucose concentrations.

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MATERIALS AND METHODS

Studies were approved by Wandsworth and Royal Brompton Hospital Local Research Ethics Committees and were conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent for inclusion in the study.

Participants

Healthy volunteers were nonsmokers without cystic fibrosis (CF), lung disease, diabetes mellitus, or first-degree relatives with diabetes. Patients with Type 1 or 2 diabetes mellitus were nonsmokers with no history of lung disease and were recruited from diabetic outpatient clinics at St George’s Hospital.

CF patients were recruited during attendance at the Department of Cystic Fibrosis, Royal Brompton Hospital. Participants had CF diagnosed by positive sweat test and clinical features, with or without genotype confirmation, and were included in the study if they had stable disease, defined as no hospital admissions, no intravenous antibiotics, no change in systemic steroid dose, and no new enteral feeding in previous 6 wk. Pregnant CF patients were excluded. Diabetes was defined as a prior diagnosis of CF-related diabetes (CFRD) requiring treatment.

Experimental Techniques

Collection and analysis of exhaled breath condensate. Exhaled breath condensate was collected by exhalation through a one-way valve into a cooled, 22.2-cm-long RTube (Respiratory Research, Charlottesville, VA) over 10 min. Participants were seated, did not wear nose clips, rinsed their mouth with water before the experiment, and at 5 min of collection and swallowed accumulated saliva regularly. Retrieved condensate was stored at −20°C. Plastics other than RTubes were washed in deionized water for 48 h to remove ions that might influence conductivity, and they were air-dried before use.

One-milliliter condensate aliquots were lyophilized to dryness over 18 h at −50°C (Micro Modulyo, Edwards) and then reconstituted in 1 ml of deionized water. Lyophilization removes >99% NH₄⁺ from condensate, allowing measurement of total nonvolatile ionic concentrations by conductivity (9).

Temperature-corrected conductivity was measured by a CON10 meter (Oakton, Cole-Parmer, London, UK) (9). A calibration curve of conductivity values for Na⁺ standards (1–1000 μmol/l) was used to convert conductivity into total cation concentrations. Dilution of respiratory fluid in condensate was estimated by dividing serum total cation concentration (150 mmol/l) by condensate total cation concentration (9).

Previous studies have shown that respiratory fluid is diluted up to 10,000 times in condensate by water vapor (9, 10). We therefore predicted that glucose would be at submicromolar concentrations in condensate and used a sensitive assay to detect this. Glucose concentrations were measured in reconstituted condensate using high-performance anion-exchange chromatography with pulsed amperometric detection (CarboPac PA20, Dionex, CA) (28). Assay sensitivity was 0.01 μmol/l, and lower limit of glucose detection was 0.001 μmol/l. Breath glucose concentrations (estimates of respiratory fluid glucose concentrations) were obtained by multiplying condensate glucose concentrations by calculated dilution factors.

Measurement of blood and salivary glucose concentrations. Capillary blood glucose measurements were made using the Accu-Check Advantage System (Roche Diagnostics).

Salivary glucose concentrations were measured using glucose oxidase sticks (Roche, Lewes, East Sussex, UK) placed under the tongue for 30 s until coated with saliva and then read compared with a visual color indicator chart.
these samples. Samples with glucose at concentrations between 0.001 and 0.01 μmol/l were designated as containing 0.001 μmol/l glucose.

Repeatability Studies

Within day. Ten healthy volunteers each made four to five repeat 10-min condensate collections on the same day. Individual repeat measurements are shown in Fig. 1A. Mean breath glucose in 45 samples was 0.41 mmol/l (SD 0.38). The within-subject within-day value was 0.26 mmol/l (SD 0.30).

Between days. Six healthy volunteers each made a 10-min condensate collection on 2–3 separate days. Individual repeat measurements are shown in Fig. 1B. One breath condensate value exceeded blood glucose values and was therefore assumed to be an error and excluded from the analysis. Without this value mean breath glucose in 16 samples was 0.54 mmol/l (SD 0.40). The within-subject between-day was 0.28 mmol/l (SD 0.24).

Effect of Changes in Salivary Glucose on Breath Glucose Concentrations

Six volunteers [3 women, age 27.3 yr (SD 3.6)] underwent breath glucose measurement before and after elevation of salivary glucose by glucose mouthwash (Fig. 2). At baseline, salivary glucose was 0 mmol/l (SD 0) and breath glucose was 0.49 mmol/l (SD 0.12). After glucose mouthwash, salivary glucose concentrations increased by 26.3 mmol/l (SD 6.5) (P < 0.0001) without change in breath glucose [change −0.05 mmol/l (SD 0.22); P = 0.602]. After repeat water mouthwashes salivary glucose fell back to 0 mmol/l (SD 0) (P < 0.0001), but breath glucose remained unchanged [0.46 mmol/l (SD 0.27); P = 0.875]. Blood glucose was 5.5 ± 0.4 mmol/l at baseline and did not change during the experiment (P = 0.191).

Effect of Changes in Blood Glucose on Breath Glucose Concentrations

Glucose ramp. Six healthy volunteers [2 women, age 28.2 yr (SD 4.8)] underwent repeat breath glucose measurements during stepwise elevation of blood glucose (Fig. 3). Breath glucose concentrations increased significantly as blood glucose rose (P = 0.012).

Rise and fall. In six healthy volunteers [1 woman, age 24.2 yr (SD 2.0)] at baseline, blood glucose concentrations were 5.4 mmol/l (SD 0.9) and breath glucose concentrations were 0.36 mmol/l (SD 0.27). When blood glucose was elevated rapidly to 14.6 mmol/l (SD 1.6), breath glucose concentrations increased to 0.75 mmol/l (SD 0.39). After removal of the clamp, blood glucose fell to 5.3 mmol/l (SD 0.8) and breath glucose to 0.38 mmol/l (SD 0.27). Breath glucose concentrations were significantly higher when blood glucose was 14.6 mmol/l (SD 1.6) than at the beginning and end of the experiment (P = 0.03).

Effect of Chronic Hyperglycemia (Diabetes Mellitus) and Lung Inflammation (CF) on Breath Glucose Concentrations

Demographic characteristics of participants and condensate collection and glucose concentration and dilution factor are given in Table 1. Clinical characteristics of CF patients with and without diabetes are compared in Table 2.

In 23 healthy volunteers blood glucose was 5.4 mmol/l (SD 0.7) and breath glucose was 0.40 mmol/l (SD 0.24). In 17 people with diabetes mellitus without lung disease, blood glucose was 13.9 mmol/l (SD 3.5) and breath glucose was 1.20 mmol/l (SD 0.69). In eight people with CF without a diagnosis of diabetes, blood glucose was 7.0 mmol/l (SD 1.40) and breath glucose was 2.04 mmol/l (SD 1.14). In eight people with CFRD, blood glucose was 8.7 mmol/l (SD 5.7) and breath glucose was 4.00 mmol/l (SD 2.07).

On two × two between-groups analysis of covariance, both cystic fibrosis (η² = 0.538, P < 0.0001) and diabetes mellitus (η² = 0.307, P < 0.0001) independently determined breath glucose concentrations. Additionally, there was a small interaction effect for CF with diabetes mellitus (η² = 0.074, P = 0.047).
Effect of Chronic Hyperglycemia (Diabetes Mellitus) and Lung Inflammation (CF) on the Breath-to-Blood Glucose Ratio

Breath. Blood glucose ratios were as follows: healthy volunteers, 0.08 (SD 0.05); diabetes mellitus alone, 0.09 (SD 0.06); CF alone, 0.29 (SD 0.17); and CFRD 0.54 (SD 0.28). Breath glucose concentrations differed significantly between the four groups (1-way analysis of variance \( P < 0.0001 \)). On post hoc Bonferroni analysis, the breath-to-blood glucose ratio was significantly greater in CFRD patients than in all other groups (\( P = 0.001 \)) and was significantly greater in CF patients without a diagnosis of diabetes than in healthy volunteers (\( P = 0.001 \)) and diabetes mellitus patients (\( P = 0.004 \)). There was no difference in the breath-to-blood glucose ratio between healthy volunteers and diabetes mellitus patients (\( P = 1.0 \)).

DISCUSSION

The aims of our study were to develop and validate a noninvasive method of measuring glucose concentrations of fluid from the lower respiratory tract and to use this method to identify factors that alter respiratory fluid glucose concentrations. We used exhaled breath condensate collection as a method of sampling lower respiratory tract fluid and addressed three major challenges of this technique: measurement of extremely low solute concentrations, correction for dilution and determination of the contribution of saliva to samples.

We measured very low glucose concentrations using high-performance anion-exchange chromatography with pulsed amperometric detection. This technique has previously been shown to quantify saccharides in the submicromolar range (28). We were able to detect glucose at concentrations \( \geq 0.001 \) \( \mu \text{mol/l} \) in 194 of 195 condensate samples tested and to quantify condensate glucose accurately (requiring glucose concentrations \( \geq 0.01 \) \( \mu \text{mol/l} \)) in 183 samples.

Correction for dilution in condensate research is controversial. Effros and colleagues (9) argue that without correction for dilution, differences in condensate solute or inflammatory mediator concentrations over time or between groups could reflect variability in amount of respiratory fluid in samples, rather than differences in respiratory fluid composition. The American Thoracic Society and European Respiratory Society

Fig. 2. Comparison of salivary and breath glucose measurements before and after a glucose mouthwash. Individual values of salivary and breath glucose concentrations are given for 6 volunteers, each denoted by a different line type (key). Peak salivary glucose concentrations (measurement 2) are the mean of salivary measurements made before and after breath glucose measurement. Despite elevation in salivary glucose from 0 mmol/l (SD 0) to 26.3 mmol/l (SD 6.5) (95% confidence interval: 19.5–33.2 mmol/l, \( P < 0.0001 \)), there was no change in breath glucose concentration \([-0.05 \text{ mmol/l (SD 0.22); 95% confidence interval: } -0.18–0.28, P = 0.602 \]).

Fig. 3. Graph comparing blood and breath glucose concentrations. Values are given as means, and error bars are SDs for blood (x-axis) and breath (y-axis) glucose concentrations. Gray diamonds denote repeat breath glucose measurements in healthy volunteers \( n = 6 \) who underwent gradual experimental blood glucose elevation. Breath glucose concentrations increased significantly as blood glucose was elevated \( (P = 0.012) \). The gray diamond marked with an asterisk denotes breath glucose concentrations in healthy volunteers \( n = 6 \) undergoing rapid elevation of blood glucose from baseline to 14.6 mmol/l (SD 1.6). The trend line describes the relationship between blood and breath glucose concentrations in healthy volunteers and is plotted through zero because it is assumed that in the absence of blood glucose breath glucose will be zero. Breath glucose concentrations are plotted against blood glucose concentrations for healthy volunteers not undergoing glucose ramp hyperglycaemic clamping (white square, \( n = 17 \)) and for diabetes mellitus patients (white triangle, \( n = 17 \)). For both groups, breath glucose concentrations are similar to those predicted for blood glucose in the hyperglycaemic clamp experiments. However, breath glucose is elevated above expected concentrations for blood glucose for both cystic fibrosis patients without a diagnosis of diabetes (black square, \( n = 8 \)) and cystic fibrosis patients with diabetes mellitus (black triangle, \( n = 8 \)), implying an abnormality in respiratory epithelial glucose homeostasis.
combined task force acknowledged desirability of correction for dilution but concluded that it was unlikely that changes in condensate mediator concentrations in inflammatory lung disease were completely explained by differences in dilution (13). In our laboratory, we used conductivity as a dilution marker because it is easy to perform using inexpensive equipment. After correction for dilution we estimated that breath glucose concentrations in healthy volunteers were 0.40 mmol/l (SD 0.24). This value is a biologically plausible estimate of respiratory fluid glucose concentrations because it is consistent with previous findings that normal nasal secretions and bronchial aspirates had glucose concentrations <1 mmol/l (18, 26) and that glucose concentrations in animal respiratory fluid were 3–20 times lower than plasma concentrations (1, 22). As further validation of our technique, breath glucose measurements were usually repeatable in healthy volunteers both on the same day and between days. We found clear differences in condensate glucose concentrations between CF and non-CF patients both with and without correction for dilution. However, correction for dilution was required to reveal elevated breath glucose concentrations in diabetes patients compared with controls. Breath glucose values for diabetic patients after correction were consistent with breath glucose concentrations in healthy volunteers during experimental hyperglycemia, supporting the use of dilution correction in our study. Respiratory fluid was more dilute in condensate samples from diabetic patients than from other groups. The cause of this is not clear, although nasal dryness and delayed mucociliary clearance have been described in diabetic patients (20, 27).

If condensate measurements are to be used to estimate glucose concentrations in respiratory fluid, it is important to exclude saliva as a source of condensate solute. We found that breath glucose measurements made using the technique described did not alter when salivary glucose was elevated by 26.3 mmol/l (SD 6.5). This indicates that our breath glucose measurements are an estimate of respiratory fluid, and not oral, glucose concentrations.

### Factors Affecting Breath Glucose Concentrations

#### Hyperglycemia.

We found that changes in blood glucose alone were sufficient to cause changes in breath glucose concentrations. Figure 3 demonstrates the relationship between breath and blood glucose in four different groups of participants without lung disease. The gray diamonds demonstrate a linear rise in breath glucose in healthy volunteers in response to experimental blood glucose elevation. Strikingly, breath glucose values in the second group undergoing hyperglycemic clamping (gray diamond with asterisk), in other healthy volunteers (white square), and in diabetic patients (white triangle) were all measured at concentrations predicted for their blood glucose measurements by the hyperglycemic clamp experiments.

The relationship between breath and blood glucose can be explained by physiological phenomena observed in animal and human lungs. Glucose appears to diffuse passively from blood to respiratory fluid by paracellular pathways. Evidence for this includes the findings that the airway epithelium is permeable to L-glucose, an isomer not carried by cellular glucose transporters (25), and that increasing paracellular permeability with protamine increases respiratory fluid glucose concentrations (21). Our observation that breath glucose increases as blood

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**Table 1. Comparison of exhaled breath condensate values between groups**

<table>
<thead>
<tr>
<th></th>
<th>Healthy Volunteers</th>
<th>DM</th>
<th>CF Patients Without DM</th>
<th>CFRD</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>23</td>
<td>17</td>
<td>8</td>
<td>8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>26.6 (3.5)</td>
<td>47.5 (19.1)</td>
<td>30.6 (8.8)</td>
<td>27.1 (8.1)</td>
<td>0.255</td>
</tr>
<tr>
<td>Sex, (male:female)</td>
<td>13:10</td>
<td>10:7</td>
<td>6:2</td>
<td>1:7</td>
<td>0.007</td>
</tr>
<tr>
<td>Conductivity, μS/cm</td>
<td>6.1 (4.4–19.2)</td>
<td>2.6 (2.2–8.8)</td>
<td>9.6 (8.6–10.1)</td>
<td>10.6 (9.4–12.7)</td>
<td>0.004</td>
</tr>
<tr>
<td>Total cations, mmol/l</td>
<td>35.3 (22.8–128.6)</td>
<td>9.7 (6.8–54.3)</td>
<td>66.4 (59.2–70.1)</td>
<td>73.5 (65.2–89.1)</td>
<td>0.002</td>
</tr>
<tr>
<td>Dilution factor</td>
<td>4,248 (1,166–6,577)</td>
<td>15,520 (2,786–22,121)</td>
<td>2,259 (2,140–2,540)</td>
<td>2,043 (1,684–2,299)</td>
<td>0.002</td>
</tr>
<tr>
<td>Condensate glucose, mmol/l</td>
<td>0.11 (0.04–0.31)</td>
<td>0.11 (0.07–0.23)</td>
<td>0.85 (0.64–1.39)</td>
<td>2.11 (1.73–2.88)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**Values are means (SD) for normally distributed variables and median (interquartile range) for variables that are not normally distributed. n, no. of subjects; CF, cystic fibrosis; DM, diabetes mellitus; CFRD, cystic fibrosis-related diabetes.**

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**Table 2. Comparison of clinical features of patients with CF without DM and of patients with CFRD**

<table>
<thead>
<tr>
<th></th>
<th>CF without DM (n = 8)</th>
<th>CFRD (n = 8)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>30.6 (8.8)</td>
<td>27.1 (8.1)</td>
<td>0.423</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.1 (2.4)</td>
<td>21.3 (3.9)</td>
<td>0.629</td>
</tr>
<tr>
<td>FEV₁, %predicted</td>
<td>43.5 (12.9)</td>
<td>47.6 (22.1)</td>
<td>0.657</td>
</tr>
<tr>
<td>FEV₁/FVC ratio, %</td>
<td>51 (12)</td>
<td>61 (12)</td>
<td>0.101</td>
</tr>
<tr>
<td>HbA₁C, %</td>
<td>6.0 (0.7)</td>
<td>8.7 (3.1)</td>
<td>0.028</td>
</tr>
<tr>
<td>CRP, mg/l</td>
<td>14.7 (14.4) (n=3)</td>
<td>41.6 (34.5) (n=5)</td>
<td>0.255</td>
</tr>
<tr>
<td>WCC, ×10³/l</td>
<td>13.1 (6.7) (n=3)</td>
<td>13.2 (4.9) (n=7)</td>
<td>0.989</td>
</tr>
<tr>
<td>Oral steroids (taking/not taking)</td>
<td>0.8</td>
<td>1.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Inhaled steroids (taking/not taking)</td>
<td>3.5</td>
<td>5.3</td>
<td>0.5</td>
</tr>
<tr>
<td>No. of oral antibiotics taken</td>
<td>0.75 (0.9)</td>
<td>1.0 (0.8)</td>
<td>0.554</td>
</tr>
<tr>
<td>No. of antibiotics nebulized</td>
<td>1.1 (0.6)</td>
<td>1.1 (0.6)</td>
<td>1.0</td>
</tr>
<tr>
<td>Oral hypoglycemias (taking/not taking)</td>
<td>0.8</td>
<td>2.6</td>
<td>0.233</td>
</tr>
<tr>
<td>Insulin (taking/not taking)</td>
<td>0.8</td>
<td>6.2</td>
<td>0.003</td>
</tr>
</tbody>
</table>

**Values are means (SD) or as no. of patients. n, no. of patients in each group. BMI, body mass index; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; HbA₁C, glycosylated hemoglobin; CRP, C reactive protein; WCC, white cell count.**

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glucose rises can by explained by an increased glucose gradient driving glucose diffusion from plasma to respiratory fluid. However, passive diffusion is not sufficient to account for the finding that breath glucose concentrations are consistently 12.5 times lower than blood glucose in people with normal lungs. Furthermore, when blood glucose falls, breath glucose also falls against the plasma-lumen glucose gradient. In animal models, glucose is actively cleared from the lung lumen and glucose removal is prevented by phloridzin, which blocks sodium-glucose cotransporter (SGLT) (1, 22). Our laboratory has demonstrated mRNA and protein for SGLT and phloridzin-sensitive glucose transport in human epithelial cells (17), which may contribute to maintenance of low glucose concentrations in human respiratory fluid.

Lung disease. Patients with CF without a diagnosis of diabetes had marked elevation of their breath glucose to concentrations greatly in excess of those predicted from their blood glucose measurements. Patients in this group had impaired lung function, had elevated inflammatory markers, and were on maintenance antibiotics, indicating severe lung disease (Table 2). Lung disease could elevate breath glucose concentrations by increasing paracellular permeability and glucose leak into respiratory fluid or by impairing glucose removal from the lung lumen. CF patients had increased $^{99m}$Tc-labeled diethylenetriamine pentaacetate uptake, indicating increased pulmonary epithelial permeability (5). Structural abnormalities of tight junctions were seen in inflamed CF airway epithelium by freeze-fracture electron microscopy (4) and were reproducible in vitro by exposure of primary cultured, non-CF, human airway epithelial cells to proinflammatory cytokines (6). Furthermore these tight junction abnormalities were shown to increase paracellular permeability to hydrophilic solutes (6). The effect of lung inflammation on glucose transport by lung epithelial cells has not been studied. However, in rabbit intestine, inflammation reduced epithelial glucose transport, both by inhibiting Na$^+$/K$^+$-ATPase pumps, which generate the Na$^+$ gradient driving SGLT transport, and by reducing SGLT protein expression (23).

CF is caused by mutations in the gene encoding the cystic fibrosis transmembrane regulator (CFTR). CFTR both functions as a chloride channel and regulates the activity of other membrane transport proteins. If CFTR upregulates glucose transport by SGLT, then CF mutations could disrupt glucose transport and account for elevated breath glucose in CF patients. In support of this, SGLT-1 was found to be positively regulated by Cl$^-$, and optimal CFTR activity was dependent on the presence of glucose in cultured Caco-2 (human colon carcinoma) cells (16). By contrast active Na$^+$-linked glucose transport was greater in jejunal biopsies from children with CF than in controls (2).

An alternative explanation for our findings is that lung or respiratory fluid abnormalities in CF alter the collection of respiratory fluid droplets in condensate, resulting in artificial elevation of breath glucose concentrations. All our CF patients had airflow obstruction, shown by their reduced percent forced expiratory volume in 1 s and forced expiratory volume in 1 s-to-forced vital capacity ratios (Table 2). It is possible that airflow obstruction could alter the aerosolization of respiratory droplets into breath condensate. However, Effros and colleagues (11) found that solute dilution in condensate was similar in patients with airflow obstruction due to chronic obstructive pulmonary disease and in normal controls. Additionally, dilution factors in CF patients in our study were not significantly different from controls. Abnormalities of respiratory fluid in CF have been widely described (14, 24) and could invalidate the assumption underlying our dilution correction, that respiratory fluid total cation concentrations are equal to plasma total cation concentrations. However, condensate glucose was elevated in CF patients, with or without dilution correction, indicating a genuine increase in respiratory fluid glucose concentrations.

**CFRD.** Breath glucose was higher in patients with CFRD than in all other groups, including CF patients without diabetes. CFRD patients also had a higher breath-to-blood glucose ratio than CF patients without diabetes, indicating that elevated breath glucose was due to additional respiratory epithelial dysfunction and not to elevated blood glucose alone. Elevated luminal glucose concentrations could simply be a marker for worsening pulmonary disease or could have a causative role. Glucose stimulates production of proinflammatory cytokines (12) and, at concentrations found in respiratory fluid, stimulates growth of CF respiratory pathogens (30). Diabetes complicates CF in up to 30% of patients and is associated with an accelerated decline in lung function, which has not previously been explained (15). Breath glucose merits further investigation as a contributing factor or marker of this accelerated pulmonary decline.

In conclusion, we have validated a noninvasive method of estimating the glucose concentrations of respiratory fluid and shown that hyperglycemia and pulmonary inflammation independently and synergistically elevate respiratory fluid glucose concentrations. We have described the relationship between breath and blood glucose concentrations as a ratio and used it to distinguish whether elevated breath glucose is due to hyperglycemia (normal ratio) or lung inflammation (elevated ratio). Further studies are now required to investigate mechanisms underlying elevated breath glucose and breath-to-blood glucose ratios in CF and to establish whether elevated luminal glucose concentrations have detrimental pulmonary effects in this condition.

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**REFERENCES**


