THE INFLUENCE OF RESPIRATORY MUSCLE FATIGUE ON INACTIVE LIMB BLOOD FLOW DURING CYCLING EXERCISE

by

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Abstract

An increased work of breathing during heavy whole body exercise can lead to respiratory muscle fatigue (RMF) and decreased leg blood flow. Heavy exercise also increases inactive limb and cutaneous blood flow. It is not known, however, how RMF affects inactive limb and cutaneous blood flow. Therefore, we tested the hypothesis that RMF during heavy exercise would reduce: 1) inactive limb blood flow, 2) inactive limb vascular conductance, and 3) inactive limb cutaneous blood flow. Twelve healthy men (23 ± 2 yrs) completed baseline pulmonary function tests followed by an incremental cycle test to VO_{2max}. Subjects then cycled at both 70% and 85%VO_{2max} (randomized) for 20 minutes. Subjects performed a second 85%VO_{2max} test ingesting N-acetylcysteine (NAC) (1800mg), which has been reported to reduce RMF, 45 minutes prior the test. Maximum inspiratory pressures (P_{Imax}) were measured prior to and immediately following each exercise trial to determine RMF. During exercise, brachial artery blood flow (BABF) was measured via Doppler ultrasound and arm cutaneous blood flow was assessed by laser-Doppler flowmetry. Cutaneous vascular conductance (CVC) was calculated as flux/mean arterial pressure and scaled as % maximal CVC (sites heated to 46^{o}C). Mean arterial pressure (MAP) was measured manually. Significant RMF occurred with 85%VO_{2max} (12.8 ± 9.8%), but not with 70%VO_{2max} ($p>0.05$). BABF significantly increased from baseline to end exercise in both conditions and was significantly lower (~18%) following the 85%VO_{2max} test. The amount of RMF at 85%VO_{2max} was inversely related to the change in BABF ($r= -0.66$, $p<0.05$). BA vascular conductance was significantly higher at end exercise at 70%VO_{2max} compared to 85%VO_{2max} (2.60 ± 0.73 vs. 2.00 ± 0.42 $mLmin^{-1}mmHg^{-1}$, resp.). The amount of RMF at 85%VO_{2max} was inversely related to BA vascular conductance at end exercise ($r= -0.80$, $p<0.05$). Cutaneous vascular conductance was not different ($p>0.05$) between trials. With NAC,

RMF was reduced and BABF was consequently significantly higher (~30%) compared to 85%VO_{2max}. These data suggest that RMF during heavy whole body exercise decreases inactive arm blood flow and vascular conductance, but not cutaneous blood flow.

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I. Introduction

The pulmonary system has been reported to limit exercise tolerance in some healthy subjects. Specifically when the work of breathing is increased during heavy whole body exercise (>85%VO_{2max}), locomotor blood flow is reduced, peripheral muscles fatigue, and respiratory muscle fatigue increases. Respiratory muscle fatigue initiates an increased sympathetic outflow, reducing locomotor blood flow and vascular conductance, potentially increasing respiratory muscle blood flow. The redistribution of blood flow from the locomotor muscles to the respiratory muscles may reduce exercise tolerance.

During lower limb exercise (e.g. cycling), inactive brachial artery and cutaneous blood flow increase in proportion to exercise intensity approximately 2-4 fold during incremental and steady state exercise due to increased thermoregulatory requirements. The increase in inactive brachial artery and cutaneous blood flow typically shows a biphasic response. At the onset of cycling exercise, there is a reduction or no change in brachial artery blood flow for five minutes due to increased sympathetic vasoconstriction. With continued steady state exercise, inactive brachial artery blood flow and vascular conductance increases partly due to increased vessel diameter via shear stress. Cutaneous blood flow increases to approximately 50-60% of maximal due to reduced sympathetic vasoconstriction and increased active vasodilation. It is currently unknown if respiratory muscle fatigue during heavy whole body exercise will reduce inactive limb and cutaneous blood flow.

II. Literature Review

Respiratory Muscles

The respiratory muscles (RM) include sternomastoids, scalenes, trapezius, and external intercostals and the diaphragm, the primary RM. In humans, the diaphragm is composed of mostly oxidative fibers, approximately 55% Type I and 21% Type IIa (53). The diaphragmatic muscle fibers exhibit marked differences compared to other skeletal muscle, including greater oxidative capacity, capillary density, maximum blood flow, and resistance to fatigue (24).

At rest, the diaphragm is the main contributor of ventilation during normal breathing (110). During diaphragmatic contraction, this dome-shaped muscle pulls downward, which increases chest cavity volume and decreases intra-thoracic pressure resulting in increased lung volume. During exhalation, the diaphragm relaxes and the chest wall returns to its resting position due to elastic recoil. During exercise, the diaphragm is the main contributor of total ventilation, but the inspiratory and expiratory accessory muscles are also recruited. The recruitment of these respiratory accessory muscles increases the mechanical work of breathing due to chest wall distortion (27, 32). From rest through moderately heavy exercise, the respiratory muscles are fatigue resistant (77). However during high intensity sustained whole body exercise, the diaphragm, inspiratory, and expiratory muscles have been reported to fatigue (77).

Respiratory Muscle Fatigue

Diaphragm Fatigue

Muscle fatigue is defined as "a condition in which there is a loss in the capacity for developing force and/or velocity of a muscle, resulting from muscle activity under load which is reversible by rest" (63). Bilateral phrenic nerve stimulation (BPNS) is the preferred technique

used to assess diaphragmatic fatigue via transdiaphragmatic pressure (Pdi) from esophageal and gastric balloons before and following heavy exercise (5-8, 44, 57, 104-106). With BPNS, Babcock et al. (1995) reported the greatest diaphragmatic fatigue (26% decrease in Pdi) when subjects exercised to exhaustion at 85-90%VO_{2max} (5). This degree of diaphragmatic fatigue at this exercise intensity is in agreement with subsequent studies of Babcock et al. (6-8) and others (104); however, some investigators (44, 57) have reported slightly less fatigue (17-21%). When subjects exercised for only five minutes at 80-90% VO_{2max}, diaphragmatic fatigue was considerably less (9-20%), likely due to the shorter duration of exercise (105, 106). Additionally, Mueller and sniff maneuvers have been used to determine Pdi and found a reduction in Pdi of 28% after maximal exercise (33). Johnson et al. (1993) observed a positive relationship between exercise intensity (% VO_{2max}) and diaphragmatic fatigue (44). However, Perret et al. (2000) tested this relationship with RM fatigue and the reductions in RM fatigue were not related to intensity. The discrepancy between the results of these two studies may be due to accessory inspiratory muscle fatigue due to inspiratory resistive breathing and measuring global RM fatigue by Perret et al. (2000) (68).

During heavy exercise, the diaphragm's contribution to total inspiratory muscle force production gradually decreases (44). Despite the reduced diaphragmatic contribution, ventilation continues to rise during sustained heavy exercise due to the increased contribution of the external intercostals and accessory respiratory muscles to the ventilatory work. The recruitment of these accessory muscles likely increases the mechanical work of breathing due to chest wall distortion (27, 32) and likely contributes to RM fatigue. The contribution of work of breathing to RM fatigue was demonstrated by reducing the work of breathing via proportional assist ventilator (50% of control) during heavy exercise and finding that RM fatigue was not present (7).

Because of the recruitment and possible consequences (chest wall distortion) of accessory RM during exercise, an increased significance has been placed on the fatigability of the inspiratory (not solely the diaphragm) and expiratory muscles during heavy exercise.

Inspiratory and Expiratory Muscle Fatigue

Maximum inspiratory and expiratory pressures measured at the mouth (P_{Imax}, P_{Emax}) have been widely used to estimate the total strength of the inspiratory and expiratory muscles (i.e. diaphragm and the accessory muscles) (54). With this method, the inspiratory muscles have been reported to fatigue approximately 10-18% (decrease in P_{Imax}) following heavy exercise (80-85%VO_{2max}) (23, 50, 54, 59, 66, 78, 108). This level of fatigue is similar to that reported via BPNS (see above). The expiratory muscles (99, 100) and specifically abdominal muscles fatigue ~13-28% during heavy exercise (85-90%VO_{2max}) (99, 100, 103). Additionally, Loke et al. (1982) reported expiratory muscle fatigue (~27% reduction in P_{Emax}) after running a marathon (54).

Therefore, the respiratory muscles and not solely the diaphragm can fatigue during heavy whole body exercise. As stated earlier, the respiratory muscles are important to maintain total ventilation during heavy exercise intensities. Due to the negative implications for exercise tolerance that may arise from respiratory muscle fatigue, previous research has been conducted to determine the contributing factors to respiratory muscle fatigue.

Mechanisms for Respiratory Muscle Fatigue during Heavy Exercise

Inspiratory muscle fatigue has been linked to respiratory muscle work, decreased respiratory muscle blood flow, hydrogen ion production, glycogen depletion, and the production of reactive oxygen species (18, 77, 79). Although each of these factors contribute to inspiratory muscle fatigue, the most widely studied contributor is respiratory muscle work and the

subsequent redistribution of blood flow during heavy exercise (77). The oxygen cost of breathing (and thus, inspiratory muscle work) at rest is approximately 2% of total VO_2 (1, 73) and increases with ventilation during voluntary hyperpnea (14, 88). Thus, an increase in oxygen cost of breathing is observed during moderate exercise in active individuals (3-5% total VO_2) with a further increase at maximal exercise in highly-fit individuals (10-15% total VO_2) (1, 37). When inspiratory muscle work was reduced (~50%) via a proportional assist ventilator, diaphragmatic fatigue was absent during heavy exercise (7). When the exercise diaphragmatic work was mimicked at rest however, diaphragmatic fatigue did not occur, indicating that diaphragmatic work is not solely responsible for the fatigue (5). For diaphragmatic fatigue to occur at rest, diaphragmatic work is required to increase more than two-fold compared to the diaphragmatic work incurred during heavy exercise (5). A plausible explanation for the lower diaphragmatic work required to induce inspiratory muscle fatigue during heavy exercise is the competition of cardiac output by the respiratory muscles and the locomotor muscles (5, 77).

Respiratory Muscle Blood Flow

During heavy exercise, the increased oxygen cost of breathing and work of breathing increases the respiratory muscle blood flow requirement (1, 37), which if not met may exacerbate RM fatigue (Vogiatzis et al. 2008). While maximum diaphragmatic blood flow has not been directly measured in humans, radioactive microspheres and flow probes have been used to measure diaphragm blood flow changes in animal models. Musch et al. (1983) and Manohar (1986) have demonstrated that during maximal exercise in dogs and ponies respectively, blood flow to the respiratory muscles increases to ~16-25% of total cardiac output to appropriately match oxygen consumption (58, 61). Additionally during maximal exercise, Poole et al. (2000) demonstrated rat diaphragmatic blood flow increased approximately 260% from baseline (70).

The blood flow to the accessory muscles (intercostals, scalenes, and abdominal muscle) increased 6-10 fold. Furthermore, vascular conductance increased in the diaphragm and the accessory muscles during maximal exercise (70).

In humans, blood flow to the respiratory muscles has not been directly measured, but cardiac output to the respiratory muscles has been estimated by using the direct Fick method. Using this technique, Harms et al. (1998) reported that respiratory muscles require ~14-16% of the total blood flow during maximal exercise (37). Recently, respiratory muscle blood flow has been estimated using near infrared spectroscopy and indocyanine green dye (34). This technique primarily estimates blood flow in the internal and external intercostals due to the accessibility of these respiratory muscles. Using this technique, Vogiatzis et al. (2008) demonstrated intercostal blood flow increases fivefold to supply the necessary oxygen during heavy exercise (104). During heavy and maximal exercise, the inspiratory muscles compete with the locomotor muscles for Q (35) and the limited blood flow to the respiratory muscles may contribute to inspiratory muscle fatigue.

Reactive Oxygen Species

During heavy exercise, there is an increase in reactive oxygen species (ROS) produced by the contracting muscles, which also have been implicated in muscle fatigue (18, 72). Additionally, inspiratory muscle contractions also release ROS during heavy exercise (72, 96). One specific alteration by ROS is the oxidation of thiol (52, 85), which has been strongly associated with fatigue (25). N-acetylcysteine (NAC), a non-specific antioxidant and thiol donor, has been observed to attenuate diaphragmatic fatigue in situ in the rabbit (89) and during whole body exercise in humans (50) as well as attenuate whole body peripheral fatigue (16). The reduction of inspiratory muscle fatigue may negate the consequences of the inspiratory

muscle fatigue and may lead to increased exercise tolerance; however, this postulate has not yet been tested.

Implications of Respiratory Muscle Fatigue

Exercise Tolerance

The influence of RM fatigue and increased work of breathing on exercise tolerance is dramatic. Harms et al. increased the work of breathing (~128-157% of control) via inspiratory resistors and observed a decreased in time cycling at 90%VO_{2max} to exhaustion by ~15% (36). To reduce the work of breathing, a proportional assist ventilator was used to unload the inspiratory muscles. When the work of breathing was reduced (~37-45% of control) during cycling at 90%VO_{2max}, time to exhaustion was increased ~14% (36). Additionally, Romer et al. (2006) observed increased quadriceps fatigue when the work of breathing was increased ~180% of control via inspiratory resistors during cycling at 90%VO_{2peak}, which led to a reduced time to exhaustion of ~39% (75).

Prior induced expiratory muscle fatigue has been shown to reduce exercise tolerance (100, 102). Specifically, Verges et al. induced expiratory muscle fatigue by having subjects perform expiratory resistive breathing until P_{Emax} was < 50% control values prior to performance and observed a ~3% reduction in distance covered in a 12 minute run (102). Similarly, Taylor et al. (2008) induced expiratory muscle fatigue by having subjects perform expiratory resistive breathing until task failure prior to exhaustive exercise and reported a ~33% reduction in time to exhaustion (100). Also, when the inspiratory muscles were fatigued (via inspiratory resistive breathing) prior to exhaustive exercise, the time to exhaustion was reduced by ~23%, which is in

agreement with studies that increased the work of breathing (56). Collectively, these studies demonstrate that RM fatigue can significantly reduce exercise tolerance.

Ventilation

Ventilation may be constrained with RM fatigue because both the inspiratory and expiratory muscles contribute to total ventilation during exercise. A reduced ventilatory response during heavy or maximal exercise may lead to arterial desaturation which has been reported to limit performance (36). However, Babcock et al. (1995) observed that the arterial saturation was maintained despite the diaphragmatic fatigue demonstrating ventilation was not constrained (6).

Dyspnea

RM fatigue may also increase the perception of breathing or dyspnea during heavy exercise and therefore may limit exercise tolerance. Using resistive breathing, the increased recruitment of accessory respiratory muscles has been reported to increase dyspnea (26, 95, 97, 109). The increased dyspnea is most likely due to the increased cost of breathing of the expiratory muscles and to a lesser extent the inspiratory muscles (22) and the chest wall distortion resulting from the recruitment of accessory muscles (27, 32). This increased oxygen cost of breathing and work of breathing could lead to an increased blood flow requirement, which may be further exacerbated with RM fatigue. Interestingly, diaphragmatic fatigue did not increase dyspnea sensations (109) due to the inability of the fatiguing diaphragm to increase neural respiratory drive (55).

Redistribution of Blood Flow

RM fatigue leads to cardiovascular adjustments at rest and during dynamic exercise. With diaphragmatic fatigue, the diaphragm increases discharge of the unmyelinated group IV

afferents (38). In resting humans, inspiratory muscle fatigue (via task failure) leads to increased muscle sympathetic nerve activity (94) and consequently reduced blood flow and vascular conductance in the leg (86, 87). In exercising dogs, Rodman et al. (2003) confirmed the reduced leg blood flow was due to increased sympathetic nerve activity by eliminating the response via an adrenergic receptor blockade (phentolamine and propranolol) (74). During heavy exercise, Vogiatizis et al. (2008) determined greater diaphragmatic fatigue, while cycling in hypoxia compared to normoxia and hyperoxia due to the exercise induced arterial hypoxemia experienced in hypoxia (104). Despite the greater diaphragmatic fatigue, intercostal blood flow was not further increased in the hypoxic condition. This provides evidence that diaphragmatic fatigue does not increase intercostal blood flow during heavy exercise. This is in agreement with findings by Musch et al. (1993) in the rat model who found no increases in intercostal blood flow but increased diaphragmatic blood flow with an increased work of breathing during exercise (61).

Harms et al. (1997) investigated how changing the work of breathing (via a proportional assist ventilator) influences blood flow distribution to the legs and respiratory muscles during cycling exercise at VO_{2max} (35). When the work of breathing was increased 128.2 ± 25.2% of control, Q distribution to the legs was decreased by 1.3 ± 0.2 liters per minute during maximal exercise, but not at 50% and 75%VO_{2max} (111). Although these studies have reported a redistribution of blood flow with increased work of breathing, it is not known if RM fatigue will elicit a similar blood flow redistribution during exercise.

Inactive Muscle Blood Flow

During whole body exercise, blood flow is distributed throughout the body to meet the increased oxygen requirement and heat production of the exercising skeletal muscles. Exercising

limb blood flow increases with increased intensity primarily to perfuse active skeletal muscle (82). Additionally, dynamic exercise greatly increases heat production as a byproduct of cellular metabolism. This increased heat production increases the thermoregulatory demands during exercise and is met by increasing conduit and cutaneous blood flow to dissipate heat in the non-exercising limb. As a result, non-exercising limb blood flow increases with increased intensity (98). During lower limb exercise (e.g. cycling), blood flow in the inactive brachial artery increases in both incremental (29, 90, 98) and steady state exercise (30, 31, 65, 67, 91).

The increase in brachial artery blood flow during leg cycling demonstrates a biphasic response (10-12, 48, 67, 91, 101). At the start of leg cycling, there is a reduction or no change in brachial artery blood flow for five minutes due to increased sympathetic vasoconstriction (10-12, 48, 67, 91, 101). During prolonged exercise (30-60 min) at an absolute workload of 120 watts, brachial artery blood flow increases approximately 2-4 fold (67, 91). The increase brachial artery blood flow places a stress on the vessel wall known as shear stress. Due to the increased shear stress, the brachial artery diameter increases and, therefore, blood flow increases to the inactive muscle (67).

Increasing inactive limb blood flow during leg cycling has also been important for therapeutic interventions. During leg cycling, inactive limb blood flow has been reported to increase to the inactive shoulder and neck muscles (3, 4). Leg cycling is speculated to help relieve shoulder and neck muscles because of this increased blood flow. Recently, Anderson et al. (2010) reported an increased oxygenation in the inactive shoulder and neck muscles during leg cycling (3), which further contributes to the therapeutic benefits.

Cutaneous Blood Flow

At the onset of exercise, cutaneous blood flow is reduced or not changed due to increased sympathetic vasoconstriction via adrenergic alpha 2 receptors. Increased sympathetic adrenergic vasoconstriction reduced forearm (12) and cutaneous blood flow (47) by using a sympathetic inhibitor (bretylium tosylate) and observing increased forearm and cutaneous blood flow. With continued exercise, core temperature raises to a threshold at which cutaneous vasoconstriction is withdrawn and active cutaneous vasodilation begins subsequently increasing cutaneous blood flow (48, 49). However if a cutaneous vasoconstriction occurs (via cold stress) during steady state exercise, a reduction in brachial artery blood flow occurs (91). This implies that cutaneous vasodilation plays a major role in the increase of brachial artery blood flow following the initial phase. Cutaneous blood flow continues to increase up to approximately 50-60% of maximal cutaneous blood flow at which a plateau in blood flow occurs (13, 42, 47). This plateau is due to cutaneous active vasodilator withdrawal rather than increased cutaneous vasoconstriction (48). It was hypothesized the plateau in cutaneous blood flow occurs to maintain atrial filling pressure (81). Therefore, this demonstrates the importance of inactive limb blood flow in meeting the thermoregulatory demands during dynamic exercise.

Summary

During heavy exercise, several factors may influence overall blood flow responses, including respiratory muscle fatigue. During dynamic exercise, respiratory muscle (inspiratory and expiratory) fatigue occurs in healthy individuals exercising at workloads of >85% VO_{2max}. Respiratory muscle fatigue has been observed to initiate increased sympathetic vasoconstriction, reduced leg blood flow, and leg vascular conductance at rest and during exercise. Factors which contribute to respiratory muscle fatigue include respiratory muscle work, respiratory muscle

blood flow, hydrogen ion production, glycogen depletion, and the production of reactive oxygen species. The consequences of respiratory muscle fatigue include constrained ventilation, dyspnea, and redistribution of blood flow. During lower limb exercise (e.g. cycling), inactive brachial artery and cutaneous blood flow increase during steady state exercise primarily due to thermoregulatory requirements. To date, the influence of respiratory muscle fatigue on inactive muscle and cutaneous blood flow during cycling exercise has not been determined.

Statement of the Problem

The purpose of this study is to investigate the influence of respiratory muscle fatigue on inactive limb and cutaneous blood flow during dynamic cycling exercise in healthy active college-aged men.

Hypotheses

We hypothesized that, compared to exercise trials without respiratory muscle fatigue, respiratory muscle fatigue will lead to reduced inactive limb: 1) blood flow, 2) vascular conductance, and 3) cutaneous blood flow.

III. Methods

Twelve active, healthy men were recruited as subjects. Each subject provided their health history (via questionnaire) and informed consent prior to testing. All subjects were free of heart and lung disease (self-report) and had normal pulmonary function as assessed by standard pulmonary function tests (PFT). All experimental procedures were approved by the Institutional Review Board at Kansas State University, Manhattan, KS.

Experimental Design

Subjects reported to the lab on four separate occasions. During session one, subjects completed medical history questionnaires, PFT's, and an incremental cycle test to exhaustion to determine maximal oxygen uptake (VO_{2max}). Session two and three were randomized and consisted of a submaximal cycle test at a workload to elicit 70%VO_{2max} or 85%VO_{2max} for 20 minutes. During session four, subjects ingested N-acetylcysteine (NAC) to reduce inspiratory muscle fatigue 45 minutes prior to a 85%VO_{2max} cycle test for 20 minutes.

Measurements

Pulmonary Function Tests

Pulmonary function tests were assessed according to American Thoracic Society guidelines (Miller et al. 2006). Maximum flow volume loops, maximal inspiratory pressures (P_{Imax}) and maximal expiratory pressures (P_{Emax}) were assessed prior to exercise testing (SensorMedics 229 Metabolic Cart, SensorMedicsCorp., Yorba Linda, CA). These tests were performed after multiple practice sessions until valid, consistent measurements were obtained. P_{Imax} was measured at residual volume and P_{Emax} was measured from total lung capacity. All

measurements were performed in triplicate with the two values closest to each other used in analysis.

Maximal Aerobic Capacity (VO_{2max})

An incremental exercise test on an electromagnetic cycle ergometer (800S, Sensor Medics Corp., Yorba Linda, CA) to exhaustion was performed to determine VO_{2max}. Baseline metabolic and ventilatory measurements were taken for three minutes. Subjects were then instructed to remain seated throughout the test and maintain 60-70 revolutions per min (rpm). The workload increased 50 watts each two minutes. Subjects exercised until volitional fatigue despite continual verbal encouragement. The incremental exercise test ended when the subject could not maintain the pedal frequency >50 rpm for five consecutive revolutions. Fifteen minutes after the completion of the incremental exercise test, a second exercise bout was performed at a constant workload (105%VO_{2max}) to verify VO_{2max} (71). The workload for the verification was determined from the last workload of the maximal incremental test and subjects were instructed to maintain ~60 rpm until volitional fatigue (2-3 min).

Metabolic and ventilatory data were continuously monitored breath-by-breath throughout exercise. A pulse oximeter (Datex-Ohmeda, 3900P, Madison, WI) was used to estimate arterial oxygen saturation (SpO_2). This oximeter provided visual waveform of blood perfusion which helped ensure accurate measurements and was secured to the earlobe to minimize movement artifact. Heart rate (HR) was collected continuously and was recorded at the end of each stage via Polar heart rate strap (T31-Uncoded).

Submaximal Exercise Tests

Results from the VO_{2max} exercise test were used to calculate a workload which would elicit 70% and 85%VO_{2max} for each subject. The first two submaximal exercise tests (separated

by 24-96 hours) were for 20 minutes and the workload was adjusted accordingly to maintain a constant metabolic rate (VO_2). During baseline and throughout exercise, the right arm was placed on a stand at the level of the heart. The inactive arm was continuously monitored to ensure the arm remained relaxed. Baseline metabolic measurements were taken for three minutes and subjects then warmed up on the cycle ergometer for three minutes with no resistance before the workload was increased. Subjects remained seated and maintained 60-70 rpm throughout exercise. During the last exercise test, subjects ingested N-acetylcysteine (NAC) 45 minutes prior to the start of exercise (50) and then cycled at 85%VO2max for 20 minutes. A dosage of 1800mg (3 x 600mg) of NAC was used based on the study by Kelley et al. (2009), who showed significant decreases (~14%) in inspiratory muscle fatigue during heavy exercise using this level of oral dosing (50). P_{Imax} measurements were taken prior to and within three minutes following each submaximal exercise. Additionally, a subset of six subjects cycled at a reduced intensity (50%VO_{2max}) for 20 minutes on a separate day to verify increased inactive muscle blood flow at a lower exercise intensity than used with this study.

Metabolic and ventilatory data were continuously monitored breath-by-breath throughout exercise. SpO2 was estimated with a pulse oximeter and heart rate was collected continuously during exercise and the averaged 20 second value was used in analysis. Blood pressure (BP) was measured manually via stethoscope auscultation at the brachial artery. Inactive muscle blood flow and blood pressure measurements were performed at baseline and every five minutes during exercise. Vascular conductance was calculated as the ratio of brachial artery blood flow to mean arterial pressure and was determined at baseline and every five minutes during exercise. To determine if inspiratory muscle fatigue influences cutaneous blood flow, cutaneous blood flow was measured at baseline and every five minutes during exercise.

Inactive Arm Blood Flow

Doppler ultrasound (DU) was used to measure the brachial artery blood velocity. The gate of the Doppler was set to full width of the brachial artery to ensure complete insonation. Measurements in the brachial artery were made 2-5 cm above the antecubital fossa. Mean blood velocity (VMEAN; cm x sec-1) was defined as time averaged mean velocity over each complete cardiac cycle while, peak velocity (VPEAK; cm x sec-1) was defined as the time averaged peak velocity. The blood velocity profile index was expressed as the VMEAN/VPEAK ratio (Lunt et al., 2000; Osada & Radegran, 2005, 2006). All blood velocities were determined over the average of 5-6 consecutive cardiac cycles and corrected for the insonation angle. Blood flow (BF) was calculated as the product of VMEAN and vessel cross sectional area (CSA). Vessel diameters were measured at rest via two-dimensional sonography and used to calculate vessel cross sectional area ($CSA=\pi r2$; cm2).

Cutaneous Blood Flow

Laser-Doppler Flowmetry (LDF) was used as an index of red blood flow cell (RBC) flux. LDF measures the Doppler shift of a laser as it reflects off red blood cells moving through the skin. LDF is a non-invasive method of obtaining a continuous index of RBC flux and has been shown to be a reliable method of determining cutaneous blood flow without influence from blood flow in underlying muscle (83).

Two local heating units were placed on the skin of the inactive right forearm. An integrated laser-Doppler probe, (Probe 413; Perimed; Jarfalla, Sweden) each with seven emitting and receiving probes, was placed in the center of each local heating unit to estimate RBC flux in the inactive forearm. After cycling, maximal blood flow was elicited by local heating via heating units to a skin temperature of 43ºC. Cutaneous blood flow data was converted to

cutaneous vascular conductance (CVC), calculated as the ratio of cutaneous blood flow to mean arterial pressure (RBC flux/ mean arterial pressure). Cutaneous vascular conductance data was expressed as a percentage of maximal vasodilation (%CVC_{max}) via local heating to 43ºC. A 60 second average was used for baseline and every five minutes for each site and then both sites were averaged.

Statistics

SigmaSTAT statistical software (Jandel Scientific Software) was used for data analysis. Data is presented as mean ± standard deviation. Differences were determined by a 2x2 (group x time) mixed factorial ANOVA. A Tukey post hoc analysis was performed to determine where significant differences existed. Differences between 85%VO_{2max} trials (with NAC; without NAC) were determined via paired t-tests. Statistical significance was set at $p < 0.05$ for all analyses.

IV. Results

Subject Characteristics

Subject characteristics are shown in Table 1. Subjects were physically active, but not competitively trained. All subjects' pulmonary function values were within normal predictive values (17). All subjects achieved similar VO_{2max} values ($p > 0.05$) from the incremental test and the constant load verification trial. Arterial oxygen saturation (SpO_2) was maintained within 3% of resting values in all subjects throughout all exercise sessions.

Table 1 Subject Characteristics, Pulmonary Function, and VO_{2max} Data

	mean ± SD	% Predicted
Age (yr)	22.9 ± 2.2	-
Height (cm)	175.2 ± 5.8	-
Weight (kg)	73.9 ± 9.7	-
PEF (L/sec)	10.4 ± 1.5	109.4 ± 0.7
FVC (L)	5.75 ± 0.71	112.1 ± 0.4
FEV_1 (L/sec)	4.67 ± 0.55	105.2 ± 0.4
FEV_1/FVC (%)	83.3 ± 9.3	97.8 ± 1.0
FEF_{25-75} (L/sec)	4.79 ± 1.46	98.7 ± 0.3
P_{Imax} (cmH_20)	163.1 ± 31.8	119.9 ± 20.6
P_{Emax} (cmH_20)	167.3 ± 23.4	68.6 ± 10.0
VO_{2max} Data		
VO_2 (L/min)	3.47 ± 0.37	-
VO_2 (mL/kg/min)	47.4 ± 5.1	-
VE (L/min)	138.8 ± 31.6	-
VE/VO_2	39.8 ± 6.2	-
VE/VCO_2	35.0 ± 5.1	-
RER	1.13 ± 0.06	-
SpO_2 (%)	98.0 ± 0.9	-
HR (bpm)	176.9 ± 7.8	-

Table 1: Values are mean ± SD. PEF: peak expiratory flow; FVC: forced expiratory flow; FEV_1: forced expiratory volume in 1 second; FEF_{25-75}: forced expiratory flow during 25-75% of vital capacity; P_{Imax}: maximal inspiratory pressure. P_{Imax}: maximum expiratory pressure. VO2: oxygen uptake; VE: ventilation; VE/VO2: ventilatory equivalent for oxygen; VE/VCO2: ventilatory equivalent for carbon dioxide. RER: respiratory exchange ratio; SpO2: arterial oxygen saturation; HR: heart rate

Respiratory Muscle Fatigue

The averaged measured VO_2 over 20 minutes of exercise as a percent of VO_{2max} in the 70%VO_{2max} test was 70.6 ± 5.5% and 86.0 ± 5.4% in the 85%VO_{2max} test. Percent mean decrease in P_{Imax} post-exercise for 70% and 85% VO_{2max} is shown in Figure 1 and individual and mean absolute values are presented in Figure 2. There were no differences ($p > 0.05$) in pre-exercise P_{Imax} between the exercise tests. P_{Imax} did not change ($p > 0.05$) following 20 minutes at 70%VO_{2max}, but was significantly lower (~13%) following 20 minutes at 85%VO_{2max} compared to pre-exercise P_{Imax} in 11 of the 12 subjects (range 4-29%), indicating inspiratory muscle fatigue.

Figure 1 Maximal Inspiratory Pressure versus 70% and 85% VO_{2max} Test

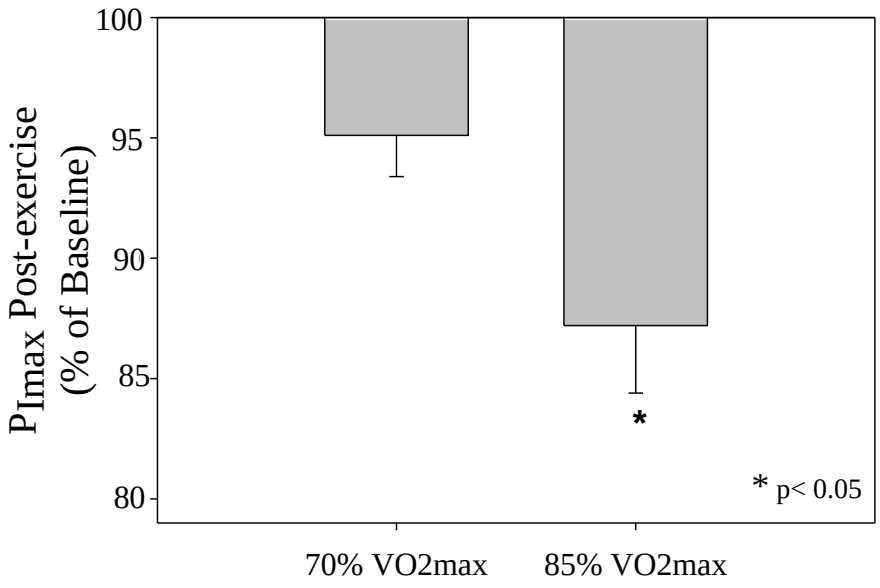
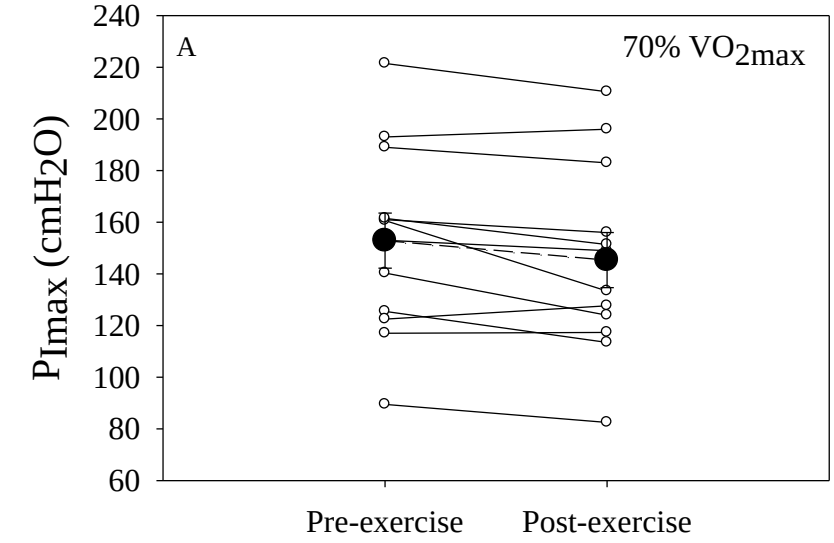


Figure 1: Maximal inspiratory pressure (P_{Imax}) post-exercise for both 70% and 85%VO_{2max} tests expressed as a % of baseline values. The 85%VO_{2max} test showed significant decreases (~13%) in P_{Imax} post- exercise.

Figure 2 Mean and Individual Maximal Inspiratory Pressure Pre-and Post-Exercise



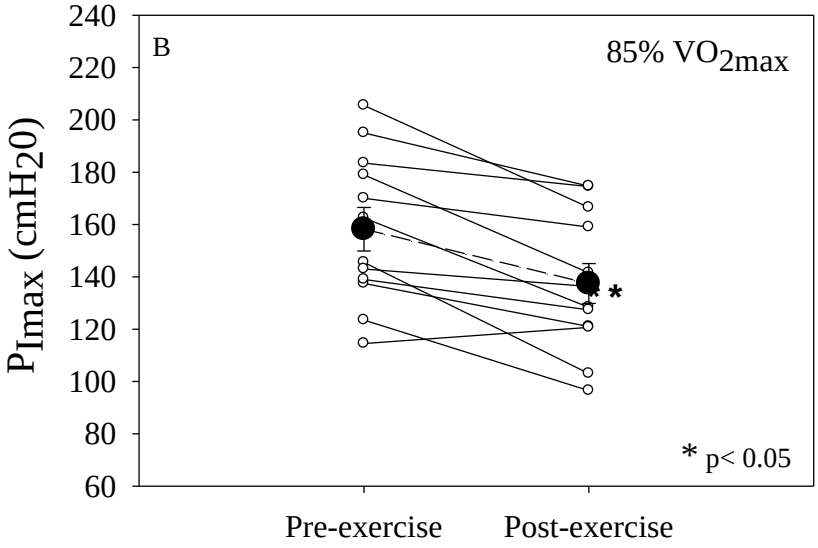


Figure 2A: Mean (filled circles) and individual (open circles) maximal inspiratory pressure (P_{Imax}) pre- and post-exercise at 70%VO_{2max}. P_{Imax} post-exercise was not different ($p > 0.05$) compared to the pre-exercise P_{Imax}. Figure 2B: Mean (filled circles) and individual (open circles) P_{Imax} pre- and post-exercise at 85%VO_{2max}. P_{Imax} post-exercise was significantly lower (~13%) following the 85%VO_{2max} test compared to the pre-exercise P_{Imax} in 11 of 12 subjects.

There were no differences ($p > 0.05$) in pre-exercise P_{Emax} between 70% and 85% VO_{2max} tests. P_{Emax} did not change ($p > 0.05$) following 20 minutes at 70% VO_{2max} but was significantly lower (~15%) following 20 minutes at 85% VO_{2max} compared to pre-exercise P_{Emax} in 11 of 12 subjects (range 5-30%), indicating expiratory muscle fatigue.

Inactive Arm Blood Flow

Mean brachial artery (BA) blood flow measured at baseline and every five minutes for the 70% and 85%VO_{2max} tests are displayed in Figure 3. Mean and individual BA blood flow values at end exercise for both tests are displayed in Figure 4. There were no differences ($p > 0.05$) in BA blood flow at baseline between the tests. Both 70% and 85% VO_{2max} led to an increase ($p < 0.05$) in BA blood flow at minute 20 compared to baseline (> 3 times resting values). In a subset (n= 6), BA blood flow increased ~twofold over resting values at 50% VO_{2max} (data not shown). There was no differences ($p > 0.05$) in BA blood flow between the exercise intensities and 5, 10 or 15 minutes of exercise At minute 20, BA blood flow was significantly higher (~22%) in the 70%VO_{2max} test compared to the 85% VO_{2max} test (241.8 ± 65.9 vs. 197.9 ± 42.9 mL/min, respectively).

Mean arterial pressure (MAP), BA diameter, and BA vascular conductance measured at baseline and every five minutes for both tests are presented in Table 2. During exercise, MAP was lower ($p < 0.05$) at 5, 10, and 20 minutes during the 70%VO_{2max} test compared to the 85%VO_{2max} test. BA diameter was not different ($p> 0.05$) at baseline or during exercise between the 70% and 85%VO_{2max} tests. BA diameter was significantly increased at 20 minutes compared to baseline in the 70%VO_{2max} test and was significantly increased at minute 15 and 20 compared to baseline in the 85%VO_{2max} test. At baseline and minute 5, 10, and 15 of exercise, BA vascular conductance was not different ($p > 0.05$) between the 70% and 85% VO_{2max} tests, but was

significantly higher in the 70%VO_{2max} test compared to the 85%VO_{2max} test (2.60 ± 0.73 vs. 2.02 ± 0.45 $mLmin^{-1}mmHg^{-1}$, respectively) at 20 minutes of exercise.

Figure 3 Mean Brachial Artery Blood Flow versus Time

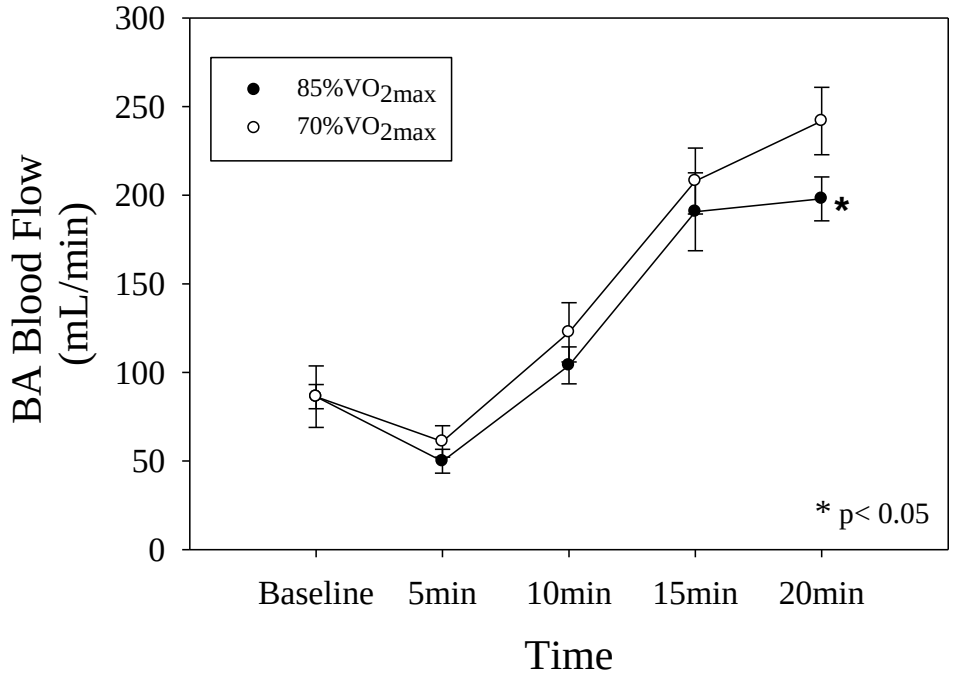


Figure 3: Mean brachial artery (BA) blood flow at baseline and during exercise at 70%VO_{2max} and 85%VO_{2max}. BA blood flow was significantly higher for 70% VO_{2max} compared to 85%VO_{2max} at 20min.

Figure 4 Mean and Individual Brachial Artery Blood Flow Values at End Exercise for 70%VO_{2max} and 85%VO_{2max} Tests

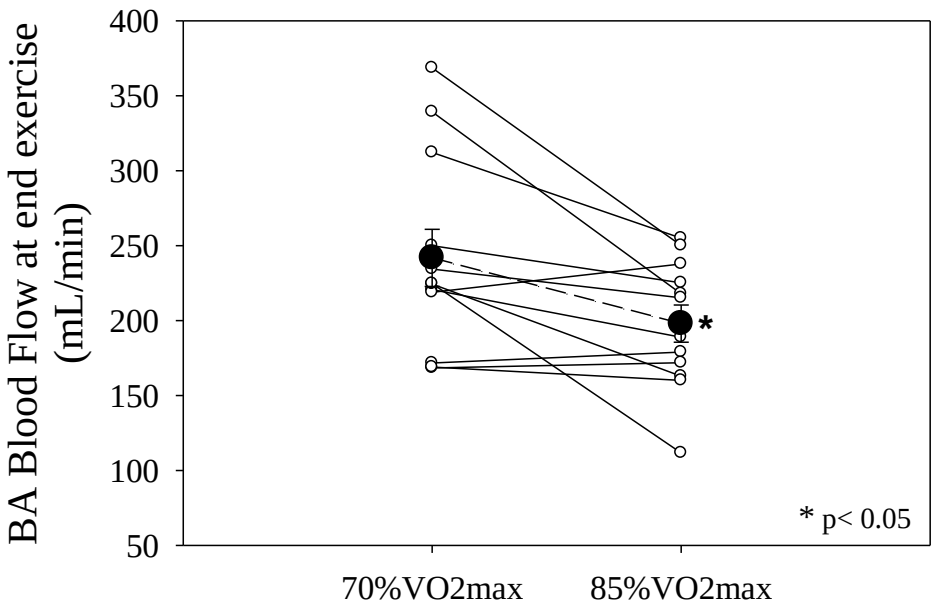


Figure 4: Mean (filled circles) and individual (open circles) brachial artery (BA) blood flow at 20 min for 70% and 85%VO_{2max}. At 20 min, BA blood flow was lower ($p < 0.05$) for 85%VO_{2max} compared to 70%VO_{2max} in 9 of 12 subjects

Table 2 Cardiovascular Measurements at Baseline and during 70%VO_{2max} and 85%VO_{2max} Tests

MAP (mmHg)	70%VO_{2max}			85%VO_{2max}		
Baseline	78.8	±	9.0	77.7	±	11.6
5min	96.0	±	11.1†	102.8	±	8.9*†
10min	96.1	±	11.1†	102.9	±	8.4*†
15min	96.0	±	10.2†	100.2	±	9.7†
20min	94.1	±	10.9†	99.3	±	9.6*†
Brachial Artery Diameter (mm)						
Baseline	4.3	±	0.3	4.3	±	0.4
5min	4.3	±	0.4	4.2	±	0.5
10min	4.4	±	0.4	4.3	±	0.5
15min	4.4	±	0.4	4.4	±	0.5†
20min	4.4	±	0.4†	4.4	±	0.5†
Vascular Conductance ($mLmin^{-1}mmHg^{-1}$)						
Baseline	1.02	±	0.31	1.08	±	0.69
5min	0.65	±	0.36	0.49	±	0.22†
10min	1.32	±	0.70	1.02	±	0.37
15min	2.18	±	0.67†	2.02	±	0.77†
20min	2.60	±	0.73†	2.02	±	0.45*†

Values are mean ± SD.

MAP= mean arterial pressure

* Significantly different from the 70% VO_{2max} trial ($p < 0.05$)

† Significantly different from baseline ($p < 0.05$)

Relationship between Respiratory Muscle Fatigue and Inactive Arm Blood Flow

Figure 5 shows the relationship between the percent change from baseline for BA blood flow at end exercise and the percent change in P_{Imax} following exercise at 85%VO_{2max}. Percent change from baseline of BA blood flow end exercise was negatively correlated with the percent change in P_{Imax} following exercise ($r = -0.66$; $p < 0.05$), suggesting those subjects with the greatest inspiratory muscle fatigue had the highest BA blood flow at end exercise. Figure 6 shows the relationship between BA vascular conductance at end exercise for the 85%VO_{2max} test and the percent change in P_{Imax} following exercise. BA vascular conductance was negatively correlated with the percent change in P_{Imax} following exercise ($r = -0.80$; $p < 0.05$), indicating those with the greatest inspiratory muscle fatigue had the highest BA vascular conductance at end exercise. There was no relationship ($p > 0.05$) between the percent change from baseline for BA blood flow or BA vascular conductance at end exercise and the percent change in P_{Emax} following 85%VO_{2max} exercise.

Figure 5 Relationship between Inspiratory Muscle Fatigue and Brachial Artery Blood Flow at End Exercise

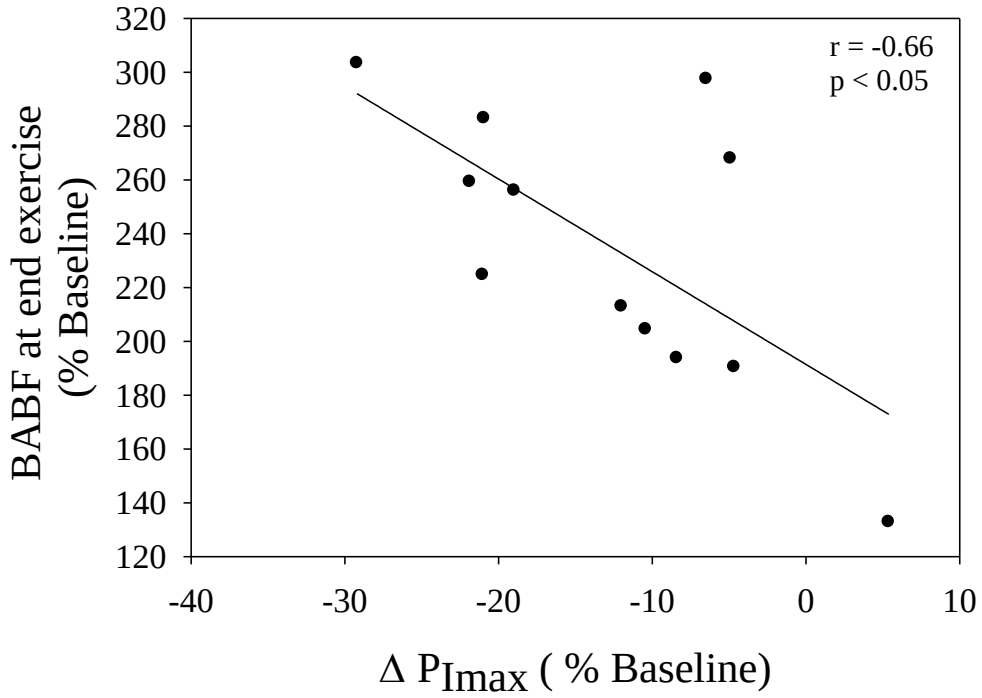


Figure 5: Relationship between percent change from baseline in brachial artery (BA) blood flow at end exercise and change in P_{Imax} during the 85%VO2max test. Subjects with the most inspiratory muscle fatigue had the highest BA blood flow.

Figure 6 Relationship between Inspiratory Muscle Fatigue and Brachial Artery Conductance at End Exercise

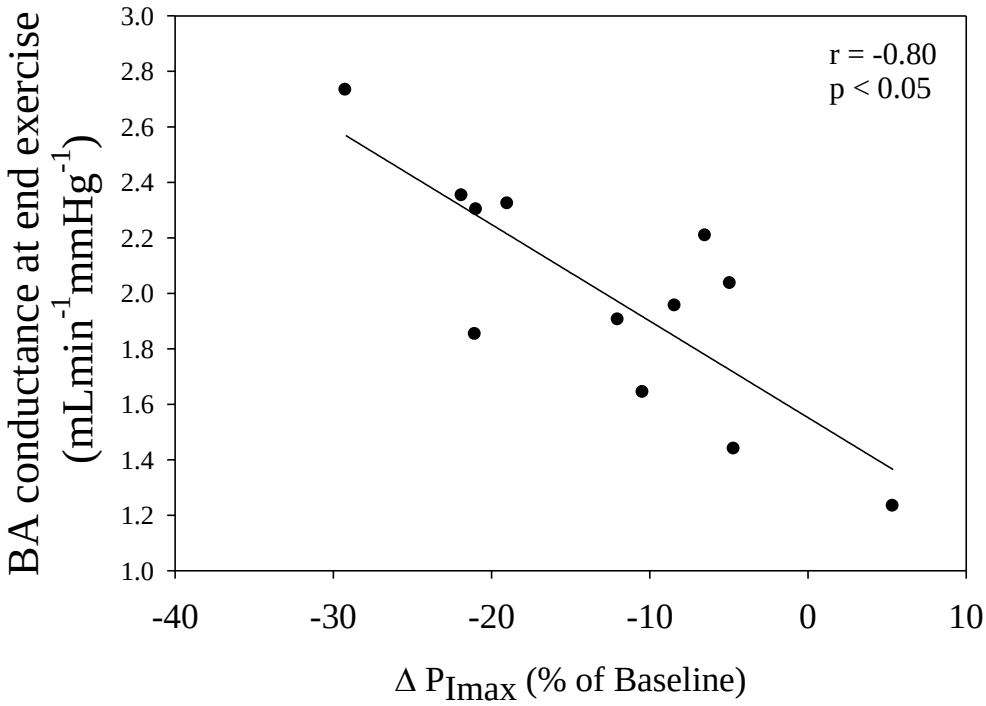


Figure 6: Relationship between brachial artery (BA) conductance at 20 min and change in P_{Imax} during the 85%VO_{2max} test. There was a negative correlation (r = -0.80; $p < 0.05$), suggesting those with the most inspiratory muscle fatigue had the highest BA conductance.

Inactive Arm Blood Flow with Removed Inspiratory Muscle Fatigue from NAC

The averaged VO_2 over 20 minutes was 83.7 ± 6.1% for the 85%VO_{2max} with N-acetylcysteine (85%VO_{2max}NAC) test and was not significantly different from the 85%VO_{2max} trial without NAC. There were no differences ($p > 0.05$) in pre-exercise P_{Imax} between the 85% and 85%VO_{2max} NAC tests. P_{Imax} did not change ($p > 0.05$) following 20 minutes of exercise at 85%VO_{2max} in the 85%VO_{2max} NAC test (Pre: 150.5 ± 30.1 cmH_20; Post: 144.8 ± 31.8 cmH_20),

indicating no inspiratory muscle fatigue confirming the results by Kelly et al. (2009). Figure 7 shows the individual and mean percent decrease in P_{Imax} post-exercise for 85% and 85% VO_{2max}NAC. The percent decrease in P_{Imax} post-exercise was significantly reduced in the 85%VO_{2max}NAC test compared to the 85%VO_{2max} test.

Pre-exercise P_{Emax} was not different ($p > 0.05$) between 85% and 85%VO_{2max} NAC tests. P_{Emax} was significantly lower (~ 10%) following 20 minutes of exercise in the 85%VO_{2max} NAC test compared to pre-exercise P_{Emax}, indicating expiratory muscle fatigue. The percent decrease in P_{Emax} post-exercise was similar ($p > 0.05$) between tests with and without NAC.

Mean BA blood flow measured at baseline and every 5 minutes for the 85% and 85%VO_{2max}NAC tests are presented in Figure 8. Mean and individual BA blood flow values at minute 20 for both tests are displayed in Figure 9. BA blood flow at baseline was similar ($p > 0.05$) between tests. Without inspiratory muscle fatigue at 85%VO_{2max}, BA blood flow was significantly higher at 10 minutes (No inspiratory muscle fatigue: 154.8 ± 106.7 vs. inspiratory muscle fatigue: 103.9 ± 36.1 mL/min) and 20 minutes (No inspiratory muscle fatigue 261.2 ± 111.2 vs. inspiratory muscle fatigue 197.9 ± 42.9 mL/min), but was similar ($p > 0.05$) to 70%VO_{2max} blood flow. At end exercise, 8 of the 12 subjects increased BA blood flow (range 4-178%) in the VO_{2max}NAC test compared to the 85%VO_{2max} test.

Figure 7 Inspiratory Muscle Fatigue at 85% versus 85%VO_{2max}NAC Tests

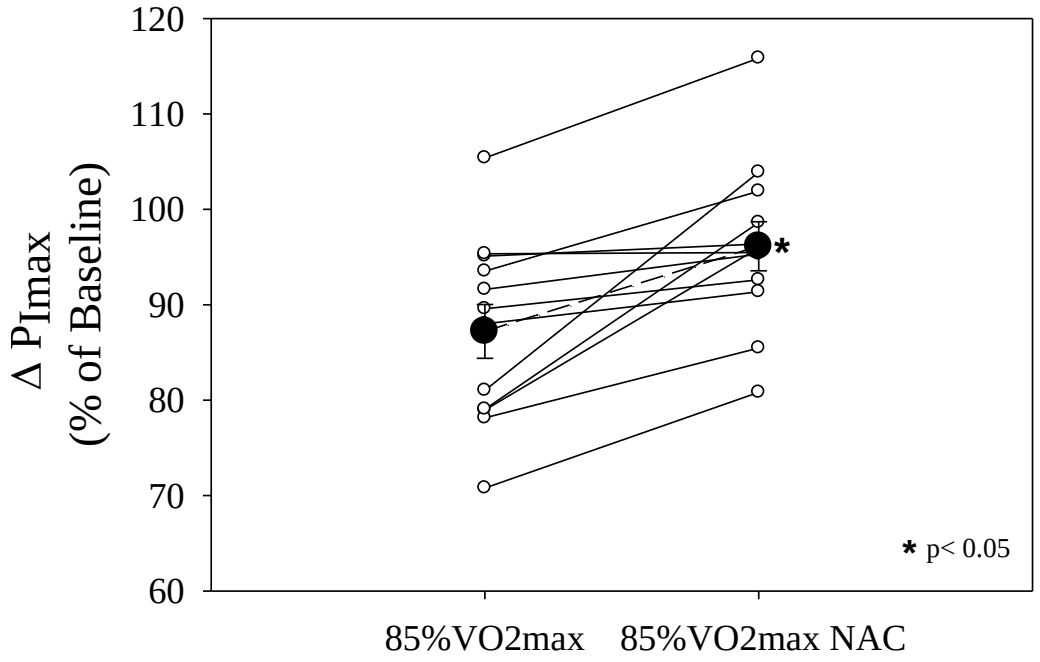


Figure 7: Mean (filled circles) and individual (open circles) maximum inspiratory pressure reported as percent of rest (% of baseline) for the 85%VO_{2max} and 85%VO_{2max} NAC post-exercise. P_{Imax} was significantly decreased following the 85%VO_{2max} test, but not for the 85%VO_{2max}NAC test in all subjects.

Figure 8 Mean Brachial Artery Blood Flow versus Time

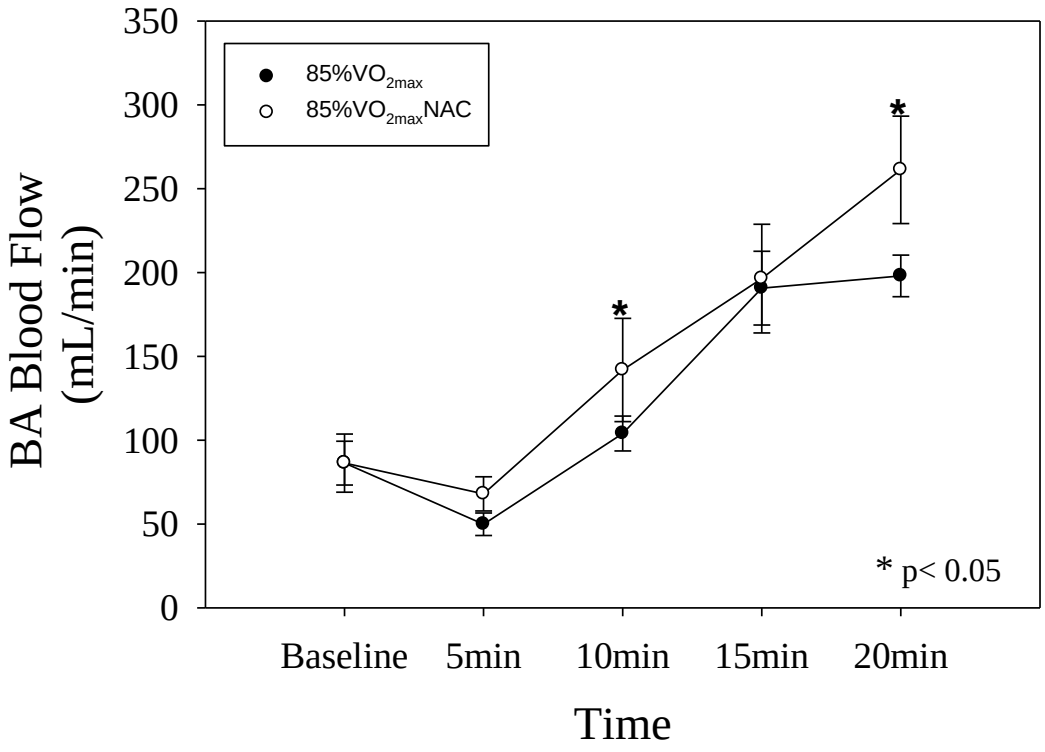


Figure 8: Mean brachial artery (BA) blood flow at baseline and during exercise at 85%VO_{2max}, and 85%VO_{2max}NAC. BA blood flow at 10 and 20 min was higher ($p < 0.05$) for 85%VO_{2max}NAC compared to 85%VO_{2max}.

Figure 9 Mean and Individual Brachial Artery Blood Flow at End Exercise for 85% VO_{2max} and 85%VO_{2max}NAC Tests

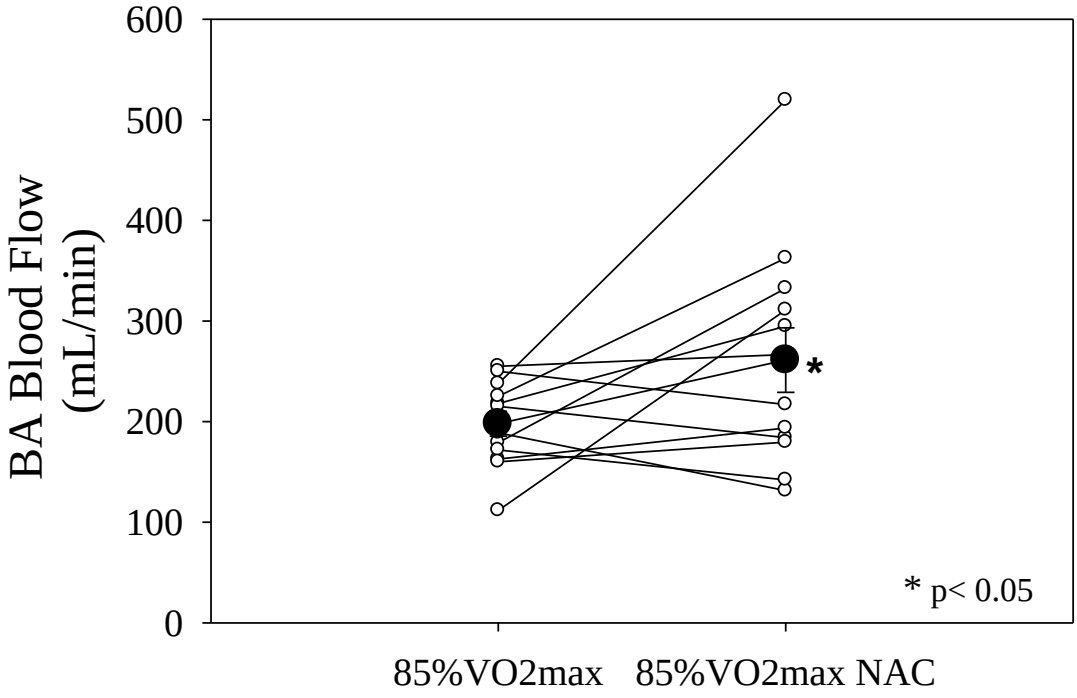


Figure 9: Mean (filled circles) and individual (open circles) brachial artery (BA) blood flow at end exercise at 85%VO_{2max} and 85%VO_{2max}NAC at end exercise. BA blood flow was higher ($p < 0.05$) for 85%VO_{2max}NAC compared to 85%VO_{2max} in 8 of 12 subjects.

MAP, BA diameter, and BA vascular conductance measured at baseline and every five minutes for both tests are presented in Table 3. At baseline, MAP was significantly higher in the 85%VO_{2max}NAC compared to the 85%VO_{2max} test, but during exercise was not different ($p > 0.05$). At baseline, the BA diameter was not different ($p > 0.05$) between tests, but during exercise was higher ($p < 0.05$) in the 85%VO_{2max}NAC test compared to the 85%VO_{2max} test. BA vascular conductance was significantly higher with the reduction of inspiratory muscle fatigue compared to the 85%VO_{2max} at 10min (No inspiratory muscle fatigue: 1.57 ± 1.11 vs. inspiratory

muscle fatigue: 1.02 ± 0.37 $mLmin^{-1}mmHg^{-1}$) and 20min (No inspiratory muscle fatigue: 2.73 ± 1.18 vs. inspiratory muscle fatigue: 2.02 ± 0.45 $mLmin^{-1}mmHg^{-1}$).

Table 3 Cardiovascular Measurements at Baseline and during 85%VO_{2max} and 85%VO_{2max}NAC Tests

MAP (mmHg)	85%VO_{2max}	85%VO_{2max} NAC
Baseline	77.7 ± 11.6	82.2 ± 9.0*
5min	102.8 ± 8.9†	103.3 ± 8.8†
10min	102.9 ± 8.4†	101.9 ± 9.0†
15min	100.2 ± 9.7†	100.8 ± 9.5†
20min	99.3 ± 9.6†	96.9 ± 9.4†
Brachial Artery Diameter (mm)		
Baseline	4.3 ± 0.4	4.4 ± 0.5
5min	4.2 ± 0.5	4.4 ± 0.5*
10min	4.3 ± 0.5	4.5 ± 0.5*
15min	4.4 ± 0.5†	4.5 ± 0.5*†
20min	4.4 ± 0.5†	4.5 ± 0.5*†
Vascular Conductance ($mLmin^{-1}mmHg^{-1}$)		
Baseline	1.08 ± 0.69	1.16 ± 0.55
5min	0.49 ± 0.22†	0.67 ± 0.36
10min	1.02 ± 0.37	1.57 ± 1.11*
15min	2.02 ± 0.77†	1.99 ± 1.19†
20min	2.02 ± 0.45†	2.73 ± 1.18*†

Values are mean ± SD.

MAP= mean arterial pressure

* Significantly different from the 85% VO_{2max} trial ($p < 0.05$)

† Significantly different from baseline ($p < 0.05$)

Cutaneous Vascular Conductance

Cutaneous vascular conductance (%CVC_{max}) was not different ($p > 0.05$) at rest or between exercise intensities throughout exercise for the 70% and 85% VO_{2max} tests. Figure 10 shows the increase in cutaneous vascular conductance for 70%VO_{2max} and 85%VO_{2max}. Cutaneous vascular conductance increased ~5 fold from rest in both conditions. Cutaneous vascular conductance at end exercise was negatively correlated with the percent change in P_{Imax} at 85%VO_{2max} ($r= -0.61$; $p < 0.05$), suggesting that those subjects with the greatest inspiratory muscle fatigue had the highest cutaneous vascular conductance at end exercise. In the 85% VO_{2max} test, cutaneous vascular conductance at end exercise was not related ($p > 0.05$) with the percent change in P_{Emax} or BA blood flow. However, Figure 11 shows cutaneous vascular conductance at end exercise in the 85%VO_{2max} test was positively related with BA vascular conductance at end exercise ($r = 0.65$; $p < 0.05$), suggesting those with the highest BA vascular conductance had the highest cutaneous vascular conductance. Cutaneous vascular conductance was not different ($p > 0.05$) at baseline or during exercise between the 85%VO_{2max} or 85%VO_{2max}NAC.

Figure 10 Cutaneous Vascular Conductance versus Time

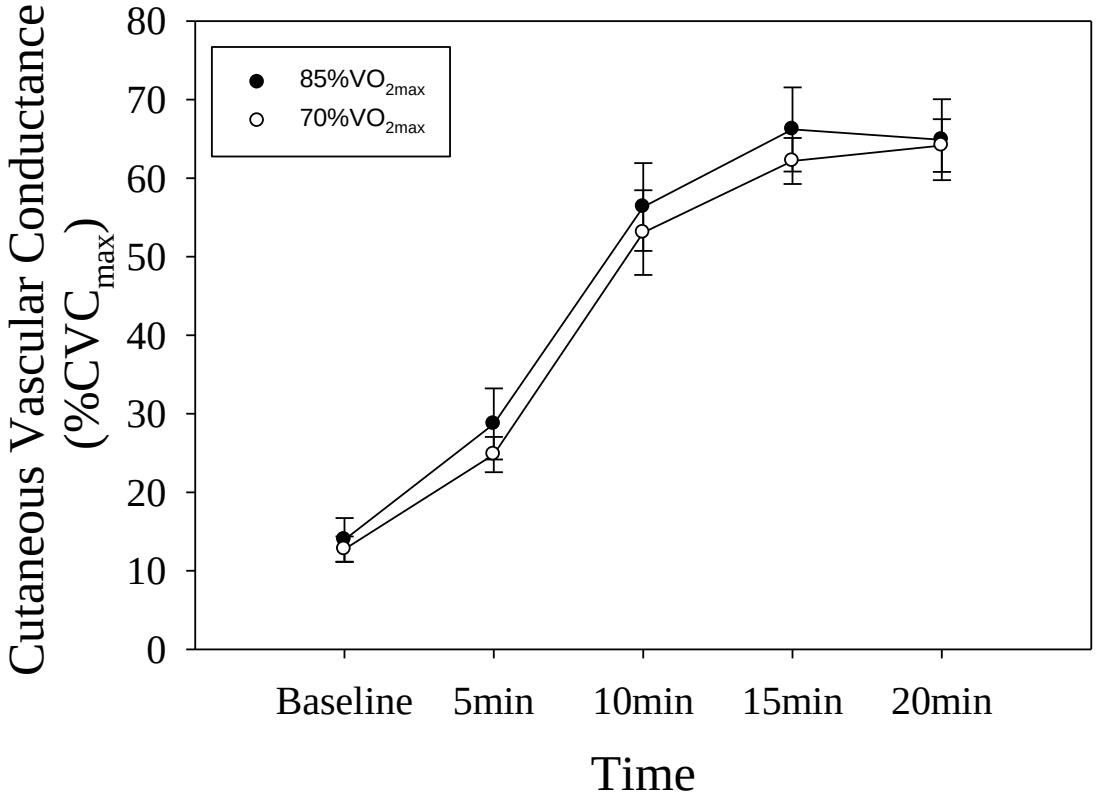


Figure 10: Mean cutaneous vascular conductance (%CVC_{max}) at baseline and during exercise at 70%VO_{2max} and 85%VO_{2max}. There was no difference ($p > 0.05$) at baseline or during exercise.

Figure 11 Relationship between Cutaneous Vascular Conductance and Brachial Artery Vascular Conductance at End Exercise

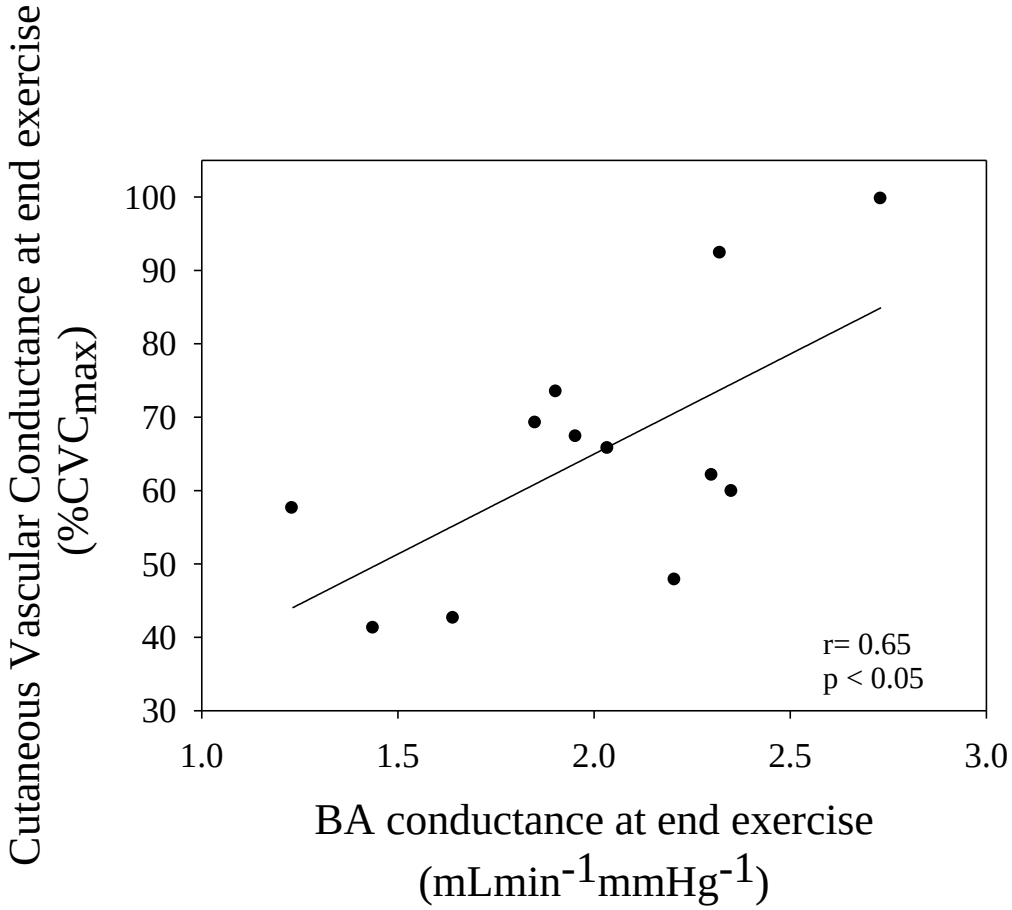


Figure 11: Relationship between cutaneous vascular conductance ($\%CVC_{max}$) and brachial artery (BA) vascular conductance at end exercise during the 85%VO_{2max} test. There was a positive correlation ($r=0.65$, $p < 0.05$) suggesting those with the highest BA conductance had the highest cutaneous vascular conductance at end exercise.

V. Discussion

Major Findings

The purpose of this study was to determine the influence of respiratory muscle fatigue on inactive limb blood flow during heavy exercise. Our major original findings support our hypothesis that respiratory muscle fatigue led to a reduced inactive limb blood flow and vascular conductance. However, against our hypothesis, cutaneous vascular conductance did not change with respiratory muscle fatigue during heavy exercise. These results combined with previous reports suggest that increased respiratory muscle work and respiratory muscle fatigue during heavy whole body exercise leads to a redistribution of blood flow from both active and inactive skeletal muscles to the respiratory muscles that does not impair thermoregulation.

Respiratory Muscle Fatigue

Inspiratory and expiratory muscle fatigue following heavy exercise is well documented (Romer et al. 2008). Specifically, decreases in respiratory muscle strength indicating fatigue have been reported with shuttle runs (10.5%) (59), 6-minute all-out rowing effort (11%) (108), incremental exercise to exhaustion (17%) (66), marathon running (16.5-18%) (54, 78), and heavy exercise to exhaustion (17%) (23). The amount of inspiratory muscle fatigue in the present study (~ 13%) is in accordance with these previous studies. Decreases in expiratory muscle strength have been reported with marathon running (27%) (54) and 20 minutes of running at 85% HR_{max} (6%) (15). The expiratory muscle fatigue in the present study (15%) also aligns with these previous studies.

Variable degrees of respiratory muscle fatigue are often experienced for a given exercise intensity (6, 23). With high intensity exercise (85% VO_{2max}), our subjects also demonstrated a wide range of respiratory muscle fatigue. This variable amount of respiratory muscle fatigue

may be due to differences between subjects in diaphragmatic duty cycle (9), respiratory muscle length/ velocity shortening (21), and/or respiratory muscle VO_2 (60).

Redistribution of Blood Flow from Inactive Muscle

Increased blood flow to inactive muscle during moderate exercise is well established (10-12, 48, 67, 91, 101), which was consistent with what we observed. During prolonged (30-60 minutes) cycling exercise at 60-69%VO_{2max}, inactive limb blood flow has been reported to increase 2-4 fold (65, 67, 91) and tends to increase with increased intensity (98). Our data confirms that the increase in inactive arm blood flow is intensity dependent as we demonstrated ~2 fold increase in inactive arm blood flow over baseline with a small subset of our subjects exercising at 50%VO_{2max}, and ~3 fold increase at 85%VO_{2max}.

During incremental and steady state exercise, inactive blood flow increases partly due to increased vessel diameter (67, 98). Tanaka et al. calculated shear stress of the brachial artery during incremental cycling exercise and reported an exercise intensity dependent increase in shear stress (98).Recently, Padilla et al. compared a forearm heating protocol, known to increase shear stress, and steady state cycling exercise (120 watts) and demonstrated that shear stress was primarily responsible for the dilation of the inactive brachial artery during exercise due to the increase in mean arterial pressure (67). The results of the present study are in agreement with these previous studies showing an increased conduit diameter during cycling exercise likely due to shear stress.

Our findings that the increased blood flow to the inactive arm was reduced with respiratory muscle fatigue is consistent with previous studies that have also reported reductions in inactive muscle blood flow with respiratory muscle fatigue in animal and human models (74, 87). However, these previous studies have all been performed under resting conditions.

Specifically, lactic acid was injected into the diaphragm (via phrenic artery) and internal abdominal expiratory muscles (via deep circumflex iliac artery) in resting awake dogs (74). Lactic acid has a similar action as diaphragmatic fatigue on stimulating phrenic IV afferent nerve activity (41). The injection of lactic acid in resting awake dogs led to a reduction in resting hind limb blood flow (74). Similar effects have been shown in humans. Sheel et al. (2001) demonstrated that inspiratory muscle task failure induced by resistive breathing at rest led to increased leg vascular resistance and reduced leg blood flow by ~30% (87). Our results now extend these findings to heavy exercise.

During heavy exercise, approximately 80-85% of cardiac output is distributed to the locomotor muscles (51, 69). Secher et al. (1977) was the first to demonstrate that the addition of arm exercise to cycling heavy exercise actually reduced blood flow and vascular conductance to the legs, indicating a "competition" between different groups of active muscle (84). Harms et al. (1997) extended these findings by increasing the work of breathing (~128% of control) via inspiratory resistors and measured the locomotor blood flow during maximal intensity exercise. With the increased work of breathing, locomotor blood decreased by ~1.3 liters per minute (~7%) and locomotor vascular resistance was increased. Conversely when the work of breathing was reduced during maximal exercise via proportional assist ventilator, locomotor blood flow increased ~0.8 liters per minute (~4%) and locomotor vascular resistance was reduced (35). This supports the premise that high respiratory muscle work during heavy exercise leads to redistribution of blood flow from active muscle to the respiratory muscles. The present study is in agreement with these previous studies and extends them to the inactive limb during heavy exercise. Specifically when respiratory muscle fatigue occurred during heavy exercise, inactive limb blood flow was reduced presumably due to vasoconstriction of the brachial artery. Also,

we were able to confirm our results by reducing respiratory muscle fatigue via N-acetylcysteine (NAC). NAC was effective in reducing respiratory muscle fatigue, which is in agreement with Kelly et al. (50), and consequently inactive limb blood flow and vascular conductance was not attenuated. Therefore, we are confident that respiratory muscle fatigue leads to reduced vascular conductance and blood flow to inactive muscle.

Why was inactive muscle blood flow reduced with respiratory muscle fatigue? Increased respiratory muscle work and respiratory muscle fatigue lead to increased sympathetic outflow eliciting vasoconstriction of both resting and exercising active muscle vasculature (87, 94). In resting humans, inspiratory and expiratory muscles were fatigued by breathing against an inspiratory and expiratory resistor (until task failure) (20, 94). Both inspiratory and expiratory muscle fatigue were reported to increase muscle sympathetic nerve activity (MSNA) in the resting limb leading to vasoconstriction of the femoral artery. Recently, Katayma et al. (2012) fatigued the inspiratory muscles via resistive breathing during submaximal (40%VO_{2max}) cycling exercise and measured MSNA in the inactive arm (45). With inspiratory muscle fatigue, MSNA was increased leading to inactive muscle vasoconstriction during submaximal cycling exercise. Although we did not measure sympathetic nerve activity, it is likely that the redistribution of blood flow to the respiratory muscles was due to sympathetically mediated vasoconstriction in the inactive arm muscle.

Unexpectedly, the relationship between respiratory muscle fatigue and brachial artery blood flow suggested that subjects who experienced the most respiratory muscle fatigue were not able to redistribute inactive blood flow to the respiratory muscles as well as subjects with the least amount of respiratory muscle fatigue. One possible explanation for this finding is subjects with the highest end exercise inactive blood flow simply did not respond to the increased

inactive arm limb sympathetic outflow, thereby the less respiratory muscle blood flow possibly exacerbated the respiratory muscle fatigue. This premise is supported by the increase in diaphragmatic fatigue reported when intercostal blood flow is not increased in hypoxia compared to normoxia during heavy exercise (106). Another possible explanation is that other factors (e.g. respiratory compensation point, critical power) may be contributing to this variability. Subjects with a lower critical power or respiratory compensation point, which are speculatively similar (19), might have an increased ventilatory response, consequently increased respiratory muscle work and respiratory muscle fatigue compared to subjects with a higher critical power or respiratory compensation point. Future studies are needed to substantiate these ideas and to help explain how the degree of respiratory muscle fatigue contributes to blood flow distribution in inactive muscle.

Respiratory Muscle Fatigue and Cutaneous Vascular Conductance

Why did the apparent sympathetic outflow elicited by the respiratory muscle fatigue not lead to a vasoconstriction of the cutaneous circulation as hypothesized? During steady state exercise, active cutaneous vasodilation is responsible for the increases and plateau in cutaneous blood flow (47). This was demonstrated by Kellogg et al. (1993) using bretylium tosylate, a vasoconstrictor blockade (47). These investigators observed the increase and plateau in cutaneous blood flow during exercise was not different, suggesting that active cutaneous vasodilation is responsible for this increase and plateau of cutaneous blood flow (47). As previously stated, respiratory muscle fatigue initiates an increase in sympathetic outflow leading to vasoconstriction; however, this sympathetic outflow appears to not elicit vasoconstriction of the cutaneous circulation during steady state exercise.

Non-thermoregulatory reflexes, such as cardiopulmonary and perhaps arterial baroreflexes, can also play a role in regulating the plateau in cutaneous blood flow during exercise (92). This is demonstrated by the absence of a cutaneous blood flow plateau during heat stress (42), suggesting that the competition for cardiac output between active muscle beds and cutaneous circulation during exercise may limit the magnitude of the cutaneous blood flow plateau. Additionally, the plateau in cutaneous blood flow was present during hypohydration (62) and absent with saline infusion (64) during exercise indicating central filling pressure plays a role in regulating the cutaneous blood flow plateau during exercise. However, MAP was maintained when respiratory muscle fatigue occurred in our study, so it is unlikely the cardiopulmonary baroreceptors influenced the plateau in cutaneous vascular conductance.

Interestingly, the reduced inactive limb blood flow with respiratory muscle fatigue did not lead to reduced cutaneous vascular conductance. A similar finding was previously reported during 30-60 minutes of leg cycling where approximately 75-80% of brachial artery blood flow was directed to the cutaneous circulation (43, 65) and presumably a reduction in brachial artery blood flow would reduce cutaneous circulation. In the present study, it is likely that much of the increased inactive muscle blood flow during exercise was distributed to the cutaneous circulation. Therefore, the reduced inactive limb blood flow with respiratory muscle fatigue did not limit the cutaneous vascular conductance response. However in the present study, we also show subjects with the highest inactive arm vascular conductance had the highest inactive arm cutaneous vascular conductance. This relationship is supported by Simmons et al. (2011) who applied local cooling to the forearm cutaneous circulation during steady state cycling exercise and measured the brachial artery blood flow and cutaneous blood flow response. These investigators observed a reduction in brachial artery vascular conductance due to the

vasoconstriction of the cutaneous circulation (91). In the present study, we propose that the subjects with the decreased cutaneous vascular conductance was due to a reduction in inactive limb vascular conductance. Therefore, the inactive muscle blood flow was redistributed to the cutaneous circulation and the reduction in inactive limb blood flow was not severe enough to elicit a decreased cutaneous vascular conductance response.

Implications of Respiratory Muscle Fatigue on Inactive Limb Blood Flow

Respiratory muscle fatigue may limit exercise performance by redistributing both active and inactive blood flow during dynamic exercise. The respiratory muscles require 14-16% of the total cardiac output during high intensity exercise (35). High intensity exercise leads to respiratory muscle fatigue which initiates a respiratory muscle metaboreflex leading to increased sympathetic outflow and consequently reduced locomotor blood flow (35). This premise is supported by the increase in time to exhaustion at 90%VO_{2max} when the work of breathing was reduced ~50% due to decreased respiratory muscle blood flow and consequently increased locomotor blood flow (36). Conversely, when the work of breathing was increased via inspiratory resistors, time to exhaustion was decreased, most likely due to increased respiratory muscle blood flow and reduced locomotor blood flow. The reduction of blood flow to the locomotor muscles has been implicated in increasing locomotor muscle fatigue due to reduced oxygen transport (2) impairing exercise performance.

The primary role of increased inactive limb blood flow during dynamic exercise is believed to be for thermoregulation via dissipation of heat (80). During exercise, the thermoregulatory demands are met by increasing conduit and cutaneous blood flow and as a result inactive limb blood flow increases with exercise intensity (98). The findings of this study demonstrate that respiratory muscle fatigue reduces inactive limb blood flow but cutaneous

circulation was not reduced. However if there was a further increased sympathetic vasoconstriction, the further reduced inactive limb blood flow may lead to reduction in cutaneous blood flow. Recently, Kayatymo et al. (2013) demonstrated inspiratory muscle fatigue led to an increased MSNA in the inactive limb during leg cycling in hypoxic conditions and therefore increased work of breathing compared to normoxic conditions (46). Therefore, it is likely that respiratory muscle fatigue in populations with a higher work of breathing (e.g. aging, COPD, CHF) will be associated with a reduced cutaneous blood flow response during exercise due to the reduced inactive limb blood flow. The reduction in inactive cutaneous circulation would lead to a reduction in heat dissipation and increased internal temperature consequently influencing exercise performance. Furthermore, González-Alonso et al. (1999) measured cycling time to exhaustion with different beginning body temperatures. When the body temperature was raised to 40ºC, the cycling time to exhaustion was reduced by ~50% compared to a beginning body temperature of 36ºC (28). These findings suggest that thermoregulation may be compromised with respiratory muscle fatigue and therefore may further reduce exercise tolerance.

The increased inactive limb blood flow during whole body exercise has also been used for therapeutic interventions. Anderson et al. (2008, 2010) have reported increased blood flow during exercise to inactive shoulders and neck muscles (3, 4). The authors speculate that this may be beneficial in relieving chronic pain to these areas. Anderson et al. (2010) reported that submaximal leg cycling also increased oxygenation in the inactive shoulders and neck, which further contributed to the therapeutic benefits (3). Our data suggests that if an individual exercised at high intensity (>85%VO_{2max}) or has a condition that would lead to respiratory muscle fatigue, a reduction in inactive muscle blood flow and thus reduced oxygenation would occur which would negatively influence this therapeutic intervention.

Limitations

Two potential limitations may have influenced our results. Previously, the gold standard for assessing diaphragmatic fatigue is bilateral phrenic nerve stimulation (BPNS) (44), while gastric pressures have been used to assess abdominal fatigue (99). In the present study, changes in maximal mouth pressures were used to measure respiratory muscle fatigue. Maximal mouth pressures have been demonstrated to show similar results as BPNS and gastric pressures and have been used in previous studies (23, 39, 40, 76, 93, 108). Maximal mouth pressures maneuvers are effort-dependent and, therefore, subjects were highly encouraged to perform the maneuver with maximal effort. Also, we performed pre- and post- exercise maximal mouth pressures tests in triplicate and lung volume measurements were made for each trial to help ensure consistency. The number of trials of maximal mouth pressures was limited to ≤ 3 with a warm-up session because this has been shown to reduce subject variability (107) and not lead to respiratory muscle fatigue. Additionally, each subject practiced maximal mouth pressures during a session before the exercise trials to help reduce variability.

Secondly, there is a possibility the inactive arm muscle periodically contracted during cycling exercise. Previously, electromyography (EMG) has been used to assure the" inactivity" of the inactive limb during incremental cycling exercise (98). Although a EMG was not used in the present study, the inactive limb was continuously monitored to ensure that it was not contracting throughout each exercise test and every effort was made to ensure the arm remained in a rested state.

Future Directions

Future research is worthwhile to expand our understanding of the influence of respiratory muscle fatigue on blood flow distribution during dynamic exercise. First, it would be interesting

to determine the influence of respiratory muscle fatigue on both active and inactive muscle blood flow concurrently during dynamic exercise. Does respiratory muscle fatigue lead to similar or different reductions in both inactive and active muscle blood flow? If so, under what conditions or subject populations? Why did some subjects not respond to the sympathetic outflow elicited by the respiratory muscle fatigue and not redistribute inactive limb blood flow? Specifically, how do factors such as critical power and the respiratory compensation point contribute to respiratory muscle fatigue? If respiratory muscle fatigue is alleviated, via NAC, does this eliminate the blood flow redistribution of both inactive and active muscle blood flow? If so, can NAC assist in improving exercise tolerance? Furthermore, it would be interesting to determine the influence of temperature on both inactive and cutaneous blood flow during exercise. Specifically, does high external temperature lead to increased inactive blood flow and cutaneous blood flow despite the influence of respiratory muscle fatigue? Conversely, does a low external temperature lead to a reduced inactive muscle and cutaneous blood flow possibly reducing respiratory muscle fatigue? Finally, subject selection is important in future work because the present study only involved healthy college-aged men subjects. Would women, older individuals, or diseased populations (i.e. COPD, CHF) have similar reductions in inactive blood flow? When the work of breathing is increased as in a diseased populations (COPD, CHF), does exercise in a hypoxic environment lead to greater reductions in both inactive and active muscle blood flow? The results from the present study certainly open the possibility of many additional questions to be addressed.

Summary

Respiratory muscle fatigue may limit exercise performance via several different mechanisms. Much research of our understanding on the effects of respiratory muscle fatigue on blood flow distribution and vascular conductance have come from studies performed at rest. We have shown for the first time that respiratory muscle fatigue during whole body heavy exercise also reduces inactive limb blood flow and vascular conductance. The present study in combination with previous studies supports the premise that respiratory muscle fatigue during heavy exercise leads to redistribution of both active and inactive vascular beds towards the respiratory muscles. These findings have important implications to both the healthy and clinical populations for exercise tolerance. The present study is also the first to demonstrate that respiratory muscle fatigue did not affect cutaneous blood flow during whole body heavy exercise, which suggests that thermoregulation is not affected by respiratory muscle fatigue. Future research is warranted to determine specific mechanisms and conditions to help explain and extend these findings.

VI. References

1. **Aaron EA, Seow KC, Johnson BD, Dempsey JA.** Oxygen cost of exercise hyperpnea: implications for performance. *J Appl Physiol* 72: 5: 1818-1825, 1992.

2. **Amann M, Calbet JA.** Convective oxygen transport and fatigue. *J Appl Physiol* 104: 3: 861-870, 2008.

3. **Andersen LL, Blangsted AK, Nielsen PK, Hansen L, Vedsted P, Sjøgaard G, Søgaard K.** Effect of cycling on oxygenation of relaxed neck/shoulder muscles in women with and without chronic pain. *Eur J Appl Physiol* 110: 2: 389-394, 2010.

4. **Andersen LL, Kjaer M, Sögaard K, Hansen L, Kryger AI, Sjögaard G.** Effect of two contrasting types of physical exercise on chronic neck muscle pain. 59: 1: 84-91, 2008.

5. **Babcock M, Pegelow D, McClaran S, Suman O, Dempsey J.** Contribution of diaphragmatic power output to exercise-induced diaphragm fatigue. *J Appl Physiol* 78: 5: 1710-1719, 1995.

6. **Babcock MA, Johnson BD, Pegelow DF, Suman OE, Griffin D, Dempsey JA.** Hypoxic effects on exercise-induced diaphragmatic fatigue in normal healthy humans. *J Appl Physiol* 78: 1: 82-92, 1995.

7. **Babcock MA, Pegelow DF, Harms CA, Dempsey JA.** Effects of respiratory muscle unloading on exercise-induced diaphragm fatigue. *J Appl Physiol* 93: 1: 201-206, 2002.

8. **Babcock MA, Pegelow DF, Johnson BD, Dempsey JA.** Aerobic fitness effects on exercise-induced low-frequency diaphragm fatigue. *J Appl Physiol* 81: 5: 2156-2164, 1996.

9. **Bellemare F, Grassino A.** Effect of pressure and timing of contraction on human diaphragm fatigue. *J Appl Physiol* 53: 5: 1190-1195, 1982.

10. **Bevegaard BS, Shepherd JT.** Reaction in man of resistance and capacity vessels in forearm and hand to leg exercise. *J Appl Physiol* 21: 1: 123-132, 1966.

11. **Bishop J, Donald K, Taylor S, Wormald P.** The blood flow in the human arm during supine leg exercise. *J Physiol (Lond)* 137: 2: 294-308, 1957.

12. **Blair DA, Glover WE, Roddie JC.** Vasomotor responses in the human arm during leg exercise. *Circ Res* 9: 2: 264-274, 1961.

13. **Brengelmann GL, Johnson JM, Hermansen L, Rowell LB.** Altered control of skin blood flow during exercise at high internal temperatures. *J Appl Physiol* 43: 5: 790-794, 1977.

14. **Coast JR, Krause KM.** Relationship of oxygen consumption and cardiac output to work of breathing. *Med Sci Sports Exerc* 25: 335-335, 1993.

15. **Cordain L, Rode E, Gotshall R, Tucker A.** Residual lung volume and ventilatory muscle strength changes following maximal and submaximal exercise. *Int J Sports Med* 15: 03: 158-161, 2008.

16. **Corn SD, Barstow TJ.** Effects of oral N-acetylcysteine on fatigue, critical power, and< i> W</i>′ in exercising humans. 178: 2: 261-268, 2011.

17. **Crapo RO, Morris AH, Gardner RM.** Reference values for pulmonary tissue volume, membrane diffusing capacity, and pulmonary capillary blood volume. *Bull Eur Physiopathol Respir* 18: 6: 893-899, 1982.

18. **Davies KJ, Quintanilha AT, Brooks GA, Packer L.** Free radicals and tissue damage produced by exercise. *Biochem Biophys Res Commun* 107: 4: 1198-1205, 1982.

19. **Dekerle J, Baron B, Dupont L, Vanvelcenaher J, Pelayo P.** Maximal lactate steady state, respiratory compensation threshold and critical power. *Eur J Appl Physiol* 89: 3-4: 281-288, 2003.

20. **Derchak PA, Sheel AW, Morgan BJ, Dempsey JA.** Effects of expiratory muscle work on muscle sympathetic nerve activity. *J Appl Physiol* 92: 4: 1539-1552, 2002.

21. **Dodd D, Collett P, Engel L.** Influence of inspiratory flow rate and frequency on O2 cost of resistive breathing in humans. *J Appl Physiol* 65: 2: 760-766, 1988.

22. **Dodd D, Yarom J, Loring S, Engel L.** O2 cost of inspiratory and expiratory resistive breathing in humans. *J Appl Physiol* 65: 6: 2518-2523, 1988.

23. **Downey AE, Chenoweth LM, Townsend DK, Ranum JD, Ferguson CS, Harms CA.** Effects of inspiratory muscle training on exercise responses in normoxia and hypoxia. 156: 2: 137-146, 2007.

24. **Edwards R, Faulkner J.** Structure and function of the respiratory muscles. 85: 185-185, 1995.

25. **Ferreira LF, Reid MB.** Muscle-derived ROS and thiol regulation in muscle fatigue. *J Appl Physiol* 104: 3: 853-860, 2008.

26. **Gandevia S, Killian K, Campbell E.** The effect of respiratory muscle fatigue on respiratory sensations. *Clin Sci* 60: 463-466, 1981.

27. **Goldman MD, Grimby G, Mead J.** Mechanical work of breathing derived from rib cage and abdominal VP partitioning. *J Appl Physiol* 41: 5: 752-763, 1976.

28. **González-Alonso J, Teller C, Andersen SL, Jensen FB, Hyldig T, Nielsen B.** Influence of body temperature on the development of fatigue during prolonged exercise in the heat. *J Appl Physiol* 86: 3: 1032-1039, 1999.

29. **Green DJ, Bilsborough W, Naylor LH, Reed C, Wright J, O'Driscoll G, Walsh JH.** Comparison of forearm blood flow responses to incremental handgrip and cycle ergometer exercise: relative contribution of nitric oxide. *J Physiol (Lond)* 562: 2: 617-628, 2005.

30. **Green D, Cheetham C, Mavaddat L, Watts K, Best M, Taylor R, O'Driscoll G.** Effect of lower limb exercise on forearm vascular function: contribution of nitric oxide. 283: 3: H899-H907, 2002.

31. **Green D, Cheetham C, Reed C, Dembo L, O'Driscoll G.** Assessment of brachial artery blood flow across the cardiac cycle: retrograde flows during cycle ergometry. *J Appl Physiol* 93: 1: 361-368, 2002.

32. **Grimby G, Goldman M, Mead J.** Respiratory muscle action inferred from rib cage and abdominal VP partitioning. *J Appl Physiol* 41: 5: 739-751, 1976.

33. **Gudjonsdottir M, Appendini L, Baderna P, Purro A, Patessio A, Vilianis G, Pastorelli M, Sigurdsson S, Donner C.** Diaphragm fatigue during exercise at high altitude: the role of hypoxia and workload. 17: 4: 674-680, 2001.

34. **Guenette JA, Vogiatzis I, Zakynthinos S, Athanasopoulos D, Koskolou M, Golemati S, Vasilopoulou M, Wagner HE, Roussos C, Wagner PD.** Human respiratory muscle blood flow measured by near-infrared spectroscopy and indocyanine green. *J Appl Physiol* 104: 4: 1202-1210, 2008.

35. **Harms CA, Babcock MA, McClaran SR, Pegelow DF, Nickele GA, Nelson WB, Dempsey JA.** Respiratory muscle work compromises leg blood flow during maximal exercise. *J Appl Physiol* 82: 5: 1573-1583, 1997.

36. **Harms CA, Wetter TJ, Croix CMS, Pegelow DF, Dempsey JA.** Effects of respiratory muscle work on exercise performance. *J Appl Physiol* 89: 1: 131-138, 2000.

37. **Harms CA, Wetter TJ, McClaran SR, Pegelow DF, Nickele GA, Nelson WB, Hanson P, Dempsey JA.** Effects of respiratory muscle work on cardiac output and its distribution during maximal exercise. *J Appl Physiol* 85: 2: 609-618, 1998.

38. **Hill JM.** Discharge of group IV phrenic afferent fibers increases during diaphragmatic fatigue. *Brain Res* 856: 1: 240-244, 2000.

39. **Huang CH, Martin AD, Davenport PW.** Effect of inspiratory muscle strength training on inspiratory motor drive and RREP early peak components. *J Appl Physiol* 94: 2: 462-468, 2003.

40. **Inbar O, Weiner P, Azgad Y, Rotstein A, Weinstein Y.** Specific inspiratory muscle training in well-trained endurance athletes. *Med Sci Sports Exerc* 32: 7: 1233-1237, 2000.

41. **Jammes Y, Balzamo E.** Changes in afferent and efferent phrenic activities with electrically induced diaphragmatic fatigue. *J Appl Physiol* 73: 3: 894-902, 1992.

42. **Johnson JM, Proppe DW.** Cardiovascular adjustments to heat stress. 2011.

43. **Johnson JM, Rowell LB.** Forearm skin and muscle vascular responses to prolonged leg exercise in man. *J Appl Physiol* 39: 6: 920-924, 1975.

44. **Johnson BD, Babcock MA, Suman OE, Dempsey JA.** Exercise-induced diaphragmatic fatigue in healthy humans. *J Physiol* 460: 385-405, 1993.

45. **Katayama K, Iwamoto E, Ishida K, Koike T, Saito M.** Inspiratory muscle fatigue increases sympathetic vasomotor outflow and blood pressure during submaximal exercise. 302: 10: R1167-R1175, 2012.

46. **Katayama K, Yamashita S, Ishida K, Iwamoto E, Koike T, Saito M.** Hypoxic effects on sympathetic vasomotor outflow and blood pressure during exercise with inspiratory resistance. 304: 5: R374-R382, 2013.

47. **Kellogg D, Johnson J, Kenney W, Pergola P, Kosiba W.** Mechanisms of control of skin blood flow during prolonged exercise in humans. 265: 2: H562-H568, 1993.

48. **Kellogg D, Johnson J, Kosiba W.** Competition between cutaneous active vasoconstriction and active vasodilation during exercise in humans. 261: 4: H1184-H1189, 1991.

49. **Kellogg D, Johnson JM, Kosiba W.** Control of internal temperature threshold for active cutaneous vasodilation by dynamic exercise. *J Appl Physiol* 71: 6: 2476-2482, 1991.

50. **Kelly MK, Wicker RJ, Barstow TJ, Harms CA.** Effects of N-acetylcysteine on respiratory muscle fatigue during heavy exercise. 165: 1: 67-72, 2009.

51. **Knight DR, Poole DC, Schaffartzik W, Guy HJ, Prediletto R, Hogan MC, Wagner PD.** Relationship between body and leg VO2 during maximal cycle ergometry. *J Appl Physiol* 73: 3: 1114-1121, 1992.

52. **Lew H, Pyke S, Quintanilha A.** Changes in the glutathione status of plasma, liver and muscle following exhaustive exercise in rats. *FEBS Lett* 185: 2: 262-266, 1985.

53. **Lieberman DA, Faulkner JA, Craig A, Maxwell L.** Performance and histochemical composition of guinea pig and human diaphragm. *J Appl Physiol* 34: 2: 233-237, 1973.

54. **Loke J, Mahler DA, Virgulto JA.** Respiratory muscle fatigue after marathon running. *J Appl Physiol* 52: 4: 821-824, 1982.

55. **Luo Y, Hart N, Mustfa N, Lyall R, Polkey M, Moxham J.** Effect of diaphragm fatigue on neural respiratory drive. *J Appl Physiol* 90: 5: 1691-1699, 2001.

56. **Mador MJ, Acevedo FA.** Effect of respiratory muscle fatigue on subsequent exercise performance. *J Appl Physiol* 70: 5: 2059-2065, 1991.

57. **Mador MJ, Magalang UJ, Rodis A, Kufel TJ.** Diaphragmatic fatigue after exercise in healthy human subjects. 148: 6 Pt 1: 1571-1575, 1993.

58. **Manohar M.** Blood flow to the respiratory and limb muscles and to abdominal organs during maximal exertion in ponies. *J Physiol (Lond)* 377: 1: 25-35, 1986.

59. **McConnell A, Caine M, Sharpe G.** Inspiratory muscle fatigue following running to volitional fatigue: the influence of baseline strength. *Int J Sports Med* 18: 03: 169-173, 2007.

60. **McCool FD, Tzelepis GE, Leith D, Hoppin F.** Oxygen cost of breathing during fatiguing inspiratory resistive loads. *J Appl Physiol* 66: 5: 2045-2055, 1989.

61. **Musch TI.** Elevated diaphragmatic blood flow during submaximal exercise in rats with chronic heart failure. *Am J Physiol* 265: 5 Pt 2: H1721-6, 1993.

62. **Nadel ER, Fortney SM, Wenger CB.** Effect of hydration state of circulatory and thermal regulations. *J Appl Physiol* 49: 4: 715-721, 1980.

63. **National Heart, Lung, and Blood Institute.** NHLBI workshop summary: respiratory muscle fatigue; report of the Respiratory Muscle Fatigue Workshop Group. *Am Rev Respir Dis* 142: 474-480, 1990.

64. **Nose H, Mack GW, Shi X, Morimoto K, Nadel ER.** Effect of saline infusion during exercise on thermal and circulatory regulations. *J Appl Physiol* 69: 2: 609-616, 1990.

65. **Ooue A, Ichinose TK, Inoue Y, Nishiyasu T, Koga S, Kondo N.** Changes in blood flow in conduit artery and veins of the upper arm during leg exercise in humans. *Eur J Appl Physiol* 103: 3: 367-373, 2008.

66. **Ozkaplan A, Rhodes EC, Sheel AW, Taunton JE.** A comparison of inspiratory muscle fatigue following maximal exercise in moderately trained males and females. *Eur J Appl Physiol* 95: 1: 52-56, 2005.

67. **Padilla J, Simmons GH, Vianna LC, Davis MJ, Laughlin MH, Fadel PJ.** Brachial artery vasodilatation during prolonged lower limb exercise: role of shear rate. *Exp Physiol* 96: 10: 1019-1027, 2011.

68. **Perret C, Spengler CM, Egger G, Boutellier U.** Influence of endurance exercise on respiratory muscle performance. *Med Sci Sports Exerc* 32: 12: 2052-2058, 2000.

69. **Poole DC, Gaesser GA, Hogan MC, Knight DR, Wagner PD.** Pulmonary and leg VO2 during submaximal exercise: implications for muscular efficiency. *J Appl Physiol* 72: 2: 805-810, 1992.

70. **Poole DC, Sexton WL, Behnke BJ, Ferguson CS, Hageman KS, Musch TI.** Respiratory muscle blood flows during physiological and chemical hyperpnea in the rat. *J Appl Physiol* 88: 1: 186-194, 2000.

71. **Poole DC, Wilkerson DP, Jones AM.** Validity of criteria for establishing maximal O 2 uptake during ramp exercise tests. *Eur J Appl Physiol* 102: 4: 403-410, 2008.

72. **Reid MB, Haack KE, Franchek KM, Valberg PA, Kobzik L, West MS.** Reactive oxygen in skeletal muscle. I. Intracellular oxidant kinetics and fatigue in vitro. *J Appl Physiol* 73: 5: 1797-1804, 1992.

73. **Robertson Jr CH, Foster GH, Johnson Jr R.** The relationship of respiratory failure to the oxygen consumption of, lactate production by, and distribution of blood flow among respiratory muscles during increasing inspiratory resistance. *J Clin Invest* 59: 1: 31, 1977.

74. **Rodman JR, Henderson KS, Smith CA, Dempsey JA.** Cardiovascular effects of the respiratory muscle metaboreflexes in dogs: rest and exercise. *J Appl Physiol* 95: 3: 1159-1169, 2003.

75. **Romer LM, Lovering AT, Haverkamp HC, Pegelow DF, Dempsey JA.** Effect of inspiratory muscle work on peripheral fatigue of locomotor muscles in healthy humans. *J Physiol (Lond)* 571: 2: 425-439, 2006.

76. **Romer LM, McCONNELL AK, Jones DA.** Effects of inspiratory muscle training on time-trial performance in trained cyclists. *J Sports Sci* 20: 7: 547-590, 2002.

77. **Romer LM, Polkey MI.** Exercise-induced respiratory muscle fatigue: implications for performance. *J Appl Physiol* 104: 3: 879-888, 2008.

78. **Ross E, Middleton N, Shave R, George K, McConnell A.** Changes in respiratory muscle and lung function following marathon running in man. *J Sports Sci* 26: 12: 1295-1301, 2008.

79. **Roussos C, MacKlem PT.** Inspiratory muscle fatigue. 1985.

80. **Rowell LB, O'Leary DS, Kellogg DL.** Integration of cardiovascular control systems in dynamic exercise. 1996.

81. **Rowell LB.** Human cardiovascular adjustments to exercise and thermal stress. *Physiol Rev* 54: 1: 75-159, 1974.

82. **Saltin B, Rådegran G, Koskolou M, Roach R.** Skeletal muscle blood flow in humans and its regulation during exercise. *Acta Physiol Scand* 162: 3: 421-436, 1998.

83. **Saumet JL, Kellogg D, Taylor WF, Johnson JM.** Cutaneous laser-Doppler flowmetry: influence of underlying muscle blood flow. *J Appl Physiol* 65: 1: 478-481, 1988.

84. **Secher NH, Clausen JP, Klausen K, Noer I, Trap-Jensen J.** Central and regional circulatory effects of adding arm exercise to leg exercise. *Acta Physiol Scand* 100: 3: 288-297, 1977.

85. **Sen CK, Atalay M, Hanninen O.** Exercise-induced oxidative stress: glutathione supplementation and deficiency. *J Appl Physiol* 77: 5: 2177-2187, 1994.

86. **Sheel AW, Derchak PA, Pegelow DF, Dempsey JA.** Threshold effects of respiratory muscle work on limb vascular resistance. 282: 5: H1732-H1738, 2002.

87. **Sheel AW, Derchak PA, Morgan BJ, Pegelow DF, Jacques AJ, Dempsey JA.** Fatiguing inspiratory muscle work causes reflex reduction in resting leg blood flow in humans. *J Physiol* 537: Pt 1: 277-289, 2001.

88. **Shephard R.** The oxygen cost of breathing during vigorous exercise. *Exp Physiol* 51: 4: 336-350, 1966.

89. **Shindoh C, DiMarco A, Thomas A, Manubay P, Supinski G.** Effect of N-acetylcysteine on diaphragm fatigue. *J Appl Physiol* 68: 5: 2107-2113, 1990.

90. **Shiroishi K, Kime R, Osada T, Murase N, Shimomura K, Katsumura T.** Decreased muscle oxygenation and increased arterial blood flow in the non-exercising limb during leg exercise. In: *Oxygen Transport to Tissue XXXI*Anonymous Springer, 2010, p. 379-384.

91. **Simmons GH, Padilla J, Young CN, Wong BJ, Lang JA, Davis MJ, Laughlin MH, Fadel PJ.** Increased brachial artery retrograde shear rate at exercise onset is abolished during prolonged cycling: role of thermoregulatory vasodilation. *J Appl Physiol* 110: 2: 389-397, 2011.

92. **Simmons GH, Wong BJ, Holowatz LA, Kenney WL.** Changes in the control of skin blood flow with exercise training: where do cutaneous vascular adaptations fit in? *Exp Physiol* 96: 9: 822-828, 2011.

93. **Sonetti DA, Wetter TJ, Pegelow DF, Dempsey JA.** Effects of respiratory muscle training versus placebo on endurance exercise performance. *Respir Physiol* 127: 2: 185-199, 2001.

94. **St Croix CM, Morgan BJ, Wetter TJ, Dempsey JA.** Fatiguing inspiratory muscle work causes reflex sympathetic activation in humans. *J Physiol* 529 Pt 2: 493-504, 2000.

95. **Supinski G, Clary S, Bark H, Kelsen S.** Effect of inspiratory muscle fatigue on perception of effort during loaded breathing. *J Appl Physiol* 62: 1: 300-307, 1987.

96. **Supinski G, Stofan D, Ciufo R, DiMarco A.** N-acetylcysteine administration alters the response to inspiratory loading in oxygen-supplemented rats. *J Appl Physiol* 82: 4: 1119-1125, 1997.

97. **Suzuki S, Sato M, Okubo T.** Expiratory muscle training and sensation of respiratory effort during exercise in normal subjects. *Thorax* 50: 4: 366-370, 1995.

98. **Tanaka H, Shimizu S, Ohmori F, Muraoka Y, Kumagai M, Yoshizawa M, Kagaya A.** Increases in blood flow and shear stress to nonworking limbs during incremental exercise. *Med Sci Sports Exerc* 38: 1: 81, 2006.

99. **Taylor BJ, How SC, Romer LM.** Exercise-induced abdominal muscle fatigue in healthy humans. *J Appl Physiol* 100: 5: 1554-1562, 2006.

100. **Taylor BJ, Romer LM.** Effect of expiratory muscle fatigue on exercise tolerance and locomotor muscle fatigue in healthy humans. *J Appl Physiol* 104: 5: 1442-1451, 2008.

101. **Taylor JA, Hand GA, Johnson DG, Seals DR.** Augmented forearm vasoconstriction during dynamic exercise in healthy older men. *Circulation* 86: 6: 1789-1799, 1992.

102. **Verges S, Sager Y, Erni C, Spengler C.** Expiratory muscle fatigue impairs exercise performance. *Eur J Appl Physiol* 101: 2: 225-232, 2007.

103. **Verges S, Schulz C, Perret C, Spengler CM.** Impaired abdominal muscle contractility after high-intensity exhaustive exercise assessed by magnetic stimulation. *Muscle Nerve* 34: 4: 423-430, 2006.

104. **Vogiatzis I, Athanasopoulos D, Boushel R, Guenette JA, Koskolou M, Vasilopoulou M, Wagner H, Roussos C, Wagner PD, Zakynthinos S.** Contribution of respiratory muscle blood flow to exercise-induced diaphragmatic fatigue in trained cyclists. *J Physiol (Lond)* 586: 22: 5575-5587, 2008.

105. **Vogiatzis I, Georgiadou O, Giannopoulou I, Koskolou M, Zakynthinos S, Kostikas K, Kosmas E, Wagner H, Peraki E, Koutsoukou A.** Effects of exercise-induced arterial

hypoxaemia and work rate on diaphragmatic fatigue in highly trained endurance athletes. *J Physiol (Lond)* 572: 2: 539-549, 2006.

106. **Vogiatzis I, Georgiadou O, Koskolou M, Athanasopoulos D, Kostikas K, Golemati S, Wagner H, Roussos C, Wagner PD, Zakynthinos S.** Effects of hypoxia on diaphragmatic fatigue in highly trained athletes. *J Physiol (Lond)* 581: 1: 299-308, 2007.

107. **Volianitis S, McConnell AK, Jones DA.** Assessment of maximum inspiratory pressure. *Respiration* 68: 1: 22-27, 2001.

108. **Volianitis S, McConnell AK, Koutedakis Y, McNaughton L, Backx K, Jones DA.** Inspiratory muscle training improves rowing performance. *Med Sci Sports Exerc* 33: 5: 803-809, 2001.

109. **Ward M, Eidelman D, Stubbing D, Bellemare F, Macklem P.** Respiratory sensation and pattern of respiratory muscle activation during diaphragm fatigue. *J Appl Physiol* 65: 5: 2181-2189, 1988.

110. **West JB.** *Respiratory physiology: the essentials.* Lippincott Williams & Wilkins, 2008.

111. **Wetter TJ, Harms CA, Nelson WB, Pegelow DF, Dempsey JA.** Influence of respiratory muscle work on VO(2) and leg blood flow during submaximal exercise. *J Appl Physiol* 87: 2: 643-651, 1999.