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Airway glucose concentrations and effect on growth of respiratory pathogens in cystic fibrosis ☆

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Abstract

Background: Pulmonary decline accelerates in cystic fibrosis-related diabetes (CFRD) proportional to severity of glucose intolerance, but mechanisms are unclear. In people without CF, airway glucose (AG) concentrations are elevated when blood glucose (BG) ≥ 8 mmol L^{-1} (airway threshold), and are associated with acquisition of respiratory infection.
Methods: To determine the relationship between BG and AG, 40 CF patients underwent paired BG and AG (nasal) measurements. Daily time with BG > airway threshold was compared in 10 CFRD, 10 CF patients with normal glucose tolerance (CF-NGT) and 10 healthy volunteers by continuous BG monitoring. The effect of glucose at airway concentrations on bacterial growth was determined *in vitro* by optical densitometry.
Results: AG was present more frequently (85%-vs.-19%, $p<0.0001$) and at higher concentrations (0.5–3 mmol L^{-1}-vs.-0.5–1 mmol L^{-1}, $p<0.0001$) when BG was ≥ 8 mmol L^{-1}-vs.-<8 mmol L^{-1}. Daily time with BG ≥ 8 mmol L^{-1} was CFRD (49±25%), CF-NGT (6±5%), healthy volunteers (1±3%), $p<0.0001$. *Staphylococcus aureus* growth increased at ≥ 0.5 mmol L^{-1} ($p=0.006$) and *Pseudomonas aeruginosa* growth above 1–4 mmol L^{-1} glucose ($p=0.039$).
Conclusions: BG ≥ 8 mmol L^{-1} predicted elevated AG concentrations in CF, at least in nasal secretions. CFRD patients spent ~ 50% day with BG > airway threshold, implying persistently elevated AG concentrations. Further studies are required to determine whether elevated airway glucose concentrations contribute to accelerated pulmonary decline in CFRD.

Keywords: Diabetes mellitus; *Staphylococcus aureus*; *Pseudomonas aeruginosa*; Nasal glucose; Continuous glucose monitoring

1. Introduction

In cystic fibrosis (CF), reduced clearance of viscous lung secretions causes progressive infection, inflammation and declining lung function, which is the major cause of morbidity and mortality. Changes in the composition of pancreatic secretions cause ductal obstruction, leading to loss of pancreatic tissue [1], including β islets which produce insulin [2]. CF is thus commonly complicated by diabetes mellitus and glucose intolerance. The prevalence of CF-related diabetes increases with age, affecting more than 25% CF patients over the age of 20 [3].

Abbreviations: CF, cystic fibrosis; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; CFRD, cystic fibrosis related diabetes; CF-NGT, cystic fibrosis-normal glucose tolerance; HbA_{1c}, glycosylated haemoglobin; CGMS, continuous glucose monitoring system; NPB, nutrient peptone broth; OD, optical densitometry; SGLT, sodium–glucose co-transporter; ICU, intensive care unit; MRSA, methicillin-resistant *Staphylococcus aureus*.

☆ This data was presented at a conference of the American Thoracic Society, Orlando, USA in May 2004 [34] and of the European Cystic Fibrosis Society, Birmingham, UK in June 2004 [35].

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CF patients with related diabetes have a 6-fold greater mortality than CF patients without diabetes [4]. The increase in mortality may be attributable to an effect of diabetes on pulmonary disease. On cross-sectional analysis of the European Epidemiologic CF Registry, FEV_1% predicted was lower at all age groups in patients with CF-related diabetes compared to CF patients without diabetes [5]. FEV_1 and FVC were already 20% and 10% lower in CF-related diabetes than in matched CF controls without diabetes 6 years prior to the onset of diabetes, implying pre-diabetic acceleration in pulmonary decline [6].

Mechanisms underlying pre-diabetic and diabetic effects on lung function in CF are poorly understood. However, as rate of pulmonary decline is directly proportional to severity of glucose intolerance, increased glucose concentrations may play a role [7]. We have recently shown that, whilst glucose is normally undetectable in human airway secretions, airway glucose becomes detectable in healthy volunteers when blood glucose is raised experimentally above 7.9±1.1 mmol L^{-1} [8]. We also detected glucose at concentrations of 4 (2–7, median (interquartile range)) mmol L^{-1} in nasal secretions from people with diabetes mellitus and from 1 to 11 mmol L^{-1} (range) in bronchial aspirates from people with acute hyperglycaemia intubated on intensive care [9]. Seventy percent of the intensive care patients with a blood glucose ≥ 8 mmol L^{-1} and 100% with a blood glucose >10 mmol L^{-1}, but only 16% of patients with blood glucose <8 mmol L^{-1} had glucose detected in bronchial aspirates. Taken together these results support the concept of a blood glucose 'threshold', above which glucose becomes detectable in airway secretions. Glucose in bronchial aspirates was significantly associated with acquisition of respiratory pathogens, particularly methicillin-resistant *Staphylococcus aureus* [10]. Glucose in airway secretions preceded and therefore could have precipitated bacterial colonisation or infection, possibly by promoting bacterial growth or impeding host immunity.

We hypothesised that airway glucose concentrations would be elevated in CF patients when blood glucose exceeded the airway threshold (≥ 8 mmol L^{-1}), which would occur for increasing proportions of the day as glucose intolerance worsened. Glucose at these concentrations in airway secretions could affect the growth of respiratory pathogens. To test this we confirmed that airway glucose was elevated in people with CF at blood glucose ≥ 8 mmol L^{-1} and used continuous glucose monitoring to determine the proportion of the day that blood glucose exceeded the airway threshold. We examined the relationship between airway glucose and *in vitro* growth of *S. aureus* and *Pseudomonas aeruginosa*.

2. Methods

2.1. In vivo studies

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

2.1.1. In vivo study 1. Relationship between blood and airway glucose concentrations in cystic fibrosis

2.1.1.1. Participants. Blood and nasal glucose measurements were compared to determine whether airway glucose concentrations were elevated in CF patients when blood glucose exceeded the airway threshold (≥ 8 mmol L^{-1}) [8]. Forty 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. CF patients with and without related diabetes were included to ensure a wide range of blood glucose concentrations. CF patients were excluded if they had nasal polyps, acute rhinitis or nasal symptoms or visible nasal inflammation on inspection.

2.1.1.2. Protocol. Participants were studied on a single occasion. Clinical information was collected and capillary blood and nasal glucose measurements were both made within 10 min of each other.

2.1.2. In vivo study 2. Proportion of the day spent with blood glucose above the airway threshold

2.1.2.1. Participants. The proportion of the day spent with blood glucose above the airway threshold (≥ 8 mmol L^{-1}) was compared between 10 CF patients with related diabetes, 10 CF patients with normal glucose tolerance and 10 healthy, matched controls.

CF patients (diagnosed and recruited as described) 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 weeks. Pregnant CF patients were excluded. Diabetes was defined as either a prior diagnosis of CF-related diabetes requiring treatment, or by oral glucose tolerance test [11]. Healthy volunteers were medical students or health care workers without cystic fibrosis matched for age and body mass index. Healthy volunteers did not have nasal or lung disease, diabetes mellitus or first degree relatives with diabetes.

2.1.2.2. Protocol. Participants without a prior diagnosis of diabetes mellitus underwent oral glucose tolerance testing to establish glucose tolerance [11]. All participants then underwent continuous interstitial glucose monitoring over 48 h.

2.1.3. Experimental techniques

Clinical information: Age, gender, body mass index and glycosylated haemoglobin (HbA_{1c}) were recorded for CF

Table 1
Characteristics of CF patients undergoing paired blood and nasal glucose measurements

Characteristic	Patients
Number	40
Age (years)	30.3±9.4
Gender (M:F)	22:18
Body mass index (kg/m^2)	21.9±2.6
FEV_1 % predicted	51±21
CRP (mg/l)	17.8±20.9 (n=23)
White cell count (×10^9/l)	11.9±4.8 (n=30)

patients and healthy volunteers. Additionally lung function, white cell count and C-reactive protein were documented for CF patients.

Airway glucose concentrations were measured in nasal secretions using glucose oxidase sticks. Sticks were placed on the nasal mucosa under direct vision for 30 s, checked for adequate coating with nasal secretions and read in comparison with a visual colour chart [9]. Nasal mucosa was inspected prior to stick placement to exclude participants with clinical inflammation.

Capillary blood glucose was measured using Accu-Chek Advantage (Roche Diagnostics Ltd).

Oral glucose tolerance testing was performed after ≥8 h fasting. Venous plasma glucose was measured before and 2 h after oral ingestion of 1.75 g/kg body weight (max 75 g) glucose in 250 ml water.

Continuous Glucose Monitoring was performed using Minimed Continuous Glucose Monitoring System (CGMS). A glucose oxidase-based electrode measured interstitial glucose every 10 s and the monitor reported average readings every 5 min. Interstitial values were calibrated against capillary blood glucose at least 3 times/24 h. Participants were monitored for 48 h and the middle continuous 24-hour period was used for analysis using Minimed CGMS Solutions Software version 3.0A.

2.2. Microbiology

2.2.1. Bacterial strains

S. aureus (National Collection of Type Cultures 6571) and *Ps. aeruginosa* (American Type Culture Collection 27853) were cultured from freezer stocks on Columbia agar plates with 5% horse blood. Growth was examined after overnight incubation at 37 °C in air to confirm culture purity.

2.2.2. Experimental techniques

Bacteria were cultured in air overnight at 37 °C in 20 ml nutrient peptone broth (NPB) containing 20 mmol L^{-1} glucose, centrifuged at 2400 g for 15 min, washed then re-suspended in NPB without glucose. 4×10^7 colony forming units of bacteria were suspended in 2 ml NPB in cuvettes containing glucose at final concentrations of 0–10 mmol L^{-1}. Experiments were repeated 5 times for *S. aureus* and *Ps. aeruginosa* at the full range of glucose concentrations.

After 0, 1, 3, 6, 24 and 48 h of culture, cultures were agitated to ensure suspension using a 1 ml pipette and bacterial growth was assessed using optical densitometry (OD_{620}) [12]. After 24 and 48 h of growth OD_{620} for bacteria grown at each glucose concentration was divided by OD_{620} for bacteria grown without glucose to calculate growth ratios.

2.2.3. Analysis

Values are given as mean±standard deviation and were compared between 2 groups using unpaired t tests and more than 2 groups using one way analysis of variance with post-hoc Bonferroni analysis. Categorical variables are expressed as numbers and percentages and were compared between groups using χ^2 tests. Pearson correlation was used to describe relationships between continuous variables. $P<0.05$ was considered significant. Statistical package for the social sciences version 11.5 was used for analysis.

3. Results

3.1. In vivo study 1. Relationship between blood and airway glucose concentrations

Clinical characteristics of 40 CF patients studied are given in Table 1 and paired blood and nasal glucose measurements are shown in Fig. 1. Glucose was detected in nasal secretions from 16/40 people. People with glucose in nasal secretions had significantly higher blood glucose concentrations (9.5±3.2 mmol L^{-1}) than people without glucose in nasal secretions (6.3±3.6 mmol L^{-1}, p=0.007). Thirteen out of

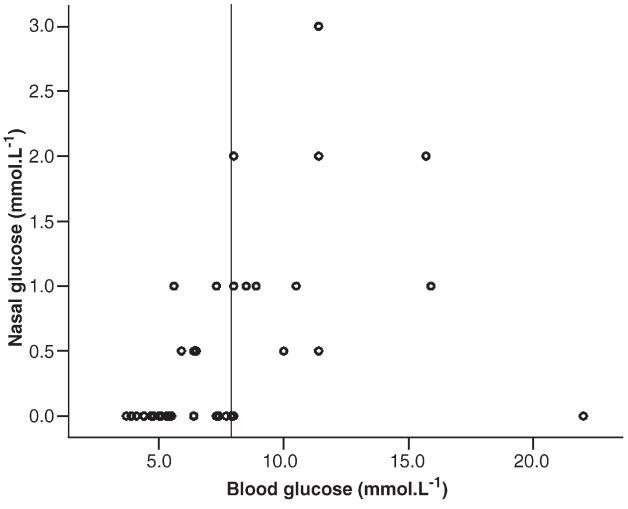

Fig. 1. Comparison between blood and nasal glucose concentrations in cystic fibrosis patients. Nasal glucose measurements are shown for 40 people with cystic fibrosis who have a range of blood glucose concentrations. The line denotes a blood glucose concentration of 7.9 mmol L^{-1}, the predicted airway glucose threshold [8]. Blood glucose was significantly correlated with nasal glucose (R=0.464, p=0.003).

Table 2
Demographic characteristics of people undergoing blood glucose monitoring

	Healthy volunteers (n=10)	CF NGT (n=10)	CFRD (n=10)	P value (analysis of variance)
Age (years)	25.6±3.3	27.7±7.0	31.7±10.3	0.198
Gender (M:F)	7:3	6:4	7:3	0.861
Body mass index (kg/m2)	22.0±2.1	22.6±2.3	21.7±2.4	0.660
FEV[1] % predicted	Not tested	64.2±22.9	51.6±18.8	0.196
Fasting blood glucose (OGTT, mmol L^{-1})	5.3±0.4	4.8±0.7	Not tested	0.073
Two-hour blood glucose (OGTT, mmol L^{-1})	5.7±1.2	5.9±1.1	Not tested	0.683
HbA_{1c} (%)	5.1±0.2	5.3±0.5	7.0±1.5[a]	<0.0001
Nasal glucose (% positive)	0 (n=6)	10	50	0.033

Oral glucose tolerance test (OGTT) results are given for CF patients with normal glucose tolerance (CF-NGT) and healthy volunteers, as OGTTs were used to confirm normal glucose tolerance in these groups. The majority of people with CF-related diabetes (CFRD) were recruited on the basis of an established diagnosis, hence did not undergo OGTT.

[a] Post-hoc Bonferroni analysis demonstrated that the difference detected between groups in HbA1c by one way analysis of variance were significant between people with cystic fibrosis-related diabetes and both cystic fibrosis-normal glucose tolerance and healthy volunteers, but not between people with cystic fibrosis-normal glucose tolerance and healthy volunteers.

40 people had blood glucose ≥ 8 mmol L^{-1}, 11 (85%) of whom had glucose in nasal secretions. 27/40 people had blood glucose < 8 mmol L^{-1}, 5 (19%) of whom had glucose in nasal secretions (p<0.0001). Nasal glucose concentrations were higher in people with blood glucose ≥ 8 mmol L^{-1} (1.2± 0.9 mmol L^{-1}, range 0.5–3 mmol L^{-1}) than in those with blood glucose < 8 mmol L^{-1} (0.1±0.3 mmol L^{-1}, range 0.5–1 mmol L^{-1}) (p<0.0001). Blood glucose was significantly correlated with nasal glucose concentrations (n=40, R=0.464, p=0.003).

3.2. In vivo study 2. Proportion of the day spent with interstitial glucose above the airway threshold

Demographic characteristics of 10 CF patients with related diabetes, 10 CF patients with normal glucose tolerance and 10 healthy volunteers undergoing continuous glucose monitoring are shown in Table 2. There were no significant differences in age, gender or body mass index between the 3 groups.

Normal glucose tolerance was confirmed in healthy volunteers and CF-normal glucose tolerance by oral glucose tolerance testing, requiring fasting blood glucose < 7.0 mmol L^{-1} and blood glucose < 7.8 mmol L^{-1} 2 h after an oral glucose load (Table 2) [11]. Nine people allocated to the CF-related diabetes group had a prior diagnosis of CF-related diabetes and one was identified by oral glucose tolerance testing, having a 2-hour blood glucose of 11.5 mmol L^{-1}. HbA_{1c} values were elevated in people with CF-related diabetes, confirming long term elevation of blood glucose, but were normal in healthy volunteers and people with CF-normal glucose tolerance (Table 2).

3.2.1. Continuous glucose monitoring

Representative interstitial glucose profiles from the middle 24 h of the monitored period are shown for healthy volunteers, CF-normal glucose tolerance and CF-related diabetes in Fig. 2. People with CF-related diabetes spent 49±25%, people with CF-normal glucose tolerance spent 6±5% and healthy volunteers spent 1± 3% of the day with interstitial glucose above the airway glucose threshold (≥8 mmol L^{-1}) [8], analysis of

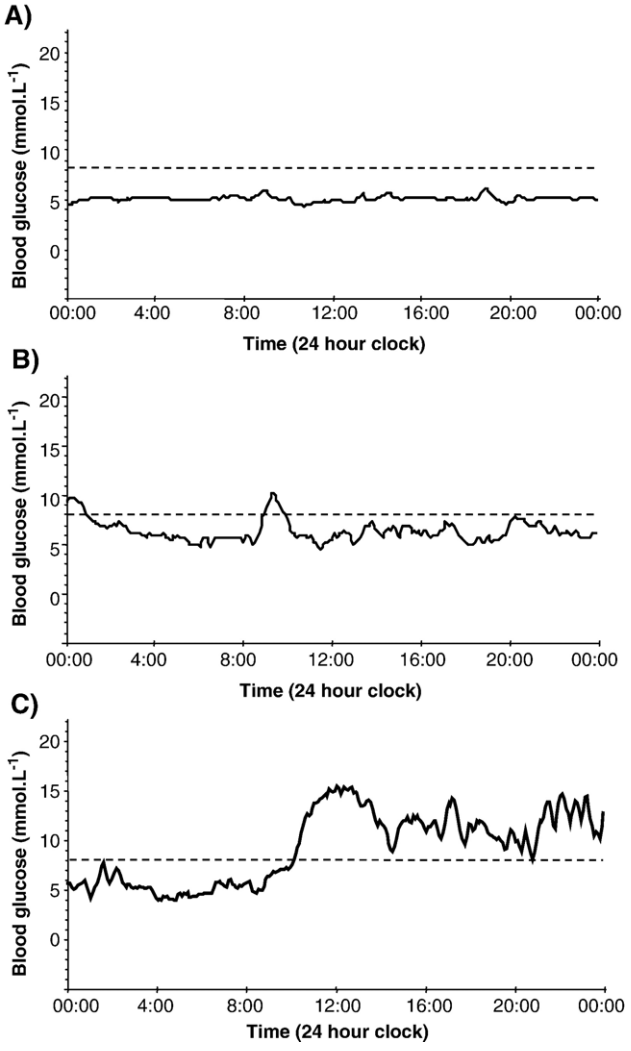

Fig. 2. Representative 24-hour blood glucose profiles. Representative 24-hour glucose profiles from the middle monitored day are shown for A) a healthy volunteer, B) a person with cystic fibrosis and normal glucose tolerance and C) cystic fibrosis related diabetes. The bold line denotes sensor blood glucose values plotted against time. The dotted line denotes the predicted airway glucose threshold.

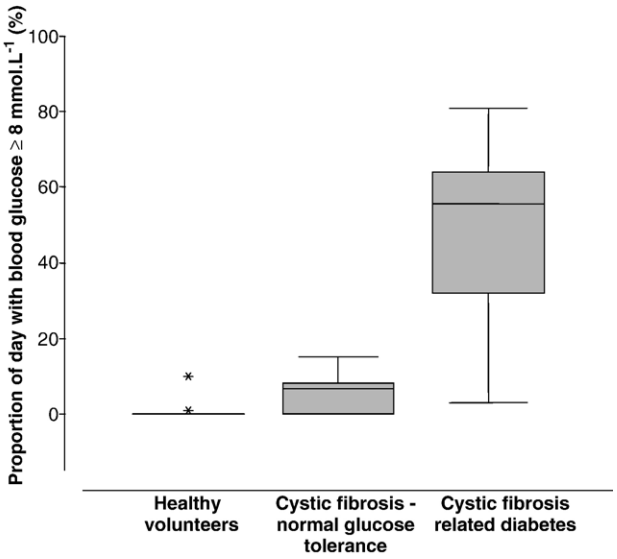

Fig. 3. Boxplot showing the proportion of the day spent by monitored groups with blood glucose above the airway glucose threshold (≥8 mmol L^{-1}). The boxplot shows median, quartiles and extreme values (*) of the proportion of 24 h spent by each group with blood glucose above the airway glucose threshold. 10 subjects were studied in each group. One way analysis of variance demonstrated significant differences in the proportion of the day spent by each group with blood glucose above the threshold ($p<0.0001$). Post-hoc Bonferroni analysis showed that the proportion of the day spent with blood glucose above the airway threshold was significantly greater in CF-related diabetes than CF-normal glucose tolerance ($p<0.0001$) and healthy volunteers ($p<0.0001$) but was not significantly different between CF-normal glucose tolerance and healthy volunteers.

variance $p<0.0001$ (Fig. 3). Post-hoc Bonferroni analysis showed that the proportion of the day spent with interstitial glucose above the airway threshold was significantly greater in CF-related diabetes than CF-normal glucose tolerance ($p<0.0001$) and healthy volunteers ($p<0.0001$) but was not significantly different between CF-normal glucose tolerance and healthy volunteers. Additionally people with CF-related diabetes spent 29±23% of the day ($n=9$) with interstitial glucose ≥ 10 mmol L^{-1}.

3.3. Effect of glucose at airway concentrations on bacterial growth in vitro

3.3.1. Staphylococcus aureus

Addition of increasing glucose concentrations (0.125–10 mmol L^{-1}) to *S. aureus* cultures in nutrient peptone broth stimulated bacterial growth, with effect apparent after 24 and 48 h of bacterial culture (Fig. 4A). Linear regression showed that growth was dependent on both glucose concentration in the range tested and time ($R=0.829$, $p<0.0001$), and that glucose ($p<0.0001$) and time ($p<0.0001$) independently affected bacterial growth. Staphylococcal growth was significantly greater than control at glucose concentrations > 0.5 mmol L^{-1} at 24 h ($p=0.006$) and >8 mmol L^{-1} at 48 h ($p<0.0001$) (Fig. 5).

3.3.2. Pseudomonas aeruginosa

Addition of increasing glucose concentrations (0.125–10 mmol L^{-1}) to *Ps. aeruginosa* cultures in nutrient peptone broth stimulated bacterial growth, with effect apparent after 24 and 48 h of bacterial culture (Fig. 4B). Linear regression showed that growth was dependent on both glucose concentration in the range tested and time ($R=0.969$, $p<0.0001$), and that glucose ($p<0.001$) and time ($p<0.0001$) independently affected bacterial growth. Pseudomonal growth was significantly greater than control at glucose

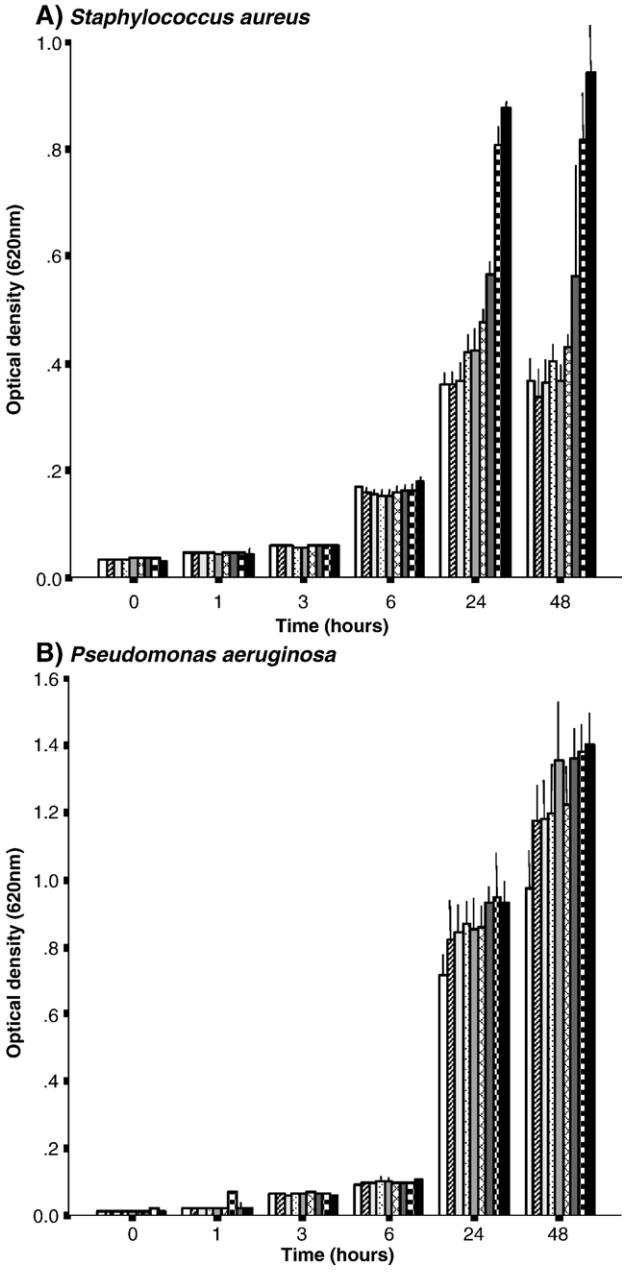

Fig. 4. Bar chart showing the effect of glucose concentration and time on bacterial growth. Bacterial growth quantified using optical density is shown for bacterial cultures at different time points and glucose concentrations: 0 mM □ 0.125 mM ▨ 0.25 mM ▩ 0.5 mM ⊡ 1 mM ▩ 2 mM ⊠ 4 mM ▩ 8 mM ⊠ 10 mM ■.

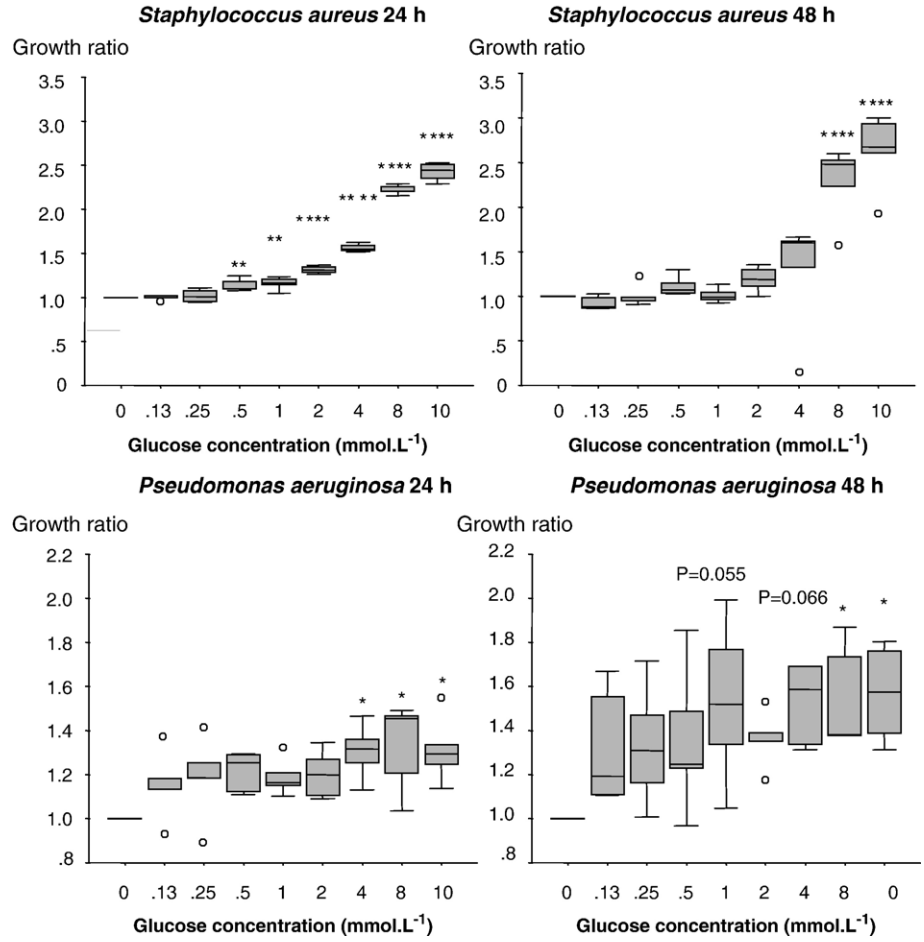

Fig. 5. Box plots of growth ratios for *Staphylococcus aureus* and *Pseudomonas aeruginosa* at different glucose concentrations compared to no added glucose after 24 and 48 h. Growth ratios were calculated by dividing OD_{620} measurements at 24 and 48 h for *Staphylococcus aureus* and *Pseudomonas aeruginosa* grown at each glucose concentration by OD_{620} for the same organism grown without glucose at each time point. Box plots indicate median, quartiles and extreme values (open circles) for OD_{620} ratios. *$p<0.05$, **$p<0.01$, ****$p<0.0001$ indicate significant differences in growth from control.

concentrations ≥ 4 mmol L^{-1} at 24 h ($p=0.039$) and at 1 mmol L^{-1} at 48 h (borderline significance, $p=0.055$) (Fig. 5).

4. Discussion

We investigated the hypothesis that airway glucose concentrations would be elevated in people with CF when blood glucose concentrations exceeded the airway threshold (≥ 8 mmol L^{-1}) identified in healthy volunteers [8] and patients on intensive care [9]. People with CF who had blood glucose ≥ 8 mmol L^{-1} had glucose in nasal secretions more often and at higher concentrations (Fig. 1) than those with blood glucose <8 mmol L^{-1}. These findings support the use of blood glucose ≥ 8 mmol L^{-1} as a surrogate marker for elevated airway glucose concentrations in CF patients. Blood glucose was ≥ 8 mmol L^{-1} for considerable proportions of the day in people with CF-related diabetes (49±25%) and for less of the day in people with CF who had normal glucose tolerance (6±5%) and in healthy volunteers (1±3%). This implies that airway glucose concentrations are elevated for around half of the day in people with CF-related diabetes.

We have previously determined the relationship between blood and nasal glucose concentrations in healthy volunteers, finding that nasal glucose was detectable above a blood glucose concentration of 7.9±1.1 mmol L^{-1} and rose in parallel with blood glucose above this concentration. Furthermore when blood glucose fell, nasal glucose was removed against a concentration gradient [8]. These observations imply that an active, saturable glucose transport process is involved in removal of glucose from the airway lumen. mRNA and protein expression of a sodium–glucose co-transporter (SGLT), which transports glucose down a sodium gradient generated by the use of ATP, has been identified in animal and human airway epithelium [13–15] and sodium glucose co-transporters contribute to glucose removal from the lung lumen in animal models [16]. Flux equations predict that movement of glucose into the lung lumen will increase as blood glucose rises [17]. SGLT activity will initially be able to increase in response to this, however when the maximum glucose transport capacity of

SGLT is exceeded luminal glucose will rise [17]. The blood glucose concentration at which this point is reached is the airway glucose threshold.

Paired blood and nasal glucose measurements in people with CF suggest that the airway glucose threshold is similar in people with CF to that previously identified in people without CF [8]. This implies that if SGLT is responsible for glucose clearance from airway secretions its function is not impaired in CF. Nasal glucose concentrations in people with CF were 0.5–3 mM, which were lower than nasal glucose concentrations in people with diabetes mellitus or intubated on intensive care [9]. This could simply be explained by differences in blood glucose concentrations between the studies, there being only 4 people in the present study with blood glucose >11 mmol L^{-1}, or by loss of activity of glucose oxidase coating sticks between studies, although all sticks were used before the manufacturers expiry date. Alternatively increased transepithelial sodium transport in people with CF could drive increased glucose clearance from CF airways by SGLT. We were unable to identify published studies of SGLT function in CF airways.

4.1. Limitations of study

We chose to measure airway glucose concentrations in nasal secretions in this study, as this is a simple, minimally-invasive technique that has been validated in our laboratory [8,9]. Glucose oxidase sticks were comparable to a glucose analyser (Analox GM9D, London, UK) in quantification of 0.5–3 mmol L^{-1} glucose in airway secretions, being less accurate outside this range [9]. This technique also changed the volume and composition of secretions less than other methods used to sample airway secretions [18]. We have previously shown strong concordance in the presence or absence of glucose between nasal and endotracheal secretions in patients intubated on intensive care ($p<0.001$), suggesting that nasal glucose can be a surrogate marker for glucose in lower airway secretions [9]. However a limitation of our study is that we did not measure lower airway glucose concentrations directly and the relationship between nasal and lower airway glucose concentrations in CF has not been established. Airway glucose concentrations are elevated where the airway epithelium is inflamed [9]. In CF, epithelial inflammation is almost invariable in pulmonary epithelium, but less prevalent in nasal epithelium [19]. The concordance between nasal and lower airway glucose may therefore be less strong in people with CF than in other groups and simple non-invasive methods for measurement of glucose in lower airway secretions are required to extend this study.

We determined the proportion of the day participants spent with blood glucose ≥ 8 mmol L^{-1} as an estimate of daily airway glucose load. Blood glucose monitoring had advantages over repeat airway glucose measurements as it did not require investigator intervention, allowing study participants to eat and take treatment normally. Repeat nasal glucose measurements could have overestimated airway glucose concentrations as nasal glucose concentrations increase after 5 or more measurements, possibly due to microscopic trauma [8]. We chose blood glucose ≥ 8 mmol L^{-1} as a surrogate marker for elevated airway glucose as the mean airway glucose threshold in healthy volunteers was 7.9 mmol L^{-1}. Although airway glucose was present more frequently and at higher concentrations in people with cystic fibrosis when blood glucose was ≥ 8 mmol L^{-1}, this threshold did not absolutely predict the presence or absence of glucose in airway secretions. As the glucose concentration of airway secretions is determined not only by the plasma–lumen glucose gradient, but also by epithelial permeability [9] and surface area and by glucose transport [17], the airway glucose threshold will vary between individuals depending on these characteristics. In healthy volunteers the airway glucose threshold varied from 6.7 to 9.7 mmol L^{-1} [8] and this variability may be similar or greater in people with CF. However as people with CF-related diabetes spent $49\pm25\%$ of the day with blood glucose ≥ 8 mmol L^{-1} and $29\pm23\%$ of the day with blood glucose >10 mmol L^{-1}, airway glucose is likely to be elevated for significant proportions of the day in this group, even allowing for individual variability in airway thresholds.

4.2. Effect of elevated airway glucose concentrations

The effect of elevated glucose concentrations in the airway is not known. We have previously shown in intubated patients on intensive care (ICU) that the presence of glucose in bronchial aspirates was significantly associated with acquisition of respiratory pathogens, particularly methicillin-resistant *S. aureus* (MRSA) [10]. We therefore determined the effect of glucose at airway concentrations on the growth of CF respiratory pathogens. Glucose at concentrations tested had an additional effect on bacterial growth in the presence of adequate supplies of other nutrients in peptone broth (Figs. 4 and 5). Growth of *S. aureus* was significantly different from control in cultures containing ≥ 0.5 mmol L^{-1} glucose at 24 h and ≥ 8 mmol L^{-1} at 48 h. Growth of *Ps. aeruginosa* was significantly different from control in cultures containing ≥ 4 mmol L^{-1} glucose at 24 h and ≥ 1 mmol L^{-1} at 48 h. Our observation that glucose affected bacterial growth after 24 h, but not 6 h of culture indicates that glucose altered the final amount but not rate of growth, although we did not assess growth rate between 6 and 24 h.

This study has a number of limitations. Optical density is an accurate method of assessing growth of both *S. aureus* and *Ps. aeruginosa* in laboratory culture [12], but is unable to distinguish between living (potentially pathogenic) and dead organisms. Furthermore studies of organisms *in vitro* may miss variations in virulence that can occur during infection *in vivo* [20]. Glucose stimulated bacterial growth in nutrient peptone broth, but may have different effects in airway secretions containing different nutrients, antimicrobial enzymes and immune proteins. Glucose was added to

cultures at the beginning of the experiment and not replenished, probably accounting for the effect of low glucose concentrations on *S. aureus* growth at 24 h, but not at 48 h. As people with CF-related diabetes spend 49±25% of the day with blood glucose above the airway glucose threshold, airway glucose concentrations may constantly be topped up *in vivo*. Culture conditions may also have accounted for the different effects of glucose on Staphylococcal and Pseudomonal growth. Cultures were stationary and only agitated for resuspension during measurement of optical density, which may have limited their aeration. These conditions may have been more suitable for growth of *S. aureus*, which is a facultative anaerobe [21], than for *Ps. aeruginosa,* which is strictly aerobic [22]. Future studies of the effects of glucose on Pseudomonal growth should ensure aeration, better to mimic conditions in human lung.

Despite their limitations our results raise the possibility that glucose in the airways could promote or sustain pathogen growth, allowing development of pulmonary infection, and may have contributed to the acquisition of MRSA in ICU patients [10]. The relevance of this is less clear in patients with cystic fibrosis. In a cross-sectional analysis of 7566 patients on the European Epidemiologic Registry of Cystic Fibrosis the difference in lung function between diabetic and non-diabetic CF patients was not linked to presence or absence of any specific pathogen in the lower respiratory tract [5]. However Lanng and colleagues found that after insulin therapy, the percentage of sputum examinations positive for *Haemophilus influenzae* and *Streptococcus pneumoniae* decreased in diabetic patients [23]. The effect of glucose or diabetes on the quantity or behaviour of bacteria in CF airways is not known. In laboratory culture glucose increased transcription of the algD gene and alginase production by a mucoid *Ps. aeruginosa* strain [24].

Elevated glucose concentrations in the airway could have effects in the lungs of CFRD patients other than through promoting bacterial growth. Increased glucose concentrations could impair host immune function. Decreased neutrophil and macrophage chemotaxis, phagocytosis and killing and impairment in complement and cytokine responses to infection have been well documented in patients with diabetes mellitus [25]. Immunoglobulins [26] and collectins [27], readily undergo non-enzymatic glycosylation when glucose concentrations are elevated, and these modifications have been associated with decreased efficacy against infection [26,28]. Alternatively elevated glucose concentrations could upregulate the inflammatory response to pulmonary infection. Experimental elevation of blood glucose stimulated acute increases in plasma IL-6, TNF-α and IL-8 in volunteers and septic patients [29–31]. Diabetes has also been associated with biochemical and structural changes in the lung, including increased levels of advanced glycation end-products, derangement of bronchial mucus production and changes in the basement membranes of alveolar and bronchial epithelium and pulmonary capillaries [32].

4.3. Implications

In CF patients, airway glucose concentrations are elevated when blood glucose exceeds the airway threshold (≥ 8 mmol L^{-1}) and are correlated with blood glucose concentrations. As patients with CF-related diabetes spend almost half of the day with blood glucose above the airway threshold, airway glucose concentrations may be persistently elevated in people with CF-related diabetes. Additionally CF patients with glucose intolerance will also have blood glucose above the airway threshold prior to the development of diabetes for between 6% (CF-normal glucose tolerance) and 48% (CF-related diabetes) of the day.

Pulmonary decline in cystic fibrosis accelerates up to 6 years prior to the development of diabetes and the rate is proportional to the degree of glucose intolerance. Further studies are required to determine whether elevated airway glucose concentrations contribute to this accelerated pulmonary decline by increasing airway bacterial load, promoting inflammation or through other mechanisms. However if glucose in airway secretions truly contributes to acceleration of pulmonary decline, then treatment targets for diabetes and glucose intolerance in patients with CF [33] may need to be revised.

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