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 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 the lower respiratory tract of human volunteers. 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 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 disease [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.

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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 diabetics 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 2.4 × 10⁻² Torr pressure (E2M2 vacuum pump, 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.

Protocols

Repeatability studies. WITHIN DAY. Breath glucose was measured in 10 healthy volunteers four to five times over 1 h on a single day. BETWEEN DAYS. Breath glucose was measured in six healthy volunteers once on each of 2–3 separate days.

Effect of changes in salivary glucose on breath glucose concentrations. Healthy volunteers underwent baseline salivary and breath glucose measurement, and then they rinsed their mouths with 75 g glucose dissolved in 100 ml water (4.2 M). Salivary glucose was remeasured before and after further breath glucose measurements. Participants then underwent three cycles of water mouthwash, salivary glucose measurement, and breath glucose measurement. Blood glucose was measured at the beginning and end of the experiment.

Effect of changes in blood glucose on breath glucose concentrations. Blood glucose concentrations were altered in healthy volunteers by a modified hyperglycemic clamp technique using 20% dextrose infusion (26). Blood glucose was measured in arterialized whole blood sampled from cannulated warmed hand veins using an Analox GM9D glucose analyzer (26). Arterialization was confirmed in all samples by oxygen saturation >85% (model ABL2000, Radiometer, Copenhagen, Denmark). Blood glucose was initially raised from nonfasting baseline to target glucose concentrations for each protocol using a weight-dependent, rapid glucose infusion based on the De Fronzo algorithm (7). Blood glucose was measured at 5-min intervals with adjustment of infusion rate to achieve subsequent target concentrations. Blood glucose was held at each target concentration for 15 min to allow equilibration of glucose concentrations between blood and respiratory fluid before commencing breath glucose measurements.

GLUCOSE RAMP. In six healthy volunteers, blood glucose was elevated from baseline in 1–2 mmol/l increments up to 12 mmol/l, and breath glucose was measured at each concentration.

RISE AND FALL. In six separate healthy volunteers breath glucose was elevated rapidly from baseline to ~14 mmol/l, the clamp was removed, and blood glucose fell back to baseline. Breath glucose was measured before, during, and after blood glucose elevation.

Effect of chronic hyperglycemia (diabetes mellitus) and lung inflammation (CF) on breath glucose concentrations. Healthy volunteers, diabetes mellitus patients, CF patients without diabetes, and CF patients with diabetes (CFRD) underwent capillary blood and breath glucose measurements over a 10-min period. Breath glucose values were divided by blood glucose to calculate the breath-to-blood glucose ratio.

Analysis

Normally distributed values are given as means (SD) and compared using unpaired or paired t-tests and between more than two independent groups using one-way analysis of variance with post hoc Bonferroni analysis. Two × two between-groups analysis of covariance was used to determine the effects of CF, diabetes mellitus, and their interaction on breath glucose concentrations. Nonnormally distributed variables are given as median (interquartile range) and compared between more than two independent groups using Kruskal Wallis tests. Categorical variables were compared between groups using χ² tests. P < 0.05 was considered significant. Statistical package for the social sciences version 11.5 was used for analysis.

RESULTS

Assay Sensitivity

The glucose assay used in this study had a sensitivity of 0.01 μmol/l glucose and lower limit of glucose detection of 0.001 μmol/l. Glucose was detected at ≥0.001 μmol/l in 194 of 195 condensate samples tested and was ≥0.01 μmol/l in 183 of
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 \([\text{change} = -0.05 \text{ mmol/l (SD 0.22); } P = 0.602]\). After repeat water mouthwashes salivary glucose fell back to 0 mmol/l (SD 0) \((P < 0.0001)\), but breath glucose remained unchanged \([0.46 \text{ mmol/l (SD 0.27); } P = 0.875]\). 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 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 \((\eta^2 = 0.538, P < 0.0001)\) and diabetes mellitus \((\eta^2 = 0.307, P < 0.0001)\) independently determined breath glucose concentrations. Additionally, there was a small interaction effect for CF with diabetes mellitus \((\eta^2 = 0.074, P = 0.047)\).

![Fig. 1. Repeat breath glucose measurements on the same day (A) and between days (B). Lines represent repeat breath glucose measurements for individual participants.](http://jap.physiology.org/doi/10.1152/jappl.00021.2007)
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).

Blood glucose ratios 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 ≥0.001 μmol/l in 194 of 195 condensate samples tested and to quantify condensate glucose accurately (requiring glucose concentrations ≥0.01 μmol/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
Factors Affecting Lung Glucose Concentrations

### 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></td>
</tr>
<tr>
<td>Sex, (male:female)</td>
<td>13:10</td>
<td>10:7</td>
<td></td>
<td></td>
<td></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, μmol/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, μmol/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.

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 previously 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) are similar to 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 αL-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...
glucose rises can be 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 phlorizin, which blocks sodium-glucose cotransporter (SGLT) (1, 22). Our laboratory has demonstrated mRNA and protein for SGLT and phlorizin-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 99mTc-labeled diethylenetriamine pentaacetic acid 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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**GRANTS**

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