

Cystic Fibrosis Transmembrane Conductance Regulator Genotype, Not Circulating Catecholamines, Influences Cardiovascular Function in Patients with Cystic Fibrosis

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ABSTRACT

BACKGROUND: Cystic fibrosis (CF) is a genetic disease affecting multiple organ systems of the body and is characterized by mutation in the gene coding for the cystic fibrosis transmembrane conductance regulator (CFTR). Previous work has shown that 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 patients with CF; however, the mechanism is unknown. Potential explanations for this decreased cardiovascular response to a β -agonist in CF include inherent cardiovascular deficits secondary to the CFTR mutation, receptor desensitization from prolonged β -agonist use as part of clinical care, or inhibited drug delivery to the bloodstream 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 the relationship between cardiovascular function and CFTR F508del mutation.

METHODS: A total of 19 patients with CF and 31 healthy control subjects completed an assessment of Q (C_2H_2 rebreathing), SV (calculated from Q and heart rate [HR]), Q and SV indexed to body surface area (BSA, QI, and SVI, respectively), SVR (through assessment of Q and mean arterial blood pressure [MAP]), and HR (from 12-lead electrocardiogram [ECG]) at rest 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 the F508del mutation (homozygous deletion for F508del, heterozygous deletion for F508del, or no deletion of F508del).

RESULTS: Cystic fibrosis patients demonstrated significantly lower BSA (CF = $1.71 \pm 0.05 \text{ m}^2$ vs healthy = $1.84 \pm 0.04 \text{ m}^2$, $P = .03$) and SVI (CF = $30.6 \pm 2.5 \text{ mL/beat/m}^2$ vs healthy = $39.9 \pm 2.5 \text{ mL/beat/m}^2$, $P = .02$) when compared with healthy subjects. Cystic fibrosis patients also demonstrated lower Q (CF = $4.58 \pm 0.36 \text{ L/min}$ vs healthy = $5.71 \pm 0.32 \text{ L/min}$, $P = .03$) and SV (CF = $54 \pm 5.5 \text{ mL/beat}$ vs healthy = $73.3 \pm 4.5 \text{ mL/beat}$, $P = .01$), and a higher HR (CF = $93.2 \pm 3.9 \text{ bpm}$ vs healthy = $80.5 \pm 2.7 \text{ bpm}$, $P < .01$) and SVR (CF = $2082 \pm 156 \text{ dynes}\cdot\text{s/cm}^{-5}$ vs healthy = $1616 \pm 74 \text{ dynes}\cdot\text{s/cm}^{-5}$, $P = .01$) compared with healthy subjects. Furthermore, CF patients demonstrated a lower SV ($P < .01$) corrected for NE when compared with healthy subjects. No significant differences were seen in HR or Q relative to NE, or SVR relative to EPI. Differences were seen in SV ($F_{(2,14)} = 7.982$, $P < .01$) and SV index ($F_{(2,14)} = 2.913$, $P = .08$) when patients with CF were stratified according to F508del mutation (number of deletions).

CONCLUSIONS: Individuals with CF have lower cardiac and peripheral hemodynamic function parameters at rest. Furthermore, these results suggest that impairment in cardiovascular function is likely the result of F508del CFTR genotype, rather than receptor desensitization or inhibited drug delivery.

KEYWORDS: cystic fibrosis transmembrane conductance regulator, F508del, cardiovascular function, catecholamines

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Introduction

Cystic fibrosis (CF) is the most common autosomal recessive disease in whites, with approximately 1 in 25 whites carrying one known mutation for CF¹ and estimates of 70% to 90% of individuals with CF having at least one deletion of the F508del allele.² The F508del mutation results in an abnormal cystic

fibrosis transmembrane conductance regulator (CFTR) protein as a consequence of the deletion of a three-nucleotide sequence on chromosome 7 between positions 507 and 508, resulting in the loss of a codon for the amino acid phenylalanine (F).³ The loss of phenylalanine yields improper folding of the CFTR protein, leading to irregular transport of chloride



(Cl⁻) and sodium (Na⁺) ions³ across epithelial cell membranes, as may be seen in human airways. The result of this is an increased viscosity and decreased depth of the airway surface fluid in the lung, leading to the development of thick, sticky mucus which is the characteristic of CF.^{4,5} Cystic fibrosis patients may express a homozygous deletion of F508 (*homo-F508del*), a heterozygous deletion of F508 (*hetero-F508del*), or no deletion of F508³ and have a diagnosis of CF.

It has been shown that mutations in CFTR affect the severity of CF lung disease, and more recently, it has been suggested that CFTR may also directly influence cardiovascular function.^{2,6-8} Cystic fibrosis transmembrane conductance regulator is expressed in both vessels and cardiomyocytes affecting both vascular reactivity and repolarization with potential to relate to arrhythmias.⁶ This seems to confirm that altered cardiac function in CF patients may influence cardiac contractility under conditions of stress, such as exercise. The majority of clinically significant cardiovascular dysfunction associated with the F508del genotype has been described as right heart failure (cor pulmonale), secondary to obstructive lung disease in CF populations.^{9,10} However, evidence of left ventricular cardiac dysfunction, particularly regarding cardiac contractility, has also been suggested in cell and animal models of CF.^{6,7} More recently, Radtke et al² demonstrated that individuals with at least one F508del (category II) plus a category-V secondary deletion had lower adjusted VO₂ output compared with individuals with two category-II mutations. Our group has previously shown attenuated cardiovascular function after the administration of an inhaled β₂ selective agonist in individuals with CF when compared with healthy subjects.¹¹ Mechanisms for this attenuation in the β₂ response may relate to the severity of CF lung disease, including decreases in diffusion capacity leading to poor drug transfer into the bloodstream, airway obstruction, and infection.^{4,12-14} Other than a simple limitation in drug delivery, this diminished β₂ response could be due to receptor desensitization¹⁵ (because of repeated stimulation as a part of regular therapy) or because of inherent effects of CFTR dysfunction on cardiac contractility. It has been demonstrated previously that the prolonged use of β₂-agonists may illicit desensitization of the β₂ receptor at the superficial level of receptor complexes.¹⁵ It is thought that further advances in medicine may continue to improve the lung function and therefore life expectancy of CF patients. Prolonged life expectancy could increase the incidence of clinical left ventricular dysfunction, which may be due to chronic low-grade hypoxia from pulmonary obstruction, or directly from altered cardiac function as a product of CFTR mutations. Emerging therapies in CF have the potential to attenuate or exacerbate left ventricular dysfunction based on the underlying mechanism of a patient's cardiac state. In addition to known effects on pulmonary function and the proposed influence on cardiovascular function, the number of F508del mutation may also play a role in estimating overall disease severity.^{1,11-13} It was found that

individuals who were homozygous for the F508del mutation (two alleles of the F508del) had a more severe disease prognosis, including diagnosis at an earlier age and more severe pancreatic dysfunction as compared with CF patients who had only one F508del allele.¹³ It remains unknown, however, whether there is a relationship between F508del genotype and the severity of cardiovascular dysfunction.

The aim of this study was to characterize the cardiovascular function in individuals with CF compared with healthy adults. We sought to determine the effects of endogenous catecholamines (epinephrine [EPI] and norepinephrine [NE], which do not have to pass through the lung/blood barrier to elicit function on the heart and vessels) on variables of left ventricular cardiac function in subjects with CF compared with healthy individuals. The responsiveness to endogenous catecholamines will allow for the determination that drug delivery plays on the attenuated cardiovascular response in CF patients, when compared with differences in receptor function. Furthermore, we wanted to explore the relationships between CFTR F508del mutations and cardiac function to address the impact of CFTR on cardiovascular function in CF.

Methods

Study population

A total of 19 individuals with CF and 31 healthy control subjects completed the study; blood samples were successfully gathered in 11 CF and 27 healthy individuals (Table 1). The University of Arizona Respiratory Center and its affiliated CF clinic at the University of Arizona Medical Center were used to recruit individuals with CF. Cystic fibrosis subjects were matched for age, height, and weight to control subjects, resulting in individuals with CF with moderate lung disease (FEV₁ of 72%). Diagnoses of CF were confirmed with a positive sweat test (≥60 mmol/L Cl⁻) and were categorized based on only genotyping of the F508del gene (patients categorized as *homo-F508del*, *hetero-F508del*, and *no F508del*). Individuals with CF who experienced a pulmonary exacerbation within the last 2 weeks or pulmonary hemorrhage within 6 months resulting in greater than 50cc of blood in the sputum, were taking any antibiotics for pulmonary exacerbation, or were taking any experimental drugs related to CF were excluded for safety reasons. 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.

Study design

Subjects completed two study visits. The first visit consisted of gathering baseline data, including height, weight, body mass index (BMI), body surface area (BSA), and F508del genotype

Table 1. Participant demographics and pulmonary function variables: CF vs healthy subjects.

	CF (19)	HEALTHY (31)
Female, n (%)	14 (74)	18 (58)
Age (years)	22.4 ± 1.8	26.9 ± 1.5
Height (cm)	167 ± 2.0	173 ± 1.9
Weight (kg)	63 ± 3.5	71 ± 2.3
Body surface area (m ²)	1.7 ± 0.05*	1.8 ± 0.03
Body mass index (kg/m ²)	22.6 ± 0.9	24 ± 0.7
Cardiac index (L/min/m ²)	2.6 ± 0.2	3.0 ± 0.2
Stroke volume index (mL/beat/m ²)	30.6 ± 2.5*	39.9 ± 2.5
Genotype		
Homozygous ΔF508 deletion	14 (74)	
Heterozygous ΔF508 deletion	3 (16)	
No ΔF508 deletion	2 (10)	
FEV ₁ (%)	72 ± 6.1**	94.8 ± 2.6
FEV ₁ / FVC _{rest} (%)	0.72 ± 0.03**	0.82 ± 0.01
VO _{2 peak} (mL/kg/min)	23 ± 2.3**	35 ± 2.1
Max. Watt (W)	101 ± 8.2**	185 ± 12
W _{MAX} (%)	52 ± 3.5**	96 ± 5.6
EPI conc. at rest (pG/mL); n = 11 CF, 30 H	72	66
NE conc. at rest (pG/mL); n = 11 CF, 30 H	386	409

Abbreviations: CF, cystic fibrosis; EPI, epinephrine; FEV₁ / FVC_{rest}, forced expiratory volume at 1 second of forced vital capacity; FVC, forced vital capacity; NE, norepinephrine; VO_{2 peak}, peak oxygen consumption during exercise; W_{MAX} (%), percent of predicted maximum wattage reached at peak exercise.

Concentration levels of EPI and NE are endogenous levels for both CF and healthy subjects. % indicates measure is a percentage of predicted value. Data are presented as mean ± standard error of mean or as n.

P* < .05; *P* < .01.

(if applicable, based on positive sweat chloride test). During this visit, subjects also completed a peak exercise test (VO_{2 peak}) to determine fitness level (data presented in previous work) and become familiarized with pulmonary function testing maneuvers.

The peak exercise test was performed on a cycle ergometer (Corival; Lode BV, The Netherlands), and how the test advanced was determined by each subject's body size and his or her reported type, speed, and intensity of exercise training. Each individual's activity was evaluated from a questionnaire given after the subjects completed consenting. Questions included subject's frequency and duration of physical activity and medical history. Subjects exercised at an initial workload ranging between 20 and 40 W (mean initial workload was as follows: healthy, 32 ± 7 W; CF, 25 ± 8 W) with the workload increasing by this initial workload (wattage) every 2 minutes until exhaustion following exercise testing standards and guidelines by the American

Heart Association.¹⁶ Subjects reached exhaustion when it was observed that they met two out of the three following factors: They were unable to maintain a pedal rate of 60 to 80 revolutions per minute; had a respiratory exchange ratio (RER) that was greater than or equal to 1.15; or a rating of perceived exertion (RPE), which ranged from 1 to 20, that was 18 or greater.¹⁷ Peripheral oxygen saturation (SpO₂) was continuously monitored by pulse oximetry using a finger sensor (Nellcor N-600 Pulse Oximeter, Bolder, CO), and blood pressure was checked by cuff auscultation which was completed by the same technician at the midpoint of each stage of exercise. To ensure accurate SpO₂ values, subjects were instructed to maintain a relaxed grip on the handle bars and subjects were monitored to confirm that there were no discrepancies in heart rate (HR) between the pulse oximeter and electrocardiogram (ECG). Recovery time was 2 minutes of pedaling. Subjects were dismissed after their HR and blood pressure returned to baseline.¹⁷

For the second visit, subjects were fitted with a 12-lead ECG (Marquette Electronics, Milwaukee, WI) to monitor HR, as well as an antecubital intravenous catheter for blood draws. In a seated upright position, standard pulmonary function testing (ie flow volume loop, forced expiratory volume in 1 second [FEV₁], 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) was completed. Spirometry was performed according to the guidelines of the American Thoracic Society.¹⁸ Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured and cardiac output (Q) was assessed.^{11,19} Resting values of cardiovascular function relative to catecholamines, Q, HR, SBP, and DBP were assessed in all subjects, and the mean arterial blood pressure (MAP), systemic vascular resistance (SVR), stroke volume (SV), stroke volume index (SVI), and cardiac index (CI) were calculated. Data from these participants have been previously reported.¹¹

Measurement of cardiac output

Cardiac output was assessed using an acetylene rebreathe technique, which is strongly associated with measures obtained using direct Fick, as described in detail elsewhere.²⁰ 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) 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 rebreathing bag (Hans Rudolph) containing 1575 mL of test gas (0.65% acetylene [C₂H₂], 9.0% helium [He], 55.0% nitrogen, and 35.0% O₂) as previously described.¹⁹ 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. Following each rebreathe measurement, the rebreathe bag was emptied with a suction device and refilled immediately prior to the next rebreathe measurement. 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.

Calculation of cardiovascular variables

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

Assessment of EPI and NE

Intravenous blood draws were used to assess resting endogenous catecholamines of EPI and NE. Serum levels of EPI and NE were assessed via high-performance liquid chromatography at the University of Arizona pathology laboratory as described previously.²¹

Data analysis

Wilcoxon rank sum tests were used to compare between the CF and healthy groups for demographic characteristics, pulmonary function variables, and cardiovascular function variables (Q, HR, SV, and SVR). Cardiac output, HR, and SV relative to NE and SVR relative to EPI were also compared between CF and healthy groups. Differences between CFTR genotypes for cardiovascular function (Q, HR, SV, SVI, and SVR) were assessed using Kruskal-Wallis rank sum test. Pearson correlation coefficients were used to test relationships between levels of catecholamines and cardiovascular function (Q, SV, and SVR) for three groups: CF + healthy combined, CF only, and healthy only. Statistical significance was determined using the alpha level for two-tailed; significance was set at $P < .05$ for all tests. Data are presented as mean \pm SEM where appropriate; all statistical analyses were performed using SPSS v.19.

Results

Subject demographics and pulmonary function

Table 1 shows the demographics for both CF and healthy subjects. There were no significant differences for age, height, weight, BMI, CI, or EPI and NE concentrations between CF and healthy subjects; there were, however, differences in BSA and SVI. The 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 (FEV₁ / FVC_{rest}) ($P < .01$), indicating obstructive lung disease. In addition, as expected, CF subjects demonstrated a lower functional capacity, as indicated by VO₂ 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} %) ($P < .01$) as compared with healthy controls.

There were significant differences between *no F508del* and *hetero-F508del* genotypes for weight ($P < .01$), BSA ($P = .01$), and BMI ($P = .02$), and between *no F508del* and *homo-F508del* genotypes for weight ($P < .01$), BSA ($P < .01$), and BMI ($P < .01$). There were no other significant differences in demographic variables dependent on CFTR F508del genotype.

Table 2. CF participant demographic and pulmonary function variables.

	NO ΔF508 DELETION (2)	HETEROZYGOUS ΔF508 DELETION (3)	HOMOZYGOUS ΔF508 DELETION (14)
Female, n (%)	2 (100)	3 (100)	9 (64)
Age (years)	20 ± 1.5	25 ± 6.9	22.3 ± 1.9
Height (cm)	179 ± 3.8	165 ± 6.6	166 ± 2.0
Weight (kg)	99 ± 4.5 ^{b,c}	66 ± 6.7 ^a	58 ± 2.2 ^a
Body surface area (m ²)	2.2 ± 0.08 ^{b,c}	1.7 ± 0.12 ^a	1.6 ± 0.04 ^a
Body mass index (kg/m ²)	31 ± 0.0 ^{b,c}	24 ± 0.07 ^a	21 ± 0.7 ^a
FEV ₁ (%)	104 ± 12	74 ± 12	67 ± 7
FEV ₁ / FVC _{rest} (%)	0.83 ± 0.04	0.71 ± 0.05	0.70 ± 0.03
VO _{2 peak} (mL/kg/min); (n = 2, 2, 12)	23 ± 0.4	21 ± 1.6	24 ± 3.0
Max. Watt (W)	150 ± 10	87 ± 6.7	98 ± 9.7
W _{MAX} (%)	52 ± 4.7	43 ± 6.1	54 ± 4.4

Abbreviations: CF, cystic fibrosis; EPI, epinephrine; FEV₁ / FVC_{rest}, forced expiratory volume at 1 second of forced vital capacity; FVC, forced vital capacity; NE, norepinephrine; VO_{2 peak}, peak oxygen consumption during exercise; W_{MAX} (%), percent of predicted maximum wattage reached at peak exercise.

Concentration levels of EPI and NE are endogenous levels for both CF and healthy. % indicates measure is a percentage of predicted value. Data are presented as mean ± standard error mean or as n.

a, unknown; b, heterozygous F508DEL (Ins/Del); c, homozygous F508DEL (Del/Del).
P < .05 between group and each other group listed.

Cardiovascular function in CF vs healthy subjects

Participants with CF demonstrated lower cardiac output (Figure 1A; $P = .03$), higher HR (Figure 1C; $P < .01$), lower calculated SV (Figure 1D; $P = .01$), and a higher SVR ($P = .01$) at rest. Although there was a trend, we found no significant difference between CF and healthy subjects for cardiac index (Figure 1B; $P = .07$). When cardiovascular variables at rest were corrected for levels of EPI and NE, individuals with CF demonstrated a significantly lower SV at rest relative to circulating NE levels (Figure 2C; $P < .01$); however, no significant differences were seen in Q relative to NE (Figure 2A), HR relative to NE (Figure 2B), or SVR relative to EPI (Figure 2D).

Cardiovascular function and CFTR stratification

When CF patients were stratified according to CFTR genotype, there were no significant differences in Q or HR between groups at rest (Figure 3A and B, respectively). Importantly, there were significant differences in SV between *no F508del* and *homo-F508del* and between *hetero-F508del* and *homo-F508del* groups at rest ($F_{(2,14)} = 7.982$, $P < .01$) as shown in Figure 3C. Differences remained between *no F508del* and *homo-F508del*, and *hetero-F508del* and *homo-F508del* in SV even when patients were indexed for BSA (SVI) as shown in Figure 3D ($F_{(2,14)} = 2.913$, $P = .08$).

Cardiovascular function and EPI correlations

There was a moderate and significant correlation between EPI levels and SV ($r = 0.44$, $P < .01$) and Q ($r = 0.45$, $P < .01$), but not in EPI and SVR ($r = -0.264$, $P = .11$) at rest, when considering the group as a whole (healthy and CF, Table 3). Within the CF group, there was a significant correlation between EPI levels and Q ($r = 0.64$, $P < .05$) and a nearly significant correlation between EPI and SV ($r = 0.66$, $P = .052$). There were no differences in SVR ($r = -0.34$, $P = .31$) as seen in Table 3. Within the healthy subject group (Table 3), there were significant correlations between EPI and SV ($r = 0.43$, $P < .05$) and EPI and Q ($r = 0.40$, $P < .05$), but not in EPI and SVR ($r = -0.24$, $P = .22$). There was no relationship between NE and cardiac function when considering the group as a whole, or when grouped by each condition (Table 3).

Discussion

In this study, CF subjects demonstrated significantly lower Q, SV, and higher SVR when compared with healthy subjects. These differences remained significant when SV was assessed relative to circulating catecholamines. In addition, when CF patients were stratified according to their CFTR F508del genotype, significant differences emerged between groups. These findings enhance our previous findings of attenuated cardiovascular responses to the inhaled β_2 -agonist, albuterol, in CF

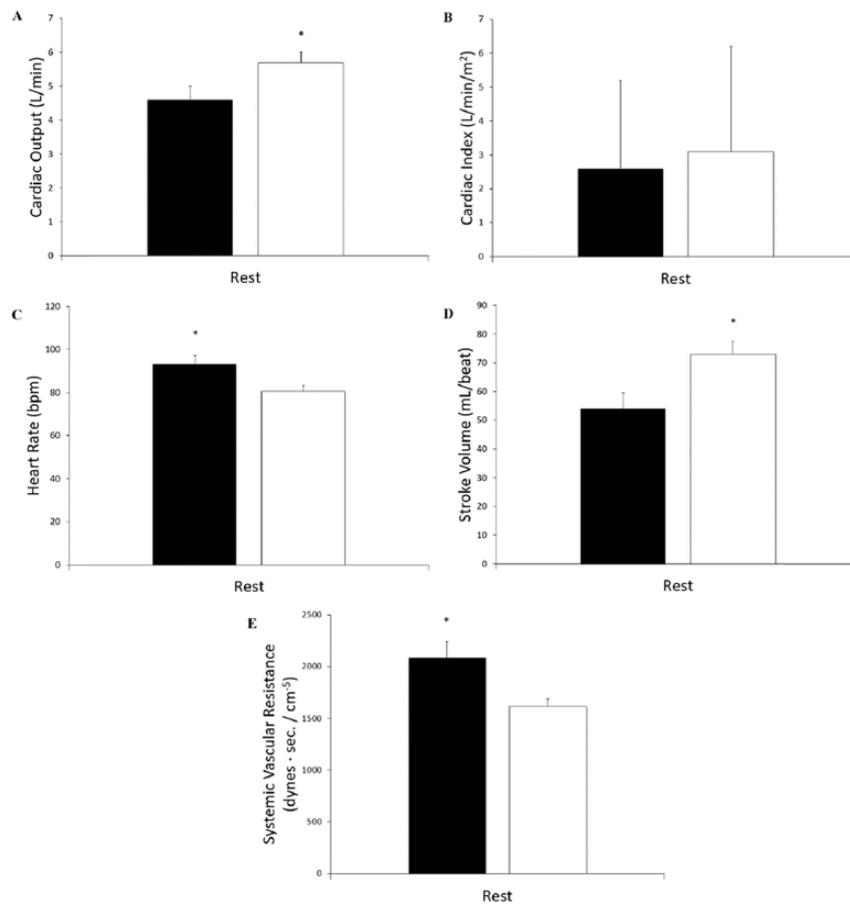


Figure 1. (A) Cardiovascular function at rest (n: 19 = CF, 30 = healthy), (B) cardiac index (n: 19 = CF, 30 = healthy), (C) heart rate (n: 18 = CF, 31 = healthy), (D) stroke volume (n: 17 = CF, 30 = healthy), and (E) systemic vascular resistance (n: 19 = CF, 30 = healthy), where black = CF at rest and white = healthy at rest \pm SEM. * $P < .05$, CF vs healthy subjects.

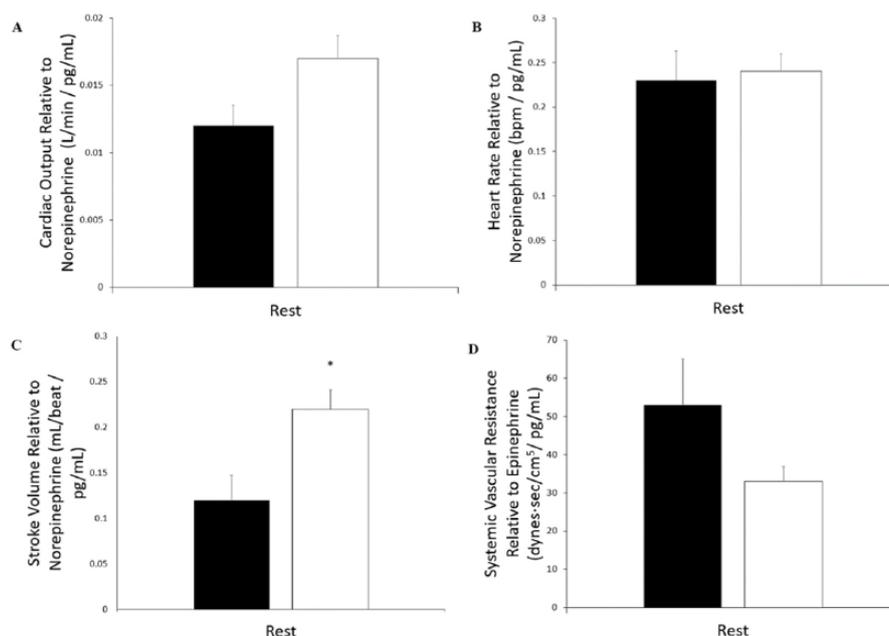


Figure 2. Variables of cardiovascular function for CF (black) vs healthy (white) at rest \pm SEM relative to catecholamines. (A) Cardiac output (n: 11 = CF, 27 = healthy), (B) heart rate (n: 11 = CF, 27 = healthy), and (C) stroke volume (n: 11 = CF, 27 = healthy) were calculated relative to norepinephrine, and (D) systemic vascular resistance (n: 11 = CF, 27 = healthy) was calculated relative to epinephrine. * $P < .05$, CF vs healthy subjects.

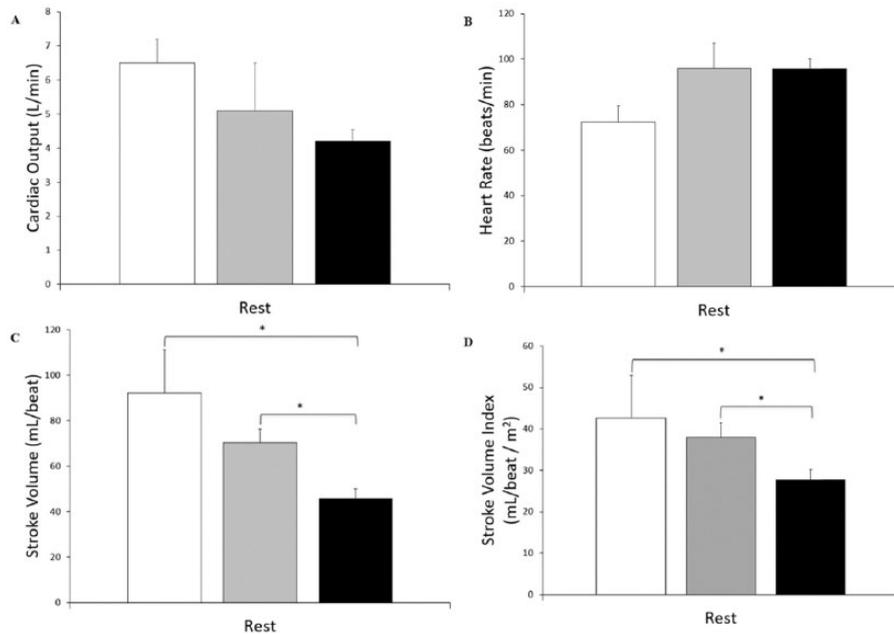


Figure 3. Subjects stratified by CFTR F508del mutation for (A) cardiac output (n: 2 = no F508del, 3 = hetero-F508del, 14 = homo-F508del), (B) heart rate (n: 2 = no F508del, 3 = hetero-F508del, 13 = homo-F508del), (C) stroke volume (n: 2 = no F508del, 2 = hetero-F508del, 13 = homo-F508del), and (D) stroke volume index (n: 2 = no F508del, 2 = hetero-F508del, 13 = homo-F508del) at rest \pm SEM. Mutation categories are as follows: no F508del (white) vs single F508del (light gray) vs double F508del (black). * $P < .05$.

patients when compared with healthy subjects and provide evidence that cardiovascular differences in CF patients are likely due to CFTR dysfunction.¹¹

We have previously demonstrated that a single dose of a β -agonist increases Q and SV and decreases SVR in healthy subjects, and that this effect is decreased in CF patient; and that there is an attenuated response in Q, SV, and SVR to acute β_2 -adrenergic receptor stimulation.^{11,22} It was not known whether the reduced response to albuterol was due to cardiovascular deficits relating to CFTR genotype, a product of receptor desensitization from prolonged use of a β -agonist^{15,23} or inhibited drug delivery and drug transfer ability from the lung periphery into the bloodstream. This study sought to re-evaluate the data by assessing cardiovascular function relative to circulating catecholamines and according to CFTR genotype to investigate the attenuated cardiovascular performance previously seen in CF subjects and take aim at the question of drug delivery inhibiting cardiovascular function.

Early studies have suggested that β_2 -adrenergic receptors are activated by neurotransmitters such as EPI and mediate vasodilation in peripheral vasculature and skeletal muscle tissues with a 10- to 30-fold higher potency than NE.²⁴⁻²⁶ Furthermore, previous work has suggested that β -adrenergic receptor-activated vasodilation in human skeletal muscle is initiated by β_2 -adrenergic receptor stimulation, and that it can lead to a decrease in SVR in human skeletal muscles, including our observation of decreased SVR response following the administration of an inhaled β_2 -agonists in CF populations compared with healthy subjects.^{22,25,27,28} From this, the present

study used resting endogenous levels of NE when comparing HR, SV, and Q between CF and healthy subjects to control for inhibited drug delivery to the cardiovascular system as a mechanism for explaining attenuated cardiovascular function, and SVR relative to EPI to explore desensitization as a cause of cardiovascular attenuation in CF patients. Another comparison that can be done in future studies as a control group is subjects with neutrophilic asthma. This group can be compared with CF subjects to control for the isolated pulmonary deficit when measuring cardiovascular changes.

In humans, both β_1 - and β_2 -adrenergic receptors are present in the heart, with several groups citing an approximate 75:25 ratio of β_1 to β_2 receptors in non-failing human heart.²⁴ Both β_1 - and β_2 -adrenergic receptors have been demonstrated to activate the adenylyl cyclase pathway in human cardiac myocytes; NE has been found to selectively activate β_1 -adrenoceptors, where EPI may activate both β_1 - and β_2 -adrenergic receptors with similar potency.^{24,25,29} Although the cardiac myocyte contains both β_1 - and β_2 -adrenergic receptors, it has been shown that left ventricular positive inotropic effects in human cardiac myocytes by NE are mediated by the activation of β_1 -adrenoceptors, and only influenced by β_2 -adrenoceptors at high non-physiological levels.²⁴ Infusion studies exploring the cardiovascular effects of β_1 - and β_2 -agonists and inhibitors have demonstrated that exercise-induced increase in HR is predominantly mediated by β_1 -adrenergic receptor stimulation.²⁴ Previous work by our group has demonstrated an increase in SV and Q following the administration of an inhaled β -agonist, suggesting that even

Table 3. Cardiovascular variable and catecholamine correlations.

			N	PEARSON COEFFICIENT	P-VALUE
Epinephrine	CF + Healthy	SV	36	0.44	<.01
		Q	38	0.45	<.01
		SVR	38	-0.26	.11
	CF	SV	9	0.66	.05
		Q	11	0.64	.03
		SVR	11	-0.34	.31
	Healthy	SV	27	0.43	.03
		Q	27	0.40	.04
		SVR	27	-0.24	.22
Norepinephrine	CF + Healthy	SV	36	0.12	.49
		Q	38	0.17	.31
		SVR	38	-0.08	.64
	CF	SV	9	-0.11	.78
		Q	11	0.30	.36
		SVR	11	-0.27	.43
	Healthy	SV	27	0.24	.24
		Q	27	0.17	.41
		SVR	27	-0.05	.81

Abbreviations: CF, cystic fibrosis; EPI, epinephrine; NE, norepinephrine; Q, cardiac output; SV, stroke volume; SVR, systemic vascular resistance.

Data are presented as number of subjects evaluated in each group (n), Pearson correlation coefficients, or a *P*-value, indicated by column at top. Subjects were split and analyzed in three groups (CF + Healthy, CF only, and Healthy only) for correlations with circulating catecholamines EPI and NE.

inhaled drug delivery can augment cardiac function through the β_2 -adrenergic receptor pathway.^{11,22}

The present results suggest that the reduced cardiovascular response to albuterol, previously observed in CF patients, was likely due to a reduced contractility, rather than decreased drug delivery and drug transfer into the bloodstream.¹¹ Furthermore, the inherent differences in cardiac contractility in patients with CF is possibly dependent on the type of CFTR mutation, and the health of endothelial tissues. Severe CFTR mutations may include class I-III mutations (including the class II F508del) that code for reduced CFTR production and function, marked by a failure to be expressed in the cell membrane.³⁰ Other severe mutations may affect regulation and turnover of CFTR proteins and are categorized as class IV-VI.³⁰ Recent work has demonstrated significant differences in adjusted VO_2 max in CF patients expressing at least one copy of F508del with a secondary class-V mutation when compared with those with two copies of a class-II mutation.² In addition, it has been suggested that endothelial function may play a role in decreased pulmonary function and exercise capacity in a cohort of relatively healthy, young CF

patients when compared with control.³¹ Their group demonstrated a significantly lower percent predicted VO_2 , a significantly lower peak workload and more severe airway disease in CF groups with more endothelial dysfunction.^{31,32} They hypothesized that the impaired cardiovascular function may be a consequence of a combination of endothelial dysfunction, deconditioning, and known pulmonary obstruction in CF patients.³¹ These findings on vascular dysfunction, along with our findings on cardiac dysfunction, can be used to explain the lower VO_2 in CF patients and in CF patients who are carriers of the F508del. In addition, it is possible that muscular function differences or differences in conditioning can also lead to differences in VO_2 or cardiovascular function.

Treatment with a β_2 -agonist is common in CF patients and has been documented to aid in sputum expectoration, bronchodilation, and mucus break-up in human lungs.^{23,24,33,34} Because of this daily use, the β -adrenergic receptors could become desensitized which could explain the lower cardiac parameters observed (and explain our previously demonstrated attenuated SV and Q response to an inhaled β -agonist) in these subjects.¹⁵ If desensitization had occurred in

this CF population, we would expect to observe attenuation in SVR for a given level of EPI, since SVR is heavily influenced by β_2 -adrenergic receptor-mediated peripheral vasodilation.¹⁵ This study found no significant differences in SVR relative to EPI, suggesting that there was no significant desensitization in this population, although more detailed analysis using cell models and vascular function in humans is needed. It was also found that there were no differences between CF and healthy subjects with respect to EPI or NE; however, these were measured on the venous side and there may be other measurements that would be useful to determine sympathetic activity differences.

Limitations

The assessment of cardiovascular function according to only one mutation of CFTR (F508del) is a limitation to this study. In doing so, we do not eliminate compounding mutations for CFTR that may contribute to limited cardiovascular function in CF individuals; and with only two subjects with no F508del CFTR mutation, we are unable to definitively say that possessing the F508del allele deletion directly correlates to a more severe cardiovascular function deficit. What we aimed to do, rather, was to determine whether CFTR is directly playing a role in cardiovascular function in CF, so we chose a common and highly functional variant; of which, nearly 70% of CF patients have at least one deletion.¹ Furthermore, the assessment of the relationship between cardiovascular function and F508del allele deletion may be limited by the small sample size of the study groups and by the relatively healthy CF population studied (Table 1).

In this study, venous antecubital blood draws were used for catecholamine measures as opposed to arterial blood draws because venous catheter placement is safer. Although previous work has demonstrated a relationship between venous and arterial catecholamines, venous levels may misrepresent arterial catecholamine values.³⁵ In addition to being a circulating hormone in peripheral tissues, NE has also been documented as a strong neurotransmitter. It has been demonstrated to have a net increase in concentration in venous blood because NE removal into surrounding tissues is often less than the overflow from sympathetic nerve synapses.²⁴ Patients with hypertension and congestive heart failure have been documented to have excessive NE spillover from cardiac and renal tissues, possibly leading to elevated levels of plasma NE.^{24,36,37} Therefore, it is possible that our venous NE levels represent a more general pool of catecholamines, culminating from different sources, rather than simply those that are binding to adrenergic receptors.

Conclusions

Collectively, our findings suggest that the reduced cardiovascular response to albuterol demonstrated in CF patients is not solely due to tachyphylaxis or reduced systemic drug delivery due to lung disease. Rather, we demonstrate that there may be inherent resting cardiovascular differences in CF patients according to CFTR F508del genotype and as a result of CFTR dysfunction.

Author contributions

Study conception and data collection: CMW, SEB, WJM, and EMS
Data analysis: ALB, ERP, EHV, TAL, and EMS
Manuscript: All authors contributed to the manuscript

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