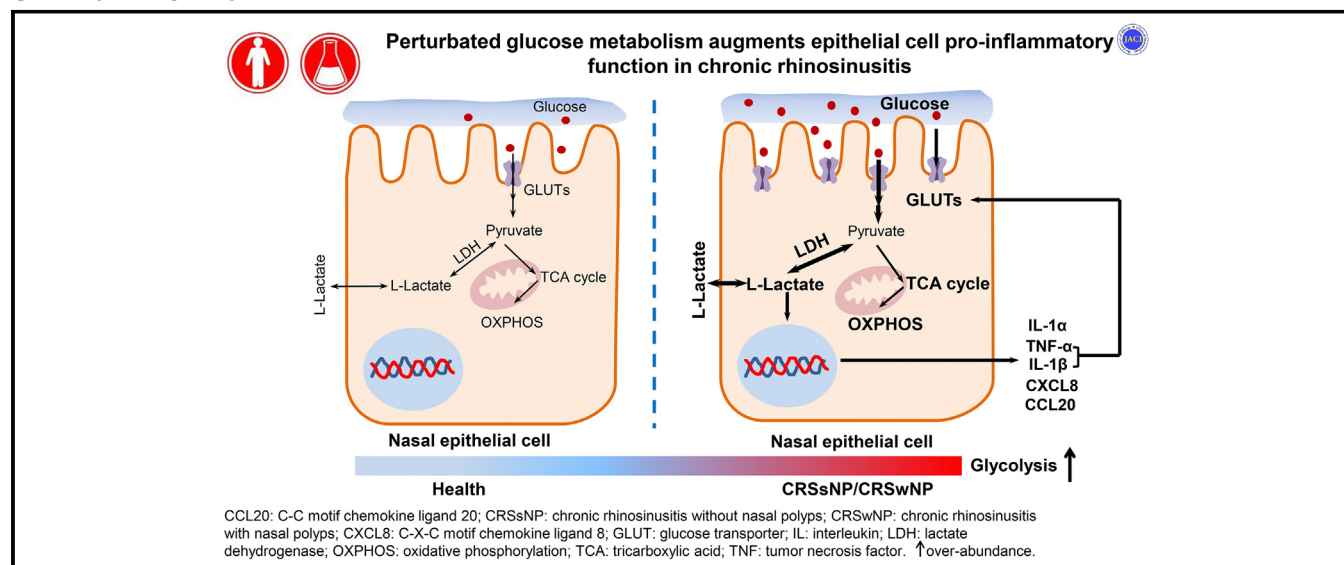


Perturbed glucose metabolism augments epithelial cell proinflammatory function in chronic rhinosinusitis



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GRAPHICAL ABSTRACT



Background: Glucose concentrations are increased in nasal secretions in chronic rhinosinusitis (CRS). However, the glucose metabolism and its contribution to disease pathogenesis in CRS remain unexplored.

Objectives: We sought to explore the glucose metabolism and its effect on the function of nasal epithelial cells in CRS with and without nasal polyps (CRSwNP and CRSsNP).

Methods: Glucose metabolites were detected with mass spectrometry. The mRNA levels of glucose transporters (GLUTs), metabolic enzymes, and inflammatory mediators were detected by quantitative RT-PCR. The protein expression of GLUTs was studied by immunofluorescence staining,

Western blotting, and flow cytometry. Glucose uptake was measured by using fluorescent glucose analog. Human nasal epithelial cells (HNECs) were cultured. Bioenergetic analysis was performed with Seahorse XF analyzer. Gene expression in HNECs was profiled by RNA sequencing.

Results: Increased glucose concentrations in nasal secretions was confirmed in both CRSsNP and CRSwNP. GLUT4, GLUT10, and GLUT11 were abundantly expressed in HNECs, whose expression was upregulated by inflammatory cytokines and D-glucose and was increased in CRS. Glucose uptake, glycolysis and tricarboxylic acid cycle metabolites, metabolic enzymes, and extracellular acidification rate and oxygen

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consumption rates were increased in HNECs in CRSsNP and CRSwNP, with a predominant shift to glycolysis. HNECs treated with high-level apical D-glucose showed enhanced glucose uptake, predominant glycolysis, and upregulated production of IL-1 α , IL-1 β , TNF- α , CCL20, and CXCL8, which was suppressed by 2-deoxy-D-glucose, an inhibitor of glycolysis.

Conclusions: Increased glucose in nasal secretions promotes glucose uptake and predominant glycolysis in epithelial cells, augmenting the proinflammatory function of epithelial cells in CRS. (J Allergy Clin Immunol 2023;151:991-1004.)

Key words: Chronic rhinosinusitis, epithelial cell, function, glucose, glycolysis, inflammation

Chronic rhinosinusitis (CRS) is an inflammatory condition of the paranasal sinuses and includes 2 main phenotypes, CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP).^{1,2} The pathophysiology of CRS is complicated and orchestrated by immune cells (eg, eosinophils, neutrophils, and lymphocytes) and structural cells (eg, epithelial cells and stromal cells).³⁻⁶ Nasal epithelial cells not only compose the first line of defense, but also, by releasing an array of cytokines, chemokines, and damage-associated molecular patterns, they are an active driver of inflammation in sinonasal mucosa.⁷⁻⁹ Despite recent progresses in understanding the importance of epithelial cells in CRS, investigation of the regulation of their function remains limited.

Metabolism fuels diverse biological programs, ranging from development, proliferation, differentiation, and the effector functions performed by cells and tissues.¹⁰ Glucose, one of the most fundamental sugar elements, has either D- or L-conformation. D-glucose is central to energy consumption and is transported into cells by glucose transporters (GLUTs) or sodium-dependent glucose transporters (SGLTs) along a concentration gradient.¹¹ Once glucose enters the cells, it is rapidly metabolized by hexokinase into D-glucose 6-phosphate, which then generates pyruvate with the help of pyruvate kinase.¹² Under the condition of hypoxia, pyruvate produces lactate by lactate dehydrogenase. While in the presence of oxygen, pyruvate enters the mitochondria for the tricarboxylic acid (TCA) cycle. Nicotinamide adenine dinucleotide and flavin adenine dinucleotide generated by the TCA cycle donate electrons to the electron transport chain for oxidative phosphorylation (OXPHOS).¹³ Nevertheless, it is increasingly recognized that inflammatory activation can occur in the context of changes in cellular metabolism toward aerobic glycolysis (the Warburg effect), in which glucose is metabolized to lactate in the presence of abundant oxygen.^{14,15}

In health, glucose concentrations in the airway surface liquid (ASL) are maintained at 0.4 mmol/L, around 12 times lower than those in blood (4.9-6.1 mmol/L).¹⁶ However, in a variety of airway pathologies, including cystic fibrosis,^{16,17} chronic obstructive pulmonary disease,¹⁸ and CRS,¹⁹ glucose concentrations in ASL are increased due to the disruption of epithelial cell tight junction and increase in paracellular glucose flux. Elevated glucose concentrations in nasal secretions have been implicated in the inhibition of bitter taste receptor-mediated innate defense in CRS.¹⁹ However, it is unclear whether increased glucose in ASL affects metabolism of nasal epithelial cells and subsequently modifies their function and shapes the inflammatory response in CRS. Recently, the IL-1/inhibitory κ B kinase signaling axis has

Abbreviations used

2-DG:	2-Deoxy-D-glucose
2-NBDG:	2-Deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl) amino]-D-glucose
ALI:	Air-liquid interface
ASL:	Airway surface liquid
CRS:	Chronic rhinosinusitis
CRSsNP:	Chronic rhinosinusitis without nasal polyps
CRSwNP:	Chronic rhinosinusitis with nasal polyps
ECAR:	Extracellular acidification rate
Foxj1:	Forkhead box protein j 1
GLUT:	Glucose transporter
HNEC:	Human nasal epithelial cell
OCR:	Oxygen consumption rate
OXPHOS:	Oxidative phosphorylation
PIMs:	Proinflammatory mediators
SGLT:	Sodium-dependent glucose transporter
SLC:	Solute carrier family
TCA:	Tricarboxylic acid

been reported to mediate house dust mite-induced glycolysis in a murine asthma model,²⁰ implicating a role of disturbed glucose metabolism in the pathogenesis of airway diseases. This study aimed to depict the glucose metabolic characteristics of nasal epithelial cells in patients with CRS and to investigate the effect of high-level D-glucose in ASL on glucose metabolism and function of nasal epithelial cells.

METHODS

Subjects and sample collection

This study was approved by the Ethics Committee of Tongji Hospital and conducted with written informed consent from all participants. A total of 99 control subjects, 188 patients with CRSwNP, and 152 patients with CRSsNP were enrolled. CRSwNP and CRSsNP were diagnosed according to European and American guidelines.^{1,2} Control subjects were those undergoing septoplasty because of anatomic variations and without other sinonasal diseases. Subjects with diabetes were excluded. Human nasal epithelial cells (HNECs) were scraped from middle meatus of patients with CRSsNP and control subjects and from polyp of patients with CRSwNP, respectively.²¹ Nasal secretions were collected using sinus packs as previously described.¹⁹ Not all samples were included in every experiment protocol because of limited quantity (see Table E1 in this article's Online Repository at www.jacionline.org).

Histology, immunohistochemistry, and immunofluorescence staining

Hematoxylin-eosin staining, immunohistochemistry, and immunofluorescence staining with specific primary (see Table E2 in this article's Online Repository at www.jacionline.org) and secondary antibodies (see Table E3 in this article's Online Repository at www.jacionline.org) were performed as previously reported.^{22,23}

Glucose and L-lactate level measurement

Nasal secretion glucose concentrations were measured using glucose colorimetric assay kit (Cell Biolabs, San Diego, Calif). Blood glucose concentrations were measured by the wet chemistry method on the Roche Cobas 8000 (Roche Diagnostics, Mannheim, Germany). L-lactate levels in cell culture supernatants were measured using the lactate assay kit (Abcam, Cambridge, UK).

Quantitative RT-PCR

Quantitative RT-PCR was performed with specific primers (see Table E4 in this article's Online Repository at www.jacionline.org).²⁴ Beta-actin was used as a housekeeping gene for normalization.

Western blotting

Western blotting analysis was performed with corresponding antibodies (see Table E5 in this article's Online Repository at www.jacionline.org).²¹

ELISA

The concentrations of cytokines and chemokines in cell lysis were measured with commercial ELISA kits.²⁴

HNEC culture

HNECs obtained from normal subjects were cultured with an air-liquid interface (ALI) method and treated with D-glucose and cytokines.^{25,26} For the purpose of comparing the differences among the control, CRSwNP, and CRSsNP groups, HNECs were cultured in submerged condition and the primary cells were used to retain the disease-specific feature.

Glucose uptake assay

Glucose uptake capacity was evaluated by using the fluorescent glucose 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl) amino]-D-glucose (2-NBDG; Invitrogen Thermo Fisher Scientific Company, Waltham, Mass) as mentioned elsewhere.²⁷ The cells treated with 2-NBDG were subject to flow cytometry with excitation light at 488 nm.

Flow cytometry

Cell staining and flow cytometric analysis were performed with corresponding antibodies (see Table E6 in this article's Online Repository at www.jacionline.org).²⁸

Bioenergetic analysis

To investigate glycolysis and OXPHOS levels of HNECs, the glycolysis-stress and mito-stress test kits (Agilent Technologies, Santa Clara, Calif) with Seahorse XF24 analyzer (Agilent Technologies) were used to monitoring extracellular acidification rate (ECAR) and oxygen consumption rate (OCR), respectively.²⁹

Metabolite measurement

Metabolomic profiles of nasal epithelial cells were measured using targeted ultraperformance liquid chromatography–mass spectrometry/mass spectrometry as previously stated.^{30,31}

RNA sequencing

Total RNA was extracted from cultured HNECs using TRIzol (TaKaRa Biotechnology, Dalian, China). Library construction and sequencing were performed by Novogene Technology (Beijing, China). All primary data have been uploaded to the Sequence Read Archive (National Center for Biotechnology Information) under the accession number PRJNA812068.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 7.0 (GraphPad Software, La Jolla, Calif). Data distribution was tested for normality using the Kolmogorov-Smirnov test. For continuous variables of *in vivo* data, results are represented in dot plots unless specifically indicated. Symbols represent individual samples; horizontal bars represent medians; and error bars show interquartile ranges. Kruskal-Wallis test with the Dunn *post hoc* test was used to

adjust for multiple comparisons. For categorical variables, a chi-square test or Fisher exact test was applied to determine differences between groups. Spearman test was performed for correlation analysis. Cell culture data are shown as mean $\pm$ SEM and were analyzed by unpaired Student *t*-test or 1-way ANOVA with the Tukey *post hoc* test. Significance was accepted at $P < .05$.

For RNA sequencing analysis, the gene expression was compared between control and treated groups using DESeq R package (version 1.26.0; R Foundation, Vienna, Austria), and genes with P adjusted value $< .05$ and $|\log_2$ (fold change)| ≥ 1 between 2 groups were considered to have significantly differential expressions.³²

More detailed information on the materials and methods used in this study is available in this article's Online Repository (available at www.jacionline.org).

RESULTS

Predominately exaggerated glycolysis in nasal epithelial cells in CRS

Confirming a previous study,¹⁹ we found that the glucose concentrations in nasal secretions were higher in both patients with CRSwNP and those with CRSsNP than in control subjects, with a further increase in patients with CRSsNP compared with in patients with CRSwNP (Fig 1, A). No correlation between nasal secretion and blood glucose concentrations was found in control, CRSsNP, or CRSwNP groups (Fig 1, B). The blood glucose concentrations were in normal range for all subjects and no difference was observed among the 3 subject groups (see Fig E1 in this article's Online Repository at www.jacionline.org).

To explore whether the increased levels of glucose in ASL would affect glycolysis and OXPHOS of epithelial cells, we first detected the glucose metabolic intermediates and enzymes (Fig 1, C) in nasal epithelial cells from different subject groups. Compared with those in control subjects, we found that the levels of the products of glycolysis (D-glucose 6-phosphate, dihydroxyacetone phosphate, glyceraldehyde 3-phosphate, 3-phosphoglycerate, phosphoenolpyruvate, and L-lactate) and intermediates in the TCA cycle (fumarate and L-malate) were increased in epithelial cells from patients with CRSwNP and those with CRSsNP (Fig 1, D and E). No significant difference in pyruvate and some other metabolites was found among 3 subject groups (see Fig E2 in this article's Online Repository at www.jacionline.org). Notably, the L-lactate–pyruvate ratio was higher in patients with CRSsNP and those with CRSwNP than in control subjects (Fig 1, E), suggesting a predominant shift toward glycolysis in nasal epithelial cells in CRS. In addition, the mRNA levels of a number of genes of glycolytic enzymes (*HK1*, *HK2*, *PFKFB3*, *PFKM*, *BPGM*, *PKM*, and *LDHA*) were increased in nasal epithelial cells from patients with CRSwNP and those with CRSsNP compared with those in control subjects, while only 1 gene of enzymes of TCA cycle (*PDHX*) was upregulated in CRS (Fig 1, F and G and see Fig E3 in this article's Online Repository at www.jacionline.org), again indicating a predominant activation of glycolysis pathway in nasal epithelial cells in CRS.

Enhanced expression of GLUTs in nasal epithelial cells in CRS and their expression regulation by inflammatory mediators

Glucose uptake into cells is mediated by glucose transport proteins including GLUT and SGLT family members. We found that *GLUT1* (*SLC2A1*), *GLUT4* (*SLC2A4*), *GLUT10* (*SLC2A10*),

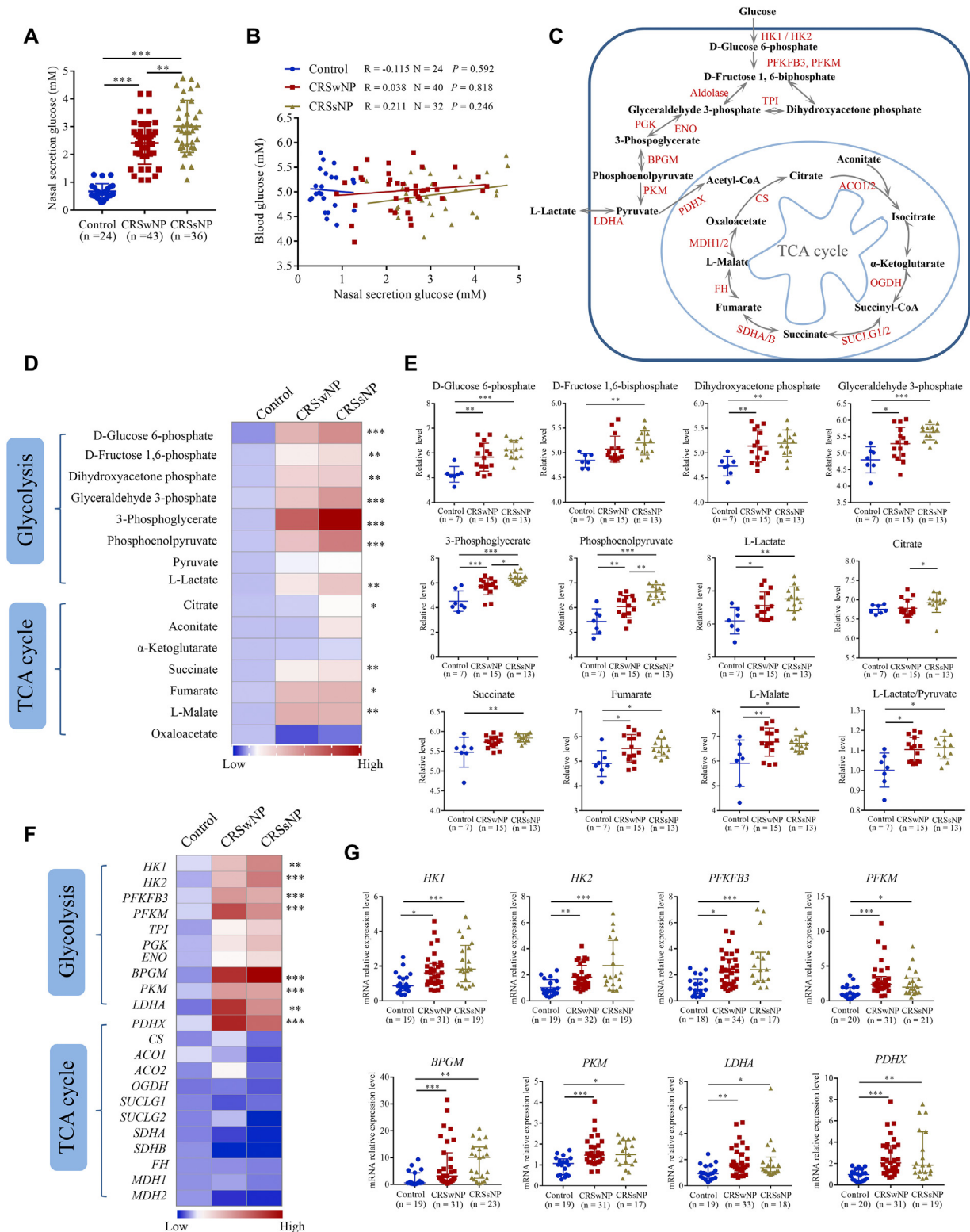


FIG 1. Predominant metabolic shift to glycolysis in the nasal epithelial cells of patients with CRS. **A**, Elevated glucose concentrations in nasal secretions of patients with CRS. **B**, No correlation between blood and nasal secretion glucose concentrations. **C**, Schematic diagram of glucose metabolism in cells, including intermediates (black) and metabolism-related enzymes (red). **D**, Heat map showing the relative abundance of metabolites in glycolysis and TCA cycle in nasal epithelial cells. **E**, Relative levels of glucose metabolites having differential abundance among the 3 study groups. **F**, Heat map showing the relative mRNA levels of genes of glycolysis- and TCA cycle-related enzymes in nasal epithelial cells. **G**, Relative mRNA levels of genes encoding glycolysis and the TCA cycle-associated enzymes with differential expression levels among the 3 study groups. * $P < .05$; ** $P < .01$; *** $P < .001$. ENO, Enolase; TPI, triosephosphate isomerase.

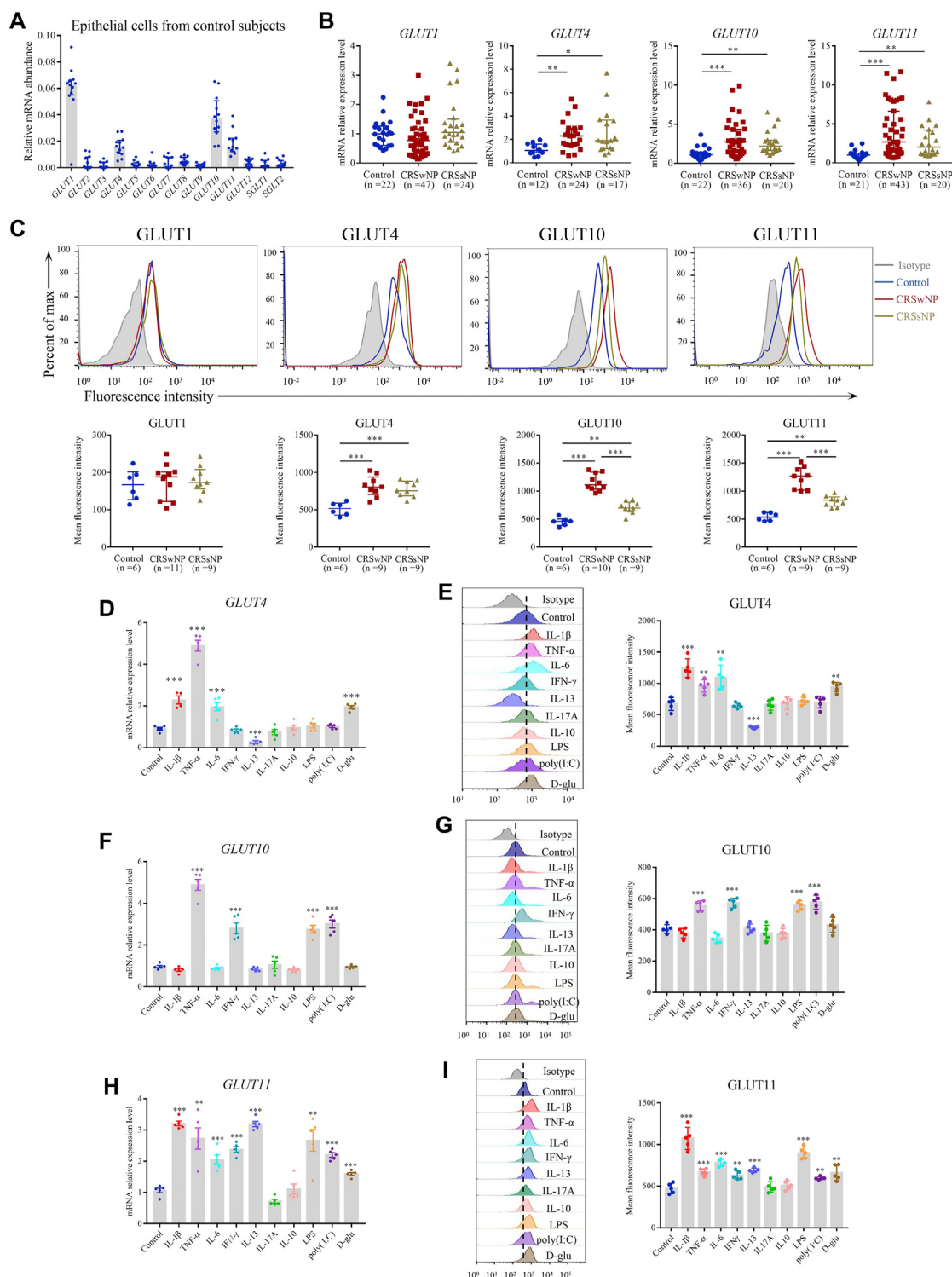


FIG 2. Elevated expression of GLUTs in nasal epithelial cells of patients with CRS. **A**, The mRNA abundance of *GLUT* (*SLC2A*) and *SGLT* (*SLC5A*) family members in nasal epithelial cells from control subjects ($n = 12$). **B**, The mRNA expression levels of genes of *GLUT1* (*SLC2A1*), *GLUT4* (*SLC2A4*), *GLUT10* (*SLC2A10*), and *GLUT11* (*SLC2A11*) in nasal epithelial cells from different study groups. **C**, Dispersed nasal tissue cells were prepared and cell surface expression of GLUT1, GLUT4, GLUT10, and GLUT11 on nasal epithelial cells was detected by flow cytometry. The representative flow cytometric histograms are shown. **D-I**, HNECs from control subjects were cultured with ALI method and stimulated with various cytokines, pathogen mimics, or D-glucose (D-glu). Twenty-four hours after treatment, the cells were subject to detection of the mRNA expression levels of *GLUT4* (*SLC2A4*, **D**), *GLUT10* (*SLC2A10*, **F**), and *GLUT11* (*SLC2A11*, **H**) by quantitative RT-PCR ($n = 5$). Forty-eight hours after treatment, the cells were subject to detection of cell surface expression of GLUT4 (**E**), GLUT10 (**G**), and GLUT11 (**I**) by flow cytometry ($n = 5$). * $P < .05$; ** $P < .01$; *** $P < .001$. poly(I:C), Polyinosinic:polycytidylic acid.

and *GLUT11* (*SLC2A11*) genes were most abundantly expressed in nasal epithelial cells in all 3 study groups (Fig 2, A and see Fig E4, A and B in this article's Online Repository at www.jacionline.org) at mRNA levels. Immunofluorescence staining confirmed the expression of GLUT1, GLUT4, GLUT10, and GLUT11 proteins in epithelial cells in nasal tissues from control subjects (Fig E4, C). We found that, except for GLUT1, the mRNA and protein levels of GLUT4, GLUT10, and GLUT11 in nasal epithelial cells were increased in patients with CRSwNP and those with CRSsNP compared with those from control subjects as detected by RT-PCR and Western blotting, respectively (Fig 2, B and Fig E4, D). Notably, the cell surface expression of these GLUT isoforms was confirmed in nasal epithelial cells by flow cytometry, which was also upregulated in patients with CRS (Fig 2, C). The gating strategies are shown in Fig E5, A-C in this article's Online Repository (available at www.jacionline.org). Moreover, patients with CRSwNP had higher protein expression of GLUT10 and GLUT11 than patients with CRSsNP did (Fig 2, C and Fig E4, D).

We then explored potential reasons for the upregulation of epithelial GLUT4, GLUT10, and GLUT11 in CRS. We treated ALI cultured HNECs obtained from control subjects with CRS-relevant cytokines, pathogen mimics, and D-glucose. We found that *GLUT4* (*SLC2A4*) mRNA expression was induced by IL-1 β , TNF- α , IL-6, and D-glucose (Fig 2, D); *GLUT10* (*SLC2A10*) mRNA expression was increased by TNF- α , IFN- γ , LPS, and polyinosinic:polycytidylic acid (Fig 2, F); and *GLUT11* (*SLC2A11*) mRNA levels were up-regulated by IL-1 β , TNF- α , IL-6, IFN- γ , IL-13, LPS polyinosinic:polycytidylic acid, and D-glucose (Fig 2, H). Flow cytometric analysis confirmed the up-regulation of GLUT4, GLUT10, and GLUT11 protein expression on cell surface by those stimulators (Fig 2, E, G, and I). The gating strategies are shown in Fig E5, D.

Localization of GLUT4, GLUT10, and GLUT11 expression in epithelium in sinonasal tissue

We further localized the GLUT4, GLUT10, and GLUT11 expression in different types of epithelial cells in sinonasal mucosa from patients with CRS and found that Foxj1⁺ ciliated cells, Mucin 5AC⁺ goblet cells, and p63⁺ basal cells all had obvious GLUT4, GLUT10, and GLUT11 expression (Fig 3). In addition, immunoreactivity of GLUT4, GLUT10, and GLUT11 was also observed in infiltrating cells and endothelial cells in lamina propria (Fig 3).

Enhanced glucose uptake, glycolysis, and OXPHOS in HNECs from patients with CRS

The upregulated epithelial expression of GLUTs in CRS prompted us to compare the difference in glucose uptake by HNECs from control subjects and patients with CRS. Primary HNECs from different subject groups were cultured, submerged to retain the disease-specific feature, and treated with 2-NBDG, which cannot be metabolized by cells. We found that glucose uptake was significantly enhanced in HNECs from patients with CRSwNP and those with CRSsNP compared with those from control subjects, with a more prominent increase in CRSwNP (Fig 4, A), which is consistent with the expression pattern of epithelial GLUTs in different groups.

We next compared bioenergetics in HNECs from different study groups using a Seahorse XF24 analyzer platform. An

increase in ECAR in response to the introduction of D-glucose reflects the basal glycolysis level (Fig 4, B). Under oligomycin treatment, which is an inhibitor of ATP synthase, the cells exert nearly full glycolytic capacity (Fig 4, B). 2-Deoxy-D-glucose (2-DG) is an inhibitor of glycolysis (Fig 4, B). In comparison with those from control subjects, the primary HNECs from patients with CRSwNP and those with CRSsNP demonstrated increased basal glycolysis, glycolysis capacity, and glycolysis reserve (Fig 4, B and C), indicating enhanced activation of glycolysis in nasal epithelial cells in CRS.

The OXPHOS in HNECs was evaluated by measuring OCR (Fig 4, D). In response to fluorocarbonyl cyanide phenylhydrazide, an uncoupling agent that collapses the proton gradient, the cells manifest maximum oxygen consumption (Fig 4, D). We found that primary HNECs from patients with CRSwNP and those with CRSsNP had increased maximal respiration and spare respiratory capacity compared to HNECs from control subjects (Fig 4, E), suggesting that OXPHOS was also enhanced in CRS. In line with the higher glucose uptake ability, primary HNECs from patients with CRSwNP, compared with those from patients from CRSsNP, showed further enhanced glycolysis and OXPHOS.

Surprisingly, we found that basal OCR/ECAR ratio was increased in submerged cultured primary HNECs from patients with CRS, suggesting a dependence more likely on OXPHOS (Fig 4, F). This is in contrast to the preferentially exaggerated glycolysis in nasal epithelial cells in CRS *in vivo* (Fig 1). This discrepancy may be caused by the submerged culture condition of HNECs, which may be unable to recapture all the metabolic features *in vivo*.

High-level apical glucose promotes glycolysis in HNECs

To study metabolic response to altered ASL glucose levels under a more physiological condition,³³ HNECs from control subjects were cultured with ALI method and stimulated with D-glucose at high level (2.5 mmol/L, consistent to the level in ASL in patients with CRS) or normal level (0.5 mmol/L, consistent to the level in ASL in control subjects) at the apical surface. We found the after high-level apical D-glucose treatment, the apical (Fig 5, A), but not basolateral (Fig 5, B), uptake of 2-NBDG was enhanced in HNECs compared to those treated with normal-level D-glucose. Moreover, we discovered that high-level D-glucose-treated HNECs had higher basal glycolysis level and glycolysis capacity than those treated with normal level D-glucose (Fig 5, C and D). In addition, the maximal respiration, spare respiratory capacity, and ATP production were all elevated in HNECs treated with high-level D-glucose (Fig 5, E and F). Notably, the basal OCR-ECAR ratio was decreased in HNECs treated with high-level D-glucose (Fig 5, G), indicating that ALI-cultured HNECs were dependent more on glycolysis than on OXPHOS in response to high-level ASL glucose.

High-level apical glucose induces proinflammatory mediator production in HNECs through glycolysis

In line with the results of bioenergetic analysis, we found that both glycolysis metabolites (D-fructose 6-phosphate, glyceraldehyde 3-phosphate, 2-phosphoglycerate, 3-phosphoglycerate, and L-lactate) and TCA cycle metabolites (aconitate, succinate,

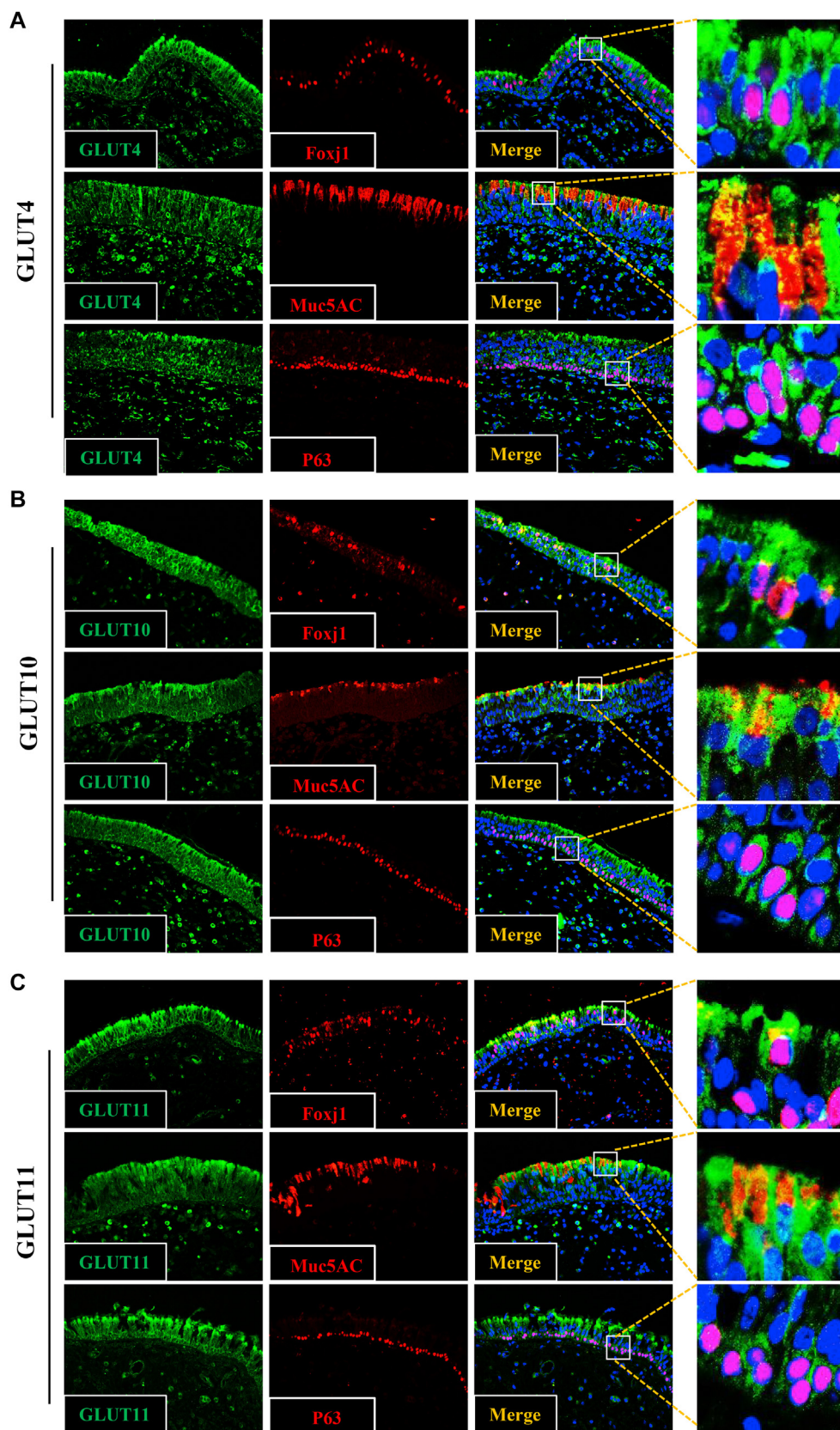


FIG 3. The immunoreactivity of GLUT4, GLUT10, and GLUT11 in different cell types in epithelium of sinonasal mucosa. Representative immunofluorescence staining of sinonasal tissue sections from patients with CRS demonstrates the immunoreactivity of GLUT4 (A), GLUT10 (B), and GLUT11 (C) in Foxj1⁺ ciliated cells, Mucin 5AC⁺ goblet cells, and p63⁺ basal cells (original magnification $\times 400$). The right images show higher magnification of the outlined area.

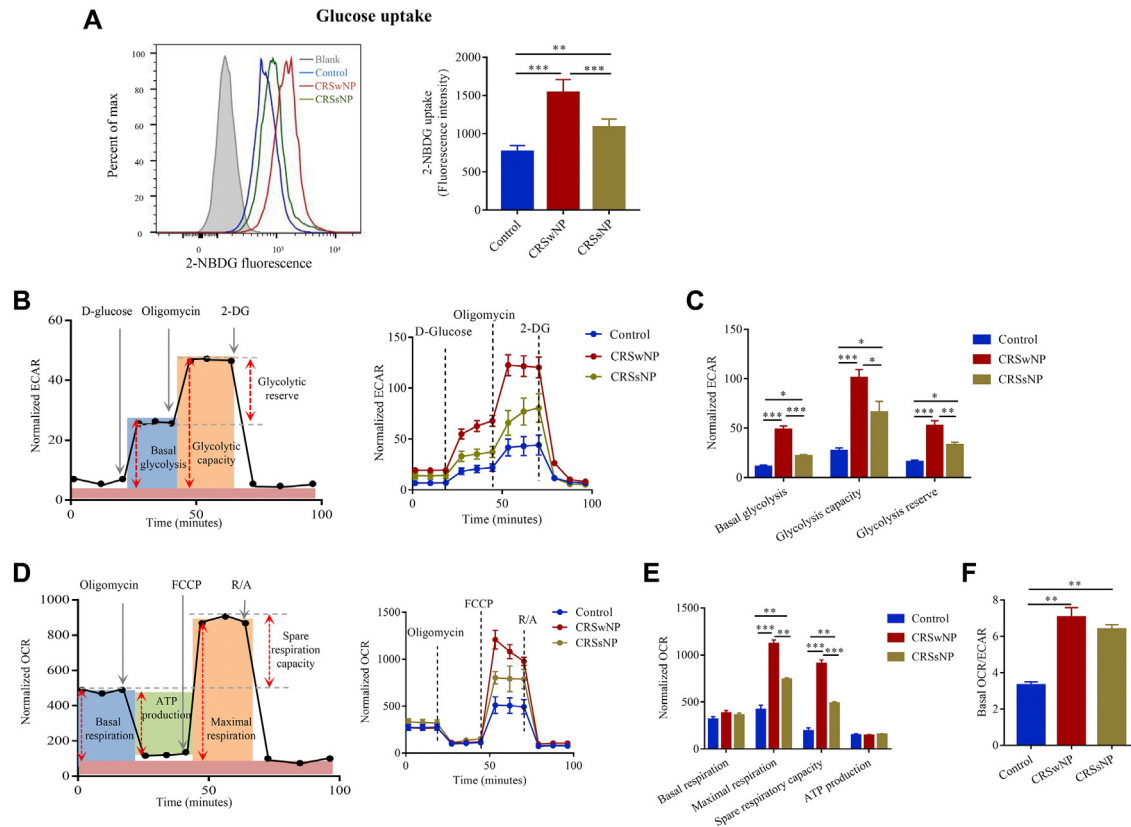


FIG 4. Enhanced glycolysis and OXPHOS in nasal epithelial cells from patients with CRS. HNECs obtained from different study groups were cultured under submerged condition to retain disease-specific features. **A**, HNECs were cultured with the 2-NBDG for 5 minutes, and glucose uptake was quantified by using flow cytometry. The gray shaded area indicates the blank control without 2-NBDG treatment. **B-F**, Bioenergetic analysis was performed with Seahorse XF24 analyzer platform. ECAR (**B-C**) and OCR (**D-E**) of cultured HNECs from different study groups were measured, and the ratios of OCR-ECAR were calculated (**F**). $n = 9-15$ wells from 3 independent experiments for each subject group. * $P < .05$; ** $P < .01$; *** $P < .001$. FCCP, Fluorocarbonyl cyanide phenylhydrazide; R/A, rotenone plus antimycin A.

fumarate, and L-malate) were increased in ALI-cultured HNECs treated with high-level apical D-glucose compared to those treated with normal-level D-glucose (Fig 6, A and see Fig E6 in this article's Online Repository at www.jacionline.org).

To investigate whether the alteration in glucose metabolism affects the function of nasal epithelial cells, we performed RNA sequencing of HNECs. Global reprogramming of transcriptome was discovered in HNECs treated with high-level D-glucose, in which 1560 genes were differentially expressed compared with HNECs treated with normal-level D-glucose (Fig 6, B). Notably, the genes of glycolysis associated enzymes, *HK2*, phosphoglycerate kinase, and *LDHA*, were upregulated in high-level glucose-treated HNECs (see Fig E7 in this article's Online Repository at www.jacionline.org), which was verified with additional cell experiments and by RT-PCR assay (Fig 6, C). No upregulation of TCA cycle-associated enzymes was found in high-level D-glucose-treated HNECs (Fig E7). Differential gene ontology analysis revealed that the top 3 upregulated pathways in high-level D-glucose-treated HNECs were regulation of signaling receptor activity, positive regulation of migration, and leukocyte migration (Fig 6, D). There were 7 overlapping genes in these 3 pathways, including *IL1A*, *IL1B*, *TNFA*, *CCL20*, *CXCL8*, *PDGFB*, and *EDN2* (Fig 6, E). Among them, the upregulation

of genes of proinflammatory cytokines and chemokines including *IL1A*, *IL1B*, *TNFA*, *CCL20*, and *CXCL8* was confirmed with additional cell experiments and by RT-PCR assay (Fig 6, F). Notably, the mRNA expression of *IL1A*, *IL1B*, *TNFA*, *CCL20*, and *CXCL8* was up-regulated in nasal epithelial cells from patients with CRSwNP and those with CRSsNP compared with those from control subjects (see Fig E8 in this article's Online Repository at www.jacionline.org).

Because IL-1 β and TNF- α upregulated the epithelial GLUT expression (Fig 2, D-I), we treated HNECs using a mixture of proinflammatory mediators (PIMs) IL-1 β and TNF- α . It was found that PIMs enhanced both glycolysis and OXPHOS in HNECs cultured with ALI method (Fig 6, G-J), with a predominant shift to glycolysis (Fig 6, K).

Inhibition of glycolysis suppresses proinflammatory mediator production in HNECs

We analyzed the correlations among nasal secretion glucose concentrations, epithelial glucose metabolite levels, epithelial metabolic enzyme, and pro-inflammatory gene expression levels and tissue eosinophil and neutrophil numbers in patients with CRS (see Fig E9 in this article's Online Repository at www.jacionline.org).

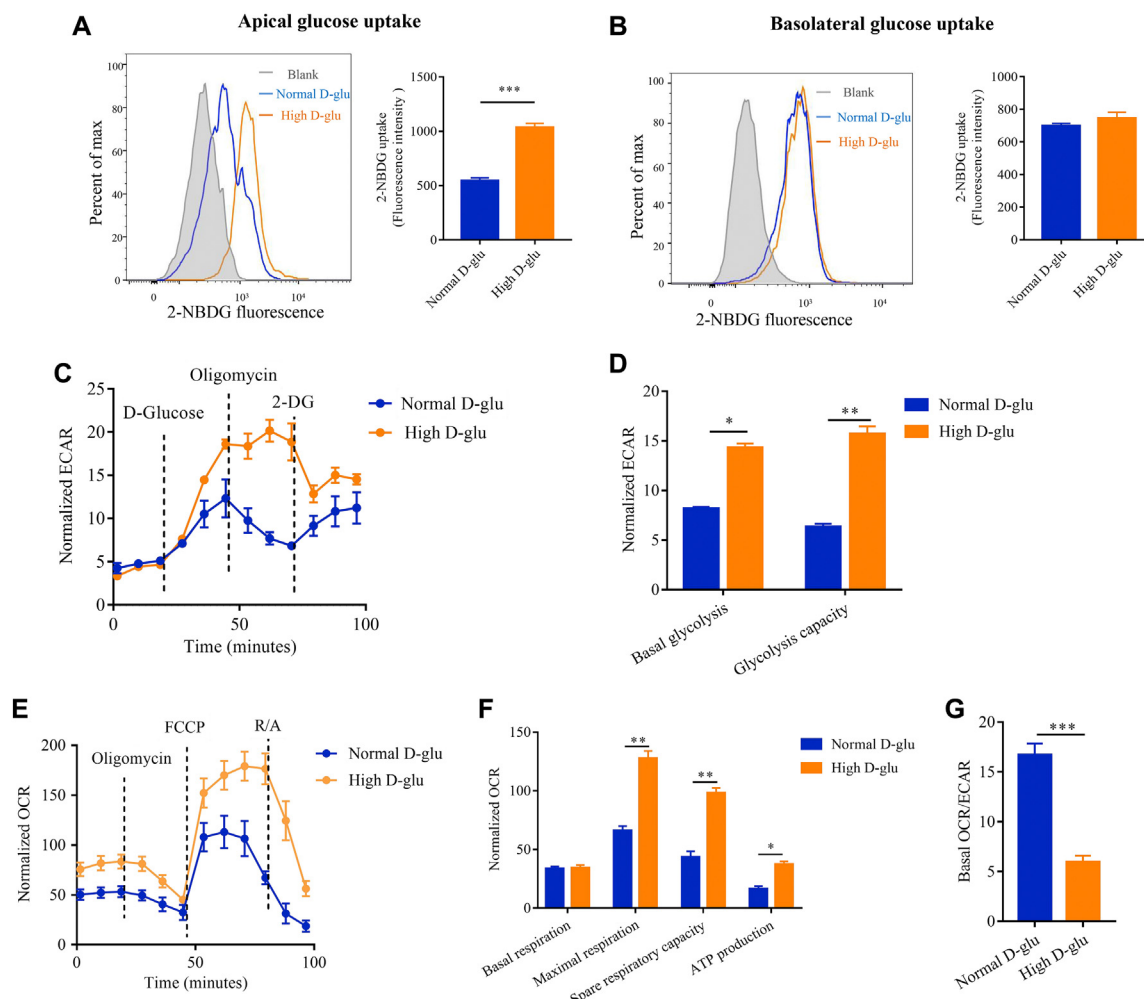


FIG 5. Predominant metabolic shift toward glycolysis in nasal epithelial cells treated with high apical levels of D-glucose. HNECs from control subjects were cultured with an ALI method and stimulated with normal or high levels of D-glucose at the apical surface for 48 hours. After that, 2-NBDG was added in the apical (A) or the basolateral (B) surface, and glucose uptake was quantified by using flow cytometry. The gray shaded area indicates the blank control without 2-NBDG treatment. C-G, Bioenergetic analysis of HNECs pretreated with normal or high levels of D-glucose with Seahorse XF24 analyzer platform. ECAR (C-D) and OCR (E-F) were measured, and the ratios of OCR/ECAR were calculated (G). $n = 9-15$ wells from 3 independent experiments. * $P < .05$; ** $P < .01$; *** $P < .001$.

[jacionline.org](http://www.jacionline.org)). We found that nasal secretion glucose concentrations were positively correlated with the epithelial levels of several glycolytic metabolites including L-lactate; the epithelial mRNA expression of genes of glycolysis enzymes and *IL1A*, *TNFA*, and *CXCL8*; and tissue neutrophil counts; but not with tissue eosinophil counts (Fig 7, A). Consistently, we found that epithelial L-lactate, one of the most important products in glycolysis, positively correlated with epithelial *IL1A*, *TNFA*, and *CXCL8* gene mRNA expression and tissue neutrophil counts (Fig 7, B). We also observed positive correlations of tissue neutrophil numbers with epithelial *IL1A*, *IL1B*, *TNFA*, *CCL20*, and *CXCL8* gene mRNA levels (Fig E9).

Finally, we inhibited glycolysis by using 2-DG and found that 2-DG suppressed high-level apical D-glucose-induced mRNA expression of *IL1A*, *IL1B*, *TNFA*, *CCL20*, and *CXCL8* in HNECs obtained from control subjects and cultured with ALI method (Fig 7, C). We also treated submerged cultured primary HNECs

obtained from patients with CRS with 2-DG and revealed that 2-DG reduced the expression of these proinflammatory mediators at both mRNA and protein level (Fig 7, D and E). Not surprisingly, 2-DG treatment suppressed L-lactate production in HNECs (see Fig E10 in this article's Online Repository at www.jacionline.org).

DISCUSSION

Ongoing inflammatory and immune responses are associated with dramatic shifts in tissue and cell metabolism. In this study, for the first time, we revealed that increased glucose in ASL-promoted GLUT expression, apical glucose uptake, and predominant glycolysis in nasal epithelial cells, which subsequently promote epithelial proinflammatory function in both CRSsNP and CRSwNP. The proinflammatory cytokines in turn upregulated GLUT expression, glucose uptake, and glycolysis in nasal

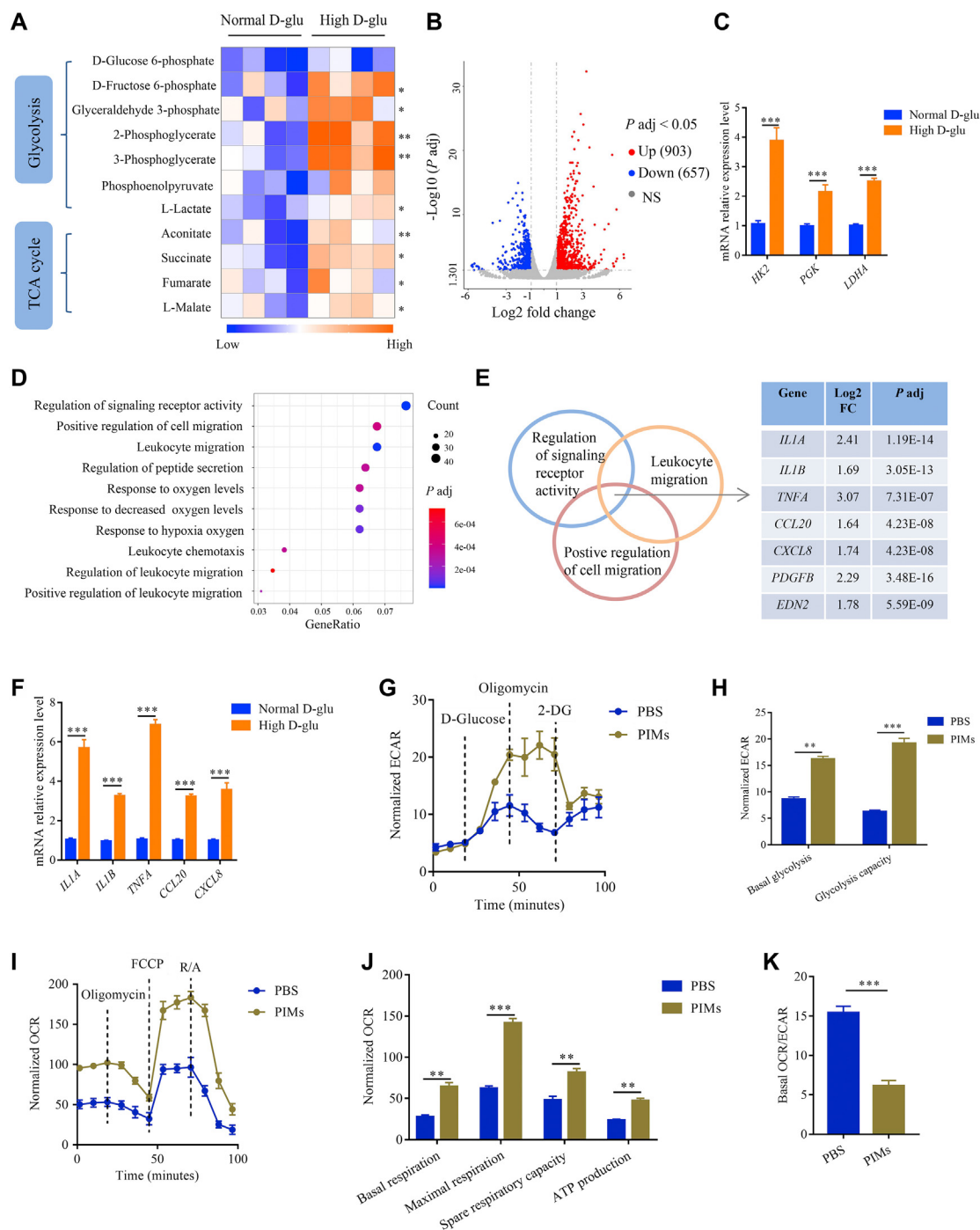


FIG 6. Change of RNA expression profiles of nasal epithelial cells treated with high apical levels of D-glucose, and the effect of proinflammatory cytokines on glucose metabolism. **A-F**, HNECs from control subjects were cultured with an ALI method and stimulated with normal or high levels of D-glucose in the apical surface for 24 hours. After culture, cells were subject to measurement of intermediates in glycolysis and TCA cycle ($n = 4$) (**A**) and RNA sequencing ($n = 3$) (**B**). The upregulation of mRNA expression of glycolysis-related enzyme was verified by additional set of cell experiments and RT-PCR assay ($n = 5$) (**C**). Gene ontology analysis demonstrated top 10 pathways of the upregulated genes (**D**). There were 7 overlapping genes in the top 3 pathways (**E**). The upregulation of mRNA expression of *IL1A*, *IL1B*, *TNFA*, *CCL20*, and *CXCL8* was verified by additional set of cell experiments and RT-PCR assay ($n = 5$) (**F**). **G-K**, HNECs from control subjects were cultured with an ALI method and stimulated with PIMs ($IL-1\beta + TNF-\alpha$) for 48 hours. ECAR (**G-H**) and OCR (**I-J**) of HNECs were measured with a Seahorse XF24 Analyzer, and the ratios of OCR/ECAR were calculated (**K**). $n = 9-15$ wells from 3 independent experiments. * $P < .05$; ** $P < .01$; *** $P < .001$. NS, Not significant.

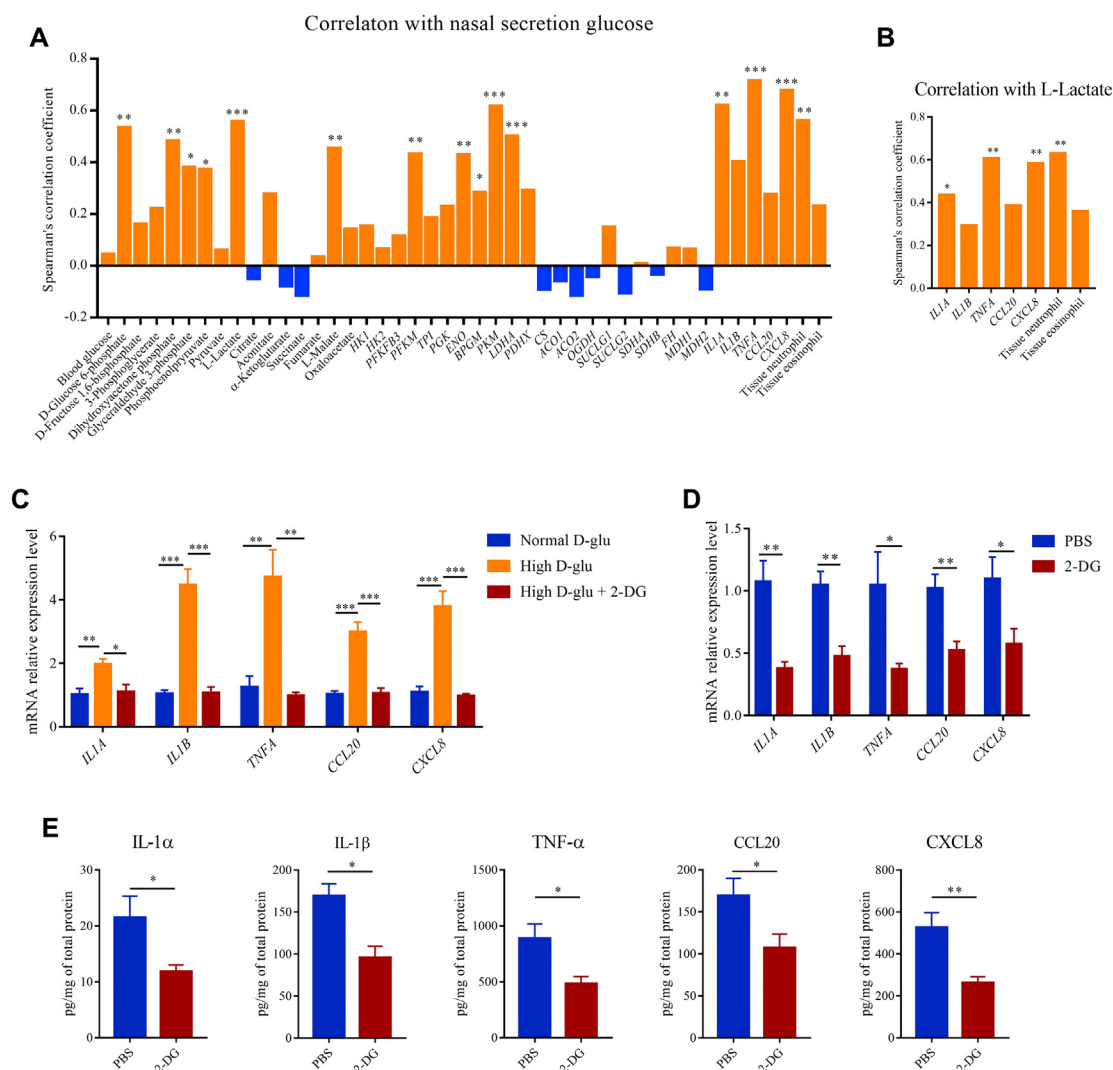


FIG 7. Inhibition of glycolysis suppresses inflammatory mediator production in nasal epithelial cells. **A**, The correlations of nasal secretion glucose concentrations with the levels of glucose metabolites, associated enzymes, and inflammatory mediators in nasal epithelial cells and the tissue eosinophil and neutrophil numbers in patients with CRS ($n = 22-44$). **B**, The correlations of L-lactate levels with inflammatory mediators in nasal epithelial cells and the tissue eosinophil and neutrophil numbers in patients with CRS ($n = 24-36$). **C**, HNECs from control subjects were cultured with an ALI method and treated with high-level apical D-glucose with or without 2-DG, and the mRNA levels of inflammatory mediators were detected 24 hours after treatment ($n = 5$). **D** and **E**, HNECs from patients with CRS ($n = 5$, including 3 with CRSsNP and 2 with CRSwNP) were cultured with submerged method and treated with 2-DG. After 24-hour and 48-hour treatments, the mRNA (**D**) and protein (**E**) levels of inflammatory mediators were detected, respectively. * $P < .05$; ** $P < .01$; *** $P < .001$.

epithelial cells, thus forming a vicious feedback loop in the pathogenesis of CRS.

Confirming a previous report,¹⁹ we found that glucose concentrations in nasal secretions were increased in both patients with CRSsNP and those with CRSwNP. In the present study, we excluded the patients with hyperglycemia and found no correlation between nasal secretion and blood glucose levels, therefore precluding a direct effect of blood glucose levels on ASL glucose concentrations. Previous studies suggest that increased paracellular glucose flux secondary to epithelial barrier disruption may contribute to increased glucose levels in ASL.¹² The increase in ASL glucose concentrations promotes glucose removal from ASL by cellular uptake through GLUTs and SGLTs and

subsequently alters cell metabolism. Previous studies have shown that *in vitro* cultured human bronchial epithelial cells only have the expression of GLUTs, but not SGLTs.^{12,34} Similarly, we found that nasal epithelial cells had low mRNA expression of *SGLTs* (*SLC5A*), but abundant expression of *GLUT1* (*SLC2A1*), *GLUT4* (*SLC2A4*), *GLUT10* (*SLC2A10*), and *GLUT11* (*SLC2A11*). GLUT4, GLUT10, and GLUT11 were expressed by ciliated cells, goblet cells, and basal cells in nasal epithelium, and their cell surface protein expression were increased in patients with CRS, especially in those with CRSwNP. We discovered that D-glucose, LPS, double-stranded RNA, and inflammatory cytokines were able to upregulate GLUT4, GLUT10, and/or GLUT11 expression on nasal

epithelial cells, which may contribute to the upregulation of GLUTs in CRS.

Consistent with GLUT expression pattern, we found that glucose uptake capacity was enhanced in epithelial cells from patients with CRS, which may reprogram the glucose metabolism. We discovered increased levels of intermediates of both glycolysis and TCA cycle in nasal epithelial cells from patients with CRS as compared to those in control subjects *in vivo*. Consistently, *in vitro* submerged cultured primary HNECs from patients with CRS had elevated ECAR and OCR, indicating enhanced activation of glycolysis and OXPHOS. Nasal epithelial cells were more likely dependent on glycolysis than OXPHOS in CRS *in vivo*, as reflected by a prominent upregulation of glycolysis, but not TCA cycle, associated enzymes, and increased ratio of L-lactate/pyruvate in nasal epithelial cells in patients with CRS.

To provide direct evidence of the effect of increased ASL glucose on the metabolism and function of nasal epithelial cells, we treated HNECs from control subjects under ALI conditions with normal or high levels of apical glucose, which mimics ASL glucose condition in control subjects or patients with CRS, respectively. We found that high-level apical D-glucose promoted glucose uptake, which may be partially due to upregulation of GLUTs in epithelial cells by D-glucose. In addition, we discovered that high-level D-glucose-treated HNECs had increased glycolysis and OXPHOS levels, while with a predominant shift to glycolysis, which was supported by the enhanced basal OCR-ECAR ratio and the upregulation of only glycolysis-associated enzymes despite the increase of metabolites of both glycolysis and TCA cycle. These *in vitro* findings are consistent with *in vivo* findings in patients with CRS. In our study, although CRS-derived primary HNECs, cultured under submerged condition, showed enhanced activation of glycolysis and OXPHOS, a higher basal OCR-ECAR ratio suggests a preference to OXPHOS. This is in contrast to the predominantly exaggerated glycolysis in nasal epithelial cells in patients with CRS *in vivo* and in control-derived HNECs cultured with ALI method and exposed to high levels of apical glucose and proinflammatory cytokines. This discrepancy may be related to the less physiological relevance of submerged culture condition for CRS-derived primary HNECs. Patients with CRSwNP had higher levels of epithelial GLUT expression and glucose uptake capacity, while those with CRSsNP displayed higher nasal secretion glucose concentrations. The net effect of those influencing factors may lead to the final, comparable change in epithelial glucose metabolism between patients with CRSsNP and those with CRSwNP *in vivo*.

Previous studies have reported that glycolysis has critical pathophysiological roles in diverse cell types, such as proliferation, growth, and invasion of cancer cells,^{35,36} and differentiation and activation of immune cells.³⁷⁻⁴⁰ In this study, we found that high-level D-glucose treatment induced remarkable reprogramming of transcriptome in nasal epithelial cells. The top 3 upregulated pathways were regulation of signaling receptor activity, leukocyte migration, and positive regulation of migration, highlighting a change of inflammation-relevant functions of nasal epithelial cells. The overlapped genes in the top 3 upregulated pathways included *IL1B*, *IL1A*, *TNFA*, *CCL20*, and *CXCL8*, whose expression was increased in epithelial cells in both CRSsNP and CRSwNP and correlated with tissue neutrophils. Interestingly, we found that IL-1 β and TNF- α not only upregulated the GLUT expression, but also enhanced both glycolysis

and OXPHOS in HNECs, also with a predominant metabolic shift to glycolysis. These data highlight a vicious feedback loop between inflammation and glucose metabolism reprogramming in nasal epithelial cells in the pathogenesis of CRS. Importantly, our *in vitro* findings were recapitulated *in vivo* as shown by the positive correlations between nasal secretion glucose, epithelial L-lactate, and epithelial proinflammatory cytokine and chemokine expression levels. Interestingly, we found that nasal secretion glucose and epithelial L-lactate levels were associated with tissue neutrophil numbers, but not eosinophil numbers, in accordance with the functional relationship between glycolysis and neutrophil-relevant chemokine and cytokine production in nasal epithelial cells. Elevated ASL glucose drives proliferation of bacteria able to use glucose as a carbon source. The enhanced neutrophil chemotaxis and activation may act as a feedback mechanism to control airway infection; however, the uncontrolled and persistent change in nasal epithelial cell glycolysis and neutrophilic inflammation will contribute to the development of CRS. It will be interesting to explore the relationships among ASL glucose, epithelial cell metabolism, microbiota, and inflammation in CRS in future. Finally, we found that inhibition of glycolysis by 2-DG downregulated the production of inflammatory cytokines and chemokines in both control-derived HNECs treated with high-level D-glucose and CRS-derived HNECs. These findings not only confirmed the role of glycolysis in promoting proinflammatory function of nasal epithelial cells, but they also suggest a therapeutic potential of glycolysis inhibition in CRS. Here, we found that nasal epithelial cells in CRS were dominated by glycolysis, which promoted inflammatory cytokine production and associated with neutrophil inflammation. Previous studies showed that neutrophilic inflammation associated with glucocorticoid resistance and refractory disease state in patients with CRS.^{41,42} Therefore, perturbed glucose metabolism may ultimately influence CRS treatment and outcome, which deserves further investigations. Several preclinical and early clinical studies have demonstrated the effectiveness of targeting glycolysis as a therapeutic approach to suppress cancer progression.^{43,44} It is interesting to explore the therapeutic effect of intervention of epithelial cell glycolysis in patients with CRS, such as intranasal application of 2-DG, particularly for those with significant neutrophilic inflammation but with limited target therapies.

It should be noted that glycolysis and mitochondrial OXPHOS are the 2 primary and overlapping metabolic pathways for generating ATP from glucose, and thus they have a universal role in controlling cell function and inflammation. However, different types of cells may prefer distinct metabolic pathways to produce energy, which also depends on disease conditions. For example, T_H17 cell polarization is linked to upregulation of glycolysis, whereas the same pathways suppress the generation of regulatory T cells.^{45,46} In colitis, glycolysis is enhanced while OXPHOS is reduced in intestinal epithelial cells, leading to impaired intestinal epithelium gap junction, reduced cell proliferation, and increased cell apoptosis.⁴⁷⁻⁴⁹ In our study, nasal epithelial cells from patients with CRS demonstrated enhancement of both glycolysis and OXPHOS, but with a shift to a glycolysis-predominant pathway. Enhanced glycolysis promoted IL-1, TNF- α , and CXCL8 production in nasal epithelial cells, thus associating with neutrophil inflammation in CRS. The efforts to dissect the cell- and disease-specific role of glucose metabolism will provide novel insights into disease pathogenesis and shed light on the disease management.

Our study has several limitations. First, the glucose levels in ASL are determined by the balance between paracellular flux across epithelium and the apical uptake by epithelial cells. The regulation of ASL glucose level in the context of CRS remains to be explored. Second, we did not determine which GLUT isoform plays the most important role in glucose uptake by nasal epithelial cells. Third, we focused on the effect of ASL glucose on glucose metabolism of nasal epithelial cells. The direct and indirect influences on lipid and amino acid metabolism require further investigations. Last, the mechanisms underlying the regulatory effect of glycolysis on nasal epithelial cell function also await future research.

In conclusion, our study reveals a previously unappreciated role of high-level ASL glucose in promoting nasal epithelial cell glycolysis and proinflammatory function in the context of CRS. There is a vicious cycle among inflammation, ASL glucose, and epithelial cell glycolysis. Inhibiting glycolysis in nasal epithelial cells may have potential therapeutic value to control neutrophilic inflammation in CRS.

Key messages

- Both glycolysis and OXPHOS pathways are enhanced in the nasal epithelial cells in patients with CRS, with a pre-dominant shift to glycolysis.
- Increased glucose in ASL promotes glucose uptake, glycolysis, as well as proinflammatory gene expression in nasal epithelial cells.
- Inhibiting glycolysis suppresses the production of proinflammatory mediators by nasal epithelial cells.

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METHODS

Subjects and sample collection

This study was approved by the Ethics Committee of Tongji Hospital and conducted with written informed consent from all participants. A total of 99 control subjects, 188 patients with bilateral CRSwNP, and 152 patients with bilateral CRSsNP were enrolled in this study. Diagnosis of CRSwNP and CRSsNP was based on European and American guidelines.^{E1,E2} Control subjects were those undergoing septoplasty because of anatomic variations and without other sinonasal diseases.

Atopic status was assessed by skin prick test with a panel of 19 common inhalant allergens in our region (Macro-Union Pharmaceutical Co, Beijing, China), and/or specific IgE against common inhalant allergens detected by using the ImmunoCAP (Phadia, Uppsala, Sweden).^{E3} Allergic rhinitis is diagnosed based on the concordance between atopic status and typical symptoms of allergy.^{E4} The diagnosis of asthma was made according to Global Initiative for Asthma guideline.^{E5} Oral and nasal glucocorticoids were discontinued at least 3 months and 1 month before surgery, respectively. Patients with acute upper respiratory tract infection and acute asthma within 4 weeks of entering this study were excluded. Patients with fungal sinusitis, cystic fibrosis, primary hair immobility syndrome, immunodeficiency, systemic vasculitis, choanal polyps, or diabetes were also excluded. The details of subjects' characteristics are summarized in Table E1.

HNECs were scraped from the middle meatus mucosa of control subjects and patients with CRSsNP and from polyps of patients with CRSwNP, respectively.^{E6,E7} Nasal secretions were collected using sinus packs and processed as previously described.^{E8} Not all samples were included in every experiment protocol because of limited quantity. The subject and experiment numbers were indicated in figures or figure legends.

Histology, immunohistochemistry, and immunofluorescence staining

Fresh tissue samples were fixed in formaldehyde solution and embedded in paraffin. After deparaffinization and rehydration, tissue sections (4 μ m) were stained with hematoxylin-eosin. For immunohistochemistry and immunofluorescence staining, sections were subject to heat-induced antigen retrieval using Target Retrieval Solution (Dako, Carpinteria, Calif). For immunohistochemistry, after blocking, sections were incubated with specific primary antibody against myeloperoxidase (MPO) or eosinophil cationic protein (ECP) (Table E2) at 4°C overnight and subsequently the corresponding secondary antibody. All antigens were detected by using the poly-horseradish peroxidase complex (Boster Biotechnology, Wuhan, China) method, and color development was achieved with 3', 3'-diaminobenzidine.^{E9} Tissue sections were finally counterstained with hematoxylin. For immunofluorescence, after blocking, tissue sections were incubated with primary antibodies (Table E2) overnight at 4°C and then incubated with fluorescence-conjugated secondary antibodies (Table E3) for 1 hour at room temperature.^{E10} The 4,6-diamidino-2-phenylindole was used as counterstain of cell nuclei. Species- and subtype-matched antibodies were used as negative controls.

The numbers of MPO⁺ neutrophils and ECP⁺ eosinophils were counted at high-power field. Ten high-power fields were randomly selected and were counted by 2 independent observers in a blinded fashion, as previously described.^{E11}

Quantitative RT-PCR

Total RNA was extracted from the samples by using TRIzol reagent (TaKaRa Biotechnology, Dalian, China) as previously mentioned.^{E12} A total of 1 μ g of RNA was reverse-transcribed to cDNA using a PrimeScript RT reagent kit (TaKaRa Biotechnology). The PCR was performed by using the SYBR Premix Ex Taq kit (TaKaRa Biotechnology) with appropriate primers (Table E4). cDNA equivalent to 50 ng of total RNA was used and quantitative PCR was performed on StepOnePlus (Applied Biosystems, Foster City, Calif). Amplification was as follows: 95°C for 2 minutes, followed by 40 cycles of 95°C for 10 seconds, specific annealing temperature for 10 seconds, and

72°C for 15 seconds. After PCR, a melting curve was constructed by increasing the temperature from 65°C to 95°C with a temperature transition rate of 0.1°C/s. *ACTB* was used as a housekeeping gene for normalization of gene expression. The relative abundance of mRNA expression of *GLUTs* (*SLC2A*) and *SGLTs* (*SLC5A*) in nasal epithelial cells was compared by using $2^{-\Delta\Delta CT}$ values (ΔCT = the difference in threshold cycles for target gene and housekeeping gene *ACTB*).^{E13} The higher $2^{-\Delta\Delta CT}$ value indicates the higher mRNA expression abundance of the target gene.^{E13} The relative gene expression in different samples was compared by using the $2^{-\Delta\Delta CT}$ method.^{E14}

Western blotting

HNECs were lysed in modified radio-immunoprecipitation assay buffer containing cocktail of protease inhibitors (Goodbio Technology, Wuhan, China) as previously stated.^{E7} Protein concentrations were quantified by using a bicinchoninic acid protein assay kit (Goodbio Technology). Samples containing 30 μ g of proteins were separated by 10% SDS-PAGE and transferred to polyvinylidene fluoride membrane (MilliporeSigma, Burlington, Mass). The membranes were blocked with 5% fat-free skim milk in Tris-buffered saline with 0.05% Tween-20 (Goodbio Technology) for 1 hour at room temperature and then incubated overnight with primary antibodies as listed in Table E5. After washing 3 times in Tris-buffered saline with 0.05% Tween-20 buffer, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody (goat anti-rabbit, 1:6000; Goodbio Technology) for 1 hour at room temperature. After that, the membranes were processed by using an enhanced chemiluminescence reaction kit (Abbkine, Wuhan, China) and followed by exposure on chemiluminescent film to visualize the proteins. β -actin was used as an internal standard to correct for variations in total protein loading. Densitometric analysis of blots was performed by using the software AlphaEase FC (Alpha Innotech, San Leandro, Calif).

ELISA

The cells were harvested, homogenized on ice, and centrifuged at 3000 rpm for 10 minutes at 4°C. The IL-1 α , IL-1 β , TNF- α , CCL20, and CXCL8 protein levels in cell lysate supernatants were detected using commercial ELISA kits (Boster Biotechnology). The lower detection limit was 0.5 pg/mL for IL-1 α , 0.15 pg/mL for IL-1 β , 1.0 pg/mL for TNF- α , 1.0 pg/mL for CCL20, and 1.0 pg/mL for CXCL8. The total protein levels were measured by using a bicinchoninic acid protein detection kit (Goodbio Technology). The cytokine levels in cell lysate were expressed as protein levels per milligram of total protein.

Flow cytometry

Dispersed nasal tissue cells were prepared from sinonasal tissues with mechanical dissociation as previously reported.^{E15} To detect the surface expression of GLUT11 and GLUT10 on nasal epithelial cells, cells were stained with rabbit polyclonal anti-human GLUT11 at 25°C for 25 minutes. After wash, the cells were stained with donkey anti-rabbit IgG-APC for 25 minutes in the dark. After that, the cells were stained with phycoerythrin (PE)-conjugated mouse mAb against human CD45, BV421-conjugated mouse mAb against human CD326, fluorescein isothiocyanate-conjugated mouse mAb against human GLUT10 for 25 minutes in the dark. To detect the surface expression of GLUT1 on nasal epithelial cells, the cells were stained with PE-conjugated mouse monoclonal antibody against human CD45, BV405-conjugated mouse mAb against human GLUT1, and APC-conjugated mouse mAb against human CD326 for 25 minutes in the dark. For the detection of GLUT4 on nasal epithelial cells, the cells were stained with PE-conjugated mouse mAb against human CD45, BV421-conjugated mouse mAb against human CD326, and APC-conjugated mouse mAb against human GLUT4 for 25 minutes in the dark. Cells were also stained with zombie aqua fixable viability stain (BioLegend, San Diego, Calif) to exclude dead cells. Nasal epithelial cells were gated as CD326⁺CD45⁺ cells.

After stimulation, cultured HNECs were stained with rabbit polyclonal anti-human GLUT11 at 25°C for 25 minutes, and then stained with donkey anti-rabbit IgG-APC for 25 minutes in the dark.^{E16} Or cells were directly

stained with fluorescein isothiocyanate–conjugated mouse mAb against human GLUT10 or APC-conjugated mouse mAb against human GLUT4 at 25°C for 25 minutes in the dark. Cells were also stained with zombie aqua fixable viability stain (BioLegend) to exclude dead cells.

The detailed information of antibodies used is listed in Table E6. Species and subtype-matched antibodies were used as negative controls. The stained cells were analyzed by using BD flow cytometer (BD Biosciences, San Jose, Calif). Fluorescence was determined for all cells in each sample after debris and aggregates were excluded by forward angle and side scatter gating. Data were analyzed using FlowJo software (TreeStar, Ashland, Ore).

HNEC culture and treatment

For ALI culture, nasal epithelial cells were scraped from the middle meatus mucosa of control subjects. HNECs were first cultured with bronchial epithelial cell basal medium (Lonza, Walkersville, Md) supplemented with SingleQuot Kit Suppl (Lonza) in 6-well plates coated with rat tail collagen type I (Sigma-Aldrich, St Louis, Mo) in a 5% CO₂-humidified atmosphere at 37°C.^{E12} After reaching confluence, the cells were trypsinized and plated in collagen-coated 0.4-μm pore membrane inserts of Transwell plates (Corning, Durham, NC) and were cultured with bronchial epithelial cell basal medium. When they reached confluence, medium from the apical side of cells was removed and medium below the insert was replaced with an ALI medium consisting of bronchial epithelial cell basal medium /Dulbecco modified Eagle medium H (1:1), and the glucose concentration in the ALI medium was 6 mmol/L to maintain a concentration comparable to that in blood. HNECs were cultured at ALI condition for 21 days until they were differentiated.

To retain the disease-specific features, primary HNECs obtained from control subjects and patients with CRS were cultured under submerged condition and the difference among the control subjects and patients with CRSwNP and those with CRSNP was compared.

To explore the expression regulation of GULTs, the HNECs cultured with an ALI method were stimulated with IL-1β, TNF-α, IL-6, IFN-γ, IL-13, IL-17A, and IL-10 at 20 ng/mL (R&D Systems, Minneapolis, Minn), LPS at 1 μg/mL (Invitrogen, San Diego, Calif), polyinosinic:polycytidylic acid at 25 μg/mL (Tocris Bioscience, Bristol, UK), and D-glucose at 2.5 mmol/L (Sigma-Aldrich). D-glucose was added at the apical surface of the cells to mimic the condition in patients with CRS. Other stimulators were added to both the apical and basolateral surfaces of the cells. After 24-hour stimulation, cells were harvested for quantitative RT-PCR analysis. After 48-hour stimulation, cells were collected for flow cytometry.

To explore the effect of normal-level and high-level D-glucose on HNECs, normal-level D-glucose (0.5 mmol/L D-glucose + 2.0 mmol/L L-glucose; Sigma-Aldrich) and high-level D-glucose (2.5 mmol/L; Sigma-Aldrich) were added to the apical surface of ALI-cultured HNECs for 24 hours and then HNECs were subject to the detection of intermediate products of glucose metabolism and RNA sequencing. After culture for 48 hours, HNECs were subject to glucose uptake and bioenergetics assay. In some experiments, HNECs were treated with high-level D-glucose and 2-DG (10 mmol/L, Agilent Technologies) simultaneously for 24 hours, and then subject to quantitative RT-PCR.

To explore the effect of proinflammatory mediators, the HNECs obtained from control subjects were cultured with an ALI method and stimulated with a mixture of PIMs, consisting IL-1β (R&D Systems) and TNF-α (R&D Systems) at 20 ng/mL for 48 hours. After that, HNECs were subject to glucose uptake and energy metabolism.

Primary HNECs from patients with CRSwNP were cultured under submerged conditions and treated with 2-DG (10 mmol/L; Agilent Technologies). After 24-hour treatment, cells were collected for quantitative RT-PCR. After 48-hour treatment, cells were collected for the detection of protein levels of inflammatory mediators with ELISA.

Glucose uptake assay

Glucose uptake by HNECs was evaluated by using fluorescent glucose 2-NBDG (Invitrogen, Thermo Fisher Scientific Company, Waltham,

Mass).^{E17,E18} To retain the disease-specific features, primary HNECs from different study groups were cultured submerged. HNECs were treated with 2-NBDG (100 μmol/L) at 37°C with 5% CO₂ for 5 minutes when reaching 90% confluence and washed twice with ice-cold PBS. Cells were then collected to detect fluorescence intensity by flow cytometer with excitation light at 488 nm.

HNECs cultured under ALI condition were incubated with 2-NBDG (100 μmol/L) at apical or basolateral surface at 37°C with 5% CO₂ for 20 minutes and then washed twice with ice-cold PBS. Cells were subject to detection of fluorescence intensity by flow cytometer with excitation light at 488 nm.

Bioenergetic analysis

We observed glucose metabolism of HNECs in real time by using Seahorse XF24 analyzer (Seahorse Bioscience, North Billerica, Mass). For monitoring glycolysis, the glycolysis-stress test kit (Agilent Technologies) was used with addition of glucose-free buffered Seahorse media (pH 7.4). For HNECs cultured with an ALI method, the final concentrations of drugs used were D-glucose at 10 mmol/L, oligomycin at 4.0 μmol/L, and 2-DG at 50 mmol/L. For cells cultured with submerged method, the final concentrations of drugs used were D-glucose at 10 mmol/L, oligomycin at 1.5 μmol/L, and 2-DG at 50 mmol/L. ECAR was measured, reflecting the glycolytic activity.

For examining mitochondrial function, a mito-stress test kit (Agilent Technologies) was used with addition of buffered Seahorse media (pH 7.4) as previously described.^{E19} For HNECs cultured with an ALI method, the final concentrations of drugs used were oligomycin at 4.0 μmol/L, fluorocarbonyl cyanide phenylhydrazide at 2.0 μmol/L, and rotenone plus antimycin A at 2.0 μmol/L. For cells cultured under submerged condition, the final concentrations of drugs used were oligomycin at 1.5 μmol/L, fluorocarbonyl cyanide phenylhydrazide at 2.0 μmol/L, and rotenone plus antimycin A at 0.5 μmol/L. OCR was measured, which represents the degree of OXPHOS.^{E19} Data were normalized to total protein in each well.

Targeted metabolomic analysis

The metabolites were extracted from nasal epithelial cells under sonication for 1 hour in ice baths. The mixture was incubated at −20°C for 1 hour followed by centrifugation at 14,000g, 4°C for 20 minutes, and then supernatants were transferred to the sampling vial for ultraperformance liquid chromatography–mass spectrometry/mass spectrometry (Shanghai Bioprofile Technology Co, Ltd, Shanghai, China) analysis.^{E20,E21}

Raw data files were processed by peak finding, alignment, and filtering using Xcalibur Qual browser software (Thermo Fisher Scientific). The relative levels of each metabolite were calculated from the calibration curve of corresponding standard metabolite.

RNA sequencing

Total RNA was extracted from HNECs using TRIzol (TaKaRa Biotechnology). A total amount of 2 μg total RNA per sample was used for stranded RNA sequencing library preparation using KCTM Stranded mRNA Library Prep Kit for Illumina (Wuhan Seqhealth Co, Ltd, Wuhan, China) following the manufacturer's instruction. PCR products corresponding to 200–500 bp were enriched, quantified, and finally sequenced on NovaSeq 6000 sequencer, PE150 model (Illumina, San Diego, Calif).

Genes differentially expressed between groups were identified using the edgeR package (version 1.26.0).^{E22} In comparison, genes with *P* adjusted value <.05 and |log₂ (fold change)| ≥ 1 between 2 groups were considered to have significantly different expression. Gene ontology analysis for differentially expressed genes were implemented by KOBAS software (version 2.1.1; Center for Bioinformatics, Peking University and Institute of Computing Technology, Chinese Academy of Sciences, Beijing, China) with a *P* adjusted value cutoff of .05 to judge statistically significant enrichment. All primary data have been uploaded to the National Center for Biotechnology Information Sequence Read Archive (accession number: PRJNA812068).

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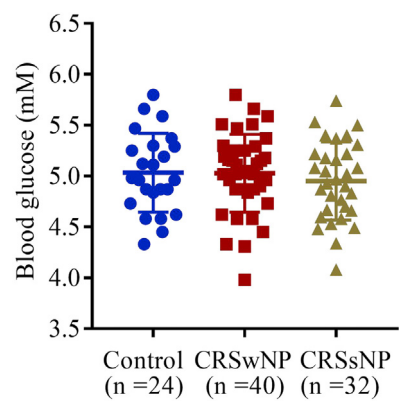


FIG E1. Blood glucose concentrations in different study groups.

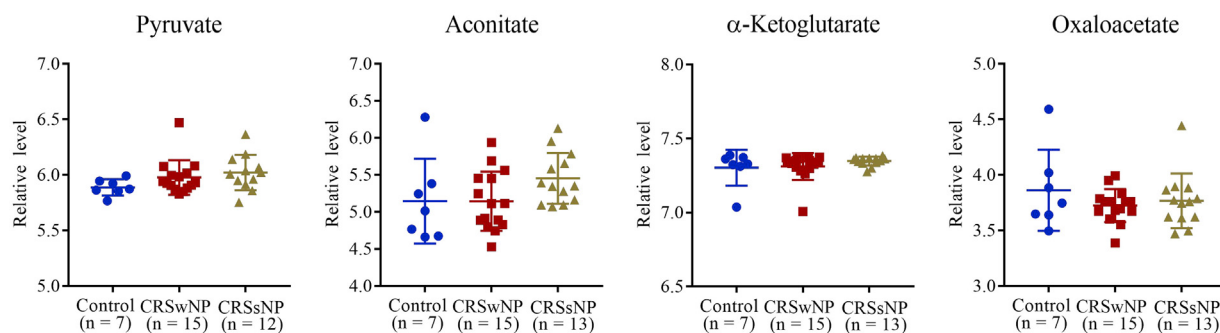


FIG E2. The metabolite levels in nasal epithelial cell having no difference among different study groups.

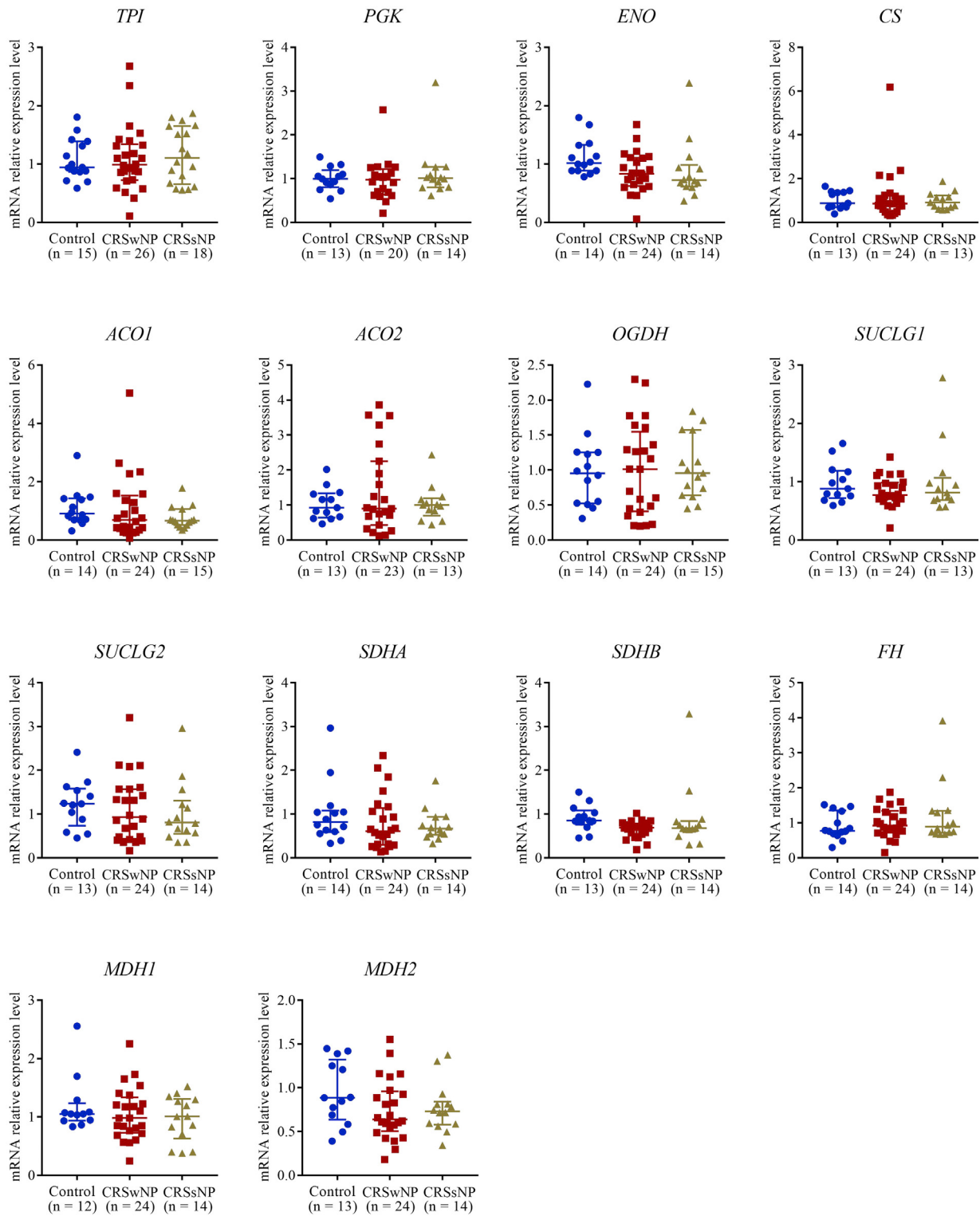


FIG E3. The mRNA expression levels of glycolysis and the TCA cycle-related enzyme genes in nasal epithelial cells with no difference among different study groups.

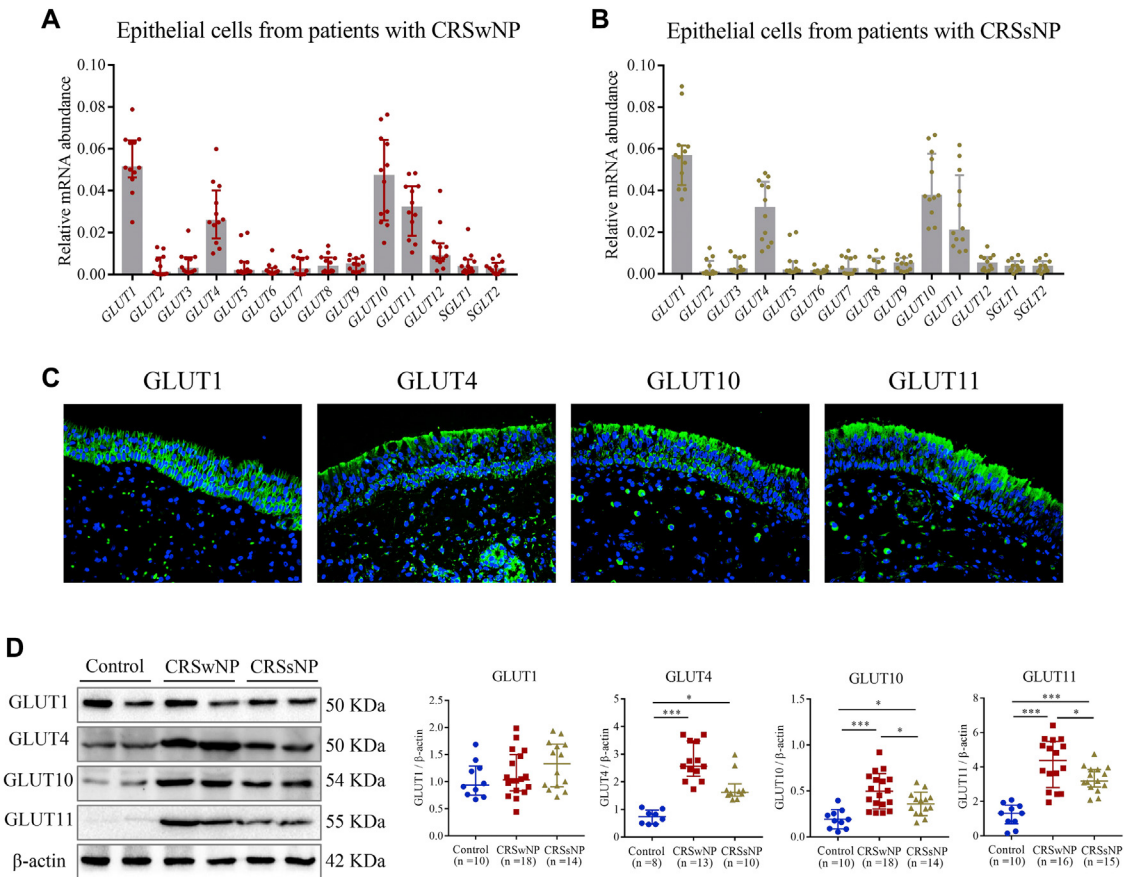


FIG E4. Glucose transporter expression in nasal epithelial cell. The mRNA expression abundance of *GLUT* (*SLC2A*) and *SGLT* (*SLC5A*) gene family members in scrapped nasal epithelial cells from patients with CRSwNP ($n = 12$) (**A**) and those with CRSsNP ($n = 12$) (**B**). **C**, Representative immunofluorescence staining of GLUT1, GLUT4, GLUT10, and GLUT11 in nasal polyp tissue sections. **D**, The protein levels of GLUT1, GLUT4, GLUT10, and GLUT11 in nasal epithelial cells as detected by Western blotting. Representative blots are shown, and densitometric analysis of blots was performed. $*P < .05$; $***P < .001$.

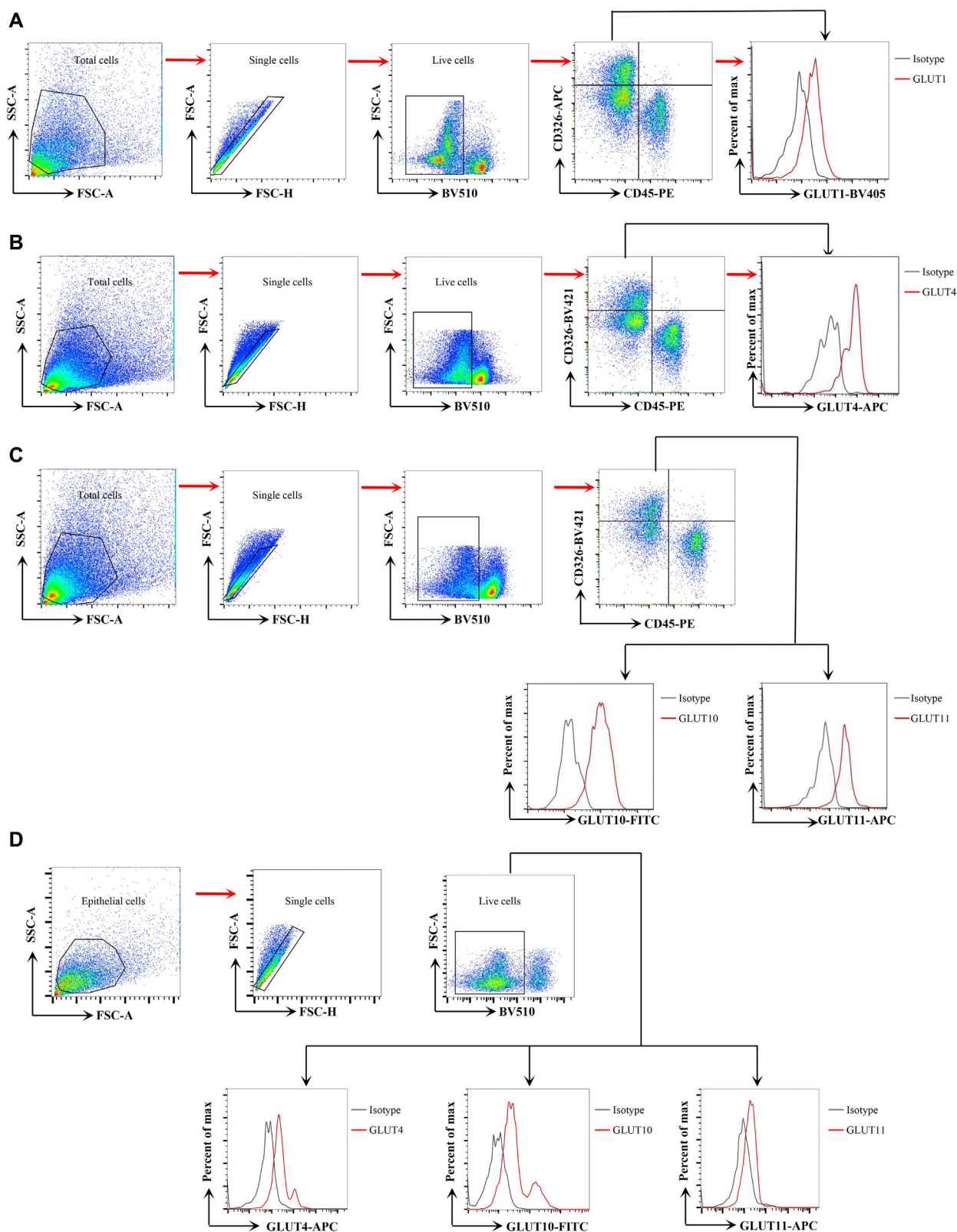


FIG E5. The gating strategies for flow cytometric analysis of GLUTs on nasal epithelial cells. **A-C**, Dispersed sinonasal tissue cells were prepared and subject to flow cytometric analysis of cell surface expression of GLUT1, GLUT4, GLUT10, and GLUT11 on epithelial cells. $CD326^{+}CD45^{-}$ epithelial cells were gated and analyzed for the GLUT expression. **D**, HNECs from control subjects were cultured with ALI method. After stimulation, cell surface expression of GLUT4, GLUT10, and GLUT11 was detected by flow cytometry. A, Area; FSC, forward scatter; H, height; SSC, side scatter.

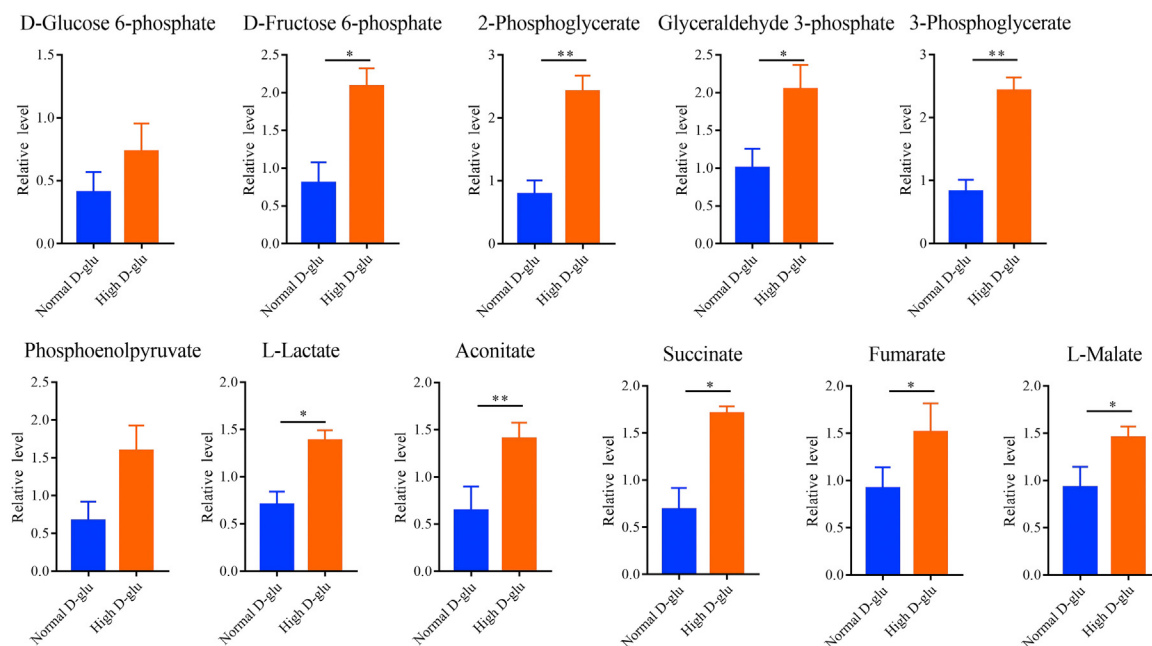


FIG E6. The relative levels of glucose metabolites in high-level D-glucose-treated nasal epithelial cells. HNECs from control subjects were cultured with an ALI method and stimulated with normal or high levels of D-glucose at the apical surface for 24 hours. After culture, cells were subject to measurement of intermediates in glycolysis and TCA cycle ($n = 4$). * $P < .05$; ** $P < .01$.

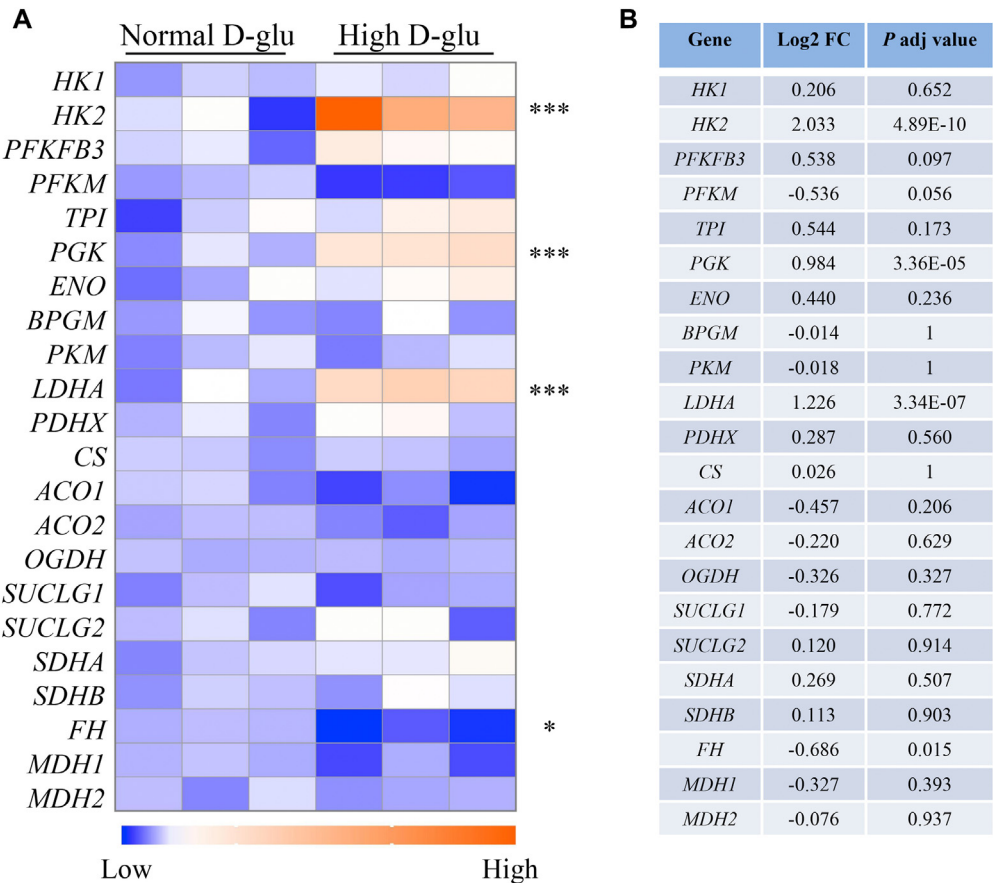


FIG E7. Relative mRNA expression levels of genes of glucose metabolism-associated enzymes in high-level D-glucose treated nasal epithelial cells. HNECs from control subjects were cultured with an ALI method and stimulated with normal or high levels of D-glucose at the apical surface for 24 hours. After culture, cells were subject to RNA sequencing. **A**, Heat map showing the relative mRNA expression of genes of metabolic enzymes. **B**, Table showing the fold changes of gene expression in high-level D-glucose-treated HNECs versus normal-level D-glucose-treated HNECs and the corresponding adjusted *P* values. **P* < .05; ***P* < .01; ****P* < .001.

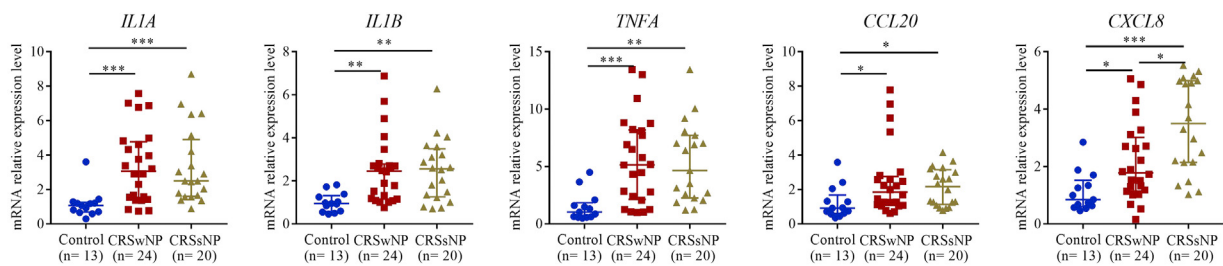


FIG E8. The mRNA expression of proinflammatory cytokines and chemokines in nasal epithelial cells in different subject groups. * $P < .05$; ** $P < .01$; *** $P < .001$.

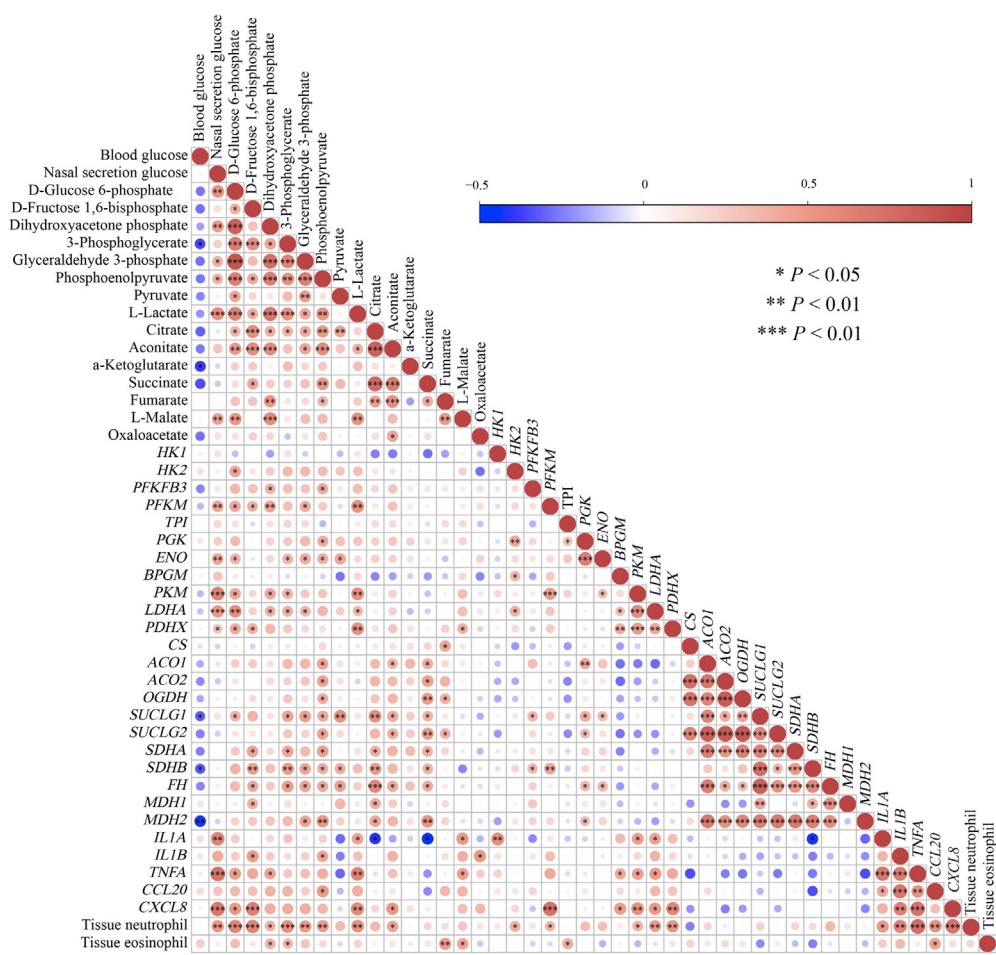


FIG E9. The correlations between nasal secretion glucose concentrations, epithelial glucose metabolite levels, epithelial proinflammatory and metabolic enzyme gene expression levels, and tissue eosinophil and neutrophil numbers in patients with CRS. The correlation matrix shows the negative (*blue*) or positive (*red*) correlation of 2 parameters in patients with CRS. Color intensity and circle size reflect correlation strength. $n = 22-44$; * $P < .05$; ** $P < .01$; *** $P < .001$.

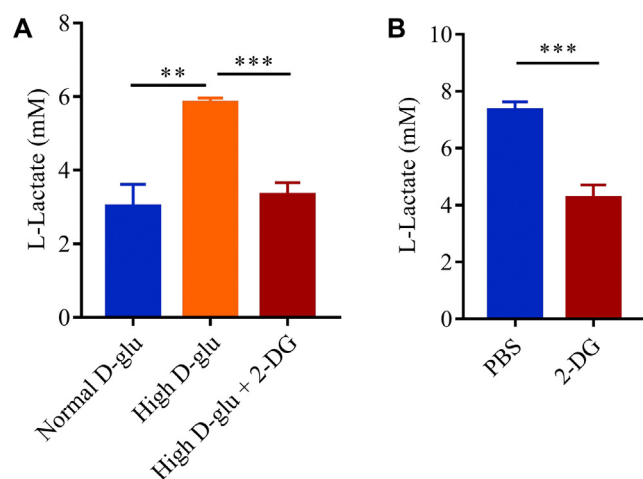


FIG E10. Decreased L-lactate levels in nasal epithelial cell culture supernatants after 2-DG treatment. **A**, HNECs from control subjects were cultured with an ALI method and treated with high apical levels of D-glucose with or without concomitant 2-DG ($n = 5$). The levels of L-lactate in culture supernatants were detected after 24-hour treatment. **B**, HNECs from patients with CRS were cultured with submerged method and treated with 2-DG. After 24-hour treatment, the levels of L-lactate in culture supernatants were detected ($n = 5$, including 3 with CRSsNP and 2 with CRSwNP). $**P < .01$; $***P < .001$.

TABLE E1. Demographic characteristics of subjects

	Control	CRSwNP	CRSSNP	P value
Total subjects enrolled	99	188	152	
Methodology used				
Immunohistochemistry and immunofluorescence				
No. of subjects		19	33	
Male		11 (57.8)	17 (51.5)	.664
Age (y)		32.0 (24.3, 57.6)	34.0 (26.5, 58.1)	.598
Patients with atopy		6 (31.6)	8 (24.3)	.575
Patients with AR		2 (10.5)	3 (9.1)	.869
Patients with asthma		3 (15.8)	5 (15.2)	.952
Nasal secretion glucose measurements				
No. of subjects	24	43	36	
Male	13 (54.2)	21 (48.8)	17 (47.2)	.774
Age (y)	41.5 (27.0, 51.0)	27.0 (20.0, 40.0)	26.0 (20., 46.8)	.143
Patients with atopy	0 (0)	10 (23.3)	8 (22.2)	.036*
Patients with AR	0 (0)	4 (9.3)	4 (11.1)	.262
Patients with asthma	0 (0)	8 (18.6)	2 (5.6)	.027*
RT-PCR				
No. of subjects	26	62	35	
Male	15 (57.7)	36 (58.1)	19 (54.3)	.935
Age (y)	31.0 (21.8, 43.3)	42.0 (32.0, 56.3)	39.0 (27.0, 50.0)	.061
Patients with atopy	0 (0)	16 (25.8)	10 (28.6)	.011*
Patients with AR	0 (0)	7 (11.3)	4 (11.4)	.202
Patients with asthma	0 (0)	13 (21.0)	4 (11.4)	.030*
Flow cytometry				
No. of subjects	7	12	11	
Male	4 (57.1)	6 (50.0)	5 (45.5)	.900
Age (y)	27.0 (20.5, 48.1)	36.0 (24.0, 57.2)	34.0 (21.9, 55.3)	.071
Patients with atopy	0 (0)	3 (25.0)	3 (27.2)	.341
Patients with AR	0 (0)	0 (0)	0 (0)	
Patients with asthma	0 (0)	1 (8.3)	0 (0)	.488
Western blotting				
No. of subjects	12	20	16	
Male	7 (58.3)	11 (55)	9 (56.3)	.984
Age (y)	36.0 (25.3, 52.5)	40.5 (34, 50)	47.5 (42.5, 56)	.091
Patients with atopy	0 (0)	5 (25.0)	2 (12.5)	.153
Patients with AR	0 (0)	2 (10.0)	1 (6.3)	.544
Patients with asthma	0 (0)	2 (10.0)	1 (6.3)	.544
Metabolite measurement				
No. of subjects	7	15	13	
Male	3 (42.9)	7 (53.3)	8 (61.5)	.667
Age (y)	40.0 (26.0, 48.0)	40.0 (27.0, 51.0)	28.0 (21.0, 43.0)	.203
Patients with atopy	0 (0)	3 (20.0)	1 (7.7)	.359
Patients with AR	0 (0)	2 (13.3)	1 (7.7)	.599
Patients with asthma	0 (0)	2 (13.3)	0 (0)	.260
Cell culture study				
No. of subjects	23	17	8	
Male	10 (43.5)	9 (52.9)	5 (62.5)	.638
Age (y)	29 (20, 50)	33 (24, 47.5)	27.5 (23, 49.5)	.103
Patients with atopy	0 (0)	3 (17.6)	0 (0)	.054
Patients with AR	0 (0)	2 (11.8)	1 (12.5)	.240
Patients with asthma	0 (0)	0 (0)	0 (0)	

Continuous variables are reported as median (interquartile range) and categorical variables as n (%).

AR, Allergic rhinitis.

*Value is statistically significant.

TABLE E2. Primary antibodies used in IHC and IF staining

Antibody	Application	Species	Concentration	Clone ID	Reference	Source
ECP	IHC	Rabbit	1:100	Polyclonal	BA4190	Boster Biotechnology
MPO	IHC	Goat	1:500	Polyclonal	AF3667	R&D Systems
GLUT1	IF	Rabbit	1:100	EPR3915	Ab202335	Abcam
GLUT4	IF	Mouse	1:100	Ag15390	66846-1-Ig	Proteintech (Wuhan, China)
GLUT10	IF	Mouse	1:100	Polyclonal	Bs-6325R-AF488	Bioss Biotechnology (Beijing, China)
GLUT11	IF	Rabbit	1:100	Polyclonal	BMP025	MBL (Nagaya, Japan)
Foxj1	IF	Mouse	1:100	CL3989	Ab220028	Abcam
Foxj1	IF	Rabbit	1:100	EPR21874	Ab235445	Abcam
Muc5ac	IF	Mouse	1:100	Polyclonal	BM0054	Boster Biotechnology
Muc5ac	IF	Rabbit	1:100	EPR16904	Ab198294	Abcam
P63	IF	Mouse	1:100	4A4	Ab735	Abcam
P63	IF	Rabbit	1:100	EPR5701	Ab124762	Abcam

IF, Immunofluorescence; IHC, immunohistochemistry.

TABLE E3. Secondary antibodies used in IF staining

Antibody	Concentration	Clone	Reference	Source
IFKine Green donkey anti-mouse IgG	1:100	Polyclonal	A24211	Abbkine Scientific Company
IFKine Green donkey anti-rabbit IgG	1:100	Polyclonal	A24221	Abbkine
IFKine Red donkey anti-rabbit IgG	1:100	Polyclonal	A24421	Abbkine
IFKine Red donkey anti-mouse IgG	1:100	Polyclonal	A24411	Abbkine

TABLE E4. Primers used for quantitative RT-PCR

Genes	Primer sequences	Expected product size (bp)	Annealing temperature (°C)
<i>ACO1</i>	F: 5'-AACCCATTGCGACACCTTG-3' R: 5'-ATGGTAAGCGCCCATATCTTG-3'	103	60
<i>ACO2</i>	F: 5'-CCCTACAGCCTACTGGTGACT-3' R: 5'-TGACTCGTTGGGCTCAAAGT-3'	121	60
<i>ACTB</i>	F: 5'-CATGTACGTTGCTATCCAGGC-3' R: 5'-CTCCTTAATGTCACGCACGAT-3'	250	60
<i>BPGM</i>	F: 5'-TGCTTGGAATAAGGAGAACCGT-3' R: 5'-CCACAGTTCGAGCTTCCTC-3'	84	60
<i>CCL20</i>	F: 5'-TGCTGTACCAAGAGTTTGCTC-3' R: 5'-CGCACACAGACAACCTTTTCTTT-3'	217	58
<i>CS</i>	F: 5'-TGCTTCCTCCACGAATTTGAAA-3' R: 5'-CCACCATACATCATGTCCACAG-3'	132	60
<i>CXCL8</i>	F: 5'-TTTTGCCAAGGAGTGCTAAAGA-3' R: 5'-AACCTCTGCACCCAGTTTTC-3'	194	60
<i>ENO</i>	F: 5'-AAAGCTGGTGCCGTTGAGAA-3' R: 5'-GGTTGTGGTAAACCTCTGCTC-3'	217	60
<i>FH</i>	F: 5'-GGAGGTGTGACAGAACGCAT-3' R: 5'-CATCTGCTGCCTTCATTATTGC-3'	133	58
<i>GLUT1 (SLC2A1)</i>	F: 5'-GGCCAAGAGTGTGCTAAAGAA-3' R: 5'-ACAGCGTTGATGCCAGACAG-3'	201	60
<i>GLUT2 (SLC2A2)</i>	F: 5'-GCTGCTCAACTAATCACCATGC-3' R: 5'-TGGTCCCAATTTTGAAAACCCC-3'	183	60
<i>GLUT3 (SLC2A3)</i>	F: 5'-GCTGGGCATCGTTGTTGGA-3' R: 5'-GCACTTTGTAGGATAGCAGGAAG-3'	123	60
<i>GLUT4 (SLC2A4)</i>	F: 5'-TGGGCGGCATGATTTCCTC-3' R: 5'-GCCAGGACATTGTTGACCAG-3'	88	60
<i>GLUT5 (SLC2A5)</i>	F: 5'-GAGGCTGACGCTTGTGCTT-3' R: 5'-CCACGTTGTACCCATACTGGA-3'	77	60
<i>GLUT6 (SLC2A6)</i>	F: 5'-CCGGACTACGACACCTTCC-3' R: 5'-GGATGTGTAGACCAGGGCATA-3'	150	58
<i>GLUT7 (SLC2A7)</i>	F: 5'-CAGTACGGCTACAACCTCTCT-3' R: 5'-TTGCGTGTGCTCAAAGTAGG-3'	88	58
<i>GLUT8 (SLC2A8)</i>	F: 5'-CTAGTGGCCCCGGTCTACAT-3' R: 5'-CCGACGACGACCATAGCTG-3'	89	60
<i>GLUT9 (SLC2A9)</i>	F: 5'-CCTCTACGGCTACAACCTGTC-3' R: 5'-AGAGTGTCTGGGTCTATTGGAC-3'	111	60
<i>GLUT10 (SLC2A10)</i>	F: 5'-CTTGCTGTATCTACGTGTCAGAG-3' R: 5'-CCAGCCAGTGCATAGTTGAGG-3'	124	60
<i>GLUT11 (SLC2A11)</i>	F: 5'-TTCACCAATGAGACATGGCAG-3' R: 5'-ACAGAGACACGATGAGGGACC-3'	88	60
<i>GLUT12 (SLC2A12)</i>	F: 5'-GAGGCTGCGGCATGTTAC-3' R: 5'-CCAAGTTCATAACCCACCAGG-3'	76	60
<i>HK1</i>	F: 5'-GCTCTCCGATGAACTCTCATAG-3' R: 5'-GGACCTTACGAATGTTGGCAA-3'	121	60
<i>HK2</i>	F: 5'-GAGCCACCACTCACCCTACT-3' R: 5'-CCAGGCATTGCGCAATGTG-3'	249	60
<i>IL1A</i>	F: 5'-TGGTAGTAGCAACCAACGGGA-3' R: 5'-ACTTTGATTGAGGGCGTCATTTC-3'	215	60
<i>IL1B</i>	F: 5'-ATGATGGCTTATTACAGTGGCAA-3' R: 5'-GTCGGAGATTCGTAGCTGGA-3'	132	60
<i>LDHA</i>	F: 5'-ATGGCAACTCTAAAGGATCAGC-3' R: 5'-CCAACCCCAACAACTGTAATCT-3'	86	60
<i>MDH1</i>	F: 5'-GGTGCAGCCTTAGATAAATACGC-3' R: 5'-AGTCAAGCAACTGAAGTTCTCC-3'	132	60
<i>MDH2</i>	F: 5'-TCGGCCCAGAACAAATGCTAAA-3' R: 5'-GCGGCTTTGGTCTCGATG-3'	170	58
<i>OGDH</i>	F: 5'-GGCTTCCCAGACTGTAAAGAC-3' R: 5'-GCAGAATAGCACCGAATCTGTTG-3'	81	58
<i>PDHX</i>	F: 5'-TTGGGAGGTTCCGACCTGT-3' R: 5'-CAACCACTCGACTGTCACTTG-3'	111	60
<i>PFKFB3</i>	F: 5'-TTGGCGTCCCCACAAAAGT-3' R: 5'-AGTTGTAGGAGCTGTACTGCTT-3'	75	60

(Continued)

TABLE E4. (Continued)

Genes	Primer sequences	Expected product size (bp)	Annealing temperature (°C)
<i>PFKM</i>	F: 5'-GGTGCCCGTGCTCTTCTTTGT-3' R: 5'-AAGCATCATCGAAACGCTCTC-3'	99	60
<i>PGK</i>	F: 5'-TGGACGTTAAAGGGAAGCGG-3' R: 5'-GCTCATAAGGACTACCGACTTGG-3'	152	60
<i>PKM</i>	F: 5'-ATAACGCCTACATGGAAAAGTGT-3' R: 5'-TAAGCCCATCATCCACGTAGA-3'	108	60
<i>SDHA</i>	F: 5'-CAAACAGGAACCCGAGGTTTT-3' R: 5'-CAGCTTGGTAACACATGCTGTAT-3'	201	58
<i>SDHB</i>	F: 5'-ACAGCTCCCCGTATCAAGAAA-3' R: 5'-GCATGATCTTCGGAAGGTCAA-3'	177	58
<i>SGLT1 (SLC5A1)</i>	F: 5'-TACCTGAGGAAGCGGTTTGGA-3' R: 5'-CGAGAAGATGTCTGCCGAGA-3'	102	60
<i>SGLT2 (SLC5A2)</i>	F: 5'-TCCTGCTGACATCCTAGTCATT-3' R: 5'-GAAGAGCGCATTCCACTCG-3'	247	60
<i>SUCLG1</i>	F: 5'-TATGGCACCAAACTCGTTGGA-3' R: 5'-GAAGCCGTTGCTCCTGTCT-3'	116	60
<i>SUCLG2</i>	F: 5'-CAAAAGACCCTAATGTTGTGGGA-3' R: 5'-TTCAGCAACCATCACCTTGTT-3'	113	50
<i>TNFA</i>	F: 5'-CCTCTCTCTAATCAGCCCTCTG-3' R: 5'-GAGGACCTGGGAGTAGATGAG-3'	220	60
<i>TPI</i>	F: 5'-CTCATCGGCACTCTGAACG-3' R: 5'-GCGAAGTCGATATAGGCAGTAGG-3'	83	58

F, Forward; R, reverse.

TABLE E5. Primary antibodies used in Western blotting

Antibody	Species	Concentration	Clone ID	Reference	Source
GLUT1	Rabbit	1:1000	EPR3915	Ab202335	Abcam
GLUT4	Mouse	1:1000	Ag15390	66846-1-Ig	Proteintech
GLUT10	Mouse	1:500	H-10	Sc-398495	Santa Cruz Biotechnology (Dallas, Tex)
GLUT11	Rabbit	1:1000	Polyclonal	BMP025	MBL
β -Actin	Mouse	1:1000	3H6G5	GB12001	Goodbio Technology

TABLE E6. Antibodies used in flow cytometry

Antibody	Species	Dilution	Clone ID	Reference	Isotype	Source
GLUT1-BV405	Mouse	1:200	202915	FAB1418V	IgG _{2b}	R&D Systems
GLUT4-APC	Mouse	1:100	Polyclonal	FAB86541R-025	IgG _{2b}	R&D Systems
GLUT10-FITC	Mouse	1:100	Polyclonal	Bs-6325R-AF488	IgG	Bioss Biotechnology
GLUT11	Rabbit	1:100	Polyclonal	BMP025	IgG	MBL
CD45-PE	Mouse	1:100	HI30	304039	IgG1, κ	BioLegend
CD326-BV421	Mouse	1:100	9C4	324220	IgG2b, κ	BioLegend
CD326-APC	Mouse	1:200	9C4	324208	IgG2b, κ	BioLegend
Anti-rabbit IgG-APC	Donkey	1:100	Polyclonal	ANT032	IgG	AntGene Biotechnology (Wuhan, China)

FITC, Fluorescein isothiocyanate.