Evidence of microvascular dysfunction in patients with cystic fibrosis

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Evidence of microvascular dysfunction in patients with cystic fibrosis. Am J Physiol Heart Circ Physiol 310: H1479–H1485, 2016. First published April 15, 2016; doi:10.1152/ajpheart.00136.2016.—Cystic fibrosis (CF) is a genetic, multisystemic disorder with broad clinical manifestations apart from the well-characterized pulmonary dysfunction. Recent findings have described impairment in conduit vessel function in patients with CF; however, whether microvascular function is affected in this population has yet to be elucidated. Using laser-Doppler imaging, we evaluated microvascular function through postocclusive reactive hyperemia (PORH), local thermal hyperemia (LTH), and iontophoresis with acetylcholine (ACh). PORH [518 ± 174% (CF) and 801 ± 125% (control), P = 0.039], LTH [1,338 ± 436% (CF) and 1,574 ± 620% (control), P = 0.045], and iontophoresis with ACh [416 ± 140% (CF) and 617 ± 143% (control), P = 0.032] were significantly lower in patients with CF than control subjects. In addition, the ratio of PORH to LTH was significantly (P = 0.043) lower in patients with CF (55.3 ± 51.1%) than control subjects (68.8 ± 3.1%). Significant positive correlations between LTH and forced expiratory volume in 1 s (%predicted) (r = 0.441, P = 0.013) and between the PORH-to-LTH ratio and exercise capacity (r = 0.350, P = 0.049) were observed. These data provide evidence of microvascular dysfunction in patients with CF compared with control subjects. In addition, our data demonstrate a complex relationship between microvascular function and classical markers of disease severity (i.e., pulmonary function and exercise capacity) in CF.

endothelium-dependent vasodilation; endothelial function; microvascular function; cystic fibrosis
adhere to the timing of their daily treatments and come to the laboratory following their morning airway clearance technique and inhaled medicines. It is worth noting that the use of inhaled β-agonists as part of routine treatment of CF does not affect cardiac or peripheral hemodynamics in these patients (52). Microvascular function was assessed; then a pulmonary function test was performed and a blood sample was collected for assessment of standard laboratory values. Subsequently, each participant completed a maximal exercise test to evaluate exercise capacity, an independent predictor of survival in CF (35).

Subjects

Sixteen patients with CF (range 13–43 yr) and 15 age-matched control subjects (range 14–42 yr) volunteered to participate in the study. The majority of the patients were homozygous ∆F508del (n = 14); however, one patient was ∆F508/621+1G→T and another was ∆F508/G551D. Participants were excluded from the study if they 1) had a forced expiratory volume in 1 s (FEV1) <50 %predicted, 2) had a resting O2 saturation <90%, 3) had a clinical diagnosis of pulmonary hypertension or cardiovascular disease, 4) were taking vasoactive medications, 5) had a clinical diagnosis of sleep apnea or any other sleep disorders, 6) were pregnant, or 7) self-reported to be a smoker. All participants and parents were informed of the objectives and possible risks of the investigation before written consent/assent for participation was obtained. The study followed the principles of the Declaration of Helsinki and was approved by the Institutional Review Board at Augusta University.

Pulmonary Function Test

A pulmonary function test was performed by all participants using the EasyOne Pro LAB spirometer (NDD Medical, Andover, MA) to evaluate FEV1, forced vital capacity (FVC), FVC-to-FEV1 ratio, and forced expiratory flow at 25–75% FVC (FEF25–75%) according to the American Thoracic Society standards. FEV1 (%predicted) was also determined following spirometric reference standards (40).

Exercise Testing

All participants performed a maximal exercise test using the Godfrey protocol on a cycle ergometer. Briefly, after a 2-min baseline and 2-min unloaded warm-up, participants started an exercise protocol that increased 15–20 W/min (depending on the height of the participant) (14). Expired gases were analyzed breath-by-breath by a metabolic cart (True One 2400, ParvoMedics, Sandy, UT). O2 consumption (VO2) was obtained and normalized for total body weight (VO2/kg) as previously described (13). Maximal exercise capacity (VO2 max) was verified using the American College of Sports Medicine exercise testing criteria (1a). Specifically, a test was considered maximal if the subject met three of the four following criteria: 1) volitional fatigue (>17 on rating of perceived exertion), 2) plateau in VO2, 3) ≥85% of predicted maximum heart rate, and 4) respiratory exchange ratio >1.1.

Microvascular Function

In a supine position, the right arm of each participant was extended laterally at ~80° of shoulder abduction and the distal forearm was secured in a vacuum-packed pillow (Vac Pac, Baltimore, MD). A forearm cuff was placed immediately distal to the medial epicondyle, and an iontophoresis gel electrode was placed on the ventral part of the wrist. Two 20-mm ring-shaped chambers (Moor Instruments, Wilmington, DE), secured with double-sided adhesive tape, were placed on the ventral surface of the forearm, with care taken to avoid any area with a tattoo or damage to the skin. The experimental setup is illustrated in Fig. 1. A laser-Doppler imager (Moor Instruments) was placed <30 cm above the forearm. After a 20-min acclimation period in a temperature-controlled (22 ± 2°C) room to achieve a hemodynamic steady state, microvascular function was determined using three different protocols: PORH followed by concurrent assessment of LTH and ACh iontophoresis. For all protocols, cutaneous blood flux is expressed in arbitrary perfusion units (PU). Baseline (BL) flux was determined by calculation of a 30-s average prior to initiation of the pertinent protocol. A biological zero (B0), to control for the Brownian movement of macromolecules in cutaneous interstitial space, was determined while the forearm cuff was inflated during the PORH protocol and subtracted from both baseline and peak responses. For each of the protocols, results are presented for 1) the peak response, 2) the relative change in flow expressed as a percentage: relative change (%) = [peak – (BL – B0) × (BL – B0)−1 × 100, and 3) the time to peak (TTP), which represents the time from the start of the stimulus to the peak flux response.

Postocclusive Reactive Hyperemia Protocol

Using a forearm cuff inflated to 250 mmHg to provoke reactive hyperemia, we determined microvascular function by recording the flux on the forearm before and after a 5-min occlusion period (44). Cutaneous flux response to this protocol was evaluated in the chamber proximal to the occlusion cuff (Fig. 1). All variables related to this protocol are defined with the subscript PORH.

Local Thermal Hyperemia Protocol

LTH was determined in the chamber placed proximal to the occlusion cuff on the solar surface of the forearm (Fig. 1). The LTH chamber was filled with 2 ml of water and then heated at >0.1°C/s to 44°C for 25 min (44). This temperature and time have been shown to elicit maximal microvascular dilation (19, 28). We have also expressed the peak reactive hyperemia values as the percentage of the peak hyperemia during local thermal heating (PORH-to-LTH ratio) to represent the hyperemic response in relation to maximal microvascular blood flow (55). All variables related to the heating protocol are defined with the subscript LTH.

ACh Iontophoresis Protocol

ACh iontophoresis was performed by filling the ventral chamber distal to the occlusion cuff with 2 ml of 2% ACh solution with a purity >99% TLC (Sigma-Aldrich, St. Louis, MO). A rectangular-shaped electrode was placed on the wrist, and both the electrode (cathode) and the chamber (anode) were connected to a battery-powered iontophoresis controller (model MIC2, Moor Instruments). After a baseline recording, ACh was delivered using an anodal current

Fig. 1. Experimental setup of microvascular function using a laser-Doppler imager with the occlusion cuff for postocclusive reactive hyperemia and the 2 chambers for local thermal hyperemia (LTH) and acetylcholine (ACh) iontophoresis.
of 100 μA for 20 s and repeated seven times at 60-s intervals. All variables related to this protocol are defined with the subscript ACh.

### Statistical Analysis

All measurements are expressed as means ± SD. All statistical analyses were performed using SPSS version 23 (SPSS, Chicago, IL), and significance was set at $P < 0.05$. The Shapiro-Wilk test was used to analyze the normality of the measurement distribution. Independent group $t$-tests were performed to identify group differences, and Pearson’s correlations were utilized to identify relationships between microvascular function and pulmonary function and between microvascular function and exercise capacity. Because of the wide age range of our participants, we have also considered age as a covariate factor for the analysis of correlations based on the close relationship of age with disease severity in CF. Effect sizes (Cohen’s $d$) were calculated for the relative change in flux response to each protocol, with 0.2, 0.5, and 0.8 representing small, medium, and large effects, respectively (31).

### RESULTS

#### Demographic Characteristics and Laboratory Values

Demographic characteristics and laboratory values for patients with CF and control subjects are presented in Table 1. No differences in age, sex, height, weight, or body mass index were observed between patients and controls. However, patients with CF exhibited significantly ($P < 0.05$) lower concentrations of lipids (total cholesterol and high- and low-density lipoproteins) than control subjects. In addition, resting $O_2$ saturation was significantly ($P = 0.01$) lower in patients than controls, although all values were still within normal limits at 98%.

### Disease Severity Markers

Spirometric dysfunction and exercise intolerance are common phenotypes in patients with CF (36) and represent classical markers of disease severity. Spirometric function values are presented in Table 2. All spirometric values [FVC, FEV$_1$,

### Table 1. Participant characteristics and laboratory values

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with CF (n = 16)</th>
<th>Control Subjects (n = 15)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M/F</td>
<td>7/9</td>
<td>7/8</td>
<td>0.621</td>
</tr>
<tr>
<td>Age, yr</td>
<td>22 ± 9</td>
<td>28 ± 8</td>
<td>0.087</td>
</tr>
<tr>
<td>Height, cm</td>
<td>158 ± 11</td>
<td>163 ± 25</td>
<td>0.626</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>63 ± 14</td>
<td>66 ± 12</td>
<td>0.729</td>
</tr>
<tr>
<td>BMI, kg/m$^2$</td>
<td>22 ± 4</td>
<td>22 ± 6</td>
<td>0.431</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>27.5 ± 7.7</td>
<td>29.5 ± 5.6</td>
<td>0.517</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>108 ± 12</td>
<td>115 ± 15</td>
<td>0.128</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>65 ± 6</td>
<td>66 ± 8</td>
<td>0.812</td>
</tr>
<tr>
<td>Resting $O_2$ saturation, %</td>
<td>98 ± 1</td>
<td>99 ± 1</td>
<td>0.010*</td>
</tr>
<tr>
<td>TC, mmol/l</td>
<td>3.3 ± 0.5</td>
<td>4.4 ± 1.1</td>
<td>0.001*</td>
</tr>
<tr>
<td>HDL, mmol/l</td>
<td>1.0 ± 0.3</td>
<td>1.5 ± 0.3</td>
<td>0.001*</td>
</tr>
<tr>
<td>LDL, mmol/l</td>
<td>1.8 ± 0.5</td>
<td>2.5 ± 0.8</td>
<td>0.008*</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>1.1 ± 0.4</td>
<td>0.9 ± 0.3</td>
<td>0.084</td>
</tr>
<tr>
<td>TC-to-HDL ratio</td>
<td>3.3 ± 0.2</td>
<td>3.0 ± 0.3</td>
<td>0.505</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>6.6 ± 2.7</td>
<td>4.8 ± 0.4</td>
<td>0.149</td>
</tr>
<tr>
<td>hsCRP, mg/l</td>
<td>3.12 ± 1.41</td>
<td>0.74 ± 0.42</td>
<td>0.125</td>
</tr>
<tr>
<td>Hemoglobin, mmol/l</td>
<td>9.5 ± 0.3</td>
<td>9.0 ± 0.3</td>
<td>0.203</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>45.6 ± 3.4</td>
<td>43.8 ± 4.2</td>
<td>0.253</td>
</tr>
</tbody>
</table>

Values are means ± SD. CF, cystic fibrosis; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL, high-density lipoproteins; LDL, low-density lipoproteins; hsCRP, high-sensitive C-reactive protein. *Statistically significant.

### Table 2. Disease severity values in patients with CF and control subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with CF</th>
<th>Control Subjects</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVC, liters</td>
<td>3.61 ± 1.24</td>
<td>4.54 ± 1.10</td>
<td>0.023*</td>
</tr>
<tr>
<td>FEV$_1$, liters</td>
<td>2.78 ± 1.01</td>
<td>3.74 ± 0.81</td>
<td>0.006*</td>
</tr>
<tr>
<td>FEF$_{25-75}$, l/s</td>
<td>2.56 ± 1.30</td>
<td>3.77 ± 0.99</td>
<td>0.007*</td>
</tr>
<tr>
<td>FEV$_1$, %predicted</td>
<td>75 ± 16</td>
<td>97 ± 9.9</td>
<td>0.001*</td>
</tr>
<tr>
<td>FEV/FVC, %</td>
<td>72 ± 8</td>
<td>91 ± 6</td>
<td>0.044*</td>
</tr>
<tr>
<td>V$_{O2peak}$ l/min</td>
<td>1.6 ± 0.5</td>
<td>2.3 ± 0.8</td>
<td>0.017*</td>
</tr>
<tr>
<td>ml/kg$^{-1}$min$^{-1}$</td>
<td>28.4 ± 4.8</td>
<td>34.5 ± 7.0</td>
<td>0.012*</td>
</tr>
<tr>
<td>%predicted</td>
<td>73 ± 12</td>
<td>98 ± 14</td>
<td>0.001*</td>
</tr>
<tr>
<td>Heart rate peak, beats/min</td>
<td>160 ± 6</td>
<td>184 ± 4</td>
<td>0.003*</td>
</tr>
<tr>
<td>Work peak, W</td>
<td>139 ± 14</td>
<td>205 ± 13</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Values are means ± SD. CF, cystic fibrosis; FEV$_1$, forced expiratory volume in 1 s; FVC, forced vital capacity; FEF$_{25-75}$, forced expiratory flow at 25-75% FVC; V$_{O2peak}$. Peak $O_2$ consumption. *Statistically significant.

### Microvascular Function

A representative trace of the following microvascular function protocols is illustrated in Fig. 2.

**Postocclusive reactive hyperemia.** Data obtained from the PORH protocol are presented in Table 3. The BL ($P = 0.748$) and peak ($P = 0.187$) flux responses were similar between patients and controls. Likewise, B0 was similar ($P = 0.355$) between patients with CF (15 ± 3 PU) and control subjects (17 ± 5 PU). The relative change PORH was significantly ($P = 0.039$, Cohen’s $d = 0.73$) reduced in patients with CF compared with control subjects.

**Local thermal hyperemia.** Data from the LTH protocol are presented in Table 3. BL and B0 values during the LTH protocol were similar ($P > 0.05$) in both groups. However, relative change LTH was significantly ($P = 0.045$, Cohen’s $d = 0.87$) lower in patients than controls. In addition, the PORH-LTH ratio (Fig. 3) was significantly ($P = 0.043$) lower in patients with CF (55.3 ± 5.1%) than control subjects (68.8 ± 3.1%).

**ACh iontophoresis.** Variables obtained from the cutaneous iontophoresis protocol with ACh are shown in Table 1. Significant correlations between microvascular function and markers of disease severity (lung function and exercise capaci-
ity) were identified. Specifically, a significant correlation ($r = 0.441, P = 0.013$) between relative change$_{LTH}$ and FEV$_1$ (%predicted) was identified. Additionally, the PORH-to-LTH ratio was significantly correlated with VO$_2$$_{peak}$ ($r = 0.350, P = 0.049$). Furthermore, TTPLTH was positively associated with FVC ($r = 0.395, P = 0.028$), FEV$_1$ ($r = 0.405, P = 0.024$), FEF$_{25-75}$ ($r = 0.373, P = 0.039$), and FEV$_1$ (%predicted) ($r = 0.384, P = 0.033$). After adjustment by age, a significant correlation between TTPLTH and FEV$_1$ (%predicted) was also observed ($r = 0.475, P = 0.046$).

**DISCUSSION**

CF is associated with many different systemic consequences. For the first time, the present study documents that patients with CF exhibit cutaneous microvascular dysfunction compared with demographically matched control subjects. Our data obtained from comprehensive assessment of microvascular function (PORH, LTH, and iontophoresis with ACh) suggest that patients with CF exhibit some evidence of microvascular dysfunction compared with control subjects. Perhaps more clinically relevant, our data support a positive relationship between microvascular function, exercise capacity, and lung function, such that the disease severity may be utilized to predict microvascular function in CF.

**Microvascular Function in Patients with CF**

The microvascular network, consisting of arterioles, capillaries, and venules, is responsible for the distribution of blood within tissues. Oxidative stress, a common phenotype in CF, reduces the bioavailability of NO in the arterioles (8, 30), allowing for a more accurate comparison not only between groups, but also between sites on the same individual. In addition, our results may suggest that the structure of the blood microvessels is preserved (41, 51), despite impairment of the mechanisms that contribute to the reactive hyperemia response. The exact mechanism is unknown; however, prostanoids are key players in the PORH response (21), and many factors, including metabolic and endothelial vasodilators, sensory nerves, and NO, have also been shown to be involved (23, 50).

In fact, abnormally elevated release of prostanooids has been observed in patients with CF (10). Considering the role of these components as vasoconstrictor factors (22), findings from the present study in patients during the PORH protocol are supported.

**Local thermal hyperemia.** After the local increase in skin temperature, the LTH response is characterized by a biphasic hyperemic response (44) and represents the maximal vasodilator capacity of the microvessels. The initial peak vasodilation is predominantly mediated by an axon reflex and substance P (44, 54), followed by a prolonged plateau phase that is mediated primarily by release of NO from the microvascular endothelium (27, 28, 44). In the present study we have identified a

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**Postocclusive reactive hyperemia.** The PORH protocol represents a sensitive assessment of microvascular function anatomically as well as functionally (15). Although no group differences were observed in the peak response or in the TTP response within the PORH protocol, findings from the present study have identified a significantly reduced relative change in reactive hyperemia in patients with CF compared with control subjects. Expression of the values as a relative change, as reported by others (55), eliminates possible spatial variations and allows for a more accurate comparison not only between sites on the same individual. In addition, our results may suggest that the structure of the blood microvessels is preserved (41, 51), despite impairment of the mechanisms that contribute to the reactive hyperemia response. The exact mechanism is unknown; however, prostanoids are key players in the PORH response (21), and many factors, including metabolic and endothelial vasodilators, sensory nerves, and NO, have also been shown to be involved (23, 50).

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**Table 3. Microvascular function in patients with CF and control subjects**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with CF</th>
<th>Control Subjects</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Postocclusive reactive hyperemia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline, PU</td>
<td>42 ± 8</td>
<td>40 ± 5</td>
<td>0.748</td>
</tr>
<tr>
<td>Peak$_{PORH}$, PU</td>
<td>167 ± 43</td>
<td>207 ± 22</td>
<td>0.187</td>
</tr>
<tr>
<td>Relative change$_{PORH}$, %</td>
<td>518 ± 174</td>
<td>801 ± 125</td>
<td>0.039*</td>
</tr>
<tr>
<td>TTP$_{PORH}$, s</td>
<td>16 ± 6</td>
<td>15 ± 2</td>
<td>0.296</td>
</tr>
<tr>
<td><strong>Local thermal hyperemia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline, PU</td>
<td>36 ± 8</td>
<td>35 ± 6</td>
<td>0.676</td>
</tr>
<tr>
<td>Peak$_{LTH}$, PU</td>
<td>302 ± 44</td>
<td>301 ± 51</td>
<td>0.275</td>
</tr>
<tr>
<td>Relative change$_{LTH}$, %</td>
<td>1,338 ± 436</td>
<td>1,574 ± 620</td>
<td>0.045*</td>
</tr>
<tr>
<td>TTP$_{LTH}$, s</td>
<td>781 ± 197</td>
<td>806 ± 182</td>
<td>0.113</td>
</tr>
<tr>
<td><strong>Iontophoresis with ACh</strong></td>
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</tr>
<tr>
<td>Baseline, PU</td>
<td>40 ± 11</td>
<td>34 ± 11</td>
<td>0.083</td>
</tr>
<tr>
<td>Peak$_{ACh}$, PU</td>
<td>129 ± 54</td>
<td>122 ± 41</td>
<td>0.094</td>
</tr>
<tr>
<td>Relative change$_{ACh}$, %</td>
<td>416 ± 140</td>
<td>617 ± 143</td>
<td>0.032*</td>
</tr>
<tr>
<td>TTP$_{ACh}$, s</td>
<td>16 ± 6</td>
<td>15 ± 2</td>
<td>0.494</td>
</tr>
</tbody>
</table>

Values are means ± SD. CF, cystic fibrosis; PORH, postocclusive reactive hyperemia; LTH, local thermal hyperemia; ACh, acetylcholine; TTP, time to peak; PU, perfusion units. *Statistically significant.
similar initial peak between patients with CF and control subjects (data not shown) followed by a significantly attenuated plateau phase in patients with CF (Table 3). In addition, a 20% reduction in the PORH-to-LTH ratio was observed in patients with CF compared with control subjects (Fig. 3). It is important to note that oxidative stress inactivates NO, attenuating the vasodilatory process (32) and, hence, playing an important role in the reaction of the microvasculature during LTH (7). Considering the strong presence of inflammation and oxidative stress in patients with CF (42), it is reasonable to suspect that these damaging mediators contribute to attenuation of microvascular vasodilatation to LTH as previously reported in other populations (12, 26). However, future studies are needed to determine the mechanistic contribution of the lower LTH cutaneous response in CF.

**ACh iontophoresis.** Cutaneous iontophoresis with ACh has previously been used to investigate microvascular function (47, 49). The infusion of ACh across the skin and into the intradermal space using an electrical charge promotes the activation of NO, although the larger response seems to be mediated by prostanoids (6, 20, 33). Findings from the present study demonstrate that patients with CF exhibit a significantly reduced cutaneous vasodilation to ACh compared with control subjects (Table 3). It is important to note that patients and controls in the present study exhibit similar ($P = 0.287$) skin resistance ($120.937 \pm 24.084$ and $135.500 \pm 18.357 \Omega$, respectively), which can impact the delivery of ACh with iontophoresis. Accordingly, it is unlikely that differences in skin resistance are contributing to the differences between groups. Bearing in mind the same considerations of elevated prostanoids in patients with CF previously described (10) and the contribution of these mediators in an impaired vascular response (21), it is not surprising that patients with CF also exhibit a lower microvascular response to the ACh iontophoresis. However, TTP values were similar between both groups, confirming that the microvascular integrity and structure are intact and unlikely affecting the ACh response (41, 51). In addition, a previously described reliability of the iontophoresis response to ACh on endothelial function was assessed through brachial artery flow-mediated dilatation, which is also impaired in CF (11). Therefore, findings from the present study confirm that patients with CF exhibit evidence of microvascular dysfunction.

**Microvascular Function and Disease Severity: Clinical Relevance**

A novel aspect of the present study is the comprehensive evaluation of microvascular function and the relationships between prognostic markers of disease severity, such as pulmonary function and exercise capacity, in patients with CF. Pulmonary function, specifically FEV$_1$ (%predicted), represents the most traditional assessment of disease severity in CF (1) and is commonly used as a predictor of survival in this patient population (43). Perhaps it is not surprising that the present study has identified positive associations between indexes of microvascular function (relative change LTH and TTP$_{LTH}$) and FEV$_1$ (%predicted). These data indicate that patients with a greater lung function may have a more robust response to the LTH protocol. A possible link between LTH and pulmonary function may rely on the role of systemic oxidative stress in the action of free radicals and NO bioavailability (2, 37, 46). In fact, excessive oxidative stress status has been observed in patients with obstructed airways and impaired lung function, including CF (5, 16). Although the present study cannot determine a cause-effect relationship, future studies are needed to elucidate the causal mechanisms.

Patients with CF also exhibit a progressive, longitudinal decline in exercise capacity, which represents an important predictor of mortality in this population (34, 35, 38). It is well known that regular aerobic exercise improves the quality of life of patients with CF through increasing expectoration of the sputum and ameliorating the decline in lung function (29, 35). Exercise also enhances NO bioavailability (3, 4, 9). In the present study a positive correlation between the PORH-to-LTH ratio and exercise capacity suggests that a greater exercise capacity positively impacts microvascular function in CF. Accordingly, it is reasonable to believe that the microvascular dysfunction identified in patients with CF can impact the transport and delivery of O$_2$ to the exercising muscles. However, future studies are needed to determine if improvement in microvascular function in CF contributes to improvements in exercise capacity.

Not all outcomes of microvascular function assessed by each protocol were related to the same markers of disease severity. It is important to remember, however, that the cutaneous response to each protocol is mediated, in part, by different molecular mechanisms, which may likely explain the different relationships between the microvascular function and the disease severity markers in the present study. Nevertheless, future studies are warranted to determine the contribution of microvascular dysfunction to both pulmonary function and exercise intolerance in CF.

**Experimental Considerations**

The present study included a relatively small sample size; therefore, the possibility of a type II error should be considered. However, we used Cohen’s $d$ statistics to identify large effect sizes in all the primary microvascular outcomes between groups. Therefore, we believe that these analyses corroborate statistical differences in microvascular function between patients with CF and control subjects.

In the present study, PORH always preceded the ACh and LTH protocols. It is plausible that the microvascular function responses to ACh iontophoresis and LTH were impacted by...
PORH preconditioning contributing to microvascular hyperactivity (18, 57); however, the same protocol was conducted in both groups. In addition, unpublished data from our lab demonstrate a similar peak response to ACh iontophoresis whether it is conducted before (158 ± 44 PU) or after (158 ± 59 PU) a PORH protocol, ruling out possible interferences among responses that may affect the present results.

In the present study, endothelium-independent microvascular dilation through iontophoresis with sodium nitroprusside was not included. Data from our lab support a similar (P = 0.207) conduit vessel endothelium-independent vasodilatory response to sublingual nitroglycerine between patients with CF and control subjects (23.1 ± 3.2% and 21.4 ± 5.8% respectively). It is plausible, however, that the endothelium-independent dilation is different in conduit and microvessels; therefore, further investigation is needed.

Conclusion

This is the first study, to our knowledge, to complete a comprehensive evaluation of microvascular function using PORH, LTH, and ACh iontophoresis in patients with CF. Findings from the present study indicate that patients with CF exhibit some evidence of microvascular function compared with demographically matched control subjects. In addition, a positive relationship between microvascular function and both exercise capacity and lung function was observed, suggesting that disease severity may contribute, at least in part, to microvascular dysfunction in this population. Future studies are necessary to elucidate whether an enhancement in NO bioavailability, and hence an improvement in microvascular function, contributes to improvements in pulmonary function and exercise capacity (or vice versa) in patients with CF.

ACKNOWLEDGMENTS

The authors thank the patients and volunteers for their commitment and participation in the study. We also acknowledge the entire Augusta University CF care team for their commitment to CF research.

GRANTS

This work was supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Grant R21 DK-100783 (R. A. Harris) and the Vertex Pharmaceuticals Investigator-Initiated Studies Program (R. A. Harris).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

P.-R.M., J.T., N.S., R.C., and R.A.H. performed the experiments; P.-R.M., J.T., N.S., R.C., K.T.M., C.F., and R.A.H. analyzed the data; P.-R.M., J.T., N.S., R.C., K.T.M., C.F., and R.A.H. interpreted the results of the experiments; P.-R.M. and R.A.H. prepared the figures; P.-R.M. and R.A.H. drafted the manuscript; P.-R.M., J.T., N.S., R.C., K.T.M., C.F., and R.A.H. edited and revised the manuscript; P.-R.M., J.T., N.S., R.C., K.T.M., C.F., and R.A.H. approved the final version of the manuscript; R.A.H. developed the concept and designed the research.

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