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Moderate intensity exercise mediates comparable increases in exhaled chloride as albuterol in individuals with cystic fibrosis

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ABSTRACT

Rationale: Despite the demonstrated advantageous systemic changes in response to regular exercise for individuals with cystic fibrosis (CF), exercise is still viewed as an elective rather than a vital component of therapy, and it is likely that these benefits extend to and are partially mediated by exercise-induced changes in ion regulation.
Objective: We sought to determine if exercise could provide comparable improvements in ion regulation in the CF lung as albuterol, measured using exhaled breath condensate (EBC) collection and nasal potential difference (NPD).
Methods: Fourteen CF (13–42 yrs.) and sixteen healthy (18–42 yrs.) subjects completed a randomized crossover study of albuterol and submaximal exercise. EBC was collected at baseline, 30- and 60-min post-albuterol administration, and at baseline and during three separate 15 min cycling exercise bouts at low, moderate, and vigorous intensity (25, 50 and 65% of the maximum workload, respectively). NPD was performed at 30- and 80-min post albuterol or following moderate and vigorous intensity exercise.
Results: CF subjects had lower EBC Cl^-, but no difference in EBC Na^+ at baseline when compared to healthy subjects. EBC Cl^- increased four-fold with moderate exercise which was similar to that seen 60-min post albuterol administration for CF subjects. Neither exercise nor albuterol altered EBC Na^+. The change in NPD voltage with amiloride (ΔAmil) was greater and there was minimal Cl^- secretion (ΔTCC) seen at baseline in the CF compared to the healthy subjects. ΔAmil was greater with both albuterol and exercise when compared to baseline within both CF and healthy groups, but there was no significant difference in the ΔTCC response with either treatment.
Conclusion: Both exercise and albuterol can alter ion regulation increasing Cl^- secretion to a significant and similar degree in individuals with CF.

Abbreviation: ASL, Airway surface liquid; CF, Cystic fibrosis; CFTR, Cystic fibrosis transmembrane conductance regulator; Cl^-, Chloride; CaCCs, Calcium-activated chloride channels; ENaC, Epithelial sodium channel; EBC, Exhaled breath condensate; FEV_1, Forced expiratory volume in one second; FEF_{25-75}, Forced expiratory flow at 25–75% of FVC; FVC, Force vital capacity; Na^+, Sodium; NPD, Nasal potential difference; $P2Y_2$, Purinergic receptor; PKA, Protein kinase A; ΔAmil, Change in NPD with amiloride; ΔTCC, Total chloride conductance: change with Cl^- free + isoproterenol in NPD; ΔATP, Change in NPD with ATP.

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1. Introduction

The principle determinant of airway surface liquid (ASL) volume is the mass of salt on the airway surface [1]. The pathologic ASL dehydration in CF is due to improper ion regulation with minimal Cl^- secretion and an increase in Na^+ absorption due to the loss or reduction in cystic fibrosis transmembrane conductance regulator (CFTR) function and its inhibitory regulation of the epithelial sodium channel (ENaC) [2–4]. Ameliorating this ion dysregulation is crucial in the treatment of CF. Although the majority of current therapies treat the ensuing symptoms, the ultimate goal in the treatment of CF and the focus of current research is to treat the basic pathophysiology due to CFTR dysfunction.

The systemic therapeutic benefits of exercise in CF are well known, demonstrating the ability to reduce the characteristic 2–3% annual decline in pulmonary function, increase respiratory muscle endurance, improve exercise tolerance, aid in facilitating sputum clearance, increase aerobic capacity, and result in improved survival for individuals with CF who have higher aerobic fitness [5–10]. More recently, the benefits of exercise have been extended to a more cellular level with demonstration of inhibition of ENaC activity with exercise, thereby potentially improving ion regulation. With the goals of treatment therapy in CF to 1) prevent or minimize decline in lung function and 2) ameliorate impairments in ion regulation, exercise has convincingly demonstrated its ability to facilitate the former and research is now suggesting it may also aid in the latter [11–13].

Albuterol, a common β_2-agonist taken by individuals with CF, has the potential to be an exogenous stimulant of the adrenergic pathway to mediate stimulation of CFTR and ENaC [14–16]. However, the resultant change in ENaC activity would be the net result of direct protein kinase A (PKA) activation of ENaC countered by PKA activation of CFTR which mediates inhibition of ENaC [17–19], with one side winning or the two canceling each other out. Due to the lack of or reduction in the activity of CFTR-mediated ENaC inhibition in individuals with CF, ENaC activity in response to albuterol should increase which would only worsen the hyperabsorption of Na^+. In contrast, exercise, in addition to activating the adrenergic pathway endogenously in response to the release of epinephrine, can also endogenously activate the purinergic pathway through the increase in nucleotide release from the airway epithelia that will follow the increase in ventilation [20]. Albuterol may be a standard medication in the CF patient's therapy regimen, but the impact on ion regulation is poorly understood in this patient population. Additionally, the potential for exercise to mediate activation of the same pathway as albuterol and also activate the purinergic pathway could be more beneficial for individuals with CF, as purinergic stimulation activates calcium-activated chloride channels (CaCC) offering a CFTR-independent means to both increase Cl^- secretion and mediate inhibition of ENaC activity [21–23]. For a summary of the ion transport pathways in the airway epithelia cells and the means of regulating the activity of CFTR, ENaC and the CFTR-independent means of Cl^- secretion through CaCC, please refer to the supplement (Fig. 7) and our hypothesis paper [24].

This study was designed to compare the effects of adrenergic and purinergic stimulation on ion regulation in the lungs by either endogenous activation through submaximal exercise, or by exogenous adrenergic activation via nebulization of the β_2-agonist albuterol. The goal of this study was to determine if exercise could provide improvements to ion regulation that are comparable to the standard pharmacological therapy of albuterol for an individual with CF. To study ion regulation, two measurement techniques were used; exhaled breath condensate (EBC) collection, a novel measure of sodium and chloride content, and nasal potential difference (NPD) measurements, a standard measure of airway ion transport. We hypothesized that moderate intensity exercise would result in a decrease in EBC Na^+ and sodium transport detected by NPD (ΔAmil) from baseline due to purinergic-mediated ENaC inhibition. With albuterol administration, we predicted an increase or no change in EBC Na^+ and ΔAmil from baseline due to albuterol's adrenergic activation of ENaC. For chloride transport, we predicted an even greater increase in EBC Cl^- and chloride transport detected by NPD (ΔTCC and ΔATP) with exercise compared to albuterol due to activation of both CFTR and CaCC with exercise. A summary of the expected changes in ion channel regulation in response to albuterol and exercise is provided in Fig. 1.

2. Methods

2.1. Subjects

Fourteen individuals with CF with mild to moderate lung disease (FEV_1 >50% predicted), clinically diagnosed with a positive sweat test (>60 mmol/L Cl^-), and who were carriers of at least one ΔF508 allele were recruited for the present study. These subjects had not participated in another clinical trial within the past 30 days, or experienced a pulmonary exacerbation within two weeks. All participants with CF abstained from taking any long acting β-agonist (e.g. salmeterol) 12 h prior to any study visit and did not take albuterol the morning of the visits. Individuals with CF continued any other medications (Table 1) or treatments (i.e. chest clearance) as normal. Additionally, sixteen healthy individuals of similar age, gender, height and weight were also recruited. Participants with CF were recruited through the University of Arizona Cystic Fibrosis Center. Control subjects were recruited through advertising posted around the University of Arizona and by word of mouth. The protocol was reviewed and approved by the University of Arizona Institutional Review Board. All participants provided written informed consent prior to study, and all aspects of the study were performed according to the Declaration of Helsinki.

2.2. Protocol

2.2.1. Visit 1

On this screening visit subjects performed basic spirometry, a NPD measurement on both nostrils and a maximal exercise test on a cycle ergometer. The specifics of the spirometry and exercise testing have been described in detail elsewhere [25]. Subjects were randomized to one of the following four sequences, A: visit 2 albuterol and visit 3 submaximal exercise low-moderate-vigorous; B: visit 2 albuterol and visit 3 submaximal exercise vigorous-moderate-low; C: visit 2 submaximal exercise low-moderate-vigorous and visit 3 albuterol; D: visit 2 submaximal exercise vigorous-moderate-low and visit 3 albuterol. A summary of the measurement sequence and timing of measurements for the albuterol and submaximal exercise visits can be found in Fig. 2.

2.2.2. Albuterol visit

A baseline exhaled breath condensate (EBC) collection was performed for 20 min and a blood sample was taken at the midpoint of the EBC collection to assess serum ions (Na^+, K^- and Cl^-). Subjects were then administered nebulized albuterol while wearing a nose piece (2.5 mg diluted in 3 mL normal saline) using a Power Neb2 nebulizer (Drive Medical, Port Washington, NY). Following nebulization two post albuterol EBC collections and NPD measurements were performed. Since EBC and NPD cannot be performed simultaneously, the NPD post measurements were performed at 30 and 80 min rather than 30 and 60. Also in order to