Supplemental Information

Heat waves elevate risks of airway hypersensitivity that inhaled endogenous ions reduce

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Supplemental Note 1. VPD and ΔP during human of extreme atmospheres

Table 1 summarizes evaporation rates based on airway VPD exposures for larynx/trachea (generation 0), primary/secondary/tertiary bronchi (generations 1, 2, 3) and first brionchiole (generations 5, 6) airways using Eq (1) of the main manuscript. The base and extreme cases of the main manuscript are considered: tidal human mouth breathing of (A) indoor cooled air during a heat wave, characterized by indoor air with VPD = 1.5 kPa, and (B) outdoor hot air with VPD = 6.0 kPa. With the characteristic mucin diffusivity $D = 2\text{x}10^{-12}$ m^2/s and mucus thickness $h_0 = 2\text{x}10^{-5}$ m, the average characteristic Pe ($=qh_0/D$) ranges from 1 to 1.6 in generations 0 to 6 to 1.9 to 3.5 in generations 0 to 3. Extremely high VPD (3.4 to 5.9) in the larynx/trachea in the A & B cases results in ultra-high Pe (3.5 to 5) highlighting the risks of laryngeal/tracheal inflammation in cooled indoor and hot outdoor conditions commonly encountered during heat waves.

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	Airway VPD & *q* on mouth breathing 20-25 °C air with 35% RH (1.5 kPa							**Mean airway values**			
Generation	0	1	2	3	4	5	6	**0-6 gen**	**0-5 gen**	**0-4 gen**	**0-3 gen**
RH (%)	40	65	75	83	89	94	98				
VPD (kPa) @35°C	3.38	1.97	1.41	0.96	0.62	0.34	0.11	1.25	1.44	1.67	1.93
q (m/s)	3.51E-07	1.48E-07	1.06E-07	7.20E-08	4.66E-08	2.54E-08	8.47E-09	1.08E-07	1.25E-07	1.45E-07	1.69E-07
	Airway VPD on mouth breathing 40 °C air with 15% RH (6.0 kPa VPD)							**Mean airway values**			
Generations	0	1	2	3	4	5	6	**0-6 gen**	**0-5 gen**	**0-4 gen**	**0-3 gen**
RH (%)	20	50	65	75	83	89	95				
VPD (kPa) @40°C	5.91	3.69	2.58	1.85	1.26	0.81	0.37	2.35	2.68	3.06	3.51
q (m/s)	5.00E-07	2.26E-07	1.58E-07	1.13E-07	7.69E-08	4.98E-08	2.26E-08	1.64E-07	1.87E-07	2.15E-07	2.49E-07

Table S1. Evaporation rates and VPD for human airways in a mild and extreme heat wave circumstance. Humidity values for airway generations 0-6 are obtained following (1). Mouth/pharyngeal heating of 20-25°C air to 35°C (Case A) and no heating of 40°C air (Case B) is assumed following (2). Pharyngeal humidification is assumed to add 5% RH at the glottal air temperature on inhalation. VPD is determined at the given relative humidity and temperature as the difference between water saturation pressure and the actual vapor pressure using the Clausius-Clapeyron Equation (3). Absence of the laryngeal jet outside of the larynx and trachea reduces the convective (ventilation rates V_R) contribution to Eq (1) in subsequent airways by a factor of 2.

For the indoor air "mild" heatwave condition (A), the average partitioning of globular protein into the PCL over the full airway generation range 0 to 6 ($Pe \sim 1$) diminishes [see Eq (8) and Eq

(9) of Methods] from 0.7 to 1.44 (VPD = 1.5 kPa), such that, ΔP = 0.98 kPa. This is the base dehydrated case on which the simulations in Fig 2 are based. In the extreme case B, over generations 0 to 4 (Pe ~ 3, VPD ~ 3.0 kPa), greater mucus thinning and sequestering of globular protein results in $K_{PCL\text{-}d}$ = 0.28, with ΔP = 2.39 kPa.

It is further instructive to consider the case of slow nasal breathing, for which, with Pe ~ 0.2, L_p ~ $0.8\text{x}10^{-10}$ m²-s/kg, $K_{PCL\text{-}d}$ ~ 0.7, and $\Pi_{gp\text{-}0}$ = 350 Pa, the globular protein osmotic water velocity is given by Eq (12) and Eq (15) of the Methods as u_{gp} ~ 0.026 μm/s. Similarly, with Π_{tm} ~ 180 Pa, Eq (8) of the Methods gives u_{tm} ~ 0.014 μm/s. Noting that u_s = 0 in the steady state, the net osmotic water velocity u ~ 0.04 μm/s exceeds the rate of evaporation q ~ 0.02 μm/s, such that the ASL hydrates, and dilutes globular protein concentration sufficiently to reach water homeostasis, with the ASL structure remaining as depicted in Fig 1B of the main manuscript. Increasing evaporation rate by 100% from q ~ 0.02 μm/s to 0.04 μm/s, as occurs on normal tidal nasal breathing (V_R ~ 15 L/min), thins the mucus hydrogel, while increasing the globular protein partitioning into the hydrogel, such that according to Eq (11) the water velocity through the hydrogel remains roughly constant at ~ 0.024 μm/s. Thus the overall osmotic water velocity u ~ 0.04 μm/s equals the evaporation rate, indicating that the base $\Pi_{gp\text{-}0}$ = 350 Pa is appropriate to support water homeostasis during nasal tidal breathing at a normal tidal ventilation rate.

Supplemental Note 2. Dehydrated ASL pressure elevation

In the steady dehydrated state, which a time-average view of the ASL over the course of the continuous breathing of air of the same atmospheric condition for approximately one minute or more — salt ions equilibrate. The average net flow of water from the airway epithelium into the ASL to support the net evaporation of water into the air lumen is entirely supported by the osmotic pressures of globular protein and tethered mucin. This gives the water mass balance

$$q = u_{gp} + u_{tm}$$

Using Eq (15) and Eqs (3)-(6) of Methods, it follows that the globular protein contribution to the osmotic water velocity is

$$u_{gp-d} = L_p K_{PCL-d} \Pi_{gp-0} \left(\frac{H_0}{H_d} \right) \approx L_p K_{PCL-d} \Pi_{gp-0} \left(\frac{h_0}{h_d} \right) \approx \frac{\frac{Pe_0}{3} L_p K_{PCL-d} \Pi_{gp-0}}{\left[\left(1 + \frac{4}{3} Pe_0 \right)^{1/4} - 1 \right]}$$

The tethered-mucin contribution to the water velocity further follows from $u_{tm\text{-}d} = L_p \, \Pi_{tm\text{-}d}$, and with

$$\Pi_{tm-d} \approx \frac{q}{L_p} - \frac{\frac{Pe_0}{3} K_{PCL-d} \Pi_{gp-0}}{\left[\left(1 + \frac{4}{3} Pe_0 \right)^{1/4} - 1 \right]}$$

The total pressure elevation [Eq (25) of Methods] is then simply

$$\Delta P_d = \frac{q}{L_p} - K_{PCL-d} \Pi_{gp-0} - \Pi_{tm-0}$$

Supplemental Note 3. Gene expression following exposure to warm dry air

Figure S1 summarizes gene expression results obtained following exposure of ALI cultures of HBE cells (Methods) to an extreme atmosphere of 4.4 kPa for 8 hours in a humidity-controlled confocal microscope chamber with air circulation: ANO1, AQP1, AQP5, CFTR, F2R1, ORAl1, P2RX3, P2RX4, P2RX7, P2RY2, PIEZO1, PIEZO2, SCNN1A, SCNN1B, SCNN1D, SCNN1G, TRPA1, TRPV1, TRPV2, TRPV3, TRPV4, TRPV5, TRPV6. All genes up-regulated at either 3h or 8h or both with the exception of AN01, CFTR, F2RL1, ORAl1, P2RX4, P2RY2, SCNN1G, TRPV1. Genes associated with chloride channel activity ANO1, CFTR (Fig S1A) down-regulate, cough provocation — notably the P2X3 cough receptor gene (Fig S1B) — up-regulate, sodium-channel activity — notably the α and β units of ENaC (SCNN1A, SCNN1B) (Fig S1C) up-regulate, TRPV cough activation — notably TRPV1 (Fig S1D) — down-regulate, and ATP secretion — notably TRPV4 (Fig S1D) — up-regulate.

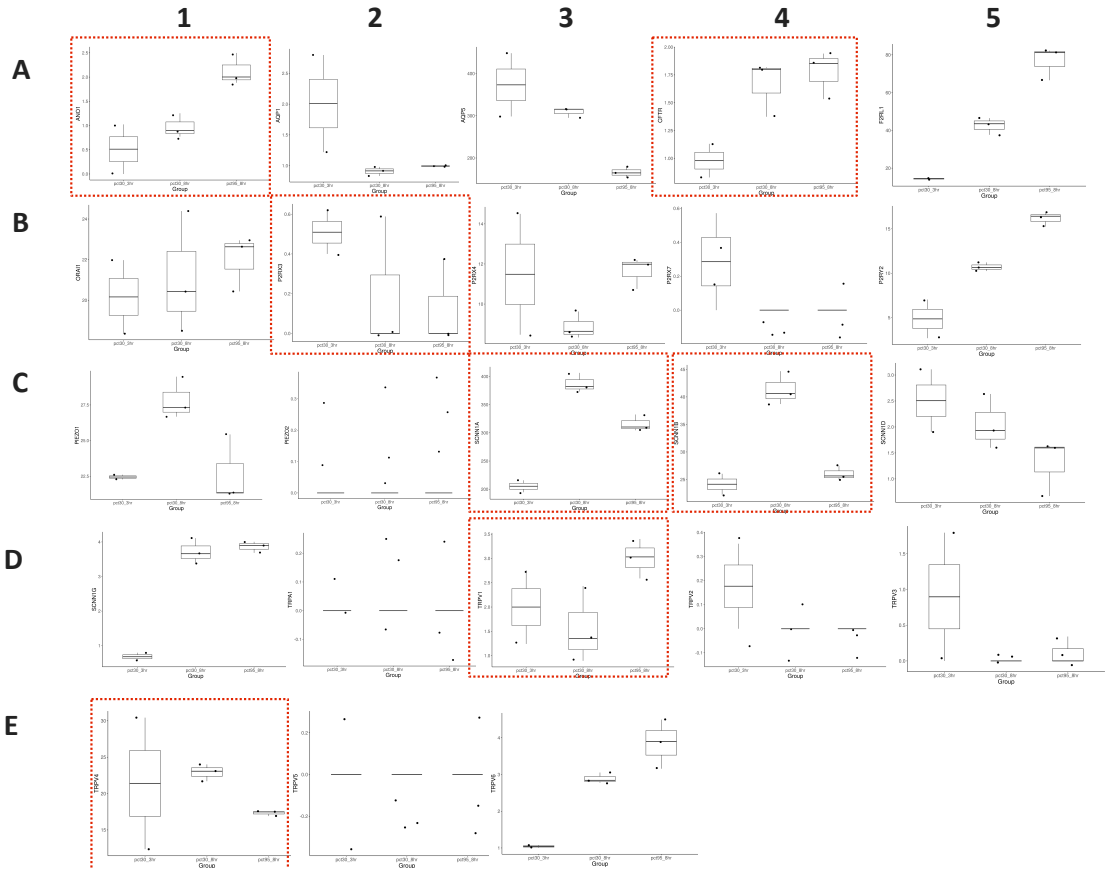

Figure S1. Gene expression following 3 to 8 hours of exposure to 37°C, 30% RH (VPD = 4.4 kPa) air. Red boxes highlight notable airway/cough hypersensitivity gene expression findings.

Figure S2 presents the results of our numerical simulations of topical deposition of alkaline (pH 9) HS (NaCl) and HDS ($MgCl_2$) aerosols on dehydrated airway mucosa following the circumstances of Fig 2B of the main article for non-alkaline aerosol deposition. Unlike the non-alkaline case of Fig 2B, water uptake in the mucus layer is significant given the disassociation of the mucus hydrogel caused by high pH. The benefit of the slow clearance of the divalent magnesium cation is to significantly prolong hydration (dotted red line) relative to the monovalent sodium cation (red solid line).

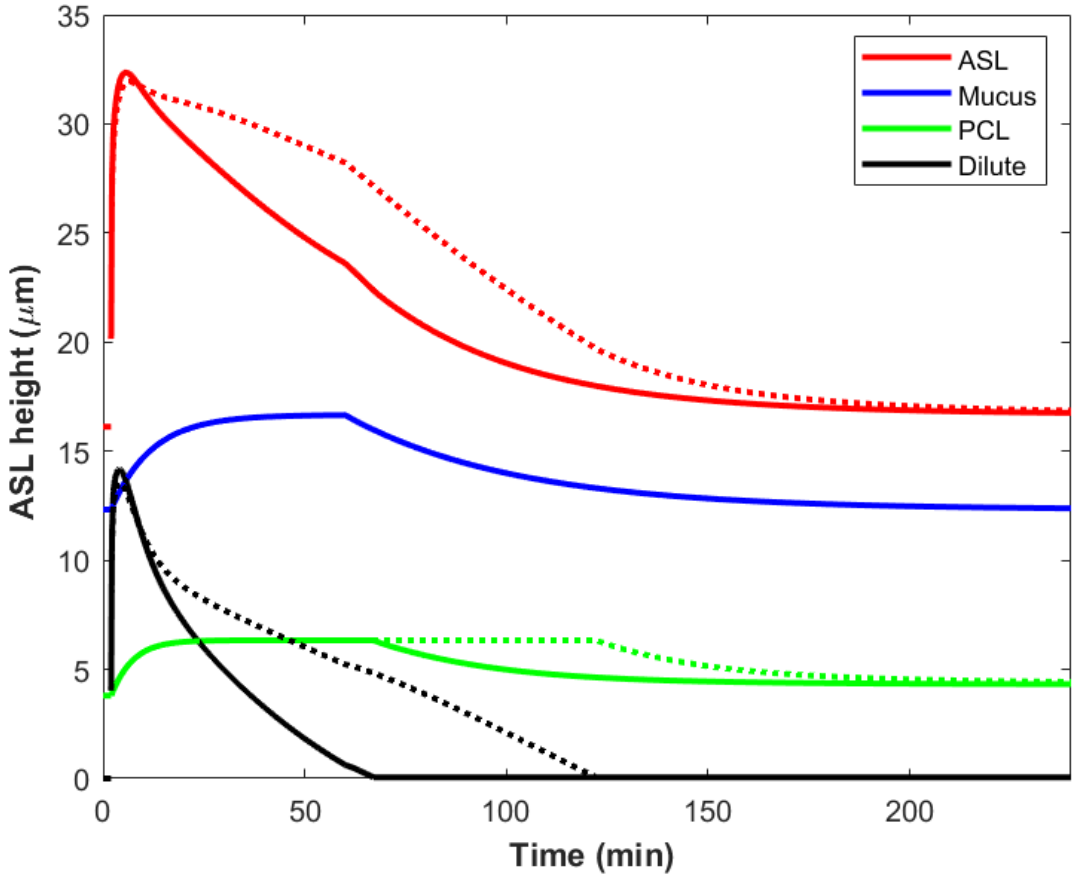

Figure S2. Rehydration of dehydrated airway mucus following deposition of HS & HDS pH 9 aerosols. Change in the heights of ASL, mucus hydrogel, dilute mucin layer, and PCL vs time post deposition of HS (NaCl, 4.3% w/w, pH 9) (solid lines) and HDS ($MgCl_2$, 4.3% w/w, pH 9) (dashed lines) with total deposited mass 25% of ASL mass. Airway generations 0 to 6 in normal tidal breathing (15 L/min), or generations 0 to 9 with fast breathing (30 L/min), are assumed dehydrated by the mouth breathing of 25°C, 30% RH air, resulting in exposure to an average VPD ~ 1.5 kPa (ranging from ~ 3.4 kPa in the larynx to ~ 0.5 kPa in the first bronchioles).

Supplemental Note 4. ASL heights following HS rehydration with partial ENaC block

Figure S3 presents the results of our numerical simulations of the effect of a 15% block of ENaC on ASL height evolution following the deposition of 4.3% NaCl pH 7 and pH 9. Minor prolongation of hydration is achieved by the 15% ENaC block relative to the hydration prolongation observed experimentally in Fig 4F of the main article with the pH 9 hypertonic aerosol relative to pH 7.

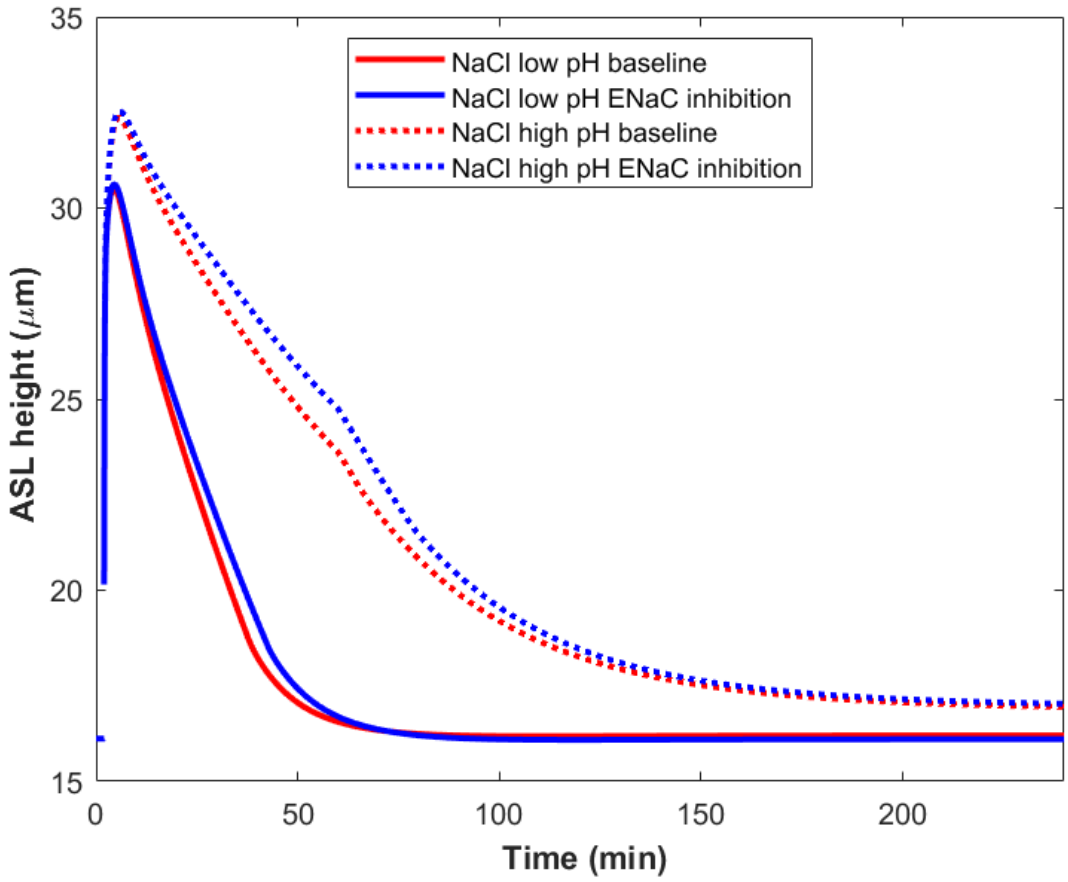

Figure S3. Rehydration of dehydrated airway mucus following deposition of HS & HDS pH 9 aerosols. Numerical simulations of ASL height post deposition of HS (4.3% NaCl w/w) pH 7 (solid lines) and pH 9 (dashed lines) aerosols with normal ENaC activity (red lines) and 15% ENaC block (dashed lines).