Effects of Pentoxifylline on Exercising Skeletal Muscle Vascular Control in Rats with Chronic Heart Failure

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Abstract: *Purpose*: Chronic heart failure (CHF) is hallmarked by cardiac and peripheral vasculature dysfunction which has been associated with elevations in tumor necrosis factor-α (TNF-α) and exercise intolerance. The pharmaceutical TNF-α synthesis suppressor pentoxifylline (PTX) reduces plasma [TNF-α] and improves left ventricular (LV) function in CHF rats, but the effects of PTX on skeletal muscle blood flow (BF) and vascular conductance (VC) during exercise are unknown. We tested the hypothesis that PTX would elevate skeletal muscle BF and VC at rest and during submaximal treadmill exercise in CHF rats and improve exercise tolerance and peak O_2 uptake.

Methods: CHF rats (coronary artery ligation) received i.p. injections of 30 $mg \cdot kg^{-1} \cdot day^{-1}$of PTX (CHF+PTX, n=13) or saline (CHF, n=8) for 21 days. BF was measured using radiolabeled microspheres at rest and during exercise (treadmill, 20 m/min^{-1}, 5% grade).

Results: Resting and exercising mean arterial pressures (MAP) were greater in CHF+PTX compared to CHF (i.e., closer to expected healthy values, both $p<0.05$). During exercise PTX increased total hindlimb BF (CHF: 83±9, CHF+PTX: 114±8 $ml \cdot min^{-1} \cdot 100g^{-1}$, $p<0.05$) and VC (CHF: 0.75±0.08, CHF+PTX: 0.88±0.06 $ml \cdot min^{-1} \cdot 100g^{-1} \cdot mmHg^{-1}$, $p<0.05$). Furthermore, exercising BF was increased in 21 and VC in 11, of the 28 individual hindlimb muscles or muscle parts with no apparent fiber-type specificity.

Conclusions: PTX administration augments skeletal muscle BF and VC during locomotory exercise in CHF rats, but the lack of increased exercise tolerance or peak O_2 uptake suggest continued peripheral O_2 pathway dysfunction.

Keywords: Cytokine, oxygen, vascular blood flow, myocardial infarction.

INTRODUCTION

Chronic heart failure (CHF) is a multifaceted clinical disorder hallmarked by left ventricular (LV) dysfunction and a reduced cardiac output. Reductions in cardiac output prompt an exaggeration in sympathetic nervous system activity, which initially attempts to maintain arterial blood pressure but precipitates progressive multiple-organ system dysfunction and impaired peripheral oxygen (O_2) transport [1]. Specifically, CHF results in enhanced sympathetically-mediated vasoconstriction and reduced peripheral vasomotor control which alters the spatial [2, 3] and temporal [4] matching of skeletal muscle O_2 delivery relative to O_2 demand during exercise. CHF-induced impairments in peripheral O_2 transport lead to exercise intolerance and a reduced quality of life [5].

One potential mediator of the impaired O_2 transport in CHF patients is elevation of the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α). TNF-α is elevated in both cachectic and non-cachectic CHF patients and plasma TNF-α concentration ([TNF-α]) correlates positively with disease severity thereby constituting a powerful prognostic indicator [6, 7]. Elevations of TNF-α in autonomic regulatory regions of the brain result in sympathoexcitation *via* alterations in superoxide and nitric oxide (NO)-mediated signaling in CHF [8]. In the heart, TNF-α up-regulates inducible NO synthase (iNOS), induces negative inotropic effects and reduces cardiac myocyte contractility [9]. In the periphery, TNF-α, in conjunction with interleukin-1 (IL-1), increases expression of adhesive molecules and associated receptors on vascular endothelial cells [10] and contributes to impaired skeletal muscle structure and function through promotion of catabolism and inhibition of contractile function [11].

Given the negative cardiovascular effects of TNF-α in CHF, the phosphodiesterase inhibitor pentoxifylline (PTX) represents a powerful therapeutic strategy based on its ability to downregulate TNF-α gene transcription and suppress TNF-α synthesis [12]. In a clinical setting, PTX administration in CHF patients improved LV ejection fraction and New York Heart Association (NYHA) functional classification due, at least in part, to reductions in plasma [TNF-α] in addition to increased exercise capacity [13]. In CHF rats, PTX administration beginning immediately following coronary artery ligation also reduces [TNF-α] and improves LV function as evidenced by reductions in LV end-diastolic pressure

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(LVEDP, 8). In addition, PTX administration lowers circulating levels of norepinephrine and epinephrine and attenuates renal sympathetic nerve activity (RSNA) [14]. However, whether PTX-induced improvements in LV function and reductions in sympathoexcitation translate to increases in blood flow (BF) within and among active skeletal muscle during exercise in CHF rats with established peripheral vascular dysfunction remains unknown. This is crucial information given that improvements in BF and O_2 delivery during exercise may improve exercise capacity and quality of life in CHF patients.

The purpose of the present investigation was to determine the effects of chronic PTX administration on active skeletal muscle BF distribution during locomotory exercise in CHF. Specifically, we tested the hypothesis that 21 days of PTX -administration in rats with CHF would elevate hindlimb skeletal muscle BF and vascular conductance (VC) during submaximal treadmill running, improve exercise tolerance, and raise peak O2 uptake ($\dot{V}O_2$ peak).

METHODS

Animals

Twenty-one male Sprague Dawley rats (body mass: 492±43 g, age: ~6 months, Charles River Laboratories, Wilmington, MA) were used in the present investigation. All rats were housed in accredited facilities and kept on a 12:12 light-dark cycle, with food and water provided ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Kansas State University and were conducted in accordance with institutional and National Institutes of Health guidelines.

Myocardial Infarction Procedure

Myocardial infarction (MI) was induced in all rats *via* left main coronary artery ligation [3]. To begin, rats were anesthetized with a 5% isoflurane (balance O_2) mixture and intubated for mechanical ventilation with a rodent respirator (Harvard Model 680; Harvard Instruments, Holliston, MA) for the length of the surgical procedure. The heart was accessed through a left thoracotomy in the fifth intercostal space. The left main coronary artery was ligated with 6-0 silk suture approximately 1-2 mm distal to the edge of the left atrium. The incision was then closed, and ampicillin (50 mg/kg i.m.) was administered to reduce risk of infection. The analgesic agents bupivacaine (1.5 mg/kg s.c.) and buprenorphine (0.01-0.05 mg/kg i.m.) were administered and isoflurane anesthesia and mechanical ventilation ceased. All rats were then extubated and monitored closely for ≥6 hours for development of cardiac arrhythmias and signs of undue distress (i.e. labored breathing, etc.) with care administered as needed. Rats were also monitored daily (i.e. appetite, weight loss/gain, gait/posture, etc.) consistent with an intensive 10-day post-operative plan carried out in conjunction with the university veterinary staff.

Intervention Protocol

21 days following the MI procedure rats were assigned randomly to either the experimental group (CHF+PTX, n=13), or control (CHF, n=8) group. The CHF+PTX group received PTX once daily for 21 days (30 mg/kg in 0.3 ml saline i.p.) whereas the CHF group received saline (0.3 ml i.p.). No healthy rat groups were utilized because: a) These would exhibit no elevated inflammatory mediators (e.g. TNF-α) that was the specific target of PTX intervention. b) Beyond providing a comparison for healthy animal mean arterial pressure (MAP), which are abundant in the scientific literature [e.g. 3, 15, 16], they would provide no mechanistic insights into the effects of PTX in CHF. c) Given “a” and “b” above and the IACUC mandate to avoid animal wastage a healthy group was not justifiable for this investigation. See *Experimental Considerations* for further discussion regarding our intervention protocol.

Performance Testing

All rats were subjected to exercise performance testing on days 19 and 20 of the 21-day intervention period (i.e. 40-41 days following MI procedure). Rats were first acclimatized to a custom-built motorized treadmill for 5 consecutive days prior to exercise tests of endurance capacity and $\dot{V}O_2$ peak, which were completed in random order on consecutive days.

The endurance capacity of each rat was measured using a progressive exercise test in which each animal ran initially at a speed of 25 m/min up a 5% grade for 15 min. Subsequently, the treadmill grade was held constant while the speed was increased incrementally by 5 m/min every 15 min until the rat was unable/unwilling to maintain pace with the treadmill belt despite manual bursts of high-pressure air aimed at the hindlimbs. At the end of each test, exhaustion was confirmed by an attenuation of the rats’ righting reflex.

Our laboratory has demonstrated previously that time-to-exhaustion with this protocol is highly accurate and reproducible within each animal [17].

$\dot{V}O_2$ peak for each rat was determined as described previously for our laboratory [17]. Briefly, each rat was placed in a custom-made metabolic chamber designed to fit into one stall on the treadmill. Standard techniques originally described by Brooks and White [18] were used for determining $\dot{V}O_2$ and carbon dioxide (CO_2) production ($\dot{V}CO_2$). Gas analysis measurements were made in real-time *via* online CO_2 and O_2 analyzers (CO_2: model CD-3A; O_2: model S-3A/I; AEI Technologies, Pittsburgh, PA) set in series. The analyzers were calibrated before and after each exercise test using precision-mixed gases that spanned the expected range of gas concentrations based on previous investigations. Each rat ran initially in the metabolic chamber at a speed of 25 m/min (5% incline) for 2-3 minutes. Subsequently, the speed of the treadmill was increased to 40 m/min for an additional 2-3 minutes. Thereafter, the treadmill speed was increased progressively in a ramp-like manner every minute until the rat was unable or unwilling to keep pace with the treadmill belt (typically after ~7-8minutes of total test time). $\dot{V}O_2$ peak was recorded as the $\dot{V}O_2$ at which the rat was no longer able/willing to run. Peak $\dot{V}CO_2$ was also recorded and the respiratory exchange ratio (RER, $\dot{V}CO_2/\dot{V}O_2$) was calculated. Criteria for a successful test were the observation of a change in gait indicative of exhaustion immediately preceding the termination of the test [17], and/or no further increase in $\dot{V}O_2$ despite continued increases in treadmill speed.

Surgical Instrumentation

The final experimental protocol was initiated immediately following the 21-day intervention period (i.e. ~42 days after MI procedure). All rats were anesthetized initially with 5% isoflurane-O_2 gas mixture. The carotid artery was cannulated and a 2-French catheter-tipped pressure transducer (Millar Instruments, Houston, TX) was advanced into the LV for measurement of diastolic pressures and LV delta pressure/delta time (dp/dt). Due to technical complications LVEDP was not determined in 1 of the 13 CHF+PTX rats. Upon completion of the measurement the transducer was removed and the carotid artery was re-cannulated with a catheter (PE-10 connected to PE-50, Intra-Medic polyethylene tubing, Clay Adams Brand, Becton, Dickinson and Company, Sparks, MD) for measurement of heart rate (HR), MAP, and arterial blood sampling. A second catheter (PE-10 connected to PE-50) was placed in the caudal (tail) artery, as described previously [3]. Both catheters were tunneled subcutaneously to the dorsal aspect of the cervical region and exteriorized through a puncture wound in the skin. Incisions were closed, anesthesia was terminated, and the animal was given >1 hour to recover.

Radiolabeled Microsphere Infusion

Following the instrumentation each rat was placed on the treadmill. The tail artery catheter was connected to a 1-ml plastic syringe that was connected to a Harvard infusion/withdrawal pump (model 907, South Nattick, MA). The carotid artery catheter was connected to a pressure transducer set at the same level as the rat for continuous measurement of MAP and HR. Exercise was initiated, and the speed of the treadmill was increased progressively during the next 30 seconds to a speed of 20 m/min (5% grade, ~60% $\dot{V}O_2$ max in healthy rats; [19]). The rat then ran steadily for another 2.5 minutes. After 3 minutes of total exercise time, blood withdrawal from the tail artery catheter was initiated at a rate of 0.25 ml/min. Simultaneously, MAP and HR were measured *via* the carotid artery catheter. The carotid artery catheter was then disconnected from the pressure transducer and ~0.5-0.6 X 10^6 microspheres with 15 μm diameter (^{57}Co or ^{85}Sr, in random order, Perkin Elmer Life and Analytical Sciences, Waltham, MA) were infused into the aortic arch *via* the carotid artery catheter to determine regional BF. After the microsphere infusion a blood sample (~0.3 ml) was taken from the carotid artery catheter for measurement of blood gases, pH, hematocrit, and blood lactate concentrations ([lactate]). Subsequently (~30 seconds after the microsphere infusion), blood withdrawal from the tail artery catheter was stopped and exercise was terminated. After a >30 minute recovery period, MAP and HR were measured as the rat sat quietly on the treadmill. A second microsphere infusion (differently labeled from the first infusion) and blood sampling procedure were then performed exactly as described above during exercise to determine BFs at rest. This strategy (exercise followed by rest) minimizes the potential for blood loss to affect the exercise response and facilitates “resting” measurements that do not reflect the pre-exercise anticipatory response [20].

Determination of Morphological Characteristics, Regional BF and VC

Following the experimental protocol rats were euthanized promptly using sodium pentobarbital overdose (≥100 mg/kg infused i.a. into the carotid artery catheter). The thorax was opened and placement of the carotid artery catheter was confirmed. The lungs were excised and weighed to determine lung/body mass ratio for each animal. The heart was removed; the right ventricle (RV) was separated from the LV and septum, and both tissues were weighed and normalized to each animal's body mass. To determine LV infarct size, an incision was made through the interventricular septum, from the base to the apex of the LV, and a digital photograph of the endocardium was taken. The image was printed, and endocardial infarct surface area was determined by planimetry. Internal organs and individual muscles and muscle portions of the hindlimb were identified and excised. Upon removal, tissues were blotted, weighed, and placed immediately into counting vials.

Radioactivity of each tissue was determined using a gamma scintillation counter (Packard Auto Gamma Spectrometer, model 5230, Downers Grove, IL). Tissue BF was then calculated using the reference sample method [3] and expressed in $ml \cdot min^{-1} \cdot 100g^{-1}$. Adequate mixing of the microspheres was confirmed for each rat by demonstrating a <15% difference in BF to the right and left kidney and/or to the right and left hindlimb musculature. VC was calculated by normalizing BF to MAP and expressed as $ml \cdot min^{-1} \cdot 100g^{-1} \cdot mmHg^{-1}$

Blood Samples and Cytokine Analysis

Pre-PTX and post-PTX administration blood samples were taken from the sub-orbital plexus using a glass capillary pipette. Pre-PTX blood samples were taken prior to group assignment, and post-PTX blood samples were taken prior to catheterization on the day of the final experimental protocol. Approximately 0.8 ml of blood was drawn into heparinized sample tubes, and centrifuged at 6000 g at 4°C for 6 minutes, plasma was extracted and frozen immediately at -80°C for later analysis of plasma cytokine concentrations. Circulating [TNF-α] was quantified in the plasma by using a commercially available rat TNF-α enzyme-linked immunosorbent assay (ELISA) kit according to manufacturer instructions (Abcam, Cambridge, MA). Due to technical complications [TNF-α] in 1 of the 13 CHF+PTX rats was not determined.

Statistical Analysis

Cardiac morphological and hemodynamic measurements were compared using unpaired Student's t-tests. Plasma [TNF-α] variance was compared using a *post hoc* F-test. All other data were compared within (rest vs. exercise) and between (CHF vs. CHF+PTX) groups using mixed 2-way ANOVAs and Student-Newman-Keuls *post hoc* tests where appropriate. Significance was set at $p<0.05$ and values are expressed as mean ± SEM.

RESULTS

Effects of PTX on Plasma [TNF-α]

When pre-intervention concentrations were compared to post-intervention concentrations, plasma [TNF-α] increased significantly in the CHF but not the CHF-PTX group. Moreover, the CHF+PTX group demonstrated significantly less variance in post-intervention plasma [TNF-α] compared with CHF ($p<0.05$, Figure **1**).

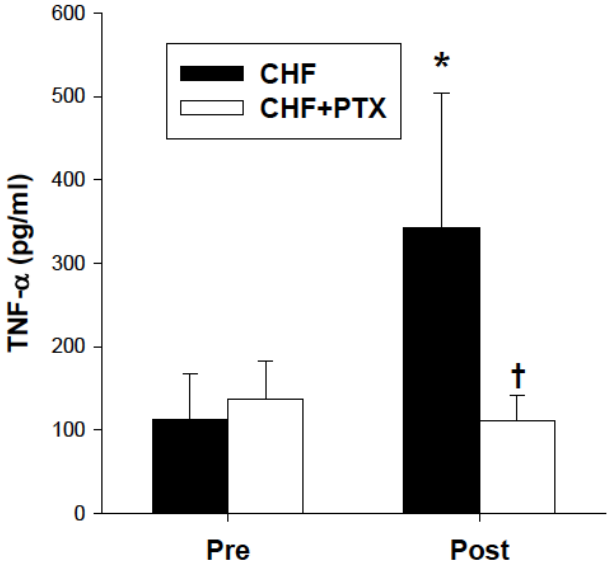

Figure 1: Effects of PTX administration on plasma [TNF-α] pre and post PTX administration. CHF: n=8; CHF+PTX: n=12 Data are mean±SEM. PTX prevented the CHF-induced increase. *p<0.05 versus pre-treatment, †F-test revealed a significant reduction in [TNF-α] variance in CHF+PTX rats.

Effects of PTX on Cardiac Indices and Exercise Performance

MI size and hemodynamic and morphological indices of cardiac function for CHF and CHF+PTX rats are presented in Table **1**. There were no differences in infarct size or RV/body mass ratio between groups. In CHF+PTX rats LVEDP and lung weight/body mass

ratio was lower compared to CHF rats. In addition, LV dp/dt was higher and LVEDP/MI size ratio was significantly lower in CHF+PTX compared with CHF ($p<0.05$, Figure **2**). There was no difference in endurance capacity between groups (CHF: 18±5, CHF+PTX: 19±2 min, $p>0.05$). $\dot{V}O_2$ peak was not different ($p>0.05$) between CHF (64±7, RER: 1.08± 0.03) and CHF+PTX (66±3 $ml \cdot kg^{-1} \cdot min^{1}$, RER: 1.02±0.02) groups.

Table 1: Morphological and Hemodynamic Characteristics of CHF and CHF+PTX Rats

	CHF	CHF+PTX
MI, %	37±4	37±3
LVEDP, mmHg	24±3	16±2*
RV/body mass, mg/g	0.77±0.08	0.72±0.04
Lung/body mass, mg/g	7.7±1.2	5.2±0.6*
LV dp/dt, mmHg/s	5663±502	6783±277*

MI, myocardial infarct size; LVEDP, left ventricular end diastolic pressure; LV dp/dt, left ventricular delta pressure/delta time. CHF: n=8; CHF+PTX: n=13 (LVEDP and LV dp/dt reflect n=12) *p<0.05 versus CHF rats.

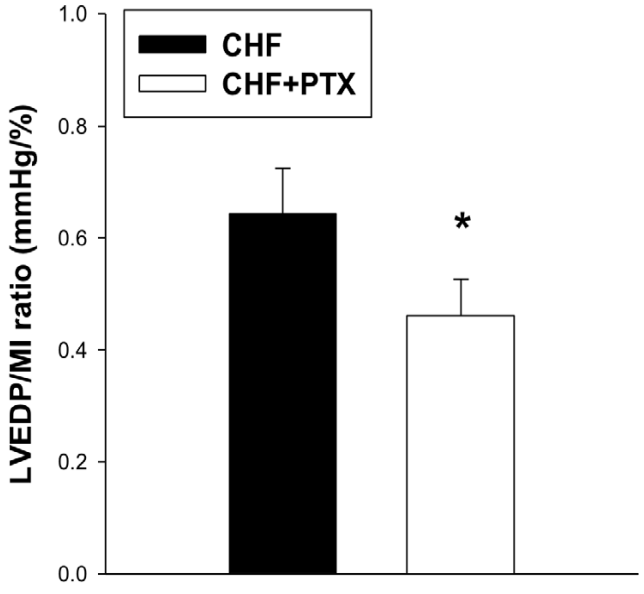

Figure 2: Effects of PTX administration on LVEDP/MI ratio. CHF: n=8; CHF+PTX n=12. Data are mean±SEM. *p<0.05 versus CHF.

Effects of PTX on HR, MAP, Arterial O_2, Saturation, and [Lactate]

The effects of PTX on resting and exercising HR and MAP are displayed in Figure **3**. CHF+PTX rats had higher MAP at rest and during exercise compared to CHF ($p<0.05$ for both). HR was not different between groups at rest but was greater in CHF+PTX compared to CHF rats during exercise ($p<0.05$). There were no differences in resting (CHF: 95±2, CHF+PTX: 97±1%, $p>0.05$) or exercising (CHF: 95±2, CHF+PTX: 96±1%, $p>0.05$) arterial O_2 saturation (and, therefore, calculated arterial [O_2]) between groups. Blood [lactate] was not different between groups at rest (CHF: 1.1±0.4, CHF+PTX: 0.9±0.1 mmol/L, $p>0.05$) or during exercise (CHF:4.5±1.5, CHF+PTX:4.7±0.7 mmol/L, $p>0.05$).

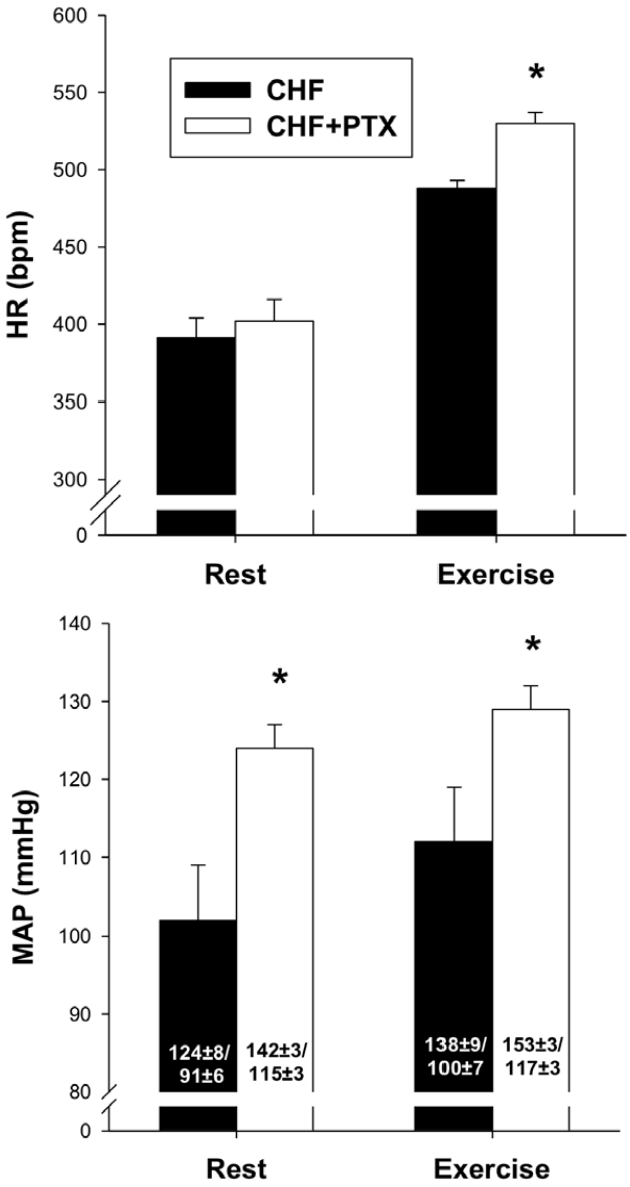

Figure 3: Effects of PTX administration on mean arterial pressure (MAP) and heart rate (HR). MAP and HR measured at rest and during submaximal treadmill exercise. Within CHF and CHF+PTX conditions exercise MAP and HR were significantly different from rest ($p<0.05$). Systolic/diastolic pressure is depicted within MAP bars and are all significantly different between CHF and CHF+PTX groups. CHF: n=8; CHF+PTX: n=13. Data are mean±SEM. *p<0.05 versus CHF.

Table 2: Effects of PTX on Resting BF (ml·min^{-1}·100 g^{-1}) and VC (ml·min^{-1}·100 g^{-1}·mmHg^{-1}) to the Individual Muscles or Muscle Parts of the Rat Hindlimb

	BF		VC	
	CHF	CHF+PTX	CHF	CHF+PTX
Ankle extensors				
Soleus (9%)	72±14	111±19	0.72±0.17	0.92±0.17
Plantaris (80%)	13±4	11±2	0.12±0.04	0.09±0.01
Gastrocnemius, red (14%)	21±6	41±10	0.22±0.07	0.34±0.09
Gastrocnemius, white (100%)	9±2	9±1	0.08±0.02	0.07±0.01
Gastrocnemius, mixed (91%)	8±2	13±2	0.08±0.02	0.11±0.02
Tibialis posterior (73%)	11±2	14±2	0.11±0.02	0.12±0.01
Flexor digitorum longus (68%)	12±2	19±3	0.12±0.02	0.15±0.03
Flexor halicus longus (71%)	9±1	10±1	0.09±0.03	0.08±0.01
Ankle flexors				
Tibialis anterior, red (63%)	22±11	24±6	0.19±0.10	0.20±0.05
Tibialis anterior, white (80%)	13±5	14±2	0.12±0.05	0.11±0.01
Extensor digitorum longus (76%)	10±2	10±1	0.10±0.02	0.08±0.01
Peroneals (67%)	12±2	15±2	0.12±0.02	0.13±0.02
Knee extensors				
Vastus intermedius (4%)	46±11	82±15	0.47±0.12	0.66±0.11
Vastus medialis (82%)	12±3	17±2	0.12±0.03	0.14±0.02
Vastus lateralis, red (35%)	27±12	43±8	0.26±0.11	0.35±0.06
Vastus lateralis, white (100%)	10±3	10±1	0.10±0.03	0.08±0.01
Vastus lateralis, mixed (89%)	12±4	13±1	0.11±0.03	0.11±0.01
Rectus femoris, red (66%)	17±6	24±5	0.15±0.06	0.18±0.04
Rectus femoris, white (100%)	10±2	13±2	0.09±0.02	0.10±0.01
Knee flexors				
Biceps femoris anterior (100%)	6±1	8±1	0.06±0.01	0.06±0.01
Biceps femoris posterior (92%)	7±2	10±2	0.07±0.02	0.08±0.02
Semitendinosus (83%)	11±3	15±3	0.10±0.03	0.12±0.02
Semimembranosus, red (72%)	11±3	12±3	0.11±0.03	0.10±0.02
Semimembranosus, white (100%)	9±2	8±1	0.09±0.02	0.07±0.01
Hip adductors				
Adductor longus (5%)	106±1	135±12	0.99±0.17	1.09±0.10
Adductor magnus & brevis (89%)	11±3	12±2	0.10±0.03	0.10±0.02
Gracilis (77%)	11±3	16±3	0.11±0.03	0.13±0.03
Pectineus (69%)	18±4	25±3	0.15±0.04	0.20±0.03

Data are mean±SEM. Values in parentheses indicate percentage type IIb + d/x according to Delp & Duan (1996). CHF: n=8; CHF+PTX: n=13.

Effects of PTX on Hindlimb BF and VC at Rest and During Exercise

There were no differences in resting total hindlimb BF or VC between CHF and CHF+PTX rats (Figure **4**). Moreover, there were no differences in resting BF or VC to any individual hindlimb muscles or muscle parts between groups (Table **2**). In contrast, exercising total hindlimb skeletal muscle BF and VC were significantly greater in CHF+PTX compared to CHF rats (Figure **4**). Specifically, exercising BF was greater in 21 of 28 and VC was greater in 11 of 28 individual hindlimb muscles or muscle parts in CHF+PTX compared to CHF rats with no apparent muscle fiber-type composition or function specificity (Table **3**).

Effect of PTX on Renal and Splanchnic BF and VC at Rest and During Exercise

Renal and splanchnic BF and VC values are presented in Table **4**. BF to the adrenals, spleen, and large intestine were greater at rest in CHF+PTX rats compared to CHF ($p<0.05$) whereas only resting adrenal and splenic VC were greater in

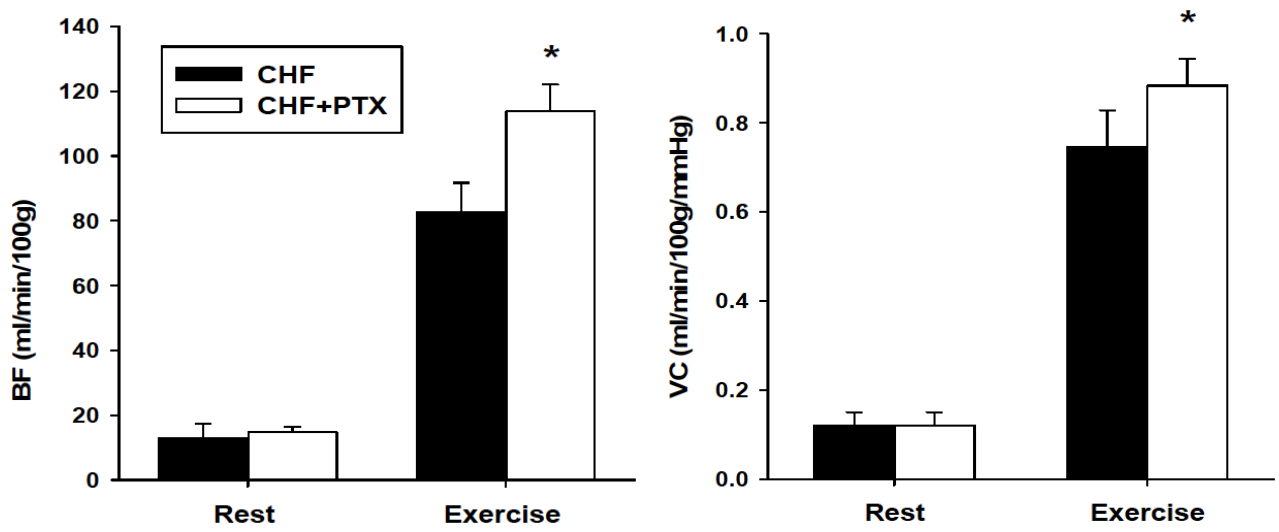

Figure 4: Total hindlimb muscle blood flow (BF) and vascular conductance (VC) at rest and during exercise for CHF and CHF+PTX rats. Within CHF and CHF+PTX groups exercising BF and VC were significantly different ($p<0.05$) from rest. CHF: n=8; CHF+PTX: n=13. Data are mean±SEM. *$p<0.05$ versus CHF.

Table 3: Exercising Individual Hindlimb Skeletal Muscle and Muscle Part BF ($ml \cdot min^{-1} \cdot 100g^{-1}$) and VC ($ml \cdot min^{-1} \cdot 100g^{-1} \cdot mmHg^{-1}$) for CHF and CHF+PTX Groups

	BF		VC	
	CHF	CHF+PTX	CHF	CHF+PTX
Ankle extensors				
Soleus (9%)	257±25	258±20	2.33±0.24	2.00±0.15
Plantaris (80%)	148±23	209±17*	1.36±0.21	1.62±0.12
Gastrocnemius, red (14%)	298±22	361±56	2.69±0.20	2.79±0.41
Gastrocnemius, white (100%)	36±10	45±4	0.36±0.12	0.35±0.03
Gastrocnemius, mixed (91%)	118±10	156±14*	1.08±0.11	2.21±0.10
Tibialis posterior (73%)	95±17	114±19	0.89±0.19	0.89±0.14
Flexor digitorum longus (68%)	54±17	108±19*	0.54±0.19	0.85±0.15*
Flexor halicus longus (71%)	50±10	88±7*	0.47±0.10	0.68±0.05*
Ankle flexors				
Tibialis anterior, red (63%)	251±29	304±22*	2.27±0.27	2.36±0.17
Tibialis anterior, white (80%)	81±12	112±7*	0.72±0.08	0.87±0.05*
Extensor digitorum longus (76%)	38±6	62±6*	0.36±0.06	0.48±0.05*
Peroneals (67%)	101±11	141±15*	0.91±0.09	1.09±0.11
Knee extensors				
Vastus intermedius (4%)	301±23	353±25*	2.71±0.22	2.76±0.21
Vastus medialis (82%)	138±20	182±15*	1.24±0.19	1.41±0.11
Vastus lateralis, red (35%)	238±32	361±29*	2.13±0.26	2.80±0.23*
Vastus lateralis, white (100%)	13±3	23±2*	0.12±0.03	0.18±0.02*
Vastus lateralis, mixed (89%)	116±12	141±12*	1.04±0.12	1.10±0.11
Rectus femoris, red (66%)	220±24	247±18	1.96±0.20	1.93±0.14
Rectus femoris, white (100%)	91±10	98±6	0.82±0.09	0.78±0.05

(Table 3). Continued.

	BF		VC	
	CHF	**CHF+PTX**	**CHF**	**CHF+PTX**
Knee flexors				
Biceps femoris, anterior (100%)	15±3	28±4*	0.14±0.03	0.22±0.03*
Biceps femoris, posterior (92%)	67±9	83±7*	0.60±0.08	0.65±0.05
Semitendinosus (83%)	33±6	60±5*	0.30±0.05	0.47±0.04*
Semimembranosus, red (72%)	92±16	126±16*	0.84±0.15	0.98±0.12
Semimembranosus, white (100%)	16±3	32±5*	0.14±0.03	0.25±0.04*
Hip adductors				
Adductor longus (5%)	254±40	297±27	2.31±0.36	2.29±0.19
Adductor magnus & brevis (89%)	64±13	92±11*	0.57±0.11	0.71±0.08
Gracilis (77%)	21±5	57±8*	0.15±0.05	0.45±0.06*
Pectineus (69%)	27±7	59±9*	0.25±0.07	0.46±0.07*

Data are mean±SEM. Values in parentheses indicate percentage type IIb + d/x according to Delp & Duan (1996). CHF: n=8; CHF+PTX: n=13. *p<0.05 versus CHF. Within CHF 24, and within CHF+PTX 28, of 28 hindlimb muscles and muscle parts demonstrated elevated exercising BF and VC above rest (p<0.05) (exceptions: gracilis, pectineus, white portions of the semimembranosus and vastus lateralis for CHF group).

Table 4: Renal and Splanchnic Organ BF ($ml \cdot min^{-1} \cdot 100g^{-1}$) and VC ($ml \cdot min^{-1} \cdot 100g^{-1} \cdot mmHg^{-1}$) at Rest and During Exercise for CHF and CHF+PTX Groups

	At rest				During exercise			
	BF		VC		BF		VC	
	CHF	**CHF+PTX**	**CHF**	**CHF+PTX**	**CHF**	**CHF+PTX**	**CHF**	**CHF+PTX**
Right kidney	542±30	576±38	5.26±0.36	4.68±0.33	379±56	350±48	2.87±0.42	2.67±0.35
Left kidney	534±32	556±33	5.19±0.35	4.53±0.30	373±61	341±48	2.72±0.42	2.59±0.34
Stomach	108±18	139±20	0.89±0.16	1.14±0.18	53±12	61±9	0.35±0.08	0.46±0.06
Adrenals	431±59	683±62*	4.04±0.72	5.52±0.51*	267±52	388±50	1.91±0.36	2.97±0.36
Spleen	254±46	398±51*	2.09±0.23	3.19±0.39*	62±16	78±18	0.43±0.13	0.59±0.13
Pancreas	139±21	166±25	1.19±0.19	1.35±0.19	93±26	113±23	0.75±0.18	0.86±0.16
Sm. intestine	377±60	460±41	3.11±0.57	3.77±0.37	220±42	238±24	1.50±0.30	1.83±0.17
Lg. intestine	168±25	223±24*	1.41±0.16	1.82±0.20	112±31	120±19	0.75±0.20	0.92±0.14
Liver[1]	16±3	27±7	0.16±0.04	0.22±0.05	14±3	25±5	0.10±0.02	0.19±0.04

Data are mean±SEM. [1]Denotes arterial not portal blood flow. CHF: n=8; CHF+PTX: n=13. *p<0.05 versus CHF.

CHF+PTX rats (p<0.05). There were no differences in renal or splanchnic organ BF and VC during exercise between groups (p>0.05).

DISCUSSION

The principal novel finding of the present investigation is that PTX administration (30 $mg \cdot kg^{-1} \cdot day^{-1}$ for 21 days) in CHF+PTX rats elevated hindlimb skeletal muscle BF and, therefore, O_2 delivery during submaximal treadmill exercise compared to CHF. The augmented O_2 delivery was associated with enhanced cardiac function, increased VC, and elevated systemic driving pressure for bulk O_2 delivery (i.e. increased MAP towards expected healthy control values e.g. [3, 15, 16]). However, despite the significant cardiovascular improvements PTX administration had no effects on treadmill exercise endurance capacity or $\dot{V}O_2$ peak.

Mechanisms of PTX-Induced BF Elevations

Whole-body exercise prompts sympathetically-mediated increases in cardiac output and peripheral BF redistribution toward working skeletal muscle. Within active skeletal muscle vascular beds BF increases *via* mechanical, humoral, and metabolic vasomotor control signals. CHF patients and animals evidence lower

skeletal muscle BF and O_2 delivery for a given level of exercise compared to healthy subjects consequent to lower cardiac output, exaggerated sympathetic vasoconstriction and impaired vascular endothelial function [1]. Importantly, elevations in TNF-α have been implicated, in part, as one mechanism underlying each of the above-mentioned perturbations [10, 21] and it has been shown that plasma [TNF-α] correlates positively with CHF severity [6].

Previous studies have shown that TNF-α administration in cancer patients can induce cardiac enlargement and pulmonary congestion, as well as reduce myocardial contractility, leading to cardiomyopathy [22, 23]. In the present investigation blockade of TNF-α production in CHF+PTX rats with the phosphodiesterase inhibitor PTX increased cardiac contractility (i.e. increased LV dp/dt) and lowered LVEDP and the lung/body mass ratio (suggesting less pulmonary congestion consequent to improvement in LV contractile function). These marked improvements in cardiac function were evident despite similar MI size between the CHF and CHF+PTX group and are consistent with previous investigations [8, 13, 24]. Importantly, CHF-induced cardiac dysfunction, particularly during exercise, challenges the ability of the cardiovascular system to regulate MAP. In this regard, the greater resting and exercising MAP values reported presently following PTX administration are similar to those observed in healthy rats [15, 25] and likely reflect improvements in cardiac function and preserved pressure regulation rather than a direct hypertensive vasoconstrictive effect of PTX on the peripheral vasculature. Moreover, the higher exercising HR value within PTX rats herein is suggestive of the reversal of the chronotropic incompetence associated with CHF. This reversal has also been achieved through exercise training [16].

PTX administration in CHF+PTX rats increased exercising hindlimb skeletal muscle BF (↑38%) and, therefore, O_2 delivery compared to CHF. This increase is due potentially to a higher cardiac output (as opposed to peripheral redistribution, as suggested by similar or increased kidney and splanchnic organ BFs in CHF+PTX versus CHF rats), elevated systemic driving pressure for bulk O_2 delivery (i.e. higher MAP), as well as modified peripheral vascular control (i.e. 18% higher VC). In regards to the latter, TNF-α participates in functional derangement of endothelial cells, that includes activation of iNOS [10], formation of adhesive molecules [10, 26], and decreased endothelial NO synthase (eNOS) messenger RNA (mRNA) due to its increased rate of degradation [27]. Stosic *et al.* [28] demonstrated that PTX administration results in reduced local iNOS expression in intra-islet and endothelial cells. This suggests that PTX administration could be increasing exercising BF through reductions in iNOS, thus lessening the systemic inflammatory response, and enhancing local NO-mediated vasodilation *via* increased eNOS expression and/or function. Moreover, PTX administration reduces RSNA [8, 24], and while there is no evidence suggesting that it was reduced in the present investigation (unchanged BF or VC to renal or splanchnic organs), it is possible that there may have been an effect of PTX on lumbar sympathetic nerve activity (LSNA), given that RSNA and LSNA are controlled independently [29]. This is consistent with the notion that PTX administration may be exerting a central effect, whereby BF is increased due to less sympathetically-meditated vasoconstriction.

Further investigation into the effects of PTX on NO bioavailability, reactive O_2 species (ROS) regulation, and sympathetic nerve activity during exercise may elucidate the specific mechanisms of PTX-induced BF elevations in CHF+PTX rats evident in the present investigation. Given the lack of muscle fiber-type specific exercising BF and VC increases found herein (Table **3**) and the fiber-type specificity of vasomotor signals including NO [30], sympathetic vasoconstriction [31], and functional sympatholysis [32], it is plausible that PTX modified multiple vascular control pathways. PTX may also impact peripheral vasodilation *via* elevations in prostacyclin formation [33] and/or intracellular concentrations of the second messenger cyclic adenosine monophosphate [34]. Aside from the neurohumoral component, PTX has also been acknowledged for its ability to increase erythrocyte deformability [35, 36] which may improve capillary hemodynamics and thus tissue O_2 delivery. This latter effect remains to be demonstrated in CHF.

Clinical Implications

In the present investigation PTX administration increased hindlimb skeletal muscle BF and VC but did not change exercise endurance capacity or $\dot{V}O_2$ peak between groups. The exercise performance values reported presently are consistent with severe CHF [37]. However, the improved indices of cardiac function following PTX administration in the CHF+PTX group more closely resemble moderate CHF [38]. This may reflect a delayed improvement in exercise tolerance despite augmented O_2 delivery similar to the delayed

reversal of impaired vasodilation seen in CHF patients post-heart transplantation [39]. However, the present data are also consistent with the fact that PTX administration improves LV function but not exercise performance in CHF patients [13, 40, 41] and suggests that PTX may not improve the ability of the active skeletal muscle to utilize the increased O_2 delivery during exercise. For example, alterations in capillary geometry [42], fewer capillaries supporting red blood cell flux at rest and during contractions [4, 43], a reduced capillary-muscle fiber ratio [42, 44], and altered microvascular O_2 pressure kinetics [38] as well as intrinsic skeletal muscle abnormalities including lower oxidative enzyme activity [45, 46], mitochondrial dysfunction [44] and impaired excitation-contractile function [37, 47-49] have been reported in CHF. The absence of PTX-induced improvements in any of these mechanisms may potentially explain the lack of improvements in exercise performance despite higher skeletal muscle BF in CHF+PTX rats found herein. For this reason, further investigation into the effects of PTX on capillary hemodynamics and microvascular O_2 pressure kinetics during muscle contraction, as well as PTX administration combined with therapies that may ameliorate structural and/or functional vascular and metabolic derangements (i.e. exercise training or nitrate supplementation [15, 50, 51, 52]), may prove most efficacious in improving exercise tolerance and quality of life in CHF patients.

Experimental Considerations

In the present investigation PTX administration reduced [TNF-α] variance in CHF+PTX rats and resulted in close-to-significant reductions in [TNF-α] (~70%↓, p=0.052) compared with CHF. Guggilam *et al.* [8, 23] reported lower tissue-bound TNF-α expression in the LV and paraventricular nucleus as well as circulating [TNF-α] following PTX treatment when administration began immediately following the MI procedure. It is important to note that our investigation differed from Guggilam *et al.* [8, 23] in that the present sample size was smaller, blood sampling was done at rest not in response to an endotoxin or stress stimulus, and 21 days were allowed (post-MI procedure) for development of CHF and completion of myocardial remodeling [53] prior to the PTX intervention. This 21-day time period may account for initial elevations in plasma [TNF-α] seen in pre-PTX blood sampling [24]. Furthermore, post-PTX plasma [TNF-α] values in CHF+PTX rats resembled closely pre-PTX administration values. This contrasts markedly with the CHF rats in which post-control intervention plasma [TNF-α] is ~2.5x greater and substantially more variable when compared to the pre-control intervention [TNF-α] measurement. Importantly, our present data is consistent with the complex nature of the TNF-α system previously described by Ferrari, Bachetti [54] as well as several studies where PTX administration in CHF patients did not reduce circulating [TNF-α] [40, 41]. The complexity of TNF-α bioassays and the effects of soluble TNF-α receptors (sTNF-Rs) on TNF-α activity as well as the conditions of our sampling are potential explanations for [TNF-α] variability found herein. The present PTX dose (30 $mg \cdot kg^{-1} \cdot day^{-1}$) as well as its administration route (i.p.) was chosen because of the successful results (i.e. reductions in RSNA, ROS, [TNF-α], and improved LV function) seen both centrally and peripherally by Guggilam *et al*. [8, 23]. Although those authors administered PTX for a 5 week period, we selected a relatively shorter 21-day intervention period in order to minimize weight gain (which hampers the treadmill running performance of the rat). In this regard, it is noteworthy that we were able to see important beneficial cardiovascular effects of the drug after only a 21-day administration protocol.

SUMMARY AND CONCLUSION

The present study is the first to investigate the effects of PTX administration on skeletal muscle BF and VC at rest and during submaximal treadmill exercise in CHF rats. PTX administration (30 $mg \cdot kg^{-1} \cdot day^{-1}$ for 21 days) improved LV function and elevated skeletal muscle BF and VC in CHF rats. These improvements were associated with PTX-induced elevations in MAP (i.e. to values similar to those observed in healthy rats) and reductions in plasma [TNF-α] variance compared with CHF. The augmented BF and VC reflect elevations in O_2 delivery which likely result in an improved ability to match skeletal muscle O_2 delivery with $\dot{V}O_2$, potentially raising microvascular and intracellular O_2 pressures and likely enhancing metabolic function. Although the specific mechanisms responsible for the increases in skeletal muscle BF and VC remain unclear, the improvements in both central cardiac and peripheral vasculature function have crucial implications for CHF patients. Specifically, they may potentiate the beneficial effects of therapies known to improve exercise tolerance and skeletal muscle BF (i.e. nitrate supplementation [15, 55]). While PTX administration did not evoke improvements in exercise tolerance or $\dot{V}O_2$ peak, enhanced metabolic function would be expected to

help patients perform exercise rehabilitation and improve post-exercise recovery. It is also possible that it is necessary to utilize PTX therapy beyond the 21-day intervention utilized herein to see improved exercise tolerance and $\dot{V}O_2$ peak.

WHAT IS THE CENTRAL QUESTION OF THIS STUDY?

The pro-inflammatory cytokine TNF-α has been implicated in the cardiac and peripheral vasculature dysfunction evident in chronic heart failure (CHF). We examined whether the pharmacological TNF-α synthesis suppressor pentoxifylline (PTX) could augment locomotor skeletal muscle blood flow during treadmill exercise in CHF rats.

WHAT IS THE MAIN FINDING AND ITS IMPORTANCE?

21 days of PTX administration elevated locomotor hindlimb skeletal muscle blood flow and vascular conductance in CHF rats compared to CHF rats that received a control intervention. Given the prevalence of impaired muscle BF responses to exercise in CHF, the present data carry important therapeutic implications for CHF patients.

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ABBREVIATIONS

ANOVA = analysis of variance

BF = blood flow

CHF = chronic heart failure

CO_2 = carbon dioxide

ELISA = enzyme-linked immunosorbent assay

eNOS = endothelial nitric oxide synthase

IL-1 = interleukin-1

iNOS = inducible nitric oxide synthase

LSNA = lumbar sympathetic nerve activity

LV = left ventricle

LV dp/dt = left ventricle diastolic pressure/diastolic time

LVEDP = left ventricular end diastolic pressure

MAP = mean arterial pressure

MI = myocardial infarction

mRNA = messenger ribonucleic acid

NO = nitric oxide

NYHA = New York Heart Association

O_2 = oxygen

PTX = pentoxifylline

RER = respiratory exchange ratio

ROS = reactive oxygen species

RSNA = renal sympathetic nerve activity

RV = right ventricle

sTNF-Rs = soluble tumor necrosis factor alpha receptors

TNF-α = tumor necrosis factor alpha

VC = vascular conductance

$\dot{V}CO_2$ = carbon dioxide production

$\dot{V}O_2$ peak = peak oxygen uptake

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