CFTR GENOTYPE, NOT CIRCULATING CATECHOLAMINES, INFLUENCE CARDIOVASCULAR FUNCTION IN CYSTIC FIBROSIS

A THESIS
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY:

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF:

MASTER OF SCIENCE

ADVISOR: Eric M. Snyder, Ph.D.

July 2015

ACKNOWLEDGEMENTS

NIH Grant HL 108962-01 supported this research. We are sincerely grateful to the subjects and physicians who were willing to donate their time and efforts to support cystic fibrosis research. The following thesis would not have been possible without the generous help and guidance of Dr. Eric Snyder.

ABSTRACT

Objective: Cystic fibrosis (CF) is a genetic disease that elicits affects throughout the body and is characterized by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) with direct, well-documented, pulmonary function consequences. It has been shown a single dose of a β-agonist increases cardiac output (Q) and stroke volume (SV) and decreases systemic vascular resistance (SVR) in healthy subjects. This effect is attenuated in CF subjects; however, it is unknown if this decreased cardiovascular response to an inhaled drug is due to inherent cardiovascular deficits from CFTR mutation, receptor desensitization from prolonged β- agonist use, or inhibited drug delivery to the blood stream due to mucus buildup in the lungs. This study sought to determine the effects of endogenous epinephrine (EPI) and norepinephrine (NE) on cardiovascular function in CF, and to evaluate cardiovascular function according to CFTR mutation (ΔF508 Ins/DEL).

Methods: Eleven CF subjects and 27 healthy control participants completed a cycle ergometery test with measures of Q, SV, SVR, and HR along with plasma measures of EPI and NE. We compared subjects by variables of cardiovascular function relative to EPI and NE, and also based on genetic variants of ΔF508 Ins/DEL.

Results: CF subjects demonstrated significantly lower Q and SV at 50% of peak exercise and peak exercise than healthy subjects, and a higher SVR at rest, 50% of peak, and peak exercise. Additionally, CF subjects also demonstrated significantly lower Q and SV relative to NE at rest, however there were no

differences in HR relative to NE or SVR relative to EPI. When SV was stratified for CFTR mutation type, there were significant differences at rest, 50% of peak exercise, and there was a trend towards significance at peak exercise. Subjects with a double deletion of the ΔF508 had lower SV when compared to single deletion subjects.

There were moderate and significant correlations found between EPI and SV and EPI and Q, but not in EPI and SVR when the study population was evaluated as a whole. Within the healthy group, there were significant correlations between EPI and SV, and EPI and Q, but none between EPI and SVR. Only correlations between EPI and Q were seen in the CF group.

Conclusion: These results demonstrate that CF subjects have lower cardiovascular function parameters. Further, these results suggest that this impairment in cardiovascular function is likely the result of impairment in CFTR function due to CFTR genotype differences of the ΔF508, rather than receptor desensitization or inhibited drug delivery.

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LIST OF ABBREVIATIONS

CF…. Cystic fibrosis

CFTR…. Cystic fibrosis transmembrane conductance regulator

CFTR Single Deletion…. Heterozygous deletion of the ΔF508 gene

CFTR Double Deletion…. Homozygous deletion of the ΔF508 gene

β_1-AR…. Beta 1 adrenergic receptor

β_2-AR…. Beta 2 adrenergic receptor

EPI…. Epinephrine, rest (pg/mL)

NE…. Norepinephrine, rest (pg/mL)

Q… Cardiac output (mL/minute)

SV…. Stroke volume (mL/beat)

HR…. Heart rate (beats per minute)

SVR…. Systemic vascular resistance

SBP…. Systolic blood pressure (mmHg)

DBP…. Diastolic blood pressure (mmHg)

MAP… Mean arterial blood pressure (mmHg)

AC…. Adenylate cyclase

cAMP…. Cyclic adenosine monophosphate

PKA…. Phosphokinase A

ENaC…. Epithelial Sodium Channel

CaCC…. Ca^{+} activated Cl^{-} channel

LTCC…. L-type Ca^{2+} channel

$CaMKII^{+}$…. Ca^{2+}/ calmodulin- dependent kinase II

COPD…. Chronic obstructive pulmonary disease

FEV_1…. Forced expiratory volume in one second (mL/sec)

FEV_1 / FVC_{rest}…. Proportion of vital capacity expired during the first second of a forced expiration (measured as a %)

$VO_{2\ peak}$…. Maximum uptake of oxygen during peak exercise (mL/kg/min)

Max. Watt.... Maximum wattage at $VO_{2\ peak}$ (W)

W_{MAX} %.... Percentage of predicted maximum wattage reached at $VO_{2\ peak}$ (%)

CHAPTER 1. INTRODUCTION

Cystic fibrosis (CF) is the most prevalent autosomal recessive disease in Caucasians, in which approximately 1 in 25 are a carrier of a known mutation for CF. Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) lead to abnormal regulation of chloride (Cl^-) and sodium (Na^+) ions, which can result in an increase of viscosity and a loss volume of airway surface fluid in the lung [1, 2]. This change in volume and viscosity can lead to the development of thick, sticky mucus in the lung that is characteristic of CF in humans. Of the 1000+ known human CFTR mutations, nearly 70% have been tied to the ΔF508 gene [3]. Additionally, research suggests that subjects who have a homozygous mutation of the ΔF508 gene may demonstrate greater disease severity and lower pulmonary function than those who have a heterozygous ΔF508 gene mutation, or CF resulting from mutations in CFTR at other sites [4].

It has been shown that mutations in CFTR play an important role in pulmonary function in subjects with CF, including decreases in gas and drug transfer ability into the bloodstream, and increases in airway obstruction and infection [1, 5-9]. The majority of suggested cardiovascular relationship in CF to date has been identified as right heart failure (cor pulmonale) and has been found to be secondary to obstructive lung disease related to ΔF508 gene mutations in these populations [10, 11]. More recently, however, it has been demonstrated that CFTR may also influence left ventricular cardiac function, particularly cardiac contractility, in cell and animal models of CF [12, 13]. Furthermore, several studies have suggested that there is an inherent impairment of cardiovascular

function in individuals with CF regardless of disease severity; however, there remain few studies investigating the direct effect of CFTR mutations on cardiovascular function in humans.

Treatment with a β_2-agonist is common in CF subjects and has been well documented to aid in sputum expectoration, bronchodilation, and mucus degradation in the lungs [14-17]. Use of a β_2-agonist must be closely cycled, as desensitization has been known to occur as a result of prolonged use of this class of drugs [12, 18]. We have previously shown that the administration of a β-agonist, results in an increase in cardiac output (Q) and stroke volume (SV), and a decrease in systemic vascular resistance (SVR) in healthy humans, suggesting systemic stimulation of the cardiovascular β-adrenergic receptors through the inhalation of this drug [19]. More recently we have demonstrated that this cardiovascular response to an inhaled β-agonist was attenuated in subjects with CF, which could be due to inhibited drug delivery as a consequence of mucus buildup, receptor desensitization due to prolonged use of a β_2 agonist, or inherent reductions due to CFTR mutations [18].

Therefore, the purpose of the present study was to determine the effects of endogenous circulating catecholamines (epinephrine and norepinephrine) on variables of left ventricular cardiovascular function at rest in subjects with CF when compared to healthy subjects. We sought to determine if the cardiovascular function with albuterol was attributable to inhibited drug delivery by measuring cardiovascular variables relative to endogenous catecholamines (which do not

have to pass through the lung/blood barrier to elicit function on the heart and vessels). Furthermore, we wanted to investigate the relationship between the degree of ΔF508 (Ins/DEL) gene mutation and variables of cardiovascular function in order to explore a relationship between CFTR mutation and inherent left ventricular cardiovascular function.

A literature review as well as a following methodology, results, discussion and conclusions pertaining to this study are detailed in the following chapters. Chapter two delves into CF as a disease, the mechanistic nature of the CFTR and how mutations in the ΔF508 gene lead to the development of CF, as well as proposed mechanisms for the regulation of cardiac contractility through CFTR function, based on early research. Chapter three addresses the methodology of this study including data collection, measurement techniques, variable calculations, and statistical analysis of variables. Chapter four outlines and details the results of the present study by analyzing cardiovascular function relative to epinephrine and norepinephrine in CF subjects as compared to healthy subjects. Chapter four also outlines the comparison of cardiovascular function in CF subjects as stratified by CFTR ΔF508 mutation degree. Chapter five discusses the importance, implications, and limitations of the present study's findings with references to pertinent current literature. Chapter six concludes the resulting study and addresses the necessity for future research on the importance of ΔF508 mutations on cardiovascular function in CF subjects.

CHAPTER 2. REVIEW OF LITERATURE

Cystic fibrosis disease

Cystic fibrosis is an inherited autosomal recessive disease that is characterized by a mutation in the gene that encodes CFTR. Cystic fibrosis results in the abnormal function of various organ systems throughout the body, but the effects on the pulmonary system are particularly prominent. Mutations of the genes that encode for CFTR lead to altered ion regulation and are typically associated with abnormal regulation of chloride (Cl^-) and sodium (Na^+) balance [1, 2]. As a consequence of mutations in the gene that encodes CFTR, there is a reduction in the volume of airway surface fluid and in increase in its viscosity, leading to the development of thick, sticky mucus in the lungs [2]. This debilitating characteristic in the pulmonary system of CF subjects can lead to increased incidence of lung infection and airway exacerbations, as well as functional impairments including increases in airway obstruction and decreases in gas and drug transferability into the bloodstream. [1, 20].

While there are over 1000 known genes coding for mutations in the CFTR, over 70% have been tied to the ΔF508 gene [3]. Genetic research has suggested that homogeneity/ heterogeneity of the ΔF508 gene deletion may be related to disease severity, and that genotype is a more important factor than a subjects' environment when assessing CF phenotype [6]. Early studies have suggested that subjects who have a homozygous DEL mutation of the ΔF508 gene demonstrate a CF diagnosis at an earlier age and a higher prevalence of pancreatic dysfunction than CF subjects that are heterozygous or have other

mutations of the CFTR [7]. Furthermore, subjects that have impairments in pancreatic function (a common clinical problem in this patient population) also experienced a more severe disease prognosis, including lower pulmonary function [3, 7]. It remains unclear if CF subjects have inherently low cardiac function (for a given disease severity) or if there is a relationship between the degree of the ΔF508 gene mutation and cardiac function in CF subjects.

CFTR ΔF508 mutations and decreases in cardiovascular function

Although there remain few studies *in vivo* investigating the direct effect of CF and CFTR mutations on left ventricular cardiovascular function in CF populations, previous research has shown that CF subjects demonstrate altered Q, SV and SVR when compared to healthy individuals [13, 18, 21-23]. Previous cardiovascular work in CF patients has characterized right heart failure (cor pulmonale) in children and adults as a secondary disease to obstructive pulmonary disease [10, 11]. Several groups have shown subclinical heart dysfunction in CF subjects, when compared to healthy individuals, as found by cardiac strain, and was found independent of pulmonary function (FEV_1), even in a relatively healthy cohort of CF subjects [23, 24]. These findings suggest an inherent impairment and remodeling of cardiovascular function, regardless of clinical disease severity. Additionally, we and others have previously demonstrated that individuals with CF have impaired cardiovascular function,

including differences in HR, Q and SV when compared to healthy subjects- however the mechanisms for these differences remain unknown [18, 22, 25].

The CFTR protein is a G_s-coupled cAMP and PKA-activated protein, as described in brief below. When a β_2 adrenergic receptor (β_2 AR) is stimulated by a β_2 agonist, (e.g. albuterol or circulating catecholamines), the stimulation travels through the body of the protein and lands on the G_s alpha subunit of the G-coupled protein inside of the cell membrane. The now activated protein binds to and activates the enzyme adenylyl cyclase and positively stimulates the adenylate cyclase (AC) pathway. The AC pathway catalyzes the conversion of adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP). An intracellular increase in cAMP in turn activates the cAMP dependent enzyme protein kinase A (PKA) to be used functionally throughout the cell. In the case of stimulation of a β_2 AR in the lung, PKA will stimulate a portion of the binding domain on the CFTR and mechanically move the R Domain from blocking the CFTR protein. This phosphorylation disrupts the CFTR complex and leads to the receptor based activation of CFTR (Figure 1) [26, 27]. In epithelial cells, the upregulation of PKA by cAMP elevation will activate CFTR dependent chloride transport (increasing Cl^- secretion), while at the same time inhibiting epithelial Na^+ channel (ENaC) function (decreasing Na+ reabsorption) (Figure 1). This combined effect of normal CFTR on Cl^- and Na^+ is necessary for maintaining ion and fluid balance and a healthy human epithelial cell and dysfunction of this system is the hallmark of CF.

In addition to its accepted role in pulmonary function in individuals with CF, it is also thought that CFTR has an influence on cardiac contractility through alterations in cardiac myocyte function. Early studies have demonstrated that CFTR expression is upregulated during times of low blood flow in the plasma membrane of CF rat ventricular myocytes, suggesting an influence of CFTR in cardiac function [28]. It has been proposed that with dysfunction in the CFTR, there is an increase in membrane potential (due to dysfunctional ion transport) and a subsequent opening of L-type Ca^{2+} channels (LTCC) to let Ca^{2+} in [12]. The opening of LTCC increases intracellular $Ca^{2+}$and inhibits PKA in favor of activating a pathway involving Ca^{2+}/ calmodulin- dependent kinase II (CaMKII) to improve intracellular Ca^{2+} ion balance. CaMKII then stimulates Ca^{+} activated Cl^{-} channels (CaCC) which work to restore the imbalance in membrane potential and decrease LTCC hyperactivity, effectively decreasing Ca^{2+} and most likely removing the inhibition of PKA to restore contraction rate [12]. However, recent work has demonstrated that in the absence of proper CFTR function, cardiac contractility can be maintained through the activation of CaCC, suggesting a correlation between normal CFTR function and cardiac contractility [12, 27-30]

In addition to CFTR regulation of the LTCC and CaCC, it is thought that a cAMP dependent PKA pathway also regulates the normal functioning of the CFTR protein in cardiovascular tissues. Although the exact pathway is unknown, the major outcome of the β-AR cAMP dependent PKA pathways in cardiac tissues is an increase in the force and rate of ventricular contraction [12, 17, 29].

The effect of β_1 and β_2-adrenergic receptors on cardiac function

In humans, both the β_1 and β_2 adrenergic receptors are present in the heart, with the β_1 receptors being the dominant form in cardiac tissue [17, 21, 31, 32]. Several groups, have cited an approximate 75:25 ratio of β_1 to β_2 receptors in the non-failing human heart [17]. It has been shown previously that both β_1 and β_2 adrenergic receptors activate the adenylate cyclase pathway in human cardiac myocytes, which subsequently leads to an inotropic effect from PKA and an increase in left ventricular cardiac contractility, as described above [17].

Norepinephrine (NE) has been found to selectively activate β_1 receptors, whereas epinephrine (EPI) may activate both β_1 and β_2 receptors with similar potency [33, 34]. It has been consistently shown that the positive inotropic effects in human cardiac myocytes by norepinephrine are mediated primarily by the activation of β_1-adrenoceptors, and only influenced by β_2-adrenoceptors at very high, non-physiological levels [17]. Early studies have suggested that β_2 adrenergic receptors are activated by neurotransmitters like EPI (adrenaline), and mediate vasodilation in peripheral vasculature and skeletal muscle tissues, including lung periphery, with a 10 to 30 fold higher potency than NE [17, 21, 33]. Previous work has shown that stimulation of β_2-adrenergic receptors can lead to a decrease in SVR in human skeletal muscle and abdominal subcutaneous tissue; and further research suggests that β-adrenergic receptor activated vasodilation in human skeletal muscle is initiated by β_2 receptors and mediated by a nitric oxide pathway [18, 33, 35, 36].

The effect of catecholamines on cardiac function

Although the mechanisms for how β adrenergic receptor agonists influence the heart are unknown and largely unclassified, there remain questions as to which receptors, β_1 or β_2, have a more potent effect in cardiac myocytes [17]. According to one review, there are several studies to support the idea that even though β_1 receptors are dominant in cardiac myocytes, β_2 adrenergic receptors more selectively stimulate AC in cardiac cells, a function which is thought to be important in maintaining cardiac contractility [17].

At rest, the parasympathetic nervous system dominates the regulation of HR in healthy humans. During exercise however, a marked increase in HR is attributed to sympathetic nervous system activation and parasympathetic withdrawal along with a noted increase in plasma norepinephrine levels [17]. From this, it may be considered that NE, a selective β1 agonist, induces increases in rate of contraction. Furthermore, infusion studies involving the use of both β_1 and β_2 agonists and inhibitors, have conclusively demonstrated that exercise induced increases in HR are predominantly mediated by β_1 adrenergic receptor stimulation [17]. Infusion studies using a measure of the shortening of the electromechanical systole (rate and afterload independent) as a surrogate for measuring inotropic effects in humans have demonstrated that positive inotropic effects in the left ventricle are predominantly mediated by β_1 adrenergic receptor stimulation [17]. Because of these findings, the present research used the

cardiovascular variables of HR, SV and Q relative to endogenous levels of NE when used in comparison between healthy and CF subject populations.

β-receptor desensitization with chronic us of albuterol

Suggested therapeutic treatments for CF subjects includes the use of physiotherapy to induce the breaking up and expectoration of sputum (mucus from the lungs) along with the more common inhaled aerosolized β_2-adrenergic receptor agonists (e.g. albuterol or salmeterol) due to their bronchodilating characteristics in CF lungs [14-16, 37, 38]. Observations in individuals with CF have demonstrated that use of a β_2 agonist, like albuterol, is associated with improvements in pulmonary function when compared to placebo after acute pulmonary exacerbations [14]. More recently, it has been shown that chronic use of a β_2-adrenergic agonist may be associated with a desensitization and possible down-regulation of β_2-adrenergic receptors in individuals with COPD and asthma [16, 39]. Whether or not this phenomenon of β_2-receptor desensitization is related to or explains lower cardiovascular function in individuals with CF remains unknown to date.

The effect of an inhaled β-agonist in cardiac function

We have previously shown that with the administration of an inhaled β_2 agonist, there is an increase in Q and SV, and a decrease in SVR in healthy subjects [18]. More recently, we have found this effect to be attenuated in

subjects with CF [19]. Additionally, our lab has shown that variables of cardiac hemodynamics (cardiac power and stroke work) are also attenuated in CF subjects following the administration of a β_2 agonist (manuscript under review).

Final argument

Therefore, for this research, we sought to determine the effects of circulating endogenous catecholamines and CFTR ΔF508 mutations on cardiovascular function in subjects with CF. Systemic vascular resistance was measured relative to epinephrine (a β_2 agonist), as a surrogate to measuring desensitization due to prolonged use of a β_2 agonist (i.e. if desensitization occurs, we would expect to see a significant attenuation in the drop in the SVR response to epinephrine in individuals with CF). Furthermore, we evaluated endogenous catecholamines in order to eliminate the possibility of concluding that decreased drug permeability due to a mucus build up in CF subjects lead to the noted decreases in measured variables of cardiovascular function.

CHAPTER 3. METHODOLOGY

Subjects/Participants

Eleven subjects with CF and 27 healthy control subjects completed the study (Table 1). Control subjects were matched for age, height, weight, and approximately matched for BSA and BMI- resulting in fairly well matched CF subjects with a relatively healthy disease prognosis. Subjects had a confirmation of CF using a positive sweat test (≥60.0 millimole/L Cl^-) and genotyping of the ΔF508 mutation of CFTR. Individuals with CF who presented with following exclusion criteria were not permitted to participate due to safety concerns: experienced a pulmonary exacerbation within the last two weeks or pulmonary hemorrhage within six months resulting in greater than 50 cc of blood in the sputum, were taking any antibiotics for pulmonary exacerbation, or were taking any experimental drugs related to CF. The Arizona Respiratory Center and its affiliated CF clinic at the University of Arizona Medical Center were used to recruit all individuals with CF. Word of mouth and posted advertising around the University of Arizona were used to recruit control participants. 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.

Testing and exercise protocol

Subjects were asked to come in for two separate visits. The first visit consisted of gathering preliminary data, including subject demographics of height,

weight, BMI, and BSA. Additionally, at this time all CF subjects were identified with a positive sweat chloride test, and genotyped for the ΔF508 gene mutation- both of which were recorded. During this preliminary visit, subjects completed a VO_2 max test in order to establish a baseline and familiarize the subjects with the protocols of pulmonary function testing during exercise, acetylene rebreathe during exercise, and the overall experience of completing a VO_2 max test during an experimental protocol.

The subjects then returned for a study visit, where data was accumulated and used for calculations. Upon arrival to the environmentally controlled physiology laboratory, participants were fitted with a 12-lead electrocardiogram (Marquette Electronics, Milwaukee, WI) to monitor HR, as well as an antecubital intravenous catheter for plasma draws throughout the exercise protocol. In a seated upright position, standard pulmonary function testing (i.e. flow volume loop: forced expiratory volume at 25 to 75% of FVC (FEF25-75), forced expiratory volume at 25% of FVC (FEF25), forced expiratory volume at 50% of FVC (FEF50), and forced expiratory volume at 75% of FVC (FEF75)) using a Medical Graphics CPFS system spirometer (Medical Graphics, St. Paul, MN). Spirometry was performed according to the guidelines of the American Thoracic Society. Pulmonary function testing was performed at a minimum of three times to ensure all measures fell within 10% of each attempt [40]. Remaining in the upright position, participants had systolic (SBP) and diastolic (DBP) blood pressures assessed using manual sphygmomanometry. Cardiac output was then assessed

using the acetylene rebreathe technique as described previously and in brief below. Resting values of cardiovascular function relative to catecholamines were assessed in all subjects. In addition to this resting data, data was successfully obtained to assess relative cardiovascular function during exercise (cardiovascular function corrected for catecholamine levels) on 12 subjects (nine with CF and three healthy subjects). Measurements of Q, HR, SBP and DBP were completed at baseline (15min post-catheter insertion), at 50% of peak exercise and at peak exercise in all subjects. Additionally, at each measurement time point mean arterial blood pressure (MAP), SVR and SV were calculated.

All progressive exercise tests were performed on the same cycle ergometer (Corival Lode B.V., The Netherlands). The testing protocol used was subject-specific and was based on the subject's body size and on the type and intensity of the subject's typical physical activity. The initial workload chosen also served as the incremental workload increase at each stage of the exercise test. Exhaustion of subjects was determined by an inability to maintain a pedal rate between 60 and 80rpm, a respiratory exchange ratio greater than 1.15, or a rating of perceived exertion of 18 or greater. The test ended when two out of the three were demonstrated, or at the subject's request.

Measurement of cardiac output

In a seated upright position, participants breathed into a non-rebreathing technician-controlled pneumatic switching Y-valve (Hans Rudolph, Kansas City,

MO) that was connected to a pneumotachometer (Hans Rudolph, Kansas City, MO) and mass spectrometer (Perkin Elmer MGA-1100, Wesley, MA). The inspiratory port of the switching valve allowed for rapid operator controlled switching for breathing room air or from a 5.0 L anesthesia rebreathe bag (Hans Rudolph, Kansas City, MO) containing 1575 mL of test gas (0.65% acetylene [C_2H_2], 9.0% helium [He], 55.0% nitrogen, and 35.0% O_2 as previously described [20, 41]. The timed-switching circuit and tank were calibrated and tested prior to each test to ensure accurate volumes, and therefore a consistent flow rate from the tank regularly produced the desired bag volume. Each rebreathe measurement period consisted of 8-10 consecutive breaths set at a cadence of 32 breaths/min using a metronome. During each rebreathe period, individuals were instructed to nearly empty the bag with each inspiration while the mass spectrometer was used to collect serial measurement of gas concentrations starting at end-expiration of the first breath during the rebreathe period which enabled rapid calculations of Q [41]. From the lungs, acetylene disappears in the blood according to the rate at which pulmonary blood flow occurs, and therefore Q is calculated from the slope of the exponential disappearance of acetylene relative to the insoluble gas He [9, 41, 42]. Because Q was assessed three times for each participant (baseline, 30min, and 60min following albuterol administration), following each rebreathe period, the rebreathe bag was emptied with a suction device and refilled immediately prior to the next rebreathe period. At the start of each new rebreathe period, there was no residual gas in the dead

space of the apparatus, nor from the exhaled air from the participants, which was confirmed via gas sampling with mass spectrometer.

Assessment of Cardiovascular Function

In addition to Q and HR, variables of cardiovascular function of SV, MAP and SVR were calculated as follows: SV = Q/HR, MAP = DBP + 1/3(SBP – DBP), and SVR = MAP/Q.

Assessment of Epinephrine and Norepinephrine

Intravenous blood draws were used to assess resting endogenous catecholamines of EPI and NE. Levels were assessed via high-performance liquid chromatography at the University of Arizona pathology laboratory.

Data analysis

Independent-samples t-tests were used to compare between CF and healthy groups for demographic characteristics, pulmonary function/ exercise performance variables, and cardiovascular function variables (Q, HR, SV and SVR), relative to catecholamines (EPI and NE). One-way analysis of variance tests (one-way ANOVA) were used to analyze subjects' cardiovascular function (Q, HR, SV and SVR) stratified by CFTR ΔF508 mutation code. Two-tailed Pearson correlation coefficients were determined for establishing relationships between resting levels of epinephrine and cardiovascular function (Q, SV, and

SVR) for three groups: CF + healthy combined, CF only, and healthy only. The alpha level for significance was set at $p<0.05$ for all tests.

CHAPTER 4. RESULTS

Subject demographics and pulmonary function variables

Table 1 shows participant characteristics for CF and healthy subjects. There were no significant differences for any of the demographic characteristics between CF and healthy subjects, including weight, BMI and resting EPI or NE concentration levels. Data is presented as the mean of each group, followed by the standard error or number of affected participants, depending on the variable. This cohort of CF subjects, although retaining similar demographic variables, demonstrated lower pulmonary function values, as expected (Table 2). Subjects with CF demonstrated a lower forced expiratory volume in 1 second at rest ($FEV_{1\ rest}$) as well as a lower proportion of vital capacity expired in the first second of a forced expiration (FEV_1 / $FVC_{\ rest}$) ($p<0.01$), indicating pulmonary function deficits. Additionally, as expected, CF subjects demonstrated a lower VO_2 peak ($VO_{2\ peak}$), lower maximum wattage at $VO_{2\ MAX}$ (Max. Watt), and lower percent predicted maximum wattage reached at peak exercise (W_{MAX} %), significant at the $p<0.01$ level.

Cardiovascular function in CF vs. healthy

Cystic fibrosis subjects demonstrated significantly lower Q at 50% of peak exercise and peak exercise when compared to healthy subjects; as well as significantly lower calculated SV at rest, 50% of peak exercise and peak exercise as seen in Figures 3A and 3C respectively ($p<0.05$). Furthermore, CF subjects demonstrated a higher SVR at rest, 50% of peak exercise and peak exercise than

their healthy counterparts (Figure 4, $p<0.05$). When analyzed relative to resting levels of epinephrine and norepinephrine, CF subjects demonstrated a significantly lower Q and SV at rest relative to NE (Figure 5A and C respectively, $p<0.05$) however no significant differences were seen in HR relative to NE (Figure 5B) or SVR relative to EPI (Figure 5D). Similarly, subjects who had successful blood sampling at rest, 50% of peak and at peak exercise demonstrated a similar pattern as described above relative to EPI (Figure 7), indicating that even when corrected for catecholamines during exercise, subjects with CF demonstrate lower cardiac function.

Cardiovascular function and CFTR stratification

When CF subjects were stratified according to CFTR genotype, there were no statistically significant differences in Q between groups at any level of exercise (Figure 6A). However, there was a statistically significant difference between groups in HR at 50% of peak exercise ($F(2, 15)= 4.182$, $p=0.036$), but not at rest or peak exercise (Figure 6B). More importantly, there was a statistical significance in SV between groups at rest ($F(2, 14)= 7.982$, $p<0.01$), 50% of peak exercise ($F(2, 14) =5.291$, $p=0.019$), and approaching significance at peak exercise ($F(2, 14)=3.502$, $p=0.058$) as shown in Figure 6C.

Cardiovascular function and epinephrine correlations

There was a moderate and significant correlation between EPI levels and SV ($r=0.44$, $p<0.01$) and Q ($r=0.45$, $p<0.01$), but not in EPI and SVR ($r=-0.264$, $p=0.11$) at rest, when considering the group as a whole (healthy and CF, Table 3A). Within the CF group there was a significant correlation between EPI levels and Q ($r=0.64$, $p<0.05$) and a nearly significant correlation between EPI and SV ($r=0.66$, $p=0.052$), but not in SVR ($r=-0.34$, $p=0.31$) as seen in Table 3A. Within the healthy subject group (Table 3A), there were significant correlations between EPI and SV ($r=0.43$, $p<0.05$) and Q ($r=0.40$, $p<0.05$), but not in EPI and SVR ($r=-0.24$, $p=0.22$). There was no relationship between NE and cardiac function when considering the group as a whole, or when considering the groups by condition (Table 3B).

CHAPTER 5. DISCUSSION

Pulmonary dysfunction attributed to CFTR mutations has been well documented in subjects with CF. More recently, research has demonstrated that CFTR may also influence cardiovascular function (specifically, cardiac contractility) in CF models, and has suggested that there are inherent cardiovascular function deficits in subjects with CF [12, 13]. We have previously demonstrated that there is an attenuated cardiovascular response to a β-agonist in subjects with CF when compared to healthy subjects [19]. The present study demonstrated that CF subjects had significantly lower variables of cardiovascular function at all three levels of exercise intensity, and differences persisted when Q and SV were compared relative to circulating norepinephrine. The present study also found significant differences in SV when CF subjects were stratified for CFTR genotype, the first such finding relating CFTR genotype and cardiac function in the human heart. Additionally, this study addresses receptor desensitization as there were no significant differences in SVR relative to levels of circulating epinephrine. If desensitization were present there would be an attenuation in SVR for a given epinephrine level. Collectively, these findings suggest inherent cardiac deficits in CF due to CFTR dysfunction.

Our group and others have previously demonstrated decrements in variables of cardiovascular function including Q, SV and SVR in patients with CF, when compared to healthy subjects, both at rest and during exercise. While some groups have suggested that detriments are due to lower workloads achieved in patients with CF, we have found that CF subjects have an inherently lower SV

and Q, even for a given relative workload [19, 22]. Additionally, cardiac strain and strain rate echocardiography have been used to determine an influence of cardiac remodeling in CF, and one group has suggested that there is an inherent impairment and remodeling of left ventricular tissues impacting cardiovascular function [23, 24]. This previous work demonstrating cardiac remodeling in CF included a relatively healthy cohort of CF subjects displaying subclinical heart dysfunction, suggesting a possible direct influence of CFTR mutation on cardiovascular function, regardless of disease severity.

With exercise, there is a marked increase in HR that has been attributed to sympathetic nervous system activation and a noted increase in plasma norepinephrine levels [17]. Studies with the use of both β_1 and β_2 agonists and inhibitors have conclusively demonstrated that exercise induced increases in HR are predominantly mediated by β_1 adrenergic receptor stimulation by β_1 agonists, that are sympathomimetic for the selective β_1 agonist, norepinephrine [17]. Furthermore, infusion studies using a surrogate measurement for inotropic effects in humans, have demonstrated that positive inotropic effects in the left ventricle are predominantly mediated by β_1 adrenergic receptor stimulation [17]. From this, the present research used the cardiovascular variables of HR, SV and Q relative to endogenous levels of NE when used in comparison between healthy and CF subject populations. We found that cardiac function was attenuated in patients with CF, despite correction for circulating catecholamines, which suggest that the

signaling pathway that causes this attenuation is downstream of catecholamine synthesis.

The CFTR protein has been demonstrated to couple to cAMP and PKA in cardiac tissues, similar to the coupling systems seen in pulmonary tissues. It is thought that through this coupling, the major outcome of abnormal or missing CFTR protein function leads to an activation of a PKA pathway and an increase in the force and rate of ventricular contraction [12, 17, 29]. Recent work has suggested a specific correlation between CFTR and cardiac contractility, showing that there is upregulation of CaCC activity with CFTR-knockout, which is thought to reestablish and maintain cardiac contractility [12, 27-29]. Individuals with a homozygous mutation of the ΔF508 gene may exhibit greater disease severity, including lower pulmonary function and greater pancreatic dysfunction [4, 7, 8]. Based on this influence and the proposed relationship between CFTR and cardiac contractility, the present study stratified CF subjects according to CFTR ΔF508 genotype in order to evaluate impairments in cardiovascular function and disease severity relative to the type of ΔF508 mutation, the first study to do so. Significant differences were seen in SV between homozygous and heterozygous groups at rest, 50% of peak exercise and approaching significance at peak exercise-suggesting a relationship between CFTR ΔF508 mutation (and, therefore, CFTR function) and cardiovascular dysfunction.

Previous research has demonstrated that there are changes in ventricular function including significant increases in Q and SV, and decreases in SVR in

healthy subjects following administration of an inhaled β-agonist [18]. Additionally, it has been shown that among the increases in Q and SV there was an increase in circulating NE, suggesting a tie between endogenous NE levels and cardiovascular function [18]. More recently, we have shown that left ventricular cardiovascular stimulation with the use of a β-agonist is attenuated in CF subjects when compared to healthy subjects, including a lower SV and Q. However, because the ability of an inhaled drug to cross the lung/blood barrier is inhibited in CF, given the mucus build up common in CF lungs, it is not clear what role drug delivery played in this attenuated left ventricular cardiovascular response [1, 2, 19, 22]. It is possible that cardiovascular function in response to a β-agonist is inhibited in CF because of receptor desensitization, due to daily use of a β-agonist. The present work sought to eliminate inhibited drug delivery as an explanation by comparing Q, SV and HR relative to endogenous levels of NE (which does not have to pass through lung/blood barrier to elicit response) and desensitization by comparing SVR relative to EPI. Even when corrected for catecholamine levels, CF subjects demonstrated significantly lower Q and SV, with no changes in SVR. These results indicate that attenuated differences in left ventricular cardiovascular function at rest are probably not due to inhibited drug delivery or desensitization but may be inherent based on CFTR ΔF508 mutation.

CHAPTER 6. CONCLUSION

Subjects with CF demonstrated significantly lower variables of left ventricular cardiac function, including lower Q and lower SV when compared to healthy subjects. These effects were attenuated even when corrected for resting endogenous NE levels. There were no differences seen in HR relative to NE or SVR relative to EPI. Additionally, there were significant differences in SV when subjects were stratified according to CFTR ΔF508 genotype. These findings suggest that there are inherent attenuations in cardiac function, likely due to altered CFTR function, in subjects with CF. Based on this information, future areas of research include determining if other CFTR gene mutations yield left ventricular cardiovascular function deficits as well, and if so, do those cardiovascular deficits negatively impact longevity and disease prognosis of CF patients.

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APPENDIX

Table 1. Participant characteristics (mean ± standard error or n)

	CF (11)	Healthy (27)
Number (%) female	8 (76)	16 (59)
Age (yrs)	24 ± 7.4	26 ± 7.3
Height (cm)	168 ± 10.4	173 ± 10.9
Weight (kg)	66 ± 15	71 ± 12
Body Surface Area (m^2)	1.7 ± 0.23	1.8 ± 0.2
Body Mass Index (kg/m^2)	23.5 ± 3.3	23.8 ± 3.6
Epinephrine conc. at rest (pG/mL)	72 ± 41	66 ± 51
Norepinephrine conc. at rest (pG/mL)	386 ± 149	409 ± 171

Data are presented as mean ± standard deviation or as n. Epinephrine and norepinephrine concentration values are endogenous levels for both CF and healthy.

Table 2. Pulmonary Function and Exercise Performance in CF and Healthy Subjects (mean ± standard error)

	CF (11)	Healthy (27)
$FEV_{1\ rest}$ (%)	72 ±6.5 **	96 ±3
FEV_1 / FVC_{rest} (%)	0.7 ±0.04 **	0.8 ±0.02
$VO_{2\ peak}$ (mL/kg/min)	23 ±4 **	37 ±2
Max. Watt (W)	103 ±12 **	188 ±13
W_{MAX} (%)	53 ±5 **	97 ±6

Data are presented as mean ± standard deviation or as n.
FVC=forced vital capacity, FEV1=forced expiratory volume at 1- second of forced vital capacity, WMAX(%) = percent of predicted maximum wattage reached at peak exercise.
*=$p<0.05$; ** =$p<0.01$.

Table 3. Cardiovascular Variable and Catecholamine Correlations

A

			n	Pearson coefficient	p-value
Epinephrine	CF + Healthy	SV	36	0.44 **	<0.01
		Q	38	0.45 **	<0.01
		SVR	38	-0.26	0.109
	CF	SV	9	0.66	0.052
		Q	11	0.64 *	<0.05
		SVR	11	-0.34	0.31
	Healthy	SV	27	0.43 *	<0.05
		Q	27	0.4 *	<0.05
		SVR	27	-0.24	0.22

B

			n	Pearson coefficient	p-value
Norepinephrine	CF + Healthy	SV	36	0.12	0.49
		Q	38	0.17	0.31
		SVR	38	-0.08	0.64
	CF	SV	9	-0.11	0.78
		Q	11	0.3	0.36
		SVR	11	-0.27	0.43
	Healthy	SV	27	0.24	0.24
		Q	27	0.17	0.41
		SVR	27	-0.05	0.81

Figure Captions

Figure 1. CFTR and ENaC function in Healthy Lung Epithelia. In healthy lung tissues, the stimulation of a β_2 adrenergic receptor will move a signal through protein and land on a G_s coupled protein. This will stimulate the AC pathway, which in juncture with ATP will increase intracellular cAMP. The increase in cAMP will increase PKA activity, which in the case of the CFTR will stimulate it to move the R-Domain and allow for upregulation of the activity of CFTR and the movement of Cl^- out of the cell. Furthermore, a functioning CFTR will control the regulation of the ENaC, allowing for normal ion balance and movement to exist within the cell.

Figure 2. CFTR and ENaC function in CF Lung Epithelia. In lung tissues of CF subjects, the CFTR is damaged, and PKA does not stimulate the movement of the R Domain, therefore movement of Cl^- out of the cell in inhibited and there is a marked increase in intracellular Cl^-. Furthermore, the protein now fails to regulate the movement of Na^+ through the ENaC channel, increasing intracellular Na^+ concentration. Water will diffuse to the concentration of ions, and move out of the interstitial space, creating thick mucus in the interstitial space due to the lack of water.

Figure 3. Comparison of variables of cardiovascular function of cardiac output **(A)** [$\mathbf{n_{rest}}$ =11CF, 27 healthy; $\mathbf{n_{50\%}}$ =11CF, 25 healthy; $\mathbf{n_{peak}}$ =11CF, 27 healthy], heart rate **(B)** [$\mathbf{n_{rest}}$ =10CF, 27 healthy; $\mathbf{n_{50\%}}$ = 10CF, 25 healthy; $\mathbf{n_{peak}}$ =10CF, 27 healthy], and stroke volume **(C)** [$\mathbf{n_{rest}}$ =9CF, 25 healthy; $\mathbf{n_{50\%}}$ =9CF, 24 healthy; $\mathbf{n_{peak}}$ =9CF, 27 healthy], for CF (dashed) vs. healthy (solid) at rest, 50%, and peak exercise levels. * = $p<0.05$ CF vs. healthy.

Figure 4. Comparison of systemic vascular resistance for CF (dashed) vs. healthy (solid) at rest, 50%, and peak exercise levels [$\mathbf{n_{rest}}$ =11CF, 27 healthy; $\mathbf{n_{50\%}}$ =11CF, 24 healthy; $\mathbf{n_{peak}}$ =11CF, 26 healthy], * = $p<0.05$ CF vs. healthy.

Figure 5. Comparison of relative variables of cardiovascular function for CF (black) vs. healthy (white) at rest. Cardiac output **(A)** [$\mathbf{n_{rest}}$ =11CF, 27 healthy],

heart rate **(B)** [$\mathbf{n_{rest}}$ =11CF, 27 healthy], and stroke volume **(C)** [$\mathbf{n_{rest}}$ =11CF, 27 healthy] were calculated relative to norepinephrine; systemic vascular resistance (D) [$\mathbf{n_{rest}}$ =11CF, 27 healthy] was calculated relative to epinephrine. * = p<0.05 CF vs. healthy.

Figure 6. Subjects stratified by CFTR mutation type for cardiac output **(A)** [$\mathbf{n_{rest}}$: no deletion =2, single deletion=3, double deletion=14, $\mathbf{n_{50\%}}$: no deletion =2, single deletion=3, double deletion=13; $\mathbf{n_{peak}}$: no deletion =2; single deletion=3; double deletion=13], heart rate **(B)** [$\mathbf{n_{rest}}$: no deletion =2, single deletion=3, double deletion=13, $\mathbf{n_{50\%}}$: no deletion =2, single deletion=3, double deletion=13; $\mathbf{n_{peak}}$: no deletion =2; single deletion=3; double deletion=13] and stroke volume **(C)** [$\mathbf{n_{rest}}$: no deletion =2, single deletion=2, double deletion=13, $\mathbf{n_{50\%}}$: no deletion =2, single deletion=2, double deletion=13; $\mathbf{n_{peak}}$: no deletion =2; single deletion=2; double deletion=13] at rest, 50% and peak exercise levels for no ΔF508 deletion (solid line with circles) vs. single ΔF508 deletion (single dash line with squares) vs. double ΔF508 deletion (double dashed with triangles). * = p<0.05 CF vs. healthy.

Figure 7. Comparison of cardiac function relative to catecholamine levels at rest and during exercise. The panels represent Q/EPI **(A)** [$\mathbf{n_{rest}}$ =9CF, 3 healthy; $\mathbf{n_{25\%}}$ =9CF, 3 healthy; $\mathbf{n_{50\%}}$ =9CF, 3 healthy; $\mathbf{n_{peak}}$ =9CF, 2 healthy], and SV/EPI **(B)** [$\mathbf{n_{rest}}$ =6CF, 2 healthy; $\mathbf{n_{25\%}}$ =6CF, 2 healthy; $\mathbf{n_{50\%}}$ =6CF, 2 healthy; $\mathbf{n_{peak}}$ =6CF, 2 healthy], for healthy (solid) vs. CF (dashed) at baseline, 25% peak exercise, 50% peak exercise, and 75% peak exercise. * = p<0.05 CF vs. healthy.

Figure 1. CFTR and ENaC Function in Healthy Lung Epithelia

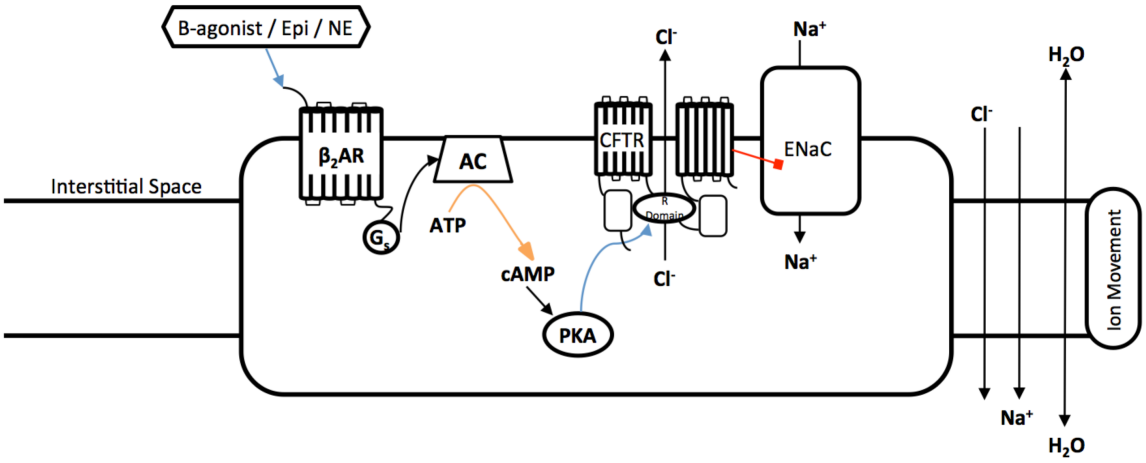


Figure 2. CFTR and ENaC Function in CF Lung Epithelia

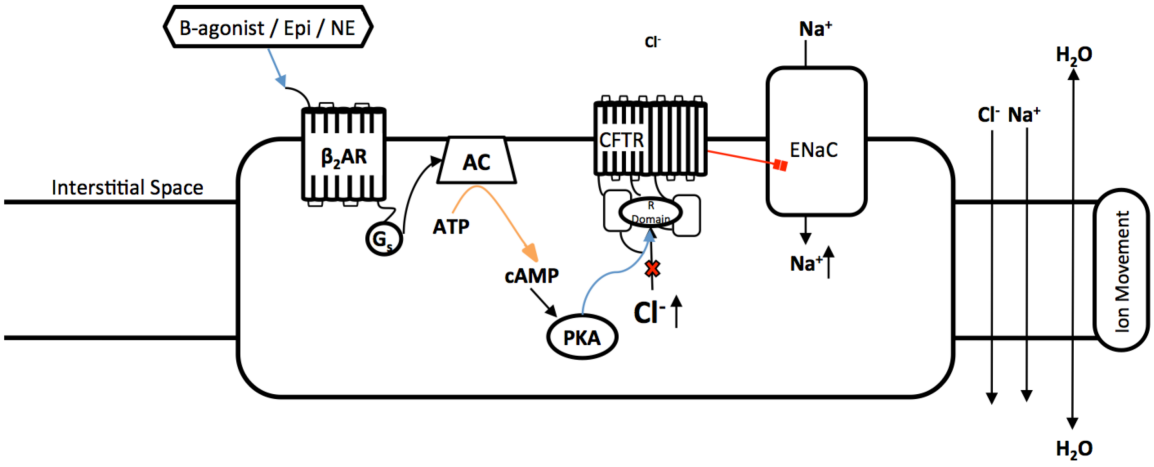


Figure 3. Variables of Cardiovascular Function: CF vs. Healthy

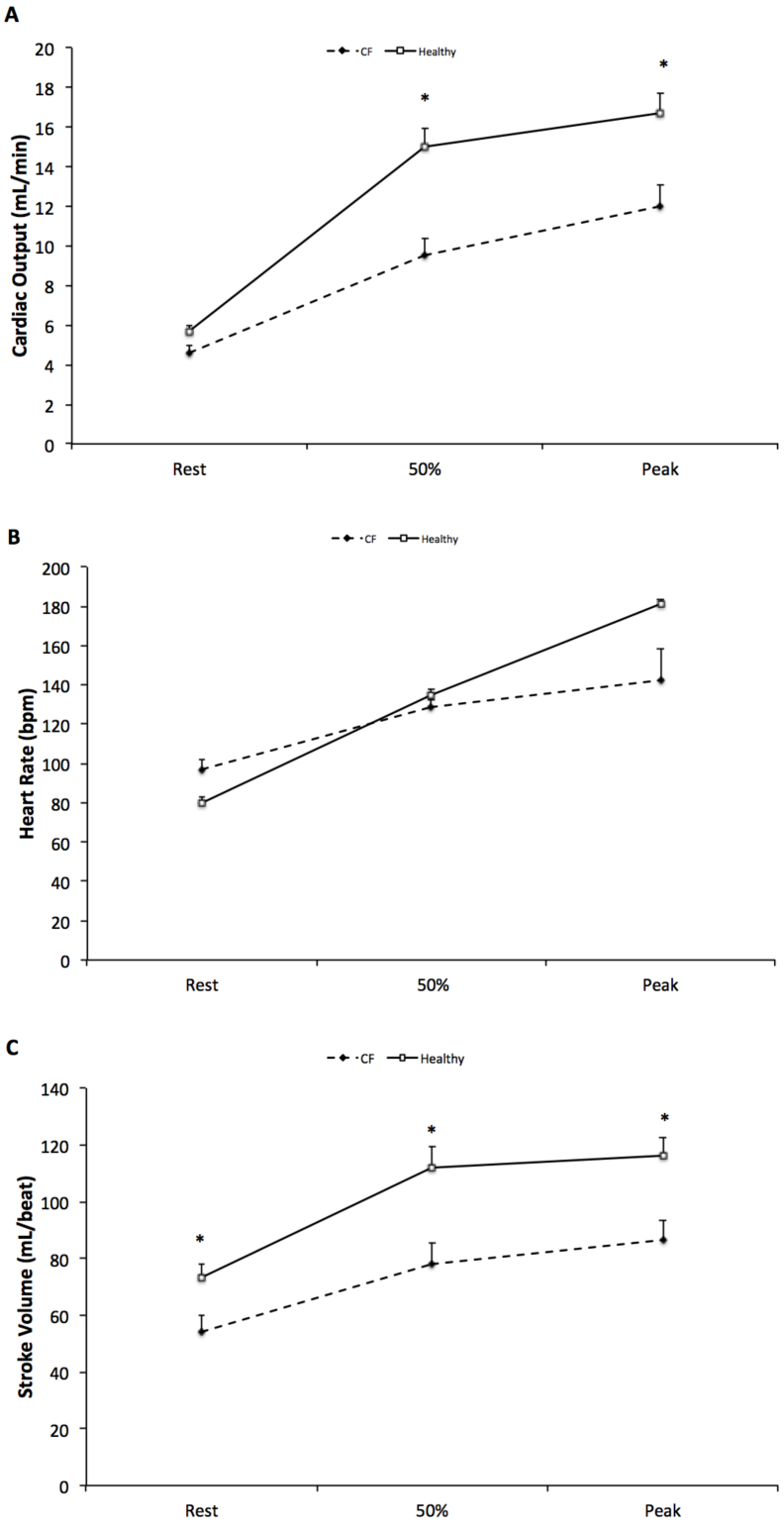


Figure 4. Systemic Vascular Resistance: CF vs. Healthy

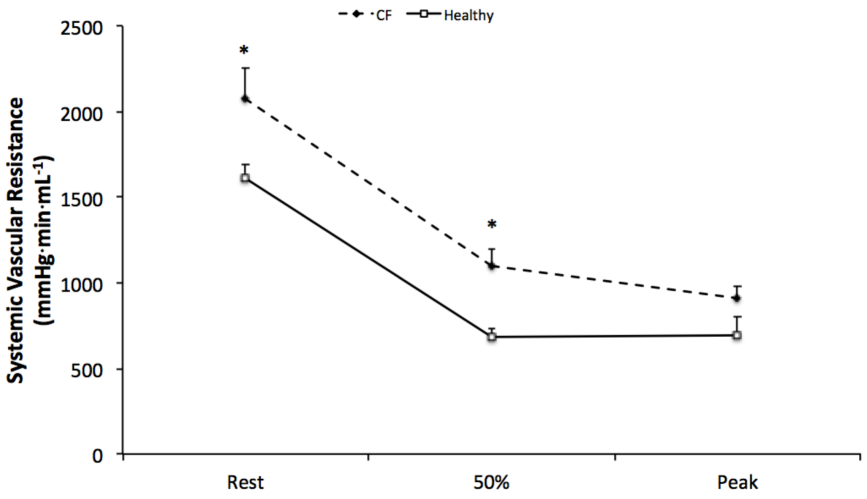


Figure 5. Relative Variables of Cardiovascular Function for Catecholamines

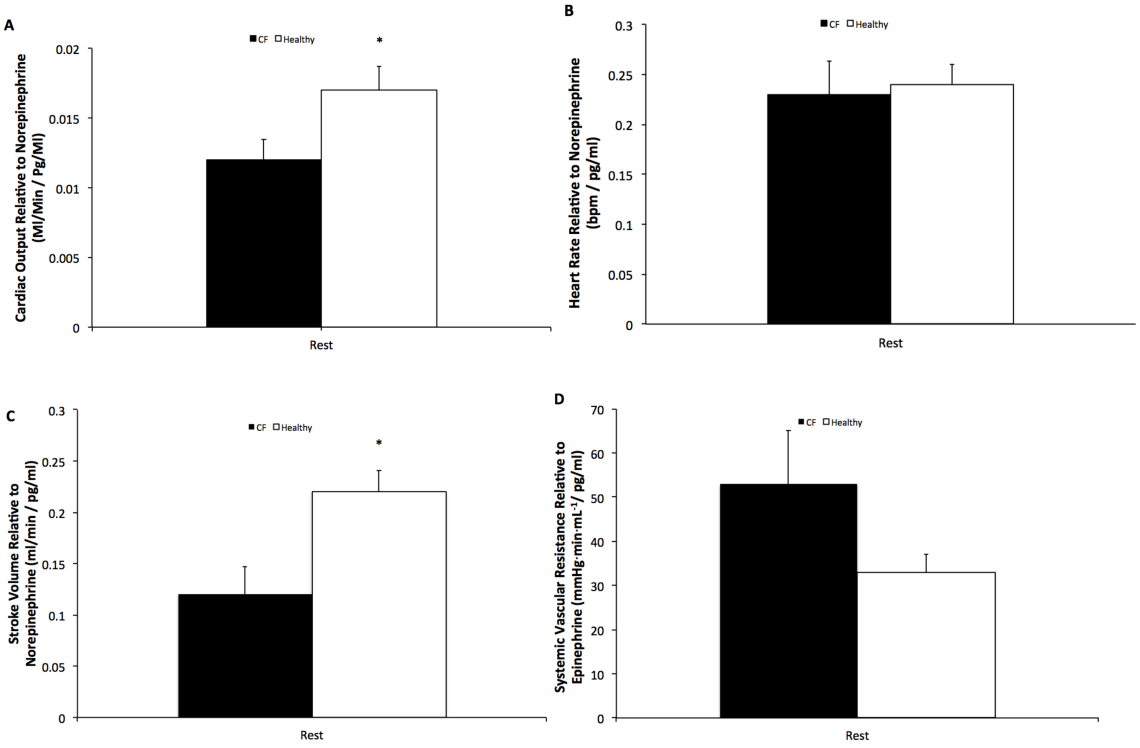


Figure 6. Cardiovascular Function Variables Stratified by CFTR ΔF508 Mutation (no deletion, single deletion and double ΔF508 deletion)

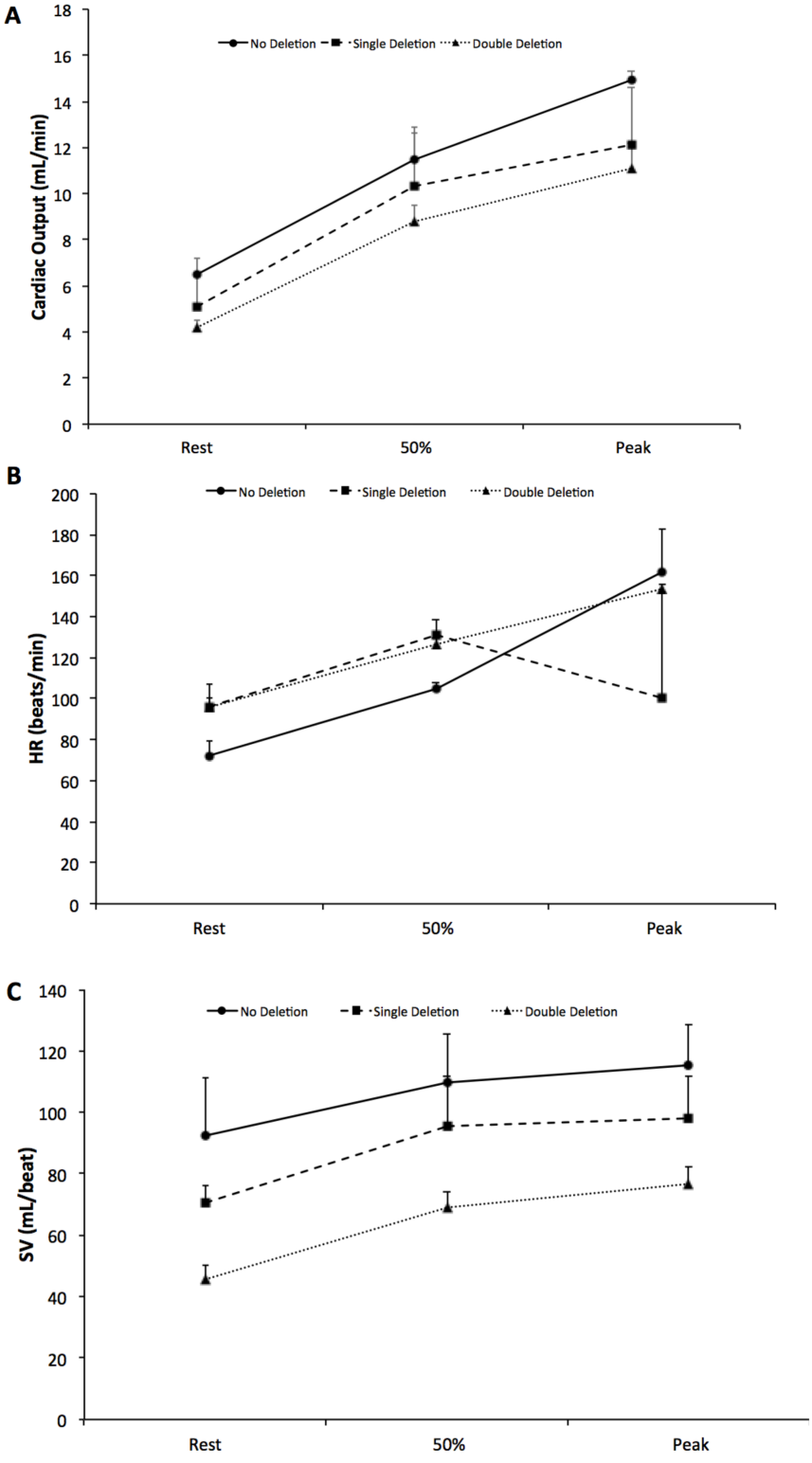
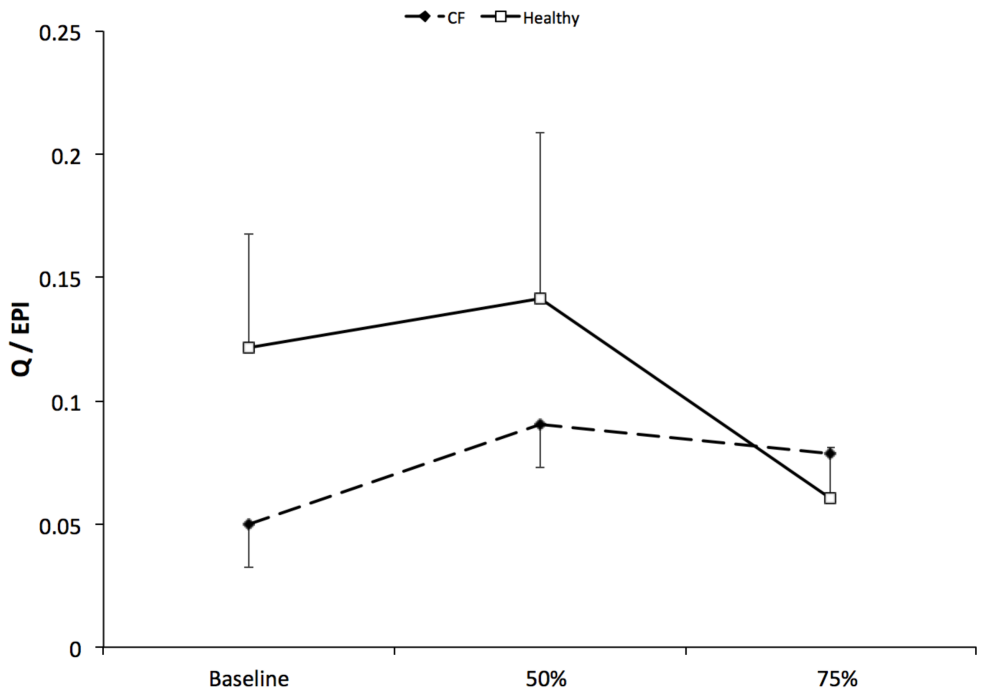


Figure 7. Cardiac Output (A) and Stroke Volume (B) Relative to Epinephrine During Exercise

A



B