Supplementary Material

Hyperglycaemia in CF adversely affects BK channel function critical for mucus clearance

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Supplementary methods

Cell culture

Culturing of human bronchial epithelial cells (HBECs) at the air-liquid interface (ALI) was performed as described [1]. For non-CF lungs, HBECs were thawed at 37°C, resuspended in warm bronchial epithelial cell growth medium (BEGM), and spun at 360 x g for 5 min at room temperature. The supernatant was discarded, and the cell pellet resuspended in BEGM with Amphotericin B (10 µg/mL). The density of viable cells plated in a 10 cm dish was approximately 1-2 x 10^6. Once the cells reached confluence, they were trypsinized and plated on Transwell and/or Snapwell inserts at a minimum of 150,000 cells/cm^2 in ALI media. Preparation of ALI media is adapted from published methods [1] and made with 50:50 Dulbecco's Modified Eagle Medium (DMEM):LHC Medium supplemented with 0.87 µM insulin (#660001; FeF Chemicals, Denmark), 0.1986 µM hydrocortisone (#H0396; MilliporeSigma, Burlington, MA, USA), 0.01 µM triiodothyronine (#T6397; MilliporeSigma), 0.125 µM transferrin (#2914-HT; R&D Systems, Minneapolis, MN, USA), 0.5 µM phosphorylethanolamine (#P0505; MilliporeSigma), 0.5 µM ethanolamine (#E0135; MilliporeSigma), 10 µg/mL bovine pituitary extract (Animal Technologies, Tyler, TX, USA), 0.5 mg/mL bovine serum albumin (#A7638; MilliporeSigma), trace elements [1], stock 4 [1], stock 11 [1], 2.73 µM epinephrine (#E4642; MilliporeSigma), 0.05 µM retinoic acid (#R2625; MilliporeSigma), 0.5 ng/mL epidermal growth factor (#354001; BD Biosciences, San Jose, CA, USA), and 100U-100µg/mL penicillin/streptomycin (#15140-122; ThermoFisher Scientific, Waltham, MA, USA). Low (5.5 mM) glucose DMEM (#11885) and high (25 mM) glucose DMEM (#11995) were acquired from ThermoFisher Scientific. A 50:50 solution of low glucose DMEM and LHC medium (5.5 mM glucose) was used to make 5.5 mM glucose ALI media. A 50:50 solution of high glucose DMEM and LHC medium was used to make high

glucose ALI media that we measured at 12.5 mM glucose. For CF lungs, HBECs were expanded in PneumaCult™-Ex Plus Medium (STEMCELL Technologies, Cambridge, MA, USA) before trypsinizing and plating on Transwell inserts. HBECs were maintained submerged for 4-6 days before exposing them to air and allowing them to re-differentiate for a minimum of three weeks before experiments were performed. ALI media was replaced every Monday, Wednesday, and Friday once HBECs were exposed to air.

Ussing chamber

Cystic fibrosis transmembrane conductance regulator (CFTR) and large conductance, Ca^{2+}-activated and voltage-dependent K^+ channel (BK) activities were recorded in Ussing chambers as previously described [2, 3]. Briefly, ENaC currents were blocked by 10 μM amiloride (MilliporeSigma) before CFTR currents were stimulated by 10 μM forskolin (MilliporeSigma) and inhibited by 10 μM $CFTR_{inh}$-172 (MilliporeSigma). Prior to measuring BK currents, the basolateral membranes were permeabilized with 20 μM amphotericin B (MilliporeSigma), 10 μM Nigericin (Tocris Bioscience, Minneapolis, MN, USA), and 10 μM Valinomycin (Tocris Bioscience). The cells were exposed to a K^+ gradient of 140 mM at the permeabilized basolateral side and 5 mM at the apical side. After ENaC currents were blocked by 10 μM amiloride, BK currents were stimulated by 10 μM ATP (Tocris Bioscience). The transepithelial membrane potential was clamped at 0 mV (model VCC MC8; Physiologic Instruments, San Diego, CA, USA), using Ag/AgCl electrodes in agar bridges. Signals were digitized and recorded using the Acquire and Analyze revision II module according to the manufacturer's instructions. The experiments were performed at 37°C using heated water jackets and bubbled with a gas mixture of 95% O_2 / 5% CO_2 to maintain physiological pH in the apical and basolateral buffers for CFTR

currents. Apical and basolateral buffers for measuring BK currents are adjusted to pH 7.4 and buffers are circulated in the Ussing chambers with air [4]. Ussing chamber experiments were performed 24 h after apical wash and basolateral media change unless otherwise noted. CFTR and BK measurements in NHBE cells on Snapwell inserts were conducted using 1.12 cm^2 aperture sliders (#P2302; Physiologic Instruments). BK measurements in CFBE cells on Transwell inserts were conducted using 0.10 cm^2 aperture sliders (#P2303; Physiologic Instruments), which may account for the larger BK currents observed in these cells. For mallotoxin experiments, 5 μM mallotoxin (#R5648; MilliporeSigma) was added 10 min after amiloride. For paxilline and TRAM-34 experiments, 10 μM paxilline (#P2928; MilliporeSigma), 1 μM TRAM-34 (#T6700; MilliporeSigma), or DMSO (control) was added 5 min after amiloride and 10 min before ATP stimulation.

Nasal cells collection

Nasal cells were collected using sterile cytology brushes (Medical Packaging Corporation, Camarillo, CA, USA). The brushes were introduced into the nasal cavity under direct visual guidance and were placed between the nasal septum and the inferior turbinate. No anesthesia was used. The cells were harvested by a few careful backward-forward and rotary movements before twirling the brush into 5 mL of sterile PBS in a 15 mL tube to release the cells. The same procedure was repeated 3 times in each nostril. Immediately after the harvest, the tube was centrifuged at 360 x g for 5 min at 4°C. The supernatant was discarded, and the remaining pellet was frozen at -80°C until qPCR experiments were performed.

Subject criteria

Inclusion criteria:

i. Patients with cystic fibrosis older than age 18 years.

ii. Subjects selected for CFRD group will have a positive OGTT

Exclusion criteria:

i. Nasal glucocorticoid use the day of Visit 1 or systemic glucocorticoid use within the past 7 days

ii. Angiotensin receptor blocker (ARB) use within the past 7 days

Withdrawal/Termination criteria:

A subject withdrawal is defined as a discontinuation from the study for any reason. Subjects may withdraw or be withdrawn from this study for the following reasons:

- As by their own request or at the request of their authorized representative at any time for any reason
- If continuation in the study would be detrimental to the subject's well-being, in the investigator's opinion

Table S1. Non-CF lung donor information.

Sex	Age
Female	15
Female	19
Female	38
Female	49
Female	49
Female	51
Female	57
Male	19
Male	21
Male	21
Male	22
Male	38
Male	38
Male	46
Male	52
Male	55
Male	56
Male	65
Male	67

Table S2. CF lung donor information.

Sex	Age	CFTR Mutation
Female	17	F508del/R1162X
Female	21	R1162X/unknown
Female	27	F508del/G542X
Female	36	F508del/F508del
Male	27	F508del/F508del
Male	29	F508del/W1145X
Male	45	F508del/F508del
Male	54	F508del/F508del

Table S3. Baseline participant characteristics.

	CF (n=5)	CFRD (n = 12)	Difference (95% CI)
Age (mean ± SEM)	22 ± 0.7	32 ± 0.5	10 (5.3, 14.7)
Female sex (%)	3 (0.6)	4 (0.3)	0.3 (-0.2, 0.8)
BMI	21.8 ± 0.48	23.6 ± 0.35	1.83 (0.49, 3.16)
F508del / F508del (%)	2 (0.4)	6 (0.5)	-0.1 (-0.41, 0.61)
F508del / minimum function (%)	3 (0.6)	4 (0.3)	0.3 (-0.2, 0.8)
CFTR modulator use (%)*	3 (0.6)	7 (0.58)	0.02 (-0.49, 0.53)
Hemoglobin A1C (mean ± SEM)	5.4 ± 0.09	6.8 ± 0.12	-1.4 (0.47, 2.33)
Baseline % predicted FEV1 (mean ± SEM)**	98% ± 2.1	64% ± 1.7	-34 (-50.77, -17.23)

Differences are expressed as difference in mean or proportion between CF and CFRD with 95% confidence intervals.
* Includes ivacaftor, lumacaftor/ivacaftor, tezacaftor/ivacaftor and elexacaftor/tezacaftor/ivacaftor.
** Based upon highest % predicted FEV1 in the 12 months preceding study enrollment.

Table S4. Comparison of CGM variables between groups

	CF (n = 5)	CFRD (n = 12)	Difference (95% CI)
Length of CGM recording (mean ± SEM)*	5 ± 2.3	7.3 ± 2.4	-2.3 (-4.99, 0.39)
Glucose management indicator	6.1 (5.9, 6.7)	7.3 (6.6, 7.9)	1.2 (0.2 to 2)
Mean amplitude of glycemic excursions	63.1 (36, 92)	115.2 (69, 129)	52.1 (-1.1 to 80.2)
% time glucose >140mg/dL	16.4 (9.3, 37.5)	60.5 (34.4, 79.8)	44.1 (11.7 to 62.1)
% time glucose >200mg/dL	2.1 (0.4, 12.6)	25.3 (6.8, 42.1)	23.2 (2.0 to 41.5)
Average AUC glucose > 180mg/dL	12848 (1590, 58853)	166523 (46199, 323090)	153675 (16235 to 333345)
Number of glucose excursions >200mg/dL	18 (6, 25)	27.5 (19.8, 31.3)	9.5 (-1 to 23)

Values reported as median (25^{th} percentile, 75^{th} percentile), differences between medians with 95% confidence intervals

Supplementary Figure S1.

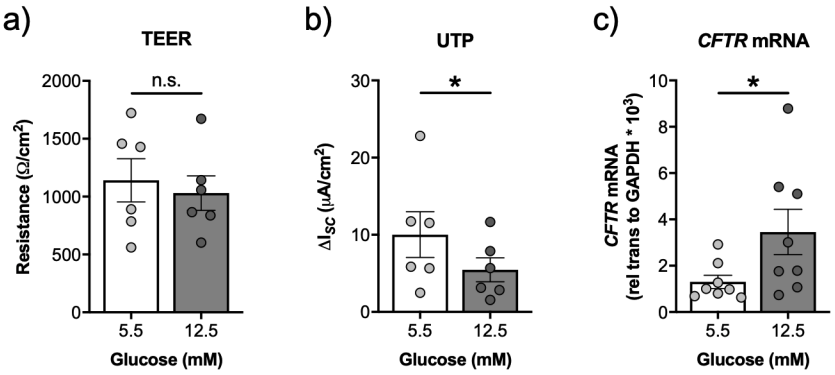

FIGURE S1. Effects of high glucose on transepithelial electrical resistance (TEER), calcium-activated chloride channel (CaCC) conductance, and *CFTR* mRNA expression in NHBE cells *in vitro*. a) TEER, as measured in Ussing chambers, is not significantly different between NHBE cells cultured in ALI media with starting glucose concentrations of 5.5 mM or 12.5 mM (n=6 lungs). **b)** CaCC current, as measured by UTP-stimulated I_{SC} in Ussing chambers, is significantly decreased in NHBE cells cultured in 12.5 mM glucose compared to NHBE cells cultured in 5.5 mM glucose (n=6 lungs). **c)** Expression of *CFTR* mRNA is significantly increased in NHBE cells cultured in 12.5 mM glucose compared to NHBE cells cultured in 5.5 mM glucose (n=8 from 6 lungs). * $p < 0.05$, Student's t-test. n.s.=not significant. Data are shown as mean ± S.E.M.

Supplementary Figure S2.

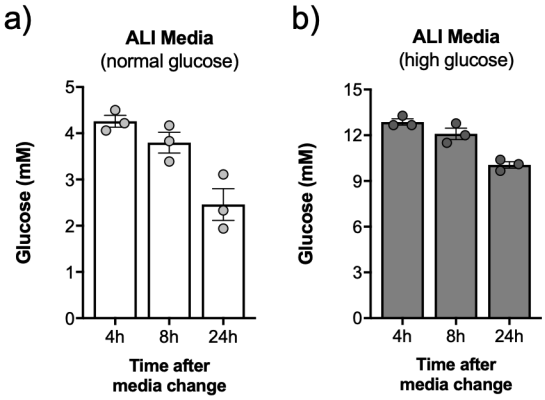

FIGURE S2. Time-course measurement of glucose concentrations in the basolateral media. **a**) Measurement of glucose levels in the basolateral media over time of CFBE cells cultured in ALI media with a starting glucose concentration of 5.5 mM (n=3 CF lungs). **b**) Measurement of glucose levels in the basolateral media over time of CFBE cells cultured in ALI media with a starting glucose concentration of 12.5 mM (n=3 CF lungs). Data are shown as mean ± S.E.M.

Supplementary Figure S3.

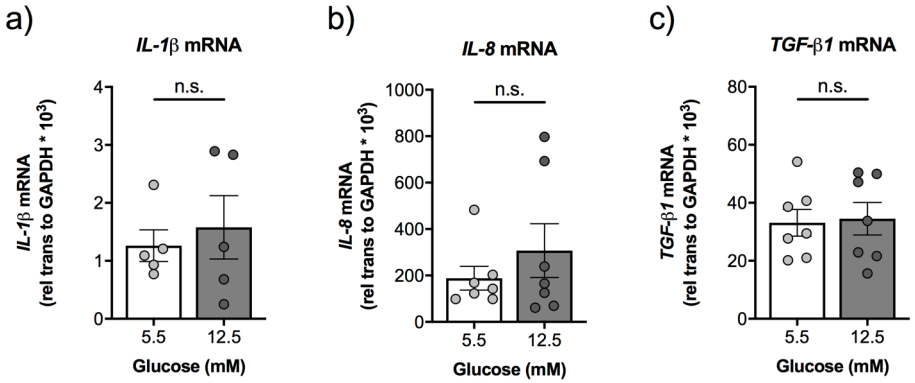

FIGURE S2. mRNA expression levels of *IL-1β*, *IL-8*, and *TGF-β1* do not change in response to elevated glucose in CFBE cells *in vitro*. a-c) mRNA expression levels of *IL-1β* (**a**), *TGF-β1* (**b**), and *IL-8* (**c**) are not significantly different between CFBE cells cultured in ALI media with starting glucose concentrations of 5 mM or 12.5 mM. n.s.=not significant, Wilcoxon or Student's t-test. Data are shown as mean ± S.E.M.

Supplementary Figure S4.

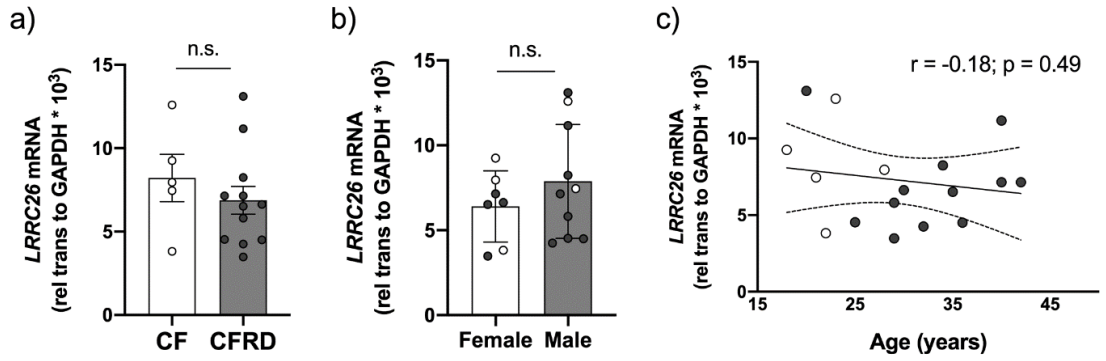

Figure S4. Comparison of clinically relevant covariates lack correlation with *LRRC26* expression *in vivo*. There was no statistically significant difference between *LRRC26* expression and (**a**) CF (open circles) and CFRD (shaded circles), (**b**) sex and (**c**) participant age ($p > 0.05$ for all). n.s.=not significant.

References

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