Exercise reduces airway sodium ion reabsorption in cystic fibrosis but not in exercise asthma

L. Schmitt*, M. Wiebel[#], F. Frese*, C. Dehnert*, C. Zugck[†],
P. Bärtsch* and H. Mairbäurl*

ABSTRACT: When ventilating large volumes of air during exercise, airway fluid secretion is essential for airway function. Since these are impaired in cystic fibrosis and exercise-induced asthma, it was the aim of this study to determine how exercise affects airway Na⁺ and Cl⁻ transport and whether changes depend on exercise intensity.

Nasal potential was measured in Ringer's solution, with amiloride to block Na⁺ transport, and in low chloride-containing isoproterenol to assess Cl⁻ channels. Nasal potential was measured at rest and during submaximal and maximal bicycle ergometer exercise in individuals with cystic fibrosis, exercise-induced asthma and controls.

At rest, nasal potential was significantly higher in cystic fibroses than in the others. Maximal exercise decreased nasal potentials in cystic fibrosis and controls but not in exercise asthma. Submaximal exercise decreased nasal potentials only in cystic fibrosis. Cl⁻ transport was not affected.

Our results indicate that nasal potentials and Na⁺ transport were decreased by maximal exercise in healthy and cystic fibrosis, whereas submaximal exercise decreased potentials in cystic fibrosis only. Exercise did not affect nasal potentials in asthmatics. Decreased reabsorption during exercise might favour airway fluid secretion during hyperpnoea. This protective effect appears blunted in patients with exercise-induced asthma.

KEYWORDS: Asthma, airway fluid balance, chloride channels, cystic fibrosis, exercise, sodium channels

Active ion transport across the respiratory epithelium plays an important role in regulating volume and composition of airway surface liquid [1]. Airway epithelial cells actively secrete Cl⁻ to generate the osmotic gradient for water transport into the direction of net ion movement [2]. Cl⁻ secretion mainly depends on the presence of the cystic fibrosis transmembrane conductance regulator (CFTR). Airway Na⁺ reabsorption involves apical epithelial Na⁺ channels (ENaC) [3, 4]. A sufficient volume of surface liquid is a prerequisite for humidification of inhaled air, for mucociliary clearance and defence against bacterial colonisation [5].

Increased ventilation during exercise can cause drying of the airway surface resulting in an increased solute concentration, cell shrinkage and fluid shifts from deeper tissue layers [6]. In healthy individuals, this fluid loss from the airways can be compensated by stimulated secretion. In patients with asthma or in those

susceptible to exercise-induced asthma (EIA), airway drying can cause acute bronchoconstriction [6]. In cystic fibrosis (CF), secretion is inadequate even at rest [7].

EIA refers to narrowing of the airways during or after intense exercise. It is related to drying and/or cooling of the airway epithelium and surrounding cells as large volumes of air pass the airway epithelium during exercise [6]. Cells shrink in response to the hyperosmolar environment. Regulatory volume increase of airway epithelial cells withdraws water from the interstitial space, thereby propagating volume shifts into deeper cell layers of the airways. This results in cell injury and release of mediators, such as histamine, prostaglandins and leukotrienes, from mast cells, causing bronchial smooth muscle contraction [8]. In healthy individuals, sympathoadrenergic activation during exercise stimulates fluid secretion by activating Cl⁻ channels such as CFTR and by decreasing fluid reabsorption from

AFFILIATIONS

*Medical Clinic VII, Sports Medicine, University of Heidelberg,

[#]Pulmonary and Critical Care Medicine, Thoraxklinik, University of Heidelberg, and

[†]Medical Clinic III, Cardiology, University of Heidelberg, Heidelberg, Germany.

CORRESPONDENCE

H. Mairbäurl
Medical Clinic VII, Sports Medicine
University of Heidelberg
Im Neuenheimer Feld 410
69120 Heidelberg
Germany
E-mail: heimo.mairbaeurl@med.uni-heidelberg.de

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the airways [9]. However, secretion may not always match the loss of water. We reasoned therefore, that in individuals susceptible to EIA, an asthma attack might be caused by inadequate fluid secretion or too high fluid reabsorption due to an imbalanced regulation of respective ion transport pathways. This appears likely since asthma has been associated with altered Cl^- transport [10]. Also in allergic asthma, attacks were related to impaired mucociliary clearance, which depends on functioning fluid secretion [11, 12], and chronic application of allergens induces hypersecretion by stimulation of Ca^{2+} -activated Cl^- channels and inhibition of amiloride-sensitive Na^+ reabsorption [13].

CF is characterised by viscous mucus caused by defective Cl^- and fluid secretion, not only in the airways but also in other secretory epithelia [7]. It is an autosomal recessive disease caused by mutations of the CFTR [14]. Many mutations of the CFTR gene associated with various degrees of the disease have been reported, the most prominent being a deletion of the amino acid phenylalanine in position 508. Defective CFTR decreases Cl^- secretion, while at the same time sodium reabsorption is increased [15, 16]. Thus, Na^+ reabsorption dominates the transepithelial potential across the nasal mucosa, which is therefore more negative than in healthy controls [17]. As a consequence, in CF patients, humidification of inspired dry air is impaired [18], which might reduce physical performance. Other exercise-related problems encountered by CF patients include insufficient thermoregulation due to decreased sweat production and metabolic disturbances [18]. Exercise-induced sympathoadrenergic activity is a known stimulator for airway fluid secretion [4]. Since this mechanism is lacking in CF, the defective CFTR channel is not activated by adrenergics and cAMP [19], and increased ventilation during exercise might decrease the airway surface film even more.

Active transport of ions is required to mediate fluid fluxes across the airway epithelium. Ion transport also generates an electrical potential difference that can be used to determine which ion transport pathways contribute to net ion movements. The potential difference across the nasal mucosa has been used to characterise for example, airway ion transport *in vivo* [20]. HEBESTREIT *et al.* [9] found a decreased nasal potential difference during submaximal exercise in healthy controls. Two studies have shown that exercise decreases the nasal potential difference in patients with CF [9, 21], which seems to be due to inhibition of nasal epithelial Na^+ reabsorption [9]. Both studies used low intensity exercise, but dependency on exercise intensity is not known.

EIA and CF have in common altered Cl^- transport. In CF airway fluid secretion is impaired, and might be aggravated during exercise. It is not clear, however, whether airway ion transport is altered in EIA and how it might be affected by exercise. Thus, we hypothesise that exercise alters ion transport across the airway epithelium, which might decrease performance and cause asthma attacks. Nasal potential differences were measured at rest and during submaximal and maximal exercise to test for changes in ion transport, which might point to adaptive or maladaptive changes in Na^+ or Cl^- transport in these patients.

MATERIALS AND METHODS

Subjects

11 healthy nonsmoking subjects with no history of respiratory disease, nine subjects with diagnosed EIA, and 10 CF patients participated in the study (anthropometric data are shown in table 1). None of the subjects had previous nasal surgery or reported an upper respiratory tract infection in the 4 weeks preceding the study. None of them suffered from diabetes mellitus, epilepsy, allergic rhinitis or heart disease. Rhinoscopy revealed a normal anatomy and ruled out mucosal inflammation. The study was approved by the University Hospital Heidelberg ethics committee (Heidelberg, Germany) and all subjects gave written informed consent.

The diagnosis of CF was confirmed by a positive sweat test and by genotyping (Thorax Clinic, University of Heidelberg, Heidelberg, Germany). None of the CF patients showed symptoms of acute infection or rhinitis. Patients were allowed to continue standard therapy consisting of pancreatic enzymes, secretolytics and/or anti-diabetics. They did not receive antibiotics or corticosteroids in the last 2 weeks prior to testing.

All participants in the study were subjected to lung function tests at rest. In patients with EIA lung function was measured before and after maximal exercise on the treadmill to confirm the diagnosis of susceptibility to EIA. Lung function had to be normal at rest. A decrease in forced expiratory volume in 1 s (FEV_1) $>20\%$ after exercise (tested twice) and a positive bronchospasmolysis test indicated EIA.

Study design

Subjects were studied on two occasions with at least 7 days between tests. On the first visit, study objectives were explained and informed written consent was obtained. A physical examination was performed to exclude any disease associated with an increased risk during physical exercise.

TABLE 1 Anthropometric data and lung function

Subjects	Males/females n	Age yrs	Height cm	Body weight kg	BMI $\text{kg}\cdot\text{m}^{-2}$	FEV_1 % pred	PEF % pred
Controls	6/5	24.9 ± 3.2	171 ± 8	68.8 ± 12.5	23.4 ± 2.6	107.3 ± 12.4	95.4 ± 9.8
EIA	6/3	22.9 ± 2.9	176 ± 9	71.1 ± 14.4	22.6 ± 2.4	103.3 ± 10.8	94.4 ± 5.0
CF	7/3	23.1 ± 4.1	169 ± 9	57.9 ± 9.7	20.1 ± 1.8	$59.5 \pm 24.3^*$	$61.3 \pm 27.8^*$

Data are presented as mean $\pm$ SD. Lung function tests were performed at rest. BMI: body mass index; FEV_1 : forced expiratory volume in 1 s; PEF: peak expiratory flow; % pred: % predicted; EIA: exercise-induced asthma; CF: cystic fibrosis. *: $p < 0.05$ compared with other groups.

After a 15-min rest in a lying position, blood was drawn from an antecubital vein for measurement of catecholamines and glucocorticoids. Then the subjects completed an incremental test on an electronically braked cycle ergometer (Ergoline GmbH, Bitz, Germany) in a semisupine position (30° angle). The upper body and head were stabilised to avoid a displacement of the nasal electrode during exercise.

Initial workload for the maximal exercise test was 50 W. The power was increased every 3 min by 25 W until volitional fatigue. Samples for lactate measurements were taken from the hyperaemic earlobe before exercise and at the end of each step. During exercise, a 12-lead-ECG was recorded. Immediately after exercise termination, blood was drawn for catecholamine measurements. Nasal potential was measured before and immediately after terminating exercise, and approximately 20 min later. Spirometric measurements could not be performed since mask and/or nose clip would limit access to the probe for nasal potential measurements.

During their second visit, the subjects performed a 20-min submaximal exercise on the semisupine bicycle ergometer. The workload was determined from the maximal test as the load where blood lactate was elevated by 1 mM above the basal value. This is an exercise intensity, which usually is below the anaerobic threshold in healthy individuals and which can be maintained for the duration of the test. Intensity was controlled by heart rate. Blood samples for measurements of catecholamines and glucocorticoids were collected at rest and about 15 min after starting the exercise. Nasal potential was measured before, after 15 min of exercise but while still cycling, and 20 min after exercise. At rest and every 5 min during exercise, samples for lactate measurements were taken from the hyperaemic ear lobe.

Measurement of transepithelial nasal potential

Nasal potential was measured as reported earlier [22] according to KNOWLES *et al.* [20] and MIDDLETON *et al.* [23]. Briefly, the nasal mucosa was superfused (100 $\mu\text{L}\cdot\text{min}^{-1}$) through an umbilical cord catheter (Sherwood Medical, Tullamore, Ireland) with the measuring electrode (WPI, Berlin, Germany) in line. It was placed at the surface of the nasal inferior turbinate. An intravenous infusion line connected to the reference electrode was placed into an antecubital vein and perfused with Ringer's solution (100 $\mu\text{L}\cdot\text{min}^{-1}$). The potential difference was measured with a high impedance voltmeter.

As indicated in figure 1, total nasal potential (NP_{tot}) was recorded during superfusion of the nasal mucosa with Ringer solution containing 10 mM HEPES, pH 7.4 (37°C). Na^+ channel mediated transport was the change in potential measured after switching to Ringer's solution containing 10 μM amiloride ($\text{NP}_{\Delta\text{amil}}$), an inhibitor of epithelial Na^+ channels. Cl^- transport ($\text{NP}_{\Delta\text{Cl}}$) was the change in potential during perfusion with a Ringer's solution where the Cl^- concentration was decreased to 5 mM by replacement with gluconate. This medium also contained amiloride (10 μM) and isoproterenol (10 μM), where the latter was added to stimulate cAMP regulated Cl^- channels, such as CFTR.

Our protocols for measuring nasal potential differed from the standard procedures for multicentre CF diagnosis [24], which would also provide measures for Ca^{2+} activated Cl^- channels.

FIGURE 1. Representative recordings of transepithelial nasal potential differences in healthy controls, exercise-induced asthma (EIA) and cystic fibrosis (CF). Subjects were seated in a semisupine position on a bicycle ergometer with their head rested stably on a pad. The transepithelial potential across the nasal mucosa was recorded under control conditions, where the nasal mucosa was rinsed by slow perfusion with sterile-filtered Ringer's solution containing 10 mM HEPES, pH 7.4. The potential recorded under these conditions was termed NP_{tot} . The proportion of active Na^+ transport to nasal potential was inhibited by switching to Ringer's buffer containing 10 μM amiloride ($\text{NP}_{\Delta\text{amil}}$). The capacity of Cl^- transport ($\text{NP}_{\Delta\text{Cl}}$) was measured as the change in potential during perfusion with a Ringer's buffer containing amiloride (10 μM), where the Cl^- concentration was decreased to 5 mM by replacement with gluconate. This medium also contained isoproterenol (10 μM) to stimulate cAMP-dependent Cl^- channels. The reference electrode was in line with a Ringer-perfusion (100 $\mu\text{L}\cdot\text{min}^{-1}$) into an antecubital vein. a) Representative tracing from a control individual showing inhibition by amiloride ($\text{NP}_{\Delta\text{amil}}$) and stimulation with low Cl^- and isoproterenol ($\text{NP}_{\Delta\text{Cl}}$). b) Representative tracings from an individual with EIA and a patient with CF showing normal total nasal potential (NP_{tot}) and a normal response to amiloride and to low Cl^- /isoproterenol in EIA, but a highly increased NP_{tot} , a large decrease in nasal potential after amiloride and no response to low Cl^- in CF.

However, this protocol takes too long for measurements during exercise.

Plasma concentrations of epinephrine, norepinephrine and cortisol were measured in samples collected at rest and during submaximal and immediately after maximal exercise from the antecubital vein, where also the catheter for the reference

TABLE 2 Results from maximal and submaximal exercise

	Controls	EIA	CF
Subjects n	11	9	10
Maximal exercise			
Workload W	198 ± 41	203 ± 46	120 ± 28 ^{*,#}
Workload W·kg ⁻¹	2.88 ± 0.36	2.87 ± 0.45	2.08 ± 0.38 ^{*,#}
Heart rate min ⁻¹	188 ± 8	180 ± 13	169 ± 12 [*]
Blood lactate mmol·L ⁻¹	7.6 ± 1.2	6.8 ± 1.6	6.5 ± 1.6
Submaximal exercise			
Workload W	124 ± 39	129 ± 36	64 ± 9 ^{*,#}
Workload W·kg ⁻¹	1.85 ± 0.66	1.66 ± 0.42	1.12 ± 0.17 ^{*,#}
Workload % maximal	59.1 ± 14.2	57.5 ± 9.2	55.5 ± 13.0
Heart rate min ⁻¹	136 ± 12	131 ± 13	128 ± 8
Heart rate % max	71.4 ± 6.2	75.7 ± 2.6	76.1 ± 7.0
Blood lactate mmol·L ⁻¹	2.3 ± 1.1	2.3 ± 0.5	3.7 ± 1.0 ^{*,#}

Data are presented as mean ± SD. Exercise was performed on a bicycle ergometer in a semisupine position. In the maximal test, beginning work load was 50 W. The load was increased by 25 W every 3 min until exhaustion. The intensity of the 20 min submaximal exercise was chosen to increase blood lactate by 1 mM above baseline values assessed in the maximal test; exercise intensity was adjusted by heart rate. Note that in cystic fibrosis (CF) patients, blood lactate increased more than in the other groups. EIA: exercise-induced asthma. *: $p \leq 0.05$ in comparison with controls. #: $p \leq 0.05$ in comparison with EIA.

electrode was placed. Measurements were performed by ELISA and radioimmunoassay (central laboratory, University Hospital Heidelberg, Heidelberg, Germany).

Statistical analysis

Except for figure 1, all transepithelial nasal potential differences (mean ± SD) are shown as positive values. Comparison between groups was performed by one-way ANOVA followed by least significant difference (LSD) tests to determine group differences. Changes during exercise were evaluated by ANOVA for repeated measures and LSD tests. Significance was accepted at $p < 0.05$.

RESULTS

Patient characteristics and exercise

At rest, FEV₁ and peak flow were in the normal range in controls and EIA (table 1) but were significantly decreased in CF patients ($p < 0.001$).

Maximal workloads (table 2) in the incremental exercise test were ~200 W in controls and EIA, but only ~120 W in CF ($p < 0.001$). Thus, duration of maximal exercise was ~21 min in the control subjects, ~24 min in EIA, but only ~11.5 min in CF patients. We do not have objective indicators of maximal workout, since spirometric data could not be obtained during the test because of the nasal potential measurements. Maximally achieved heart rates were slightly lower than the expected age-related maximum; CF patients reached only about 85% of their predicted maximum. There was no difference in maximal heart rate between controls and EIA patients ($p = 0.098$), but maximal heart rate was significantly lower in CF patients than in controls ($p < 0.001$). Maximal blood

FIGURE 2. Changes in nasal potential difference during submaximal and maximal exercise. a) NP_{tot} and its components were measured at rest, during submaximal, and after maximal exercise. b) Transport mediated by epithelial Na⁺ channels was determined as the proportion of NP_{tot} inhibited by 10 μM amiloride (NP_{Δamil}). c) The capacity of Cl⁻ transport (NP_{ΔCl}) was determined after decreasing the Cl⁻ concentration in the perfusate to 5 mM in presence of amiloride (10 μM) and isoproterenol (10 μM) to stimulate cAMP dependent Cl⁻ channels. Details are described in the methods section. Data are presented as mean ± SD of nasal potentials measured at rest (R), during submaximal (S), and immediately after maximal (M) exercise from 11 healthy individuals, nine individuals with exercise-induced asthma (EIA) and 10 patients with cystic fibrosis. *: $p < 0.05$ between rest and exercise; #: $p < 0.05$ between cystic fibrosis and the other groups.

TABLE 3 Hormonal changes during exercise

	Rest	Submaximal	Maximal
Epinephrine pM			
Controls	170 ± 150	491 ± 574*	2429 ± 2193* [#]
EIA	160 ± 105	363 ± 359*	1021 ± 920* ^{#,†}
CF	135 ± 194	198 ± 165*	440 ± 436* ^{#,†}
Norepinephrine pM			
Controls	2023 ± 1203	2426 ± 1997*	17869 ± 8850* [#]
EIA	994 ± 488	3471 ± 2633* [†]	7475 ± 6420* ^{#,†}
CF	1099 ± 825	1934 ± 1628* [#]	5043 ± 1628* ^{#,†}
Cortisol µg·dL⁻¹			
Controls	159 ± 97	144 ± 96	155 ± 71
EIA	148 ± 71	110 ± 115	122 ± 61
CF	160 ± 66	116 ± 53	143 ± 54

Data are presented as mean ± SD for 11 controls, nine patients with exercise-induced asthma and 10 with cystic fibrosis. Subjects were rested in a semisupine position for 15 min before the resting blood samples were taken. Blood was collected in the last 2 min of submaximal exercise and immediately after exercise termination in the maximal test. Resting values before the two tests were not different and were therefore averaged. *: $p < 0.05$ compared with rest; [#]: $p < 0.05$ between submaximal and maximal exercise; [†]: $p < 0.05$ relative to controls.

lactate concentrations were not different between groups. Workloads in the submaximal test were comparable in controls and EIA patients ($p = 0.925$) but much lower in CF patients ($p < 0.001$). Nevertheless, blood lactate during submaximal exercise was higher in CF patients than in controls and EIA patients ($p < 0.001$). Heart rates during submaximal exercise were not different.

Typical recordings of nasal potentials from one individual from each group at rest are shown in figure 1. It also shows the definitions used below for nasal potentials and its components (fig. 1a). Mean values of NP_{tot} and its components from each group during submaximal and maximal exercise are summarised in figure 2. It shows that NP_{tot} (fig. 2a) at rest was approximately -17 mV in controls and EIA, and that there was no difference between these two groups. In contrast, NP_{tot} was significantly more negative in CF patients ($p < 0.001$). Figure 2 also shows that in all individuals measurements were very similar on the 2 days when tests were performed.

In controls, NP_{tot} was decreased slightly after maximal exercise ($p = 0.015$) but not in submaximal exercise. A much larger decrease by ~10 mV was seen in CF both in maximal ($p = 0.018$) and submaximal exercise ($p = 0.001$). Neither maximal nor submaximal exercise affected NP_{tot} in EIA ($p = 0.972$). In all groups, recovery from either test restored pre-exercise NP_{tot} (data not shown).

Figure 2b shows that $NP_{\Delta amil}$ was comparable in controls and EIA patients ($p = 0.758$) but that it was approximately three times higher in CF patients ($p < 0.001$). During maximal exercise there was no change in $NP_{\Delta amil}$ in controls ($p = 0.593$) and EIA ($p = 0.900$), but a decrease in $NP_{\Delta amil}$ by ~10 mV in CF patients ($p = 0.049$). $NP_{\Delta amil}$ did not change significantly during submaximal exercise in either group.

Results on Cl^- transport are summarised in figure 2c. It shows that perfusion with low Cl^- in the presence of amiloride and isoproterenol caused a decrease in nasal potential by ~13 mV in controls and EIA patients, whereas this component was barely detectable in CF patients, and thus $NP_{\Delta Cl}$ was lower in CF patients than in controls and EIA patients under all experimental conditions ($p < 0.001$). $NP_{\Delta Cl}$ was not altered significantly during exercise or recovery in any of the groups.

Since epithelial Na^+ and Cl^- transport in the lung can be stimulated by stress hormones, it was important to determine changes in catecholamines and cortisol in plasma during exercise. Table 3 shows a gradual increase in epinephrine and norepinephrine with exercise intensity in all groups. Interestingly, despite similar workloads in the maximal test, plasma epinephrine was lower in EIA patients than in controls ($p = 0.047$). Epinephrine was also significantly lower in CF patients than in controls ($p = 0.010$), probably owing to the lower exercise intensity, as shown in table 2. There was no statistically significant difference between groups in epinephrine in the submaximal test. At maximal exercise, norepinephrine was lower in EIA ($p = 0.003$) and CF ($p < 0.001$) than in controls, whereas there was no difference between groups in submaximal exercise ($p = 0.347$). Plasma cortisol was not different between groups and did not change during either exercise test.

DISCUSSION

The results of our study show that, in healthy individuals only, heavy but not submaximal exercise decreased reabsorptive ion transport in the nasal epithelium, whereas it was decreased in CF in both modes of exercise. These exercise-related changes in nasal potential seem to be lacking in EIA. Decreased reabsorption can be interpreted as a means to increase the volume of the airway epithelial fluid film by favouring the secretion required for humidification of inspired air during exercise hyperpnoea. Thus, it appears that EIA patients might lack a means of optimising the volume of airway lining fluid during exercise.

Performance in the maximal test was similar in controls and EIA, where subjects reached ~2.9 W·kg⁻¹. Maximal heart rates in these groups were slightly below the predicted maximum indicating that they may not have reached full exertion, whereas maximal lactates were in a reasonable range for relatively unfit individuals. Maximal workloads and heart rates of CF patients were lower than values for controls and patients with EIA, and lactate tended to be lower. This has to be explained by airway dysfunction in this disease, which probably impaired alveolar gas exchange.

Changes in nasal potential during submaximal exercise have been reported earlier [9, 21]. In healthy individuals, ALSUWAIDAN *et al.* [21] found a gradual increase in nasal potential in the first 15 min of exercise at an intensity of ~80% of the predicted maximal heart rate, which was followed by a slow return towards baseline. In contrast, HEBESTREIT *et al.* [9], who exercised their individuals at 85% of the intensity at the ventilatory threshold, found a decrease in nasal potential and $NP_{\Delta amil}$. Our results on healthy individuals showed no change in nasal potential during 20 min exercise with an intensity of ~55% of maximal performance, whereas a small (4 mV) but

statistically significant decrease in nasal potential was found when measurements were performed immediately after terminating maximal exercise. Changes might have been bigger during intensive exercise. Based on these findings, in healthy individuals the change in nasal potential seems to depend on exercise intensity. However, the changes we found after maximal exercise were considerably smaller than those reported by HEBESTREIT *et al.* [9]. If there was a clear dependency on exercise intensity then larger changes would have to be expected. We have no explanation for this difference. Since we do not have measurements during exercise our data allow no conclusion on a dependency on exercise duration.

In CF, the transport systems controlling the volume and composition of airway lining fluid are disturbed by the lack of functioning CFTR Cl^- channels due to mutations of the channel protein [14]. CFTR channel inactivity is associated with increased Na^+ reabsorption [24]. Catecholamine-stimulated cAMP production might increase Na^+ channel activity even further [4], which might exaggerate the depletion of airway lining fluid caused by the defective CFTR. Results of nasal potential measurements of CF patients unanimously show decreases in nasal potential in the first ~20 min of exercise [9, 21]. We confirm this result and show that the decrease was much larger in CF than in healthy individuals. The decrease in nasal potential during exercise of CF patients in our study was smaller than that observed by HEBESTREIT *et al.* [9] but was in the range of changes found by ALSUWIDAN *et al.* [21] in the first 15–20 min of exercise. We have no explanation for this discrepancy. Placement of the electrode might be one possible reason as nasal potential varies considerably within small areas of the nasal mucosa. ALSUWIDAN *et al.* [21] also show that continuing exercise caused nasal potential to return to the pre-exercise nasal potential, indicating a non-linear time dependency of this response.

Our results indicate that in CF both maximal and submaximal exercise result in the same decrease in nasal potential and $\text{NP}_{\Delta\text{amil}}$. It has to be pointed out that the larger decrease in nasal potential in CF occurred at much lower exercise intensity than in healthy controls, indicating that, in CF, changes in nasal potentials did not depend on the absolute value of exercise intensity. Thus, the statement on dependency of nasal potential changes on exercise intensity does not hold true for CF. If changes in nasal potential during exercise are a threshold phenomenon, then these data would indicate that, in CF, this threshold has shifted towards lower exercise intensity. This result also indicates that altered catecholamine levels seem not to account for changes in nasal potential during exercise, since their plasma levels correlated with exercise intensity.

In EIA patients, nasal potential and its components were not different from healthy controls at rest. This is in accordance with results obtained by CHUNG *et al.* [25] on mild asthmatics. A difference might have been expected due to altered expression of Ca^{2+} -dependent Cl^- channels in asthmatics [10]. We might have missed this component, since our technique was set up mainly to detect changes in Na^+ transport and Cl^- transport by CFTR because of the short time available for measurements during exercise tests. It was not specifically aimed at detecting all types of Cl^- transport pathways.

Interestingly, regardless of the exercise intensity employed, we did not observe a change in nasal potential during exercise in EIA individuals.

Fluid balance across the airways is controlled by secretion mediated by basolateral $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransport and apical Cl^- channels, mainly CFTR, and by reabsorption of fluid driven by Na^+ transport [3, 4], thus warranting humidification of inspired air, mucociliary clearance and immune defence [26]. In nasal potential measurements, these two transport pathways are characterised by $\text{NP}_{\Delta\text{Cl}}$ and by $\text{NP}_{\Delta\text{amil}}$, respectively, where a decrease in nasal potential by decreasing $\text{NP}_{\Delta\text{amil}}$ indicates inhibition of the reabsorptive pathway. The significance of elevated Na^+ transport has been demonstrated by the beneficial effect of treatment of CF with the Na^+ channel blocker amiloride [16, 27], and decreased volume of airway lining fluid and CF-like symptoms in transgenic mice overexpressing ENaC in the airways [27, 28]. Therefore, a decrease in total nasal potential and $\text{NP}_{\Delta\text{amil}}$ indicates a decreased rate of reabsorption of airway lining fluid [9, 21], which should increase the volume of airway lining fluid and prevent airway drying when ventilating large volumes of air during exercise. The fact that decreased Na^+ reabsorption was not seen in EIA might indicate that these patients lack this potentially protective mechanism. The subsequent decreased volume of airway lining fluid during hyperpnoea, an increased osmolality, and fluid shifts are known to be key events in EIA [6, 29]. Thus, sustained rather than inhibited reabsorption might be a causal factor in the occurrence of EIA. Further studies on a larger study population are required to clarify this issue, particularly to clarify which transport processes are involved. It would also be interesting to see whether similar events can be observed in other forms of asthma.

One possible limitation of this and previous studies [9, 21], with regard to ventilation and exercise-induced changes in nasal potential, might be the placement of the electrode. Nasal airflow might be reduced during exercise due to a shift to lower resistance of ventilating through the mouth. Measuring in deeper airways would most likely much better reflect changes in the fluid balance and associated ion transport processes. The fact that potentials measured in the nose change with exercise might indicate that not the airflow *per se* causes these changes. Hormones and/or mediators released into circulation might cause these effects. Elevated nitric oxide, atrial natriuretic peptide and C-type natriuretic peptide, and extracellular nucleotides had been discussed [9]. Elevated catecholamines during exercise might stimulate secretion by cAMP and protein kinase A-dependent activation of CFTR [30] and by elevated intracellular Ca^{2+} [31]. Measuring $\text{NP}_{\Delta\text{Cl}}$ in the presence of isoproterenol might mask this effect. However, catecholamines also stimulate Na^+ reabsorption in the airways [4], whereas we found inhibition. Thus, the inhibitory pathway must have a stronger effect on Na^+ transport than the catecholamines. It is unclear why the inhibition of Na^+ transport was absent in EIA.

In conclusion, we show here an inhibition of airway reabsorption during exercise that seems to depend on exercise intensity. This effect was more pronounced in healthy individuals than in subjects with CF. The decrease in the transport might point to prevention of reabsorption, thus maintaining a high volume

of airway lining fluid. CF patients might benefit more from this effect than individuals with a high activity of Cl^- transport. In contrast, this means of increasing airway lining fluid during exercise was not seen in EIA, which might indicate a defective control of the volume of airway lining fluid that might be associated with the appearance of these symptoms.

STATEMENT OF INTEREST

None declared.

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