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Exercise hyperemia and vasoconstrictor responses in humans with cystic fibrosis

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Abstract

ATP released from circulating erythrocytes is a potential signal regulating muscle blood flow during exercise (exercise hyperemia), and intravascular ATP appears to blunt sympathetic vasoconstriction during exercise. Erythrocytes from patients with cystic fibrosis (CF) do not release ATP. The goal of the present study was to determine whether increases in forearm blood flow during exercise are blunted in CF patients and whether CF patients exhibit greater vasoconstrictor responsiveness during exercise. Nine control subjects and 10 CF patients who were free of other disease complications (~96% O₂ saturation) performed incremental rhythmic forearm exercise at 5, 10, and 15% of maximum handgrip strength for 21 min (7 min at each workload). We used a cold pressor test to evoke sympathetic vasoconstriction under resting conditions and at each exercise workload. As a control, subjects performed a second exercise bout without the cold pressor test. Continuous brachial artery blood velocity was monitored beat-to-beat, and vessel diameter was assessed by Doppler ultrasound. Artery diameter, as well as blood pressure, heart rate, and O₂ saturation, was measured at steady-state exercise and at 1 min into the cold pressor stimulus. Blood pressure and heart rate responses to the forearm exercise and each cold pressor test were similar in both groups ($P > 0.05$). Contrary to our hypothesis, forearm blood flow ($P = 0.91$) and forearm vascular conductance ($P = 0.82$) were similar at rest and at each level of exercise between CF patients and controls. Additionally, there was no difference in the degree of sympathetic vasoconstriction between groups at rest and at each level of exercise ($P = 0.22$). Our results suggest that ATP released from the deformation of erythrocytes is not an obligatory signal for exercise hyperemia in human skeletal muscle.

Keywords

Doppler ultrasound; ATP; cold pressor test; forearm

The mechanisms that elevate blood flow in contracting muscle (exercise hyperemia) to match metabolic need remain elusive, despite intensive study for over a century. One possible signal for exercise hyperemia implicated in animal (6,20,43) and human (13,14,16,32) models is ATP, a potent endothelium-dependent vasodilating substance. Intra-arterial administration of ATP in human limbs evokes skeletal muscle vasodilation (10,21,30,32) that is qualitatively and quantitatively similar to that seen during voluntary rhythmic exercise. ATP infusion also limits sympathetic vasoconstriction in exercising muscle, making it an attractive candidate as an

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important factor (32) mediating functional sympatholysis. An intriguing hypothesis is that ATP released from erythrocytes during exercise can evoke vasodilation (6,7,11,12,22,35,36) and link oxygen supply to oxygen demand in the working muscle (12,16).

Two physiological stimuli that potentially release ATP from erythrocytes are mechanical deformation (35,37,38) and hemoglobin desaturation (1,22).

Along these lines, recent evidence suggests that, for erythrocytes to release ATP on deformation, a functional CF trans-membrane regulator (CFTR) protein is required (35). In vitro studies with erythrocytes from humans with cystic fibrosis (CF) confirm that ATP release is abolished when these erythrocytes are deformed (35). In addition, flow-mediated pulmonary vasodilation is absent when erythrocytes without CFTR are studied (36). The mechanism for ATP release via hemoglobin desaturation is unknown (22). If ATP, released from the deformation of erythrocytes, is an important signal for exercise hyperemia, it is reasonable to propose that muscle blood flow in CF patients might be blunted compared with that in controls.

Therefore, to gain insight into the possible role of ATP release from human erythrocytes in exercise hyperemia, we compared muscle blood flow and functional sympatholysis during forearm exercise in healthy normal subjects and medically stable CF patients. We hypothesized that the CF patients would have blunted vasodilator and functional sympatholysis responses to exercise compared with control subjects.

METHODS

Subjects

Subjects were recruited from Rochester, MN, and surrounding areas. Ten (7 male and 3 female, 21–38 yr old) patients with CF, recruited from patients regularly seen at the Mayo Clinic Departments of Pediatrics and Pulmonary/Critical Care, were otherwise healthy and free of acute respiratory infections. All CF patients continued their normal medication regimen, which could include inhaled β -agonists, digestive enzymes, Pulmozyme, fat-soluble vitamin replacements, and itraconazole (antimicrobial). Five of the 10 patients displayed at least one copy of the $\Delta F508$ mutation; the genotype of the remaining 5 patients was not known. Nine (4 male and 5 female, 19–25 yr old) healthy subjects were recruited as a control group. All subjects were nonsmokers, nonobese (body mass index $<30 \text{ kg/m}^2$), and normotensive and were free of cardiovascular disease. Control subjects were not taking medications. All procedures were approved by the Mayo Institutional Review Board. After reviewing the protocol, all subjects provided written informed consent.

Measurements

Heart rate and blood pressure—Heart rate (HR) was measured by three-lead electrocardiography (ECG). Blood pressure was assessed (beat-to-beat) with a finger plethysmograph (Finapres) on the dominant (right, nonexercising) arm and verified with an automated blood pressure cuff on the same arm. Arterial oxygen saturation was monitored continuously on the index finger of the dominant hand with a pulse oximeter (Cardio-Cap).

Forearm blood flow—Brachial artery diameter and blood velocity were measured with a Doppler ultrasound probe (12-MHz linear array; model M12L, Vivid 7, General Electric) with a probe insonation angle previously calibrated to 60° . Diameter measurements were assessed between contractions and corresponded to the QRS complex (end diastole) of the ECG. Arterial blood velocity was continuously assessed throughout rest (baseline) and during each level of exercise. Diameter measurements typically resulted in loss of pulse-wave signal for 15–20 s.

Forearm blood flow (FBF) was calculated as brachial blood velocity multiplied by brachial artery cross-sectional area (34,41,42).

Experimental Procedures

Forearm exercise—Rhythmic forearm exercise was performed with a handgrip device by the nondominant arm lifting a weight 4–5 cm over a pulley at a duty cycle of 1 s contraction–2 s relaxation (20 contractions/min). The exercise workloads corresponded to 5, 10, and 15% of maximal voluntary contraction obtained before instrumentation. Each workload lasted for 7 min and was then immediately increased to the next workload (21 min of forearm exercise). We chose low-to-moderate workloads to minimize chance of fatigue, which may lead to increases in sympathetic nerve activity (44).

Cold pressor test—A cold pressor test was employed to evoke sympathetic nervous system stimulation (23,27,28). The subject's bare foot was passively placed in ice water (4°C) for 2 min. A cold pressor test was performed twice at rest and once at each exercise workload. From pilot experiments ($n = 4$), there was no statistical difference in blood pressure responses between five consecutive resting cold pressor tests. However, the first cold pressor tended to elicit a larger rise in blood pressure and was therefore removed from statistical analysis. Thus we used the second cold pressor test during baseline conditions compared with each subsequent cold pressor test during exercise.

Exercise Protocol

The general exercise protocol is summarized in Fig. 1. After instrumentation, subjects rested quietly for 20 min, and all testing was performed in the supine position. The experimental protocol consisted of subjects initially performing two cold pressor tests (2 min), each separated by 5 min of resting baseline. During exercise, a cold pressor test was performed for the final 2 min of each workload (*minutes 5–7*). The foot was removed from the ice water, and the workload was immediately increased. Blood flow velocity measurements were obtained continuously throughout baseline, at each exercise level, and during each cold pressor test. Brachial artery diameter was obtained after 4 min of baseline, at steady-state exercise (*minute 4*), and after the 1st min of the cold pressor stimulus.

To account for possible effects of the cold pressor test on exercise blood flow, each subject performed an identical control exercise protocol without performing the cold pressor tests. All variables during the control protocol were assessed at the same time points. The order of the control and experimental protocols was counterbalanced.

Data Collection and Statistical Analysis

All hemodynamic data were digitized and stored on a computer at 200 Hz and analyzed offline with signal-processing software (Power-lab, ADInstruments). HR was derived from the ECG signal (3-lead ECG), and mean arterial pressure (MAP) was derived from the Finapres pressure waveform. The mean blood velocity (MBV) of the Doppler signal was averaged across 30-s intervals during steady-state exercise to reduce contraction-to-contraction-induced variability in blood flow.

Forearm vascular conductance (FVC) was calculated as $(\text{FBF} / \text{MAP}) \times 100$ and expressed as milliliters per minute per 100 mmHg. MBV during the cold pressor test was averaged every 10 s to assess the dynamic nature of the FBF response to the cold pressor test. To assess vasoconstrictor effects on changes in blood flow with concurrent changes in MAP during cold pressor tests, we calculated the relative reduction in FVC from steady-state values of FBF and MAP immediately before and during the nadir of each cold pressor test (8,9). The percent

decrease in FVC was calculated as follows: $\% \Delta \text{FVC} = (\text{FVC}_{\text{nadir}} - \text{FVC}_{\text{ss}}) / \text{FVC}_{\text{ss}} \times 100$, where ss represents steady state.

Student's unpaired *t*-tests were used to compare subject characteristics between groups. Repeated-measures analysis of variance was used to compare group differences from rest to exercise, as well as effects of the cold pressor test on blood flow. Values are means $\pm$ SE. Statistical significance was set a priori at $P < 0.05$.

RESULTS

Subject Characteristics

Control subjects and CF patients displayed similar age, height, weight, body mass index, forearm volume, maximal voluntary contraction, and exercise workloads. Subject characteristics are summarized in Table 1. There were no obvious differences between the responses of male and female subjects to the cold pressor test or exercise hyperemia; therefore, data from both genders were combined.

Reproducibility of Hemodynamic Responses to Exercise

Comparison of experimental and control protocols indicated similar steady-state FBF ($P = 0.74$) and FVC ($P = 0.59$) responses when a cold pressor test preceded the increase in workload. The MAP response to each level of exercise was also similar between groups ($P = 0.98$). These results suggest that the steady-state exercise blood flow response was repeatable across exercise bouts and that a cold pressor test immediately before an increase in exercise intensity did not affect the subsequent steady-state measurements.

Systemic Response to Exercise and Cold Pressor Test

MAP and HR during each cold pressor test and at each level of exercise are summarized in Table 2. Blood pressure in CF patients tended ($P = 0.07$) to be higher throughout the study, and in both groups the cold pressor test increased MAP from steady-state levels ($P = 0.02$). However, the MAP response to each cold pressor test and the response to each level of exercise was similar between groups ($P = 0.68$). HR was slightly higher throughout the study in CF patients ($P < 0.01$), possibly because of their use of an inhaled β -agonist each morning and evening. HR increased significantly during the cold pressor tests ($P = 0.01$), but HR responses at rest and during exercise were similar between controls and CF patients ($P = 0.81$). Oxygen saturation was slightly lower in CF patients: $95 \pm 1\%$ vs. $98 \pm 1\%$ for controls ($P < 0.01$). However, neither exercise nor the cold pressor tests altered oxygen saturation in either group ($P = 0.92$).

Hemodynamic Response to Forearm Exercise and Cold Pressor Test

FBF responses to incremental exercise are summarized in Fig. 2A. FBF was similar at rest and at each level of exercise between CF patients and control subjects ($P = 0.91$). Similarly, there was no difference in FVC between the two groups (Fig. 2B; $P = 0.82$). Brachial artery diameter was smaller in CF patients (0.37 ± 0.02 vs. 0.39 ± 0.02 cm in controls, $P = 0.04$; data not shown) and did not change significantly with exercise or cold pressor tests ($P = 0.9$).

Responses to the cold pressor test at rest and each level of exercise are summarized in Table 2. The cold pressor test led to a significant reduction in FBF at rest and during exercise in both groups ($P = 0.049$), but the decrease in FBF was similar between groups ($P = 0.45$; Table 2). Furthermore, the decrease in FVC was similar between groups ($P = 0.97$; Table 2).

The percent drop in FVC associated with the cold pressor test at baseline and during steady-state exercise is displayed in Fig. 3. Both groups exhibited significant reductions in percent

drop in FVC with each cold pressor test ($P < 0.05$); however, the constriction was similar between groups ($P = 0.22$).

DISCUSSION

This study is the first to systematically examine muscle blood flow during exercise in CF patients. Contrary to our hypothesis, our results demonstrate that blood flow and vascular conductance were similar in control subjects and CF patients. The results from cold pressor tests suggest that although there was vasoconstriction in both groups, it appears that the responses at rest and during exercise were similar. Taken together, these findings do not support an obligatory role of ATP release from erythrocytes in exercise hyperemia.

Muscle blood flow during exercise increases linearly with increases in workload to meet the metabolic demand of the tissue. One potentially important vasodilator signal is ATP, inasmuch as circulating ATP (13,14,17) and interstitial ATP (17,19,25) levels increase in a dose-dependent manner during exercise. In addition, intra-arterial infusion of ATP markedly increases blood flow to levels similar to that obtained during muscle contraction (10,32).

Several potential sources of intravascular ATP during exercise include 1) the skeletal muscle itself, 2) endothelial cells, 3) circulating erythrocytes on deformation, and 4) circulating erythrocytes in response to low oxygen saturation. Striated muscle does not appear to serve as a significant source for intravascular ATP (15,29). The contribution of ATP from vascular endothelium cannot be excluded, because cultured endothelial cells exhibit shear-sensitive ATP release (15,45). Because isolated resistance arteries release only nanomolar amounts of ATP in response to increases in shear (7) and erythrocytes release micromolar amounts, the major intravascular source of ATP is likely the circulating erythrocyte (7,17,35).

Erythrocytes deform when they pass through capillaries and are likely deformed in resistance arteries and small veins during each muscle contraction. In vitro deformation of erythrocytes releases micromolar concentrations of ATP (35). The ability of erythrocytes to release ATP is dependent on a functional CFTR protein. In vitro experiments by Sprague et al. (35) clearly demonstrated the absence of ATP release on deformation of blood cells from CF patients. It is reasonable to propose that CF patients would exhibit a blunted exercise hyperemic response due to lack of ATP from erythrocytes. However, our data demonstrate that the exercise hyperemic responses in CF patients and control subjects were similar (Fig. 2).

A second signal for ATP release from erythrocytes is the deoxygenation of hemoglobin (1, 17,22). Unfortunately, we cannot distinguish between erythrocyte deformation and deoxygenation release of ATP, because both may occur during exercise. Although it is known that CFTR is required for deformation release of ATP, the mechanism for ATP release via hemoglobin deoxygenation is unknown. However, reports of ATP release during deoxygenation suggest that ATP release is associated with CFTR and/or band 4.5 and band 3 proteins, the key proteins involved with anion exchange in the erythrocyte (1,22). Although indirect, this evidence suggests that deformation- and deoxygenation-induced ATP release from erythrocytes may be mediated via the CFTR protein. This suggests that ATP release from erythrocytes (deformation and/or deoxygenation) is not obligatory for normal steady-state blood flow response to exercise.

Nitric oxide (NO) (24,33,40) and ATP-sensitive potassium channels (24,39) have been implicated in sympatholysis. In humans, definitive evidence for (4,33) or against (9) NO is lacking. Intra-arterial ATP infusions in humans appear to mimic sympatholysis, making ATP an attractive candidate (32). Our results suggest that although the cold pressor test provided sufficient stimulus for sympathetic activation and subsequent forearm vasoconstriction (Table 2, Fig. 3), the relative amount of vasoconstriction was similar between controls and CF patients

and was not attenuated with exercise (Fig. 3). Thus our findings do not support an obligatory role for ATP in functional sympatholysis at low-to-moderate levels of exercise. Although vasoconstriction in response to the sympathetic stimulus was similar between CF patients and controls, the finding that there was no sympatholysis in control subjects limits the interpretation of our results. More conclusive results regarding functional sympatholysis could be obtained using higher exercise intensities and local tyramine infusion (endogenous norepinephrine release).

Experimental Considerations and Limitations

Although the present results do not support a role for ATP in exercise hyperemia or sympatholysis, it is possible that some other vasodilator signal is upregulated in CF patients. Potential compensatory signals might include NO, prostaglandins, adenosine, or other undefined metabolites. Along these lines, vasocloses from ATP binding to purinergic (P2Y) receptors includes NO (2,26) and prostaglandins (18) released from vascular endothelium (5).

Another consideration is that ATP is an important sympatholytic factor in healthy humans, but some other substances may mediate sympatholysis in CF patients. It is also possible that the levels of ATP required to achieve sympatholysis are achieved only at higher exercise intensities than those achieved in the present study. Compared with previous studies in our laboratory using intra-arterial tyramine (9,31), cold pressor tests performed with the foot exhibit only moderate vasoconstriction at rest and during exercise. Therefore, it is possible that the cold pressor test was not a sufficient vasoconstrictor stimulus to elicit functional sympatholysis.

It is noteworthy that three CF patients were taking the antimicrobial itraconazole, which belongs to a class of drugs known to inhibit *P*-450 enzymes. If itraconazole inhibited these enzymes in the vascular wall, similar to miconazole (3), it is possible that this drug altered the balance of vasoconstrictor and vasodilator arachidonic acid metabolites.

Interpretation of our results is not likely influenced by differences in oxygen utilization, inasmuch as the relative and absolute workloads were similar between control subjects and CF patients (Table 1). Although oxygen saturation was slightly lower in the CF patients, there was no change within each group, suggesting that oxygen delivery was not a limiting factor. We did not attempt to repeat the *in vitro* erythrocyte deformation studies to analyze ATP release from erythrocytes in our subjects. However, five CF patients were known to express at least one copy of the $\Delta F508$ mutation, which is the same mutation that displayed no ATP release on deformation (35). There was no obvious difference in the exercise blood flow responses between these five patients and those whose genotype was unknown. All patients were Caucasians, who predominantly carry the $\Delta F508$ mutation. Therefore, it is likely that ATP was not released from the deformation of erythrocytes in CF patients in our study.

In conclusion, our results indicate that the hyperemic response to moderate exercise is similar between healthy controls and CF patients. Moreover, the relative vasoconstriction in response to sympathetic activation was also similar between groups. We conclude that ATP derived from the deformation of erythrocytes may not be obligatory to exercise hyperemia in humans.

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Fig 1.

Experimental time line. After resting measurements were obtained, subjects completed 7 min of exercise at 5, 10, and 15% workloads (21 min total). Subjects performed a 2-min cold pressor test at rest and during the last 2 min of each workload. A control exercise protocol was identical, except for cold pressor tests. Hemodynamic values were obtained at *minutes 4 and 6* during each workload (arrows).

Fig 2. Blood flow response to forearm exercise in healthy controls and patients with cystic fibrosis (CF). Values are means $\pm$ SE. *A*: healthy controls and CF patients displayed similar forearm blood flow at rest and each level of rhythmic exercise ($P = 0.91$). *B*: similar results were obtained for forearm vascular conductance ($P = 0.82$).

Fig 3.

Vasoconstrictor responses to cold pressor tests at rest and during forearm exercise in controls and CF patients. Values are means $\pm$ SE. Controls and CF patients displayed similar relative reductions in forearm vascular conductance (FVC) at rest and at each level of rhythmic exercise ($P = 0.22$).

Table 1

Subject characteristics

Variable	Control	CF Patient	<i>P</i>
Gender	4 M/5 F	7 M/3 F	0.4
Age, yr	23±1	25±2	0.2
Height, cm	174±3	172±3	0.5
Weight, kg	77±5	69±2	0.1
Forearm volume, ml	1,013±96	979±57	0.8
BMI, kg/m ²	25±1	23±1	0.1
MVC, kg	47±4	45±2	0.6
Workload, kg			
5%	2.0±0.2	2.0±0.2	0.9
10%	3.9±0.4	3.9±0.4	0.8
15%	5.8±0.6	5.8±0.6	0.9

Values are means ± SE. Healthy control subjects and patients with ceptic fibrosis (CF) displayed similar age, physical characteristics, and workload at 5, 10, and 15% of maximum handgrip contraction. M, male; F, female; MVC, maximal voluntary contraction; BMI, body mass index. *P* values denote unpaired *t*-tests.

Table 2

Hemodynamic responses to exercise and cold pressor tests

	Baseline		Exercise			
	Rest	CPT	5%	CPT	10%	CPT
MAP, mmHg						
Control [†]	87±4	93±3	89±2	95±3	90±2	94±3
CF [†]	96±4	103±6	97±4	105±5	99±5	103±5
HR, beats/min						
Control [†]	59±6	62±5	55±4	66±6	56±4	65±5
CF ^{†,‡}	73±5	77±4	74±5	78±4	75±4	79±4
FBF, ml/min						
Control [*]	44±6	43±6	89±17	83±15	144±27	130±27
CF [*]	55±9	40±3	106±13	92±9	161±25	152±17
FVC, units						
Control ^{*,†}	50±7	46±7	100±20	88±17	162±30	139±30
CF ^{*,†}	59±312	40±3	112±15	87±9	165±27	147±18

Values are mean ± SE. Control subjects and CF patients displayed similar responses to exercise (5, 10, and 15% of maximum handgrip contraction) and cold pressor tests. MAP, mean arterial pressure; HR, heart rate; FBF, forearm blood flow; FVC forearm vascular conductance, CPT, cold pressor test.

* Main effect of exercise;

[†] main effect of cold pressor test;

[‡] main effect between groups ($P < 0.05$).