Effect of hyperglycaemia on glucose concentration of human nasal secretions

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ABSTRACT

Glucose is not detectable in airways secretions of normoglycaemic volunteers, but is present at 1–9 mmol·l⁻¹ in airways secretions from people with hyperglycaemia. These observations suggest the existence of a blood glucose threshold at which glucose appears in airways secretions, similar to that seen in renal and salivary epithelia. In the present study we determined the blood glucose threshold at which glucose appears in nasal secretions. Blood glucose concentrations were raised in healthy human volunteers by 20% dextrose intravenous infusion or 75 g oral glucose load. Nasal glucose concentrations were measured using modified glucose oxidase sticks as blood glucose concentrations were raised. Glucose appeared rapidly in nasal secretions once blood glucose was clamped at approx. 12 mmol·l⁻¹ (n = 6). On removal of the clamp, nasal glucose fell to baseline levels in parallel with blood glucose concentrations. An airway glucose threshold of 6.7–9.7 mmol·l⁻¹ was identified (n = 12). In six subjects with normal glucose tolerance, blood glucose concentrations rose above the airways threshold and nasal glucose became detectable following an oral glucose load. The presence of an airway glucose threshold suggests that active glucose transport by airway epithelial cells normally maintains low glucose concentrations in airways secretions. Blood glucose exceeds the airway threshold after a glucose load even in people with normal glucose tolerance, so it is likely that people with diabetes or hyperglycaemia spend a significant proportion of each day with glucose in their airways secretions.

INTRODUCTION

Airway surface liquid, the thin layer of fluid that overlies the airway epithelium, is critical for lung defence. The volume and composition of this liquid are tightly controlled and appear to determine its function. Little is known about the regulation of glucose concentrations in airway surface liquid, but we have shown recently [1] that glucose is not normally present in nasal secretions from volunteers with normal blood glucose; however, glucose was present in nasal secretions from 18 out of 20 people with diabetes mellitus attending a follow-up clinic, at concentrations from 1–9 mmol·l⁻¹. Glucose was also present in lower airways secretions from people with stress hyperglycaemia intubated on our intensive care unit. All patients with blood glucose > 10 mmol·l⁻¹, but only four patients with blood glucose < 7 mmol·l⁻¹, had glucose in lower airways secretions.

Our observations [1] suggest that there may be a blood glucose threshold, above which glucose appears in upper and lower airways secretions. Blood glucose thresholds have been determined previously for appearance of glucose in urine and saliva [2–4]. Glucose appears in the urine of patients with Type I and Type II diabetes at blood glucose concentrations ranging from 6.0–14.3 mmol·l⁻¹ [2,3]. This 'renal threshold' can be explained by glucose

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transport processes in the renal tubule. At blood glucose concentrations below the renal threshold, glucose entering the urine by glomerular filtration is completely reabsorbed from the urine by glucose transport proteins. At blood glucose concentrations above the renal threshold, the capacity of the glucose transporters for reabsorption is exceeded, hence, glucose appears in the urine.

Glucose homoeostasis in epithelial tissues may serve not only to preserve glucose from excretion, but also to maintain low glucose concentrations in body fluids as part of defence against infection. People with diabetes mellitus have glucose in urine and saliva and are more likely than those without diabetes to develop urinary tract infections and dental decay [5,6]. Morbidity and mortality from pulmonary infection is greater in people with diabetes mellitus than in those with normal blood glucose [7–9]. Although the mechanisms underlying this predisposition are undoubtedly complex, glucose in airways secretions could contribute to the risk of pulmonary infection.

The aim of the present study was to investigate the relationship between glucose concentrations in blood and airways secretions in healthy volunteers, in particular to determine whether there is a blood glucose threshold at which glucose appears in airways secretions (‘airways threshold’). We have shown previously [1] that nasal (upper airways) glucose concentrations are tightly related to glucose concentrations in lower airways secretions. We therefore used the nose as a model for the airway and determined the effect of changes in blood glucose concentrations on nasal glucose concentrations.

**MATERIALS AND METHODS**

**Subjects**

Subjects were healthy volunteers attending St George’s Hospital Medical School, who were recruited by advertisement. Subjects were included in the study if they had a random blood glucose measurement <7.0 mmol·l⁻¹ and no glucose present in their nasal secretions. Subjects were excluded if they had rhinitis, a history of nasal trauma or nose bleeds, diabetes mellitus or a first-degree family relative with diabetes, asthma or other respiratory disease, serious underlying medical condition, pregnancy or oral or nasal drug use. All studies were approved by the Local Research Ethics Committee at St George’s Hospital and were in accordance with the Declaration of Helsinki. All subjects gave written informed consent for their inclusion in the study.

**Nasal glucose measurement**

Glucose concentrations in nasal airway secretions were measured using glucose oxidase sticks as described previously [1]. Briefly, nasal secretions were sampled by placement of trimmed glucose oxidase sticks (Roche, Lewes, East Sussex, U.K.) in direct contact with the nasal mucosa of the inferior turbinate under direct vision for 30 s. The sticks were then removed, checked for adequate coating with nasal secretions and read in comparison with a visual colour indicator chart. Measurements were made in alternate nostrils to minimize trauma to the nasal mucosa. We have shown previously [1] that glucose measurements made with these sticks are comparable with measurements made by the Analox GM9D analyser (London, U.K.) in lower airways secretions at glucose concentrations of 1–3 mmol·l⁻¹.

**Blood glucose measurement**

Arterialized whole blood was sampled from cannulated warmed hand veins using an established technique [10]. Arterialization was confirmed by ensuring that oxygen saturation of venous samples was >85 % using a blood gas analyser (ABL2000; Radiometer, Copenhagen, Denmark). Glucose concentrations were measured in whole blood using an Analox GM9D glucose analyser. This uses a platinum electrode to measure oxygen consumption by glucose oxidase during glucose metabolism. The coefficient of variation with the Analox analyser is 2 % at 5 mmol·l⁻¹ (S.D., 0.07–0.26) and the sensitivity is 0.01 mmol·l⁻¹.

**Hyperglycaemic clamping**

A modified hyperglycaemic clamp technique using a 20 % dextrose infusion alone was used to control blood glucose concentrations. Blood glucose concentrations were raised from non-fasting baseline concentrations to the initial desired glucose concentration for each protocol using a weight-dependent, rapid glucose infusion, based on the De Fronzo algorithm [11]. Blood glucose concentrations were subsequently controlled by measurement of blood glucose concentrations at 5 min intervals with adjustment of infusion rate to achieve the desired concentration.

**Oral glucose tolerance test (OGTT)**

A 2 h OGTT was performed after an overnight fast. A glucose load of 75 g of anhydrous glucose was dissolved in 250 ml of water and consumed over a 5 min period [12].

**Study protocols**

**Control studies**

Nasal glucose measurements may cause minor trauma and stimulate production of nasal secretions. We therefore determined the effect of repeat nasal glucose measurement and stimulation of production of nasal secretions on the glucose concentration of nasal secretions (nasal glucose). To determine the effect of repeated measurements on nasal glucose concentrations, nasal glucose was measured in alternate nostrils every 10 min for eight measurements. To determine the effect of increased volume of nasal
secretions on nasal glucose concentrations, nasal secretions were stimulated without direct manipulation of the nasal mucosa by oral stimulation with capsaicin for 5 min [13]. Nasal glucose concentrations were measured prior to the study and 1 min after stopping capsaicin stimulation.

**Temporal relationship between blood and nasal glucose concentrations**

The modified hyperglycaemic clamp technique was used to raise blood glucose concentrations rapidly from baseline to 12 mmol·l⁻¹ and maintain blood glucose at 12 mmol·l⁻¹ for 20 min. The hyperglycaemic clamp was then terminated and blood glucose concentrations allowed to fall to baseline. Nasal glucose measurements were made at 10 min intervals throughout the study.

**Identification of the blood glucose concentration at which glucose appears in nasal secretions (airways glucose threshold)**

Blood glucose concentrations were raised from baseline in 1 mmol·l⁻¹ increments and nasal glucose concentrations were measured either immediately on reaching each new concentration (protocol A) or after 15 min at each concentration (protocol B). In both protocols the airways threshold was defined as the blood glucose concentration at which glucose became detectable in nasal secretions.

**Effect of high blood glucose concentrations on nasal glucose concentrations**

Blood glucose was raised from baseline to 10–12 mmol·l⁻¹, then in further 2 mmol·l⁻¹ steps to a maximum of 18–20 mmol·l⁻¹. Blood glucose was maintained for 15 min at each concentration before nasal glucose measurements were made.

**Effect of oral glucose load on nasal glucose concentrations**

Arterialized blood and nasal glucose concentrations were measured at baseline, then at 10 min intervals for 2 h following ingestion of a 75 g glucose load.

**Statistical analysis**

Values are given as means ± S.D. Differences between variables were tested using an unpaired Student t test. Two-tailed P values of < 0.05 were considered significant.

**RESULTS**

**Subjects**

Demographic characteristics (age, random blood glucose and body mass index) of the subjects recruited for each of the study protocols are shown in Table 1.

**Control studies**

**Effect of repeated nasal glucose measurements on nasal glucose concentrations**

No glucose was detected in nasal secretions on initial measurement in four subjects. Nasal glucose was detected consistently after five repeat measurements in all four subjects. A trace of glucose was found on the second measurement in one subject and on the fourth measurement in a second subject. The maximum detected glucose concentration in nasal secretions following repeat measurement at normal blood glucose concentrations was 0.9 ± 0.5 mmol·l⁻¹ (Figure 1). Subjects experienced a slight increase in volume of nasal secretions and nasal irritation with sneezing following repeat measurements.

**Effect of oral capsaicin stimulation on volume and composition of nasal secretions**

In five subjects, oral capsaicin stimulation produced a marked increase in the volume of nasal secretions on visual inspection. Glucose was not detected in nasal secretions from any subject either before or 1 min after capsaicin stimulation.

**Temporal relationship between blood and nasal glucose concentrations**

Blood glucose concentrations were raised rapidly from baseline (5.1 ± 1.0 mmol·l⁻¹) to 12 mmol·l⁻¹ (Figure 1) and maintained at this concentration for a further 20 min before the hyperglycaemic clamp was removed and

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**Table 1  Demographic characteristics of subjects recruited for each protocol**

<table>
<thead>
<tr>
<th></th>
<th>Timing studies</th>
<th>A</th>
<th>B</th>
<th>High blood glucose clamping</th>
<th>OGTT</th>
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<td>n</td>
<td>6</td>
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<td>6</td>
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<tr>
<td>Age (years)</td>
<td>26.3 ± 5.0</td>
<td>25.7 ± 3.0</td>
<td>25.9 ± 2.3</td>
<td>26.2 ± 3.4</td>
<td>25.3 ± 2.9</td>
</tr>
<tr>
<td>Random glucose (mmol·l⁻¹)</td>
<td>5.1 ± 1.0</td>
<td>5.0 ± 0.6</td>
<td>6.1 ± 0.7</td>
<td>5.5 ± 0.5</td>
<td>5.4 ± 0.4</td>
</tr>
<tr>
<td>Body mass index (kg·m⁻²)</td>
<td>23.2 ± 5.0</td>
<td>25.3 ± 2.7</td>
<td>25.1 ± 4.5</td>
<td>22.6 ± 1.2</td>
<td>22.0 ± 1.8</td>
</tr>
</tbody>
</table>
Temporal relationship between changes in blood and nasal glucose concentrations

Blood glucose concentrations (■) were raised by hyperglycaemic clamping and nasal glucose concentrations (▲) were measured. Control nasal glucose measurements (●) are shown. Values are mean of six (hyperglycaemic clamp) or four (control) experiments and error bars show S.D.

Identification of an airways glucose threshold

Six subjects underwent protocol A and six different subjects underwent protocol B. The mean blood glucose concentration at which glucose became detectable in nasal airway secretions was 8.6 ± 0.7 mmol·l⁻¹ (range, 7.3–9.4 mmol·l⁻¹) for protocol A and 7.9 ± 1.1 mmol·l⁻¹ (range, 6.7–9.7 mmol·l⁻¹) for protocol B. There was no significant difference between airways glucose thresholds identified by the two different methods (P = 0.51). Figure 2 shows that, in protocol B, each subject had at least one negative nasal glucose measurement before the airways glucose threshold was identified.

Effect of high blood glucose concentrations on nasal glucose concentrations

In six subjects, blood glucose concentrations were raised from baseline (5.5 ± 0.5 mmol·l⁻¹) to 11.4 ± 0.4 mmol·l⁻¹ and then, in 2 mmol·l⁻¹ steps, to 19.1 ± 0.4 mmol·l⁻¹. Figure 3 shows the relationship between nasal and blood glucose concentrations at higher blood glucose concentrations.

Effect of oral glucose load on nasal glucose concentrations

Six subjects had a normal OGTT, with baseline and 2 h blood glucose concentrations of <6.1 and
Effect of oral glucose load on nasal glucose concentrations

Blood (■) and nasal (△) glucose concentrations were measured at 10 min intervals following a 75 g OGTT. Values are mean of six experiments and error bars show S.D. Dotted line represents the mean threshold for the detection of nasal glucose from protocol B.

< 7.8 mmol·l⁻¹ respectively [12]. However, in all subjects, blood glucose concentrations exceeded the airways glucose threshold range of 6.7–9.7 mmol·l⁻¹ (Figure 4). Nasal glucose concentrations rose as blood glucose concentrations increased and fell to control concentrations as blood glucose concentrations returned to baseline.

DISCUSSION

We have shown previously [1] that glucose can be detected in airways secretions from people with diabetes mellitus and stress hyperglycaemia. In the present study, we have elucidated the dynamic relationship between blood and nasal glucose concentrations in normal volunteers. We have shown that, when blood glucose concentrations are raised from baseline to 12 mmol·l⁻¹, glucose moves into airways secretions within 10 min. Furthermore, nasal glucose concentrations fall within 10 min of a fall in blood glucose concentration. An ‘airways glucose threshold’ exists, since glucose does not appear in airways secretions until blood glucose reaches a concentration of 6.7–9.7 mmol·l⁻¹. Above the airways glucose threshold, nasal glucose concentrations increase almost in parallel with blood glucose concentrations up to a blood glucose concentration of 20 mmol·l⁻¹. Even people with normal glucose tolerance have blood glucose concentrations above the airways glucose threshold and glucose in airways secretions after an oral glucose load.

These observations imply that glucose can move readily into and out of airways secretions, and that the glucose content of airways secretions is actively regulated. Renal glucose physiology suggests mechanisms that could also underlie glucose homeostasis in the airway. In the kidney, glucose moves into the urine by glomerular filtration, where it is at concentrations similar to blood glucose concentrations. If blood glucose concentrations are normal, glucose transporters, such as sodium-glucose co-transporters (SGLT), in the proximal tubular epithelium are able to reabsorb filtered glucose completely. However, if glucose concentrations in blood and urinary filtrate are raised above the capacity of the transporters to reabsorb glucose (renal threshold), then glucose appears in the urine.

In the nose, glucose moves down a concentration gradient from blood to luminal secretions. It is likely that this is a passive process which could be accounted for by paracellular or facilitated transcellular glucose diffusion. Paracellular permeability of airway epithelium has been demonstrated previously by transepithelial movement of mannitol across rat whole lung, excised bovine nasal mucosa and human cultured nasal epithelium [14–16]. Airway epithelium was also permeable to l-glucose, a glucose analogue which is not carried by glucose transporters [14,17,18]. It is not yet known whether facilitated diffusion could account for passive glucose movement from blood to airway lumen. In rat intestine, after 10 days of streptozotocin-induced diabetes, expression of the facilitative transporter GLUT2 was increased in jejunal brush border membranes [19]. If apical GLUT2 expression is increased in airway epithelium in response to hyperglycaemia, then facilitated diffusion could account for movement of glucose into airways secretions as blood glucose rises.

As blood glucose always exceeds nasal glucose, active processes must be responsible for removal of glucose from airways secretions. The presence of an airways glucose threshold implies that, like renal glucose transport, airways glucose reabsorption becomes saturated. Sodium glucose transporters capable of saturable glucose absorption against a glucose gradient could account for airways glucose reabsorption, and there is some evidence that these transporters are present in airway epithelium. mRNA transcripts encoding SGLT isoforms have been identified in rat (SGLT 1 and 2) whole lung tissue and rat alveolar type II cells (SGLT 1) [20–22]. Sodium-dependent phloridzin-inhibited glucose transport, characteristic of SGLT transport, has been demonstrated in fetal sheep and isolated rat whole lungs [14,23]. Phloridzin-inhibited sodium transport has also provided some evidence of SGLT activity in human tracheal tissue in vitro [24]. Alternatively, glucose could be removed from airways secretions by mucociliary clearance. However, glucose concentrations in nasal secretions fall more rapidly than could be attributed to mucociliary clearance [25], and glucose is cleared from lower airways secretions in intensive care patients where ciliary function may be paralysed by opiates and other drugs [26].
It is important to consider methods used for measuring nasal glucose and for raising blood glucose when interpreting the results of the present study. Nasal glucose measurements were made using a trimmed glucose oxidase stick, wetted with nasal secretions by direct application to the nasal mucosa and read by comparison with a visual colour indicator chart. We optimized the accuracy of this technique, firstly, by applying the stick to the nasal mucosa under direct vision and, secondly, by visual inspection of the stick after sampling to ensure wetting had occurred. Where the stick remained dry it was discarded and sampling was repeated to ensure that negative results were due to glucose-free secretions rather than sampling error. We chose to measure glucose in nasal secretions directly by this method rather than by stimulating and collecting secretions for laboratory analysis as all measures used to stimulate secretion change the nature of the secretions [27]. Glucose oxidase sticks were designed for measurement of glucose concentrations in blood, not airways secretions. However, we have shown previously [1] that the strips reliably indicate the presence or absence of glucose and accurately measure low concentrations of glucose (<3 mmol·l⁻¹) in airways secretions. Glucose oxidase sticks are less accurate at high glucose concentrations (>3 mmol·l⁻¹) and may not have measured higher nasal glucose concentrations accurately in the high glucose protocol.

In previous studies, we have detected 1–2 mmol·l⁻¹ glucose in nasal secretions from normoglycaemic people with nasal epithelial inflammation due to viral colds [1]. We were therefore concerned that repeat nasal glucose measurements might themselves increase nasal glucose through microtrauma or by stimulating production of glucose-rich secretions. Our control studies showed that, although glucose does appear consistently in nasal secretions after repeat measurements, at least five measurements were required and the maximum nasal glucose concentration was only 0.9 ± 0.5 mmol·l⁻¹. As stimulation of nasal secretions with oral capsaicin did not increase nasal glucose concentrations, we concluded that repeat measurements increase nasal glucose concentrations through trauma, although this must be microscopic as no damage could be seen on visual inspection and nasal samples were not blood stained. Protocols were designed to minimize the number of nasal glucose measurements made to ensure that glucose detected, particularly in the threshold studies, was attributable to changes in blood glucose rather than trauma. Figure 2 illustrates the number of nasal glucose measurements made before the airways glucose threshold was detected using protocol B. In five subjects, nasal glucose was detected long before glucose could have appeared through trauma.

We used a modified hyperglycaemic clamp technique to control blood glucose concentrations. Blood glucose was raised or maintained using a variable rate of 20% dextrose infusion. This technique was sufficient to raise blood glucose accurately in 1 mmol·l⁻¹ steps and to maintain blood glucose at each concentration. In the high glucose experiments, we increased blood glucose concentrations in 2 mmol·l⁻¹ steps to reduce the duration of the experiment for the volunteers. We did not attempt to clamp or control plasma insulin concentrations in any of the experiments and it is likely that these rose considerably as blood glucose concentrations were increased in healthy volunteers. It is not clear whether insulin affects glucose homoeostasis in the airways, although in rat alveolar type II cells insulin increased cellular uptake of 2-deoxyglucose by 67.8 ± 15.7% in a dose-dependent manner [28].

Two different protocols were used to determine the airways glucose threshold. In protocol A, subjects had blood glucose concentrations raised in 1 mmol·l⁻¹ steps and nasal glucose was measured immediately on achieving each blood glucose concentration. Our timing studies showed that nasal glucose changes within 10 min of a change in blood glucose. We were, therefore, concerned that protocol A may have overestimated the airways glucose threshold. In protocol B, blood glucose concentrations were, therefore, maintained at each concentration before measurement of nasal glucose. This method ensured that changes in nasal glucose were complete before measurements were made and should have given a more accurate estimate of the airways glucose threshold. However, airways glucose thresholds measured by protocols A and B were not significantly different.

The airway glucose threshold for normal volunteers identified in the present study ranged from 6.7–9.7 mmol·l⁻¹ (n = 12). These values are similar, but not identical, to those obtained in patients with diabetes for the renal threshold (Type I, 6.0–14.3 mmol·l⁻¹, n = 23; Type II, 6.2–12.3 mmol·l⁻¹, n = 24) [2,3] and the salivary glucose threshold (10–15 mmol·l⁻¹, n = 11) [4]. Differences in thresholds may purely reflect the small number of subjects studied. Alternatively, differences may reflect the ease of sampling of different body fluids or diversity of study populations. We measured the airways threshold in healthy volunteers, whereas renal and salivary thresholds were determined in people with established diabetes mellitus. Chronic hyperglycaemia could alter epithelial glucose thresholds, for example, by changing epithelial permeability through basement membrane thickening [29,30].

Our OGTT results show that even people with normal glucose tolerance briefly achieve blood glucose concentrations above the airways glucose threshold and have glucose transiently in their nasal secretions after a glucose load. People with diabetes mellitus are, therefore, likely to exceed the airways glucose threshold and have glucose in airways secretions for a considerable proportion of the day.
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REFERENCES


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