ERJ Express. Published on June 4, 2010 as doi: 10.1183/09031936.00197309

Exercise reduces airway Na-reabsorption in cystic fibrosis but not in exercise asthma

Laurenz Schmitt[1], Matthias Wiebel[2], Falko Frese[1], Christoph Dehnert[1], Christian Zugck[3], Peter Bärtsch[1], Heimo Mairbäurl[1]

1) Medical Clinic VII, Sports Medicine, University of Heidelberg, Heidelberg, Germany
2) Pulmonary and Critical Care Medicine, Thoraxklinik, University of Heidelberg, Heidelberg, Germany
3) Medical Clinic III, Cardiology, University of Heidelberg, Heidelberg, Germany

Short title: **NP in exercise induced asthma and cystic fibrosis during exercise**

Corresponding author
Prof. Dr. Heimo Mairbäurl
Medical Clinic VII, Sports Medicine
University of Heidelberg
Im Neuenheimer Feld 410
69120 Heidelberg

Tel.: +49 6221 56 8103
Fax: +49 6221 56 7307
e-Mail: heimo.mairbaeurl@med.uni-heidelberg.de

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, with amiloride to block Na-transport, and in low chloride containing isoproterenol to assess Cl^- channel. NP 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 favor airway fluid secretion during hyperpnoea. This protective effect appears blunted in patients with exercise induced asthma.

Key words
Airway fluid balance, asthma, chloride-channels, cystic fibrosis, exercise, sodium-channels

***Introduction*:**
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) (for review see (3;4)). A sufficient volume of surface liquid is a prerequisite for humidification of inhaled air, for mucociliary clearance, and defense against bacterial colonization (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 susceptibles to exercise induced asthma (EIA) airway drying can cause acute broncho-constriction (for review see (6)). In cystic fibrosis 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 interstitial space thereby propagating volume shifts into deeper cell layers of the airways, resulting in cell injury, release of mediators such as histamine, prostaglandins and leukotrienes from mast cells causing bronchial smooth muscle contraction (8). In healthy individuals, sympatho-adrenergic activation during exercise stimulates fluid secretion by activating Cl^- channels such as CFTR and by decreasing fluid reabsorption from 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 hyper-secretion by stimulation of Ca^{2+}-activated Cl^--channels and inhibition of amiloride-sensitive Na^{2+}-reabsorption (13).
Cystic fibrosis (CF) is characterized by viscous mucus caused by defective Cl^- and fluid secretion not only in the airways but also in other secretory epithelia (for review see (7)). It is an autosomal recessive disease caused by mutations of the cystic fibrosis transmembrane conductance regulator (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 of CF patients include insufficient thermoregulation due to decreased sweat production and metabolic disturbances (18). Exercise-induced sympatho-adrenergic 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 cyclic AMP (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 (NP) has been used to characterize airway ion transport in vivo (e.g. (20)).

Hebestreit et al. found a decreased NP during submaximal exercise in healthy controls (9). 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 that Cl^- transport is altered. In CF airway fluid secretion is impaired, which 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 hypothesize that exercise alters ion transport across the airway epithelium, which might decrease performance and which might 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 ion Na^+ or Cl^- transport in these patients.

Materials and Methods:

Subjects

Eleven healthy nonsmoking subjects (CO) with no history of respiratory disease, nine subjects with diagnosed exercise induced asthma (EIA), and ten cystic fibrosis (CF) patients participated in the study (anthropometric data in **tab.1.**). None of the subjects had previous nasal surgery or reported an upper respiratory tract infection in the four 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; 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). 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 two weeks prior to testing.

All participants in the study were subjected to lung function tests at rest. In patients with exercise induced asthma (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 $FEV_1 > 20\%$ 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. After a 15-minute 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, Germany) in a semi supine position (30° angle). Upper body and head were stabilized to avoid a displacement of the nasal electrode during exercise.

Beginning work load for the maximal exercise test was 50 watts. The power was increased every 3 minutes by 25 watts until volitional fatigue. Samples for lactate measurements were taken from the hyperemic earlobe before exercise and at the end of each step. During exercise, a 12-lead-electrocardiogram (ECG) was recorded. Immediately after exercise termination, blood was drawn for catecholamine measurements. NP was measured before, immediately after terminating exercise, and approximately 20 minutes later. Spirometric measurements could not be performed since mask and/or nose clip would limit access to the probe for NP measurements.

During their second visit, the subjects performed a 20 min submaximal exercise on the semi supine bicycle ergometer. The work load 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 measurement of catecholamines and glucocorticoids were collected at rest and about 15 min after starting the exercise. NP 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 hyperemic ear lobe.

Measurement of transepithelial nasal potential (NP)

NP was measured as reported earlier (22) according to Knowles et al. (20) and Middleton et al. (23). Briefly, the nasal mucosa was superfused (100 µl/min) through an umbilical cord

catheter (Sherwood Medical, Ireland) with the measuring electrode (WPI, 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 solution (100 µl/min). The potential difference was measured with a high impedance voltmeter.
As indicated in **fig.1A**, total nasal potential (NP_{tot}) was recorded during superfusion of the nasal mucosa with Ringer solution containing 10 mM N-[2-hydroxyethyl]-piperazine-n'-[2-ethane-sulphonic-acid] (HEPES), pH 7.4 (37°C). Na^+ channel mediated transport was the change in potential measured after switching to Ringer containing 10 µM amiloride ($NP_{\Delta amil}$), an inhibitor of epithelial Na^+ channels. Cl^- transport ($NP_{\Delta Cl}$) was the change in potential during perfusion with a Ringer 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 NP differed from the standard procedures for multicenter CF diagnosis (24), which would also provide measures for Ca^{2+} activated Cl^- channels. 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 electrode was placed. Measurements were performed by ELISA and RIA (central laboratory, University Hospital Heidelberg).

Statistical analysis
Except for fig.1 all transepithelial nasal potential differences (means ± SD) are shown as positive values. Comparison between groups was performed by one way ANOVA followed by 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, FEV 1 and peak flow were in the normal range in CO and EIA (**tab.1**) but were significantly decreased in CF patients (P<0.001).

Maximal work loads (**tab.2.**) in the incremental exercise test were ~200 W in CO and EIA, but only ~120 W in CF (P<0.001). Thus, duration of maximal exercise was ~21 min in the controls subjects, ~24 min in EIA, but only ~11.5 min in CF. We do not have objective indicators of maximal workout since spirometric data could not be obtained during the test because of the NP measurements. Maximally achieved heart rates were slightly lower than the expected, age-related maximum; CF reached only about 85% of their predicted maximum. There was no difference in maximal heart rate between CO and EIA (P=0.098), but maximal heart rate was significantly lower in CF than in CO (P<0.001). Maximal blood lactate concentrations were not different between groups. Work loads in the submaximal test were comparable in CO and EIA (P=0.925) but much lower in CF (P<0.001). Nevertheless, blood lactate during submaximal exercise was higher in CF than in CO and EIA (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 **fig.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 summarized in **fig.2**. It shows that NP_{tot} (**fig.2A**) at rest was approximately -17 mV in CO and EIA, and that there was no difference between these two groups. In contrast, NP_{tot} was significantly more negative in CF (P<0.001). Fig.2A,B, and C also show that in all individuals measurements were very similar on the two days when tests were performed.

In CO, NP_{tot} was decreased slightly after maximal exercise (P=0.015) but not in submaximal exercise. A much larger decrease by approximately 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} (not shown).

Figure 2B shows that $NP_{\Delta amil}$ was comparable in CO and EIA (P=0.758) but that it was about 3-times higher in CF (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 about 10 mV in CF (P=0.049). In neither group $NP_{\Delta amil}$ changed significantly during submaximal exercise.

Results on Cl^- transport are summarized in **fig.2C**. It shows that perfusion with low Cl^- in presence of amiloride and isoproterenol caused a decrease in nasal potential by about 13mV in CO and EIA, whereas this component was barely detectible in CF and thus $NP_{\Delta Cl}$ was lower in CF than in CO and EIA under all experimental conditions (P<0.001). In none of the groups, $NP_{\Delta Cl}$ was altered significantly during exercise or recovery.

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. **Tab.3** shows a gradual increase in epinephrine and norepinephrine with exercise intensity in all groups. Interestingly, despite similar work loads in the maximal test, plasma epinephrine was lower in EIA than in CO (P=0.047). Epinephrine was also significantly lower in CF than in CO (P=0.010), probably owing to the lower exercise intensity as shown in tab.2. There was no statistically significant difference between groups in epinephrine in the submaximal test. At maximal exercise, also norepinephrine was lower in EIA (P=0.003) and CF (P<0.001) than in CO, whereas there was no difference between groups in submaximal exercise (P=0.347). Plasma cortisol was not different between groups 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 nasal epithelium, whereas it was decreased in CF in both modes of exercise. These exercise-related changes in nasal potential seem to lack in EIA. Decreased reabsorption can be interpreted as a means to increase the volume of the airway epithelial fluid film by favoring secretion required for humidification of inspired air during exercise hyperpnoea. Thus it appears that EIA might lack a means of optimizing the volume of airway lining fluid during exercise.

Performance in the maximal test was similar in CO and EIA, where subjects reached about 2.9 Watts/kg. 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 work loads and heart rates of CF patients were lower than values of CO and EIA, lactate tended to be lower. This has to be explained by airway dysfunction in this disease which probably impaired alveolar gas exchange.

Changes in NP during submaximal exercise have been reported earlier (9;21). In healthy individuals, Asluwaidan et al. found a gradual increase in NP 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 (21). In contrast, Hebestreit et al., who exercised their individuals at 85% of the intensity at the ventilatory threshold, found a decrease in NP and $NP_{\Delta amil}$ (9). Our results on healthy individuals showed no change in NP during 20 min exercise with an intensity of about 55% of maximal performance, whereas a small (4 mV) but statistically significant decrease in NP 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 NP 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 were 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 NP measurements of CF patients unanimously show decrease in NP 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 NP 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 Alsuwaidan et al. in the first 15 to 20 min of exercise (21). We have no explanation for this discrepancy. Placement of the electrode might be one possible reason since NP varies considerably within small areas of the nasal mucosa. Alsuwaidan et al. also show that continuing exercise caused NP to return to pre-exercise NP (21) indicating a non-linear time dependency of this response.

Our results indicate that in CF both the maximal and the submaximal exercise result in the same decrease in NP and $NP_{\Delta amil}$. It has to be pointed out that the larger decrease in NP 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 NP changes on exercise intensity does not hold true for CF. If changes in NP 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 NP 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 accordance with results obtained by Chung et al. on mild asthmatics (25). 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 NP during exercise in EIA individuals.

Fluid balance across the airways is controlled by secretion mediated by basolateral $Na^+/K^+/2Cl^-$ cotransport and apical Cl^- channels, mainly CFTR, and by reabsorption of fluid driven by Na^+ transport (for recent reviews see (3;4)), thus warranting humidification of

inspired air, mucociliary clearance and immune defense (26). In NP measurements, these two transport pathways are characterized by $NP_{\Delta Cl}$ and by $NP_{\Delta amil}$, respectively, where a decrease in NP by decreasing $NP_{\Delta amil}$ 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 over-expressing ENaC in the airways (27;28). Therefore a decrease in total NP and $NP_{\Delta amil}$ indicates 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 hyperpnea, an increased osmolarity, and fluid shifts are known as key events in EIA (6;29). Thus, sustained rather than inhibited reabsorption might be in causal relation to 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 NP 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 air flow per se causes these changes. Hormones and/or mediators released into circulation might cause these effects. Elevated NO, ANP and CNP, and extracellular nucleotides had been discussed (9). Elevated catecholamines during exercise might stimulate secretion by cAMP and PKA-dependent activation of CFTR (e.g. (30)) and by elevated intracellular Ca^{2+} (for review see (31)). Measuring $NP_{\Delta Cl}$ in presence of isoproterenol might mask this effect. However, catecholamines also stimulate Na^+ reabsorption in the airways (for review see (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 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.

Acknowledgements: We thank Mrs.Sonja Engelhardt, Mrs.Christiane Herth, and Mrs.Judith Strunz for excellent technical assistance.

Reference List

1. Kilburn, K. H. A Hypothesis for Pulmonary Clearance and Its Implications. Am Rev.Respir.Dis. 1968;98(3):449-63.

2. Olver, R. E., Davis, B., Marin, M. G., and NADEL, J. A. Active Transport of Na^+ and Cl- Across the Canine Tracheal Epithelium in Vitro. Am Rev.Respir.Dis. 1975;112(6):811-5.

3. Boucher, R. C. Regulation of Airway Surface Liquid Volume by Human Airway Epithelia. Pflugers Arch. 2003;445(4):495-8.

4. Chambers, L. A., Rollins, B. M., and Tarran, R. Liquid Movement Across the Surface Epithelium of Large Airways. Respir.Physiol Neurobiol. 15-12-2007;159(3):256-70.

5. Boucher, R. C. Molecular Insights into the Physiology of the 'Thin Film' of Airway Surface Liquid. J Physiol 1-5-1999;516 (Pt 3):631-8.

6. Anderson, S. D. and Daviskas, E. The Mechanism of Exercise-Induced Asthma Is ... J.Allerg.Clin.Immunol. 2000;106:453-9.

7. Welsh, M. J. Abnormal Regulation of Ion Channels in Cystic Fibrosis Epithelia. Faseb J 1990;4(10):2718-25.

8. Anderson, S. D. and Holzer, K. Exercise-Induced Asthma: Is It the Right Diagnosos in Elite Athletes? J.Allerg.Clin.Immunol. 2000;106:419-28.

9. Hebestreit, A., Kersting, U., Basler, B., Jeschke, R., and Hebestreit, H. Exercise Inhibits Epithelial Sodium Channels in Patients With Cystic Fibrosis. Am.J.Respir.Crit.Care Med. 1-8-2001;164(3):443-6.

10. Hoshino, M., Morita, S., Iwashita, H., Sagiya, Y., Nagi, T., Nakanishi, A., Ashida, Y., Nishimura, O., Fujisawa, Y., and Fujino, M. Increased Expression of the Human Ca^{2+}-Activated Cl^- Channel 1 (CaCC1) Gene in the Asthmatic Airway. Am.J.Respir.Cell.Molec.Biol. 2002;165:1132-6.

11. Button, B. and Boucher, R. C. Role of Mechanical Stress in Regulating Airway Surface Hydration and Mucus Clearance Rates. Respir.Physiol Neurobiol. 30-11-2008;163(1-3):189-201.

12. Messina, M. S., O'Riordan, T. G., and Smaldone, G. C. Changes in Mucociliary Clearance During Acute Exacerbations of Asthma. Am Rev.Respir.Dis. 1991;143(5 Pt 1):993-7.

13. Anagnostopoulou, P., Dai, L., Schatterny, J., Hirtz, S., Duerr, J., and Mall, M. A. Allergic Airway Inflammation Induces a Prosecretory Epithelial Ion Transport Phenotype in Mice. Eur.Respir.J. 22-4-2010.

14. Riordan, J. R., Rommens, J. M., Kerem, B., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., and Chou, J. L. Identification of the Cystic Fibrosis Gene: Cloning and Characterization of Complementary DNA. Science 8-9-1989;245(4922):1066-73.

15. Ko, Y. H. and Pedersen, P. L. Frontiers in Research on Cystic Fibrosis: Understanding Its Molecular and Chemical Basis and Relationship to the Pathogenesis of the Disease. J.Bioenerg.Biomembrane. 1997;29(5):417-27.

16. Knowles, M. R., Church, N. L., Waltner, W. E., Yankaskas, J. R., Gilligan, P., King, M., Edwards, L. J., Helms, R. W., and Boucher, R. C. Aerosolized Amiloride As Treatment of Cystic Fibrosis Lung Disease: a Pilot Study. Advances in Experimental Medicine and Biology 1991;290:119-28.

17. Knowles, M. R., Paradiso, A. M., and Boucher, R. C. In Vivo Nasal Potential Difference: Techniques and Protocols for Assessing Efficacy of Gene Transfer in Cystic Fibrosis. Hum.Gene Ther. 1995;6(4):445-55.

18. Primiano, F. P., Jr., Saidel, G. M., Montague, F. W., Jr., Kruse, K. L., Green, C. G., and Horowitz, J. G. Water Vapour and Temperature Dynamics in the Upper Airways of Normal and CF Subjects. Eur.Respir.J 1988;1(5):407-14.

19. Li, M., McCann, J. D., Liedtke, C. M., Nairn, A. C., Greengard, P., and Welsh, M. J. Cyclic AMP-Dependent Protein Kinase Opens Chloride Channels in Normal but Not Cystic Fibrosis Airway Epithelium. Nature (London) 28-1-1988;331(6154):358-60.

20. Knowles, M. R., Carson, J. L., Collier, J. T., Gatzy, J. T., and Boucher, R. C. Measurements of Nasal Transepithelial Electric Potential Differences in Normal Human Subjects in Vivo. Am.Rev.Respir.Dis. 1981;124:484-90.

21. Alsuwaidan, S., Li Wan Po, A., Morrison, G., Redmond, A., Dodge, J. A., McElnay, J., Stewart, E., and Stanford, C. F. Effect of Exercise on the Nasal Transmucosal Potential Difference in Patients With Cystic Fibrosis and Normal Subjects. Thorax 1994;49:1249-50.

22. Mairbäurl, H., Weymann, J., Möhrlein, A., Swenson, E. R., Maggiorini, M., Gibbs, J. S. R., and Bärtsch, P. Nasal Epithelium Potential Difference at High Altitude (4559 M): Evidence for Secretion. Am.J.Respir.Crit.Care Med. 2003;167:862-7.

23. Middleton, P. G., Geddes, D. M., and Alton, E. W. F. W. Protocols for in Vivo Measurements of the Ion Transport Defects in Cystic Fibrosis Nasal Epithelium. Eur.Resp.J. 1994;7:2050-6.

24. Standaert, T. A., Boitano, L., Emerson, J., Milgram, L. J., Konstan, M. W., Hunter, J., Berclaz, P. Y., Brass, L., Zeitlin, P. L., Hammond, K., Davies, Z., Foy, C., Noone, P. G., and Knowles, M. R. Standardized Procedure for Measurement of Nasal Potential Difference: an Outcome Measure in Multicenter Cystic Fibrosis Clinical Trials. Pediatr.Pulmonol. 2004;37(5):385-92.

25. Chung, N. C., Illek, B., Widdicombe, J. H., and Fischer, H. Measurement of Nasal Potential Difference in Mild Asthmatics. Chest 2003;123(5):1467-71.

26. Guggino, W. B. Cystic Fibrosis and the Salt Controversy. Cell 5-3-1999;96(5):607-10.

27. Zhou, Z., Treis, D., Schubert, S. C., Harm, M., Schatterny, J., Hirtz, S., Duerr, J., Boucher, R. C., and Mall, M. A. Preventive but Not Late Amiloride Therapy Reduces Morbidity and Mortality of Lung Disease in BetaENaC-Overexpressing Mice. Am J Respir.Crit Care Med. 15-12-2008;178(12):1245-56.

28. Mall, M. A. Role of the Amiloride-Sensitive Epithelial Na+ Channel in the Pathogenesis and As a Therapeutic Target for Cystic Fibrosis Lung Disease. Exp.Physiol 2009;94(2):171-4.

29. Anderson, S. D. How Does Exercise Cause Asthma Attacks? Curr.Opin.Allergy Clin.Immunol. 2006;6(1):37-42.

30. Knowles, M., Gatzy, J., and Boucher, R. Relative Ion Permeability of Normal and Cystic Fibrosis Nasal Epithelium. J Clin.Invest 1983;71(5):1410-7.

31. Salathe, M. Effects of Beta-Agonists on Airway Epithelial Cells. J Allergy Clin.Immunol. 2002;110(6 Suppl):S275-S281.

Figure legends:

Figure 1. Representative recordings of transepithelial nasal potential differences in CO, EIA, and CF. Subjects were seated in a semi-supine 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 solution containing 10 mM N-[2-hydroxyethyl]-piperazine-n'-[2-ethane-sulphonic-acid] (HEPES), pH 7.4. The potential recorded under these conditions was termed NP_{tot}. The proportion of active Na^+ transport to NP was inhibited by switching to Ringer buffer containing 10 µM amiloride ($NP_{\Delta amil}$). The capacity of Cl^- transport ($NP_{\Delta Cl}$) was measured as the change in potential during perfusion with a Ringer 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 (100µl/min) into an antecubital vein. (A) Representative tracing from a control individual showing inhibition by amiloride ($NP_{\Delta amil}$) and stimulation with low Cl^- and isoproterenol ($NP_{\Delta Cl}$). (B) Representative tracings from an individual with exercise induced asthma (EIA) and a patient with cystic fibrosis (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 NP after amiloride and no response to low Cl^- in CF.

Figure 2. Changes in nasal potential difference during submaximal and maximal exercise. NP_{tot} (A) and its components were measured at rest, during submaximal, and after maximal exercise. Transport mediated by epithelial Na^+ channels was determined as the proportion of NP_{tot} inhibited by 10µM amiloride (B; $NP_{\Delta amil}$). The capacity of Cl^- transport (C; $NP_{\Delta Cl}$) 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. Mean values ± SD of nasal potentials measured at rest (R), during submaximal (S), and immediately after maximal (M) exercise from 11 healthy individuals (CO), 9 individuals with EIA, and 10 patients with CF. * $P<0.05$ between rest and exercise, # $P<0.05$ between CF and the other groups.

Table 1: Anthropometric data and lung function. Lung function tests were performed at rest. CO, healthy controls; EIA, exercise induced asthma; CF, cystic fibrosis; m, males; f, females; BMI, body mass index; FEV1, forced expiratory volume in first second; PEF, peak flow. Results are expressed as mean ± SD. * $P < 0.05$ compared to other groups.

subjects	gender	age (years)	height (cm)	body weight (kg)	BMI ($kg*m^{-2}$)	FEV 1 (% predicted)	PEF (% predicted)
CO	6m, 5w	24.9 ± 3.2	171 ± 8	68.8 ± 12.5	23.4 ± 2.6	107.3 ± 12.4	95.4 ± 9.8
EIA	6m, 3w	22.9 ± 2.9	176 ± 9	71.1 ± 14.4	22,6 ± 2.4	103.3 ± 10.8	94.4 ± 5.0
CF	7m, 3w	23.1 ± 4.1	169 ± 9	57.9 ± 9.7	20.1 ± 1.8	59.5 ± 24.3*	61.3 ± 27.8*

Table 2: Results from maximal and submaximal exercise. Exercise was performed on a bicycle ergometer in a semi-supine position. In the maximal test, beginning work load was 50 Watts. 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 1mM above baseline values assessed in the maximal test; exercise intensity was adjusted by heart rate. Note that in CF-patients blood lactate increased more than in the other groups. CO: Healthy controls. EIA: Exercise induced asthma. CF: Cystic fibrosis. Mean results ± SD of CO: n=11, EIA: n=9, CF: n=10. *: $p \leq 0.05$ in comparison to CO. #: $p \leq 0.05$ in comparison to EIA.

maximal exercise		**CO**	**EIA**	**CF**
work load	Watt W/kg	198 ± 41 2.88 ± 0.36	203 ± 46 2.87 ± 0.45	120 ± 28*# 2.08 ± 0.38*#
heart rate	min^{-1}	188 ± 8	180 ± 13	169 ± 12*
blood lactate	mmol/l	7.6 ± 1.2	6.8 ± 1.6	6.5 ± 1.6
submaximal exercise		**CO**	**EIA**	**CF**
work load	Watt W/kg % maximal	124 ± 39 1.85 ± 0.66 59.1 ± 14.2	129 ± 36 1.66 ± 0.42 57.5 ± 9.2	64 ± 9*# 1.12 ± 0.17*# 55.5 ± 13.0
heart rate	min^{-1} % max	136 ± 12 71.4 ± 6.2	131 ± 13 75.7 ± 2.6	128 ± 8 76.1 ± 7.0
blood lactate	mmol/l	2.3 ± 1.1	2.3 ± 0.5	3.7 ± 1.0*#

Table 3. Hormonal changes during exercise. Subjects were rested in a semi-supine position for 15 min before the resting blood sampling was 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. Mean values ± SD of 11 controls (CO), 9 individuals with exercise induced asthma (EIA) and 10 patients with cystic fibrosis (CF). * P<0.05 compared with rest; # P<0.05 between submaximal and maximal exercise; + P<0.05 relative to controls

		Rest	**Submaximal**	**Maximal**
Epinephrine (pM)	**CO**	170 ± 150	491 ± 574*	2429 ± 2193*#
	EIA	160 ± 105	363 ± 359*	1021 ± 920*#+
	CF	135 ± 194	198 ± 165*	440 ± 436*#+
Norepinephrine (pM)	**CO**	2023 ± 1203	2426 ± 1997*	17869 ± 8850*#
	EIA	994 ± 488	3471 ± 2633*+	7475 ± 6420*#+
	CF	1099 ± 825	1934 ± 1628*#	5043 ± 1628*#+
Cortisol (µg/dl)	**CO**	159 ± 97	144 ± 96	155 ± 71
	EIA	148 ± 71	110 ± 115	122 ± 61
	CF	160 ± 66	116 ± 53	143 ± 54

Figure 1. Representative recordings of nasal potential differences in CO, EIA, and CF.

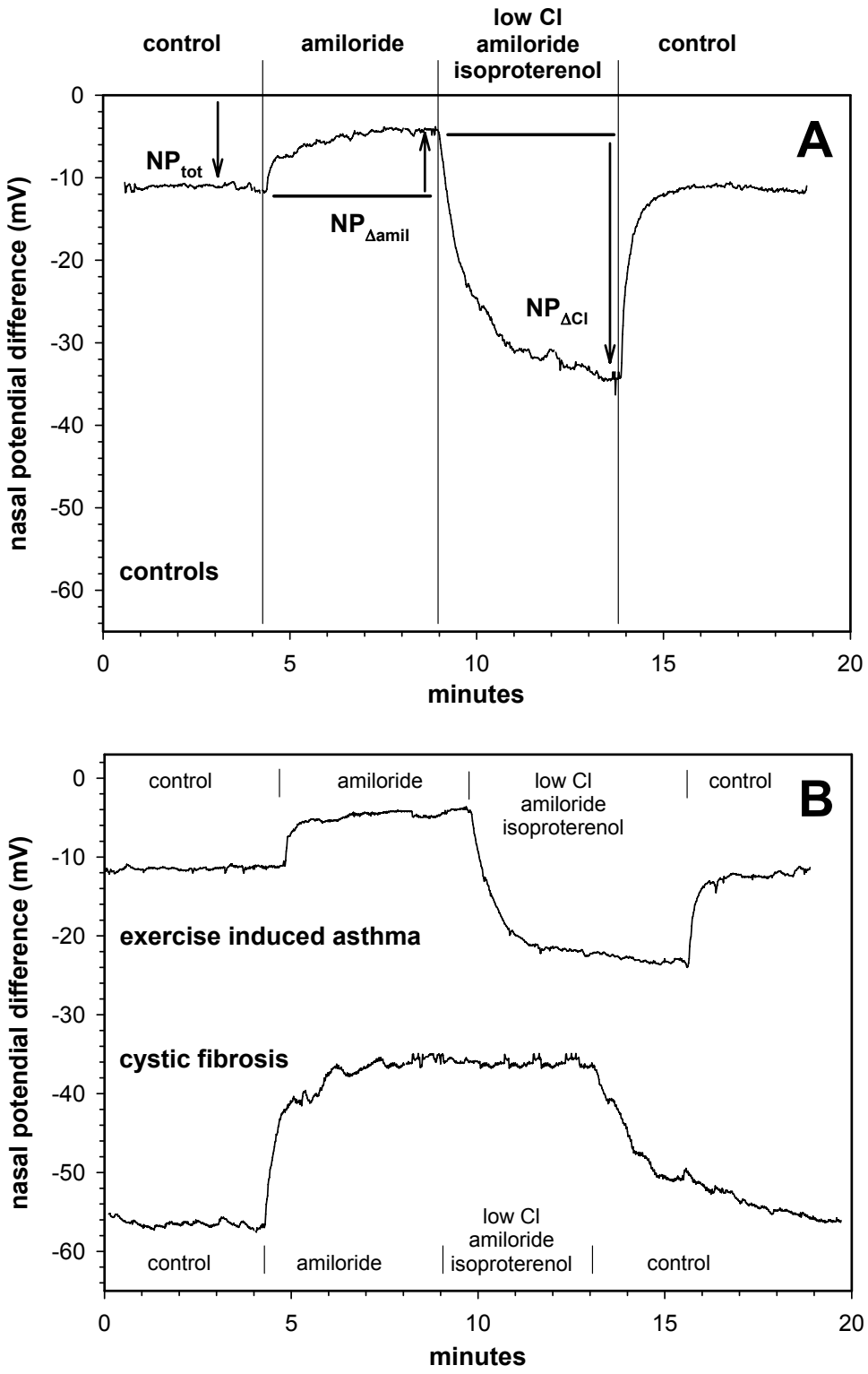

Figure 2. Changes in nasal potential difference during submaximal and maximal exercise.