EFFECTS OF DIETARY FISH OIL ON SKELETAL MUSCLE VASCULAR CONTROL IN CHRONIC HEART FAILURE RATS: REST AND EXERCISE

by

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Abstract

Impaired vasomotor control in chronic heart failure (CHF) limits the delivery of O_2 to skeletal muscle during exercise. Previous results demonstrate significant increases in skeletal muscle blood flow (BF) during exercise with omega-3 polyunsaturated fatty acid (PUFA) supplementation via fish oil (FO) versus safflower oil (SO) in healthy rats (Stebbins CL et al., Int J Sport Nutr Exerc Metab 20:475-86, 2010). Whether PUFA supplementation with FO will improve vasomotor control in CHF and skeletal muscle BF during exercise remains to be determined. This investigation tested the hypothesis that PUFA supplementation with FO would augment the skeletal muscle BF response to exercise in rats with CHF when compared to SO. CHF was induced in male Sprague-Dawley rats by myocardial infarction produced via left coronary artery ligation. Rats were then randomized to dietary FO (20% docosahexaenoic acid and 30% eicosapentaenoic acid, n = 8) or SO (5% safflower, n = 6) supplementation for 6 weeks. Rats remained on their respective diets until final experiments were conducted. Following acute instrumentation and recovery (> 1 hour), mean arterial pressure (MAP), skeletal muscle BF to the total hindlimb and individual muscles (via radiolabeled microspheres), and blood lactate concentration were determined during rest, submaximal treadmill exercise and exercise+LNAME (20 $m \cdot min^{-1}$, 5% incline). Left ventricular end-diastolic pressure (LVEDP) measured in the SO and FO groups during instrumentation were similar and demonstrated moderate CHF (LVEDP; SO: 14 ± 2; FO: 11 ± 1 mmHg, $P>0.05$). During submaximal exercise, MAP (SO: 128 ± 3; FO: 132 ± 3 mmHg) and blood lactate (SO: 3.8 ± 0.4; FO: 4.6 ± 0.5 $mmol \cdot l^{-1}$) were similar ($P>0.05$) between groups. Exercising hindlimb skeletal muscle BF was higher in SO compared to FO (SO: 120 ± 11; FO: 93 ± 4 $ml \cdot min^{-1} \cdot 100\ g^{-1}$). Specifically, 17 of 28 individual hindlimb muscle BF's were higher ($P<0.05$) in SO. These data suggest that PUFA supplementation with FO in rats with moderate CHF decreases the skeletal muscle BF response to submaximal whole body exercise.

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Chapter 1 - Introduction

The relationship between ω-3 polyunsaturated fatty acid (PUFA) intake and a reduced incidence of mortality associated with cardiovascular disease has incited significant clinical interest (Bonaa, 1989). The primary focus has been on the cardioprotective effects of PUFAs with particular concern for mechanisms explaining the anti-arrhythmic properties they exhibit in cardiovascular disease (Marchioloi, 2002; Kromhout, 2010; Tavazzi, 2008; Yokoyama, 2007). A relatively unexplored, yet potentially key component of PUFA utilization is their role outside of the central cardiac domain.

Dietary supplementation with PUFAs augments blood flow (BF) through conduit vessels supplying healthy human forearm muscle via increases in vascular conductance (VC; Walser, 2006), likely consequent to an increase in endothelial nitric oxide synthase (eNOS) and NO bioavailability (Stebbins, 2008). However, limiting the focus to conduit vessels ignores highly heterogeneous muscle BF distribution which varies according to muscle function and/or fiber type composition independent of upstream conduit vessel regulation. In this regard, our laboratory utilized radiolabelled microspheres during submaximal treadmill exercise to demonstrate that PUFA supplementation significantly augmented rat hindlimb muscle BF with the increases occurring principally within muscles and muscle portions composed of predominantly type I and IIa fibers (Stebbins, 2010).

The recent findings associated with chronic PUFA administration found in healthy individuals raises important implications for cardiovascular disease populations, particularly patients with chronic heart failure (CHF). In addition to their obvious decrements in cardiac performance, patients can present with significant endothelial dysfunction and depressed vasomotor control which are important mechanisms responsible for the impaired hyperemic response to exercise characteristic of CHF (Katz, 2005; McAllister, 1993). Consequences of the failure to appropriately redistribute BF during exercise are a greater reliance on substrate level phosphorylation and glycogenolysis, partially accounting for the fatigue and exercise intolerance emblematic of this population (Poole, 2012). Unfortunately, the diminished capacity to perform work severely impacts the efficacy of exercise rehabilitation; a primary therapeutic modality in the treatment of CHF.

The deranged skeletal muscle BF distribution found in CHF may be a consequence of decrements in NO-mediated vasodilation (Hirai, 1995). Thus, changes in NO bioavailability underlie both the etiology of CHF and the therapeutic effect of PUFAs. This presents an intriguing non-pharmacological potential for improving the exercising skeletal muscle BF response in CHF.

Therefore, the purpose of the present study was to test the hypothesis that PUFA supplementation via dietary fish oil (FO) would increase exercising skeletal muscle BF and VC in a rat model of CHF. Additionally, due to the evidence for a PUFA induced increase in eNOS activity, we expected higher BF and VC to be related to an improved NO-mediated vasodilation.

Chapter 2 - Methods

Animal selection and care

25 adult male Sprague-Dawley rats (initial body mass = ~275g) were maintained in accredited animal facilities at Kansas State University on a 12hr/12hr light-dark cycle with food and water provided ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee of Kansas State University under the guidelines established by the National Institutes of Health.

Myocardial infarction procedures

All rats underwent induction of a myocardial infarction (MI) via ligation of the left main coronary artery which has been shown to result reliably in the development of CHF (Musch, 1992). Briefly, each rat was anesthetized with a gas mixture of 5% isoflurane-O_2 and intubated for mechanical ventilation on a rodent respirator (model 680, Harvard Instruments, Holliston, MA) with subsequent maintenance on a 3% isoflurane-O_2 gas mixture for the duration of the procedure. A left thoracotomy was performed to expose the heart through the fifth intercostal space. Exteriorization of the heart provided access to the left main coronary artery which was ligated with a 6-0 silk suture ~1-2 mm distal to the edge of the left atrium. The muscles of the thorax were then closed with 4-0 gut, and the skin incision was closed with 3-0 silk followed by an administration of the analgesic agents bupivacaine (1.5 $mg \cdot kg^{-1}$ subcutaneously) and buprenorphine (0.01–0.05 $mg \cdot kg^{-1}$ i.m.) as well as ampicillin (50 $mg \cdot kg^{-1}$, i.m.) to reduce the risk of infection. Upon removal from mechanical ventilation and withdrawal of anesthesia the rats were monitored ~8-12 hrs post-operatively for the development of arrhythmias and undue stress with care administered as needed. The recovery duration prior to pre-intervention performance testing was ≥21 days which is consistent with the time course for complete remodeling of necrotic myocardial tissue (Fishbein, 1978). During this time the rats were monitored daily (appetite, weight loss, gait/posture, etc.) according to a plan conducted in conjunction with the university veterinary staff.

Treadmill acclimatization

A familiarization period was used to acclimate the rats to high speed running. It was comprised of 5-7 sessions on a custom built motor-driven treadmill set at an incline of 5%. Each session involved progressive increases in treadmill speed from ~20 $m \cdot min^{-1}$ to ~45 $m \cdot min^{-1}$ over a total duration of no more than 5 min.

Exercise performance testing

Rats were evaluated both pre- and post-diet for maximal oxygen uptake (O_{2peak}) and endurance capacity. The test sequence was randomized to avoid an ordering effect, and a minimum of 24 h was maintained between sessions. O_{2peak} was determined using a protocol shown to elicit highly reproducible VO_{2peak} measurements in untrained rats (Musch, 1988; Copp, 2009). Briefly, each rat was placed inside a single stall metabolic chamber on the motorized treadmill. Ambient air was drawn, in sequence, through the chamber, Dririte and a flow meter (Fischer-Porter, model 10A1378, Burr Ridge, IL) at a rate of 5000 $ml \cdot min^{-1}$ via a vacuum pump (Neptune-Dyna, model 4K, Dover, NJ). Effluent gas from the chamber was continuously measured for O_2 and CO_2 percentages by online O_2 and CO_2 analyzers (AEI Technologies, models S-3A/I and CD-3A, Pittsburg, PA) calibrated using gravimetrically-analyzed gas concentrations. O_2 and CO_2 were calculated according to the method described in Brooks and White (1978). The protocol consisted of increasing treadmill speed by ~5-10 $m \cdot min^{-1}$ in a ramp fashion while monitoring and recording O_2 until a plateau was observed and/or the rat was unable to keep pace with the treadmill. Total test duration was ~5-8 min, and the criterion for a valid test required satisfying one of the following conditions; 1) a plateau in O_2 values despite an increasing treadmill speed or 2) an alteration in gait immediately preceding the termination of the test. For a respiratory exchange ratio of <1.0 neither of the above criteria could independently validate the test and a second trial was repeated after a minimum of 24 h.

The endurance capacity protocol was an incremental exercise test on the motorized treadmill set at a 5% grade. The rat was brought to an initial speed of 25 $m \cdot min^{-1}$ for the first 15 min. At each 15 min interval the speed was increased by 5 $m \cdot min^{-1}$ until exhaustion (i.e. the inability to move off the back of the treadmill lane despite encouragement from manual bursts of high pressure air towards the hindlimbs). Considerations for a valid test included the animal displaying a dramatically altered gait involving lowered hindquarters and raised snout.

Additionally, immediately upon completion of the test the investigator evaluated the righting reflex by placing the rat in the palm of their hand in the supine position. Failure to elicit the righting reflex in ~10 seconds was regarded as an indicator of exhaustion.

Dietary supplementation

Upon completion of the pre-diet testing all rats were allocated randomly to either the experimental group fed a FO diet (n = 15), or to the control group fed a safflower oil (SO) diet (n = 10). The diet for both groups contained the following in grams·kg^{-1}: casein 225, cornstarch 446, sucrose 223, cellulose 31, DL-methionine 1, standard mineral mix 14, standard vitamin mix 10, oil 50, and butylhydroquinone 0.08. Included in the control diet was 5% SO which has previously been shown to have no significant effect on the hemodynamic variables of BF, VC, mean arterial pressure (MAP) or cardiac output during exercise (Walser et al. 2006). The FO diet included 5% menhaden oil of which ~20% is docosahexaenoic acid and ~30% is eicosapentaenoic acid. The diets were isocaloric with an administration period of 6-8 weeks.

Surgical instrumentation

On the day of the final protocol the rats were anesthetized with a 5% isoflurane-O_2 mixture and maintained on a 3% isoflurane-O_2 mixture for the duration of the surgical instrumentation. The carotid artery was cannulated and a two-French-catheter-tipped pressure transducer (Millar Instruments, Houston, TX) was advanced into the left ventricle (LV) for the measurement of left ventricular end diastolic pressure (LVEDP). Subsequently, cannulation of both the carotid and caudal arteries was performed with PE-10 connected to PE-50 (Intra-Medic polyethylene tubing, Clay Adams, Spark, MD). The catheters were then tunneled subcutaneously to the dorsal aspect of the cervical region where they were exteriorized through a puncture wound in the skin. Following closure of incisions the rat was removed from anesthesia and given a minimum recovery period of 2 h.

Subsequent to the recovery period the final protocol was performed with the treadmill set at an incline of 5%. The rat was placed on the treadmill and the carotid catheter was attached to a pressure transducer (Gould Statham P23ID) for the measurement of MAP and heart rate (HR) while the caudal catheter was connected to a 1 ml syringe attached to a Harvard pump (model

907, Cambridge, MA). Exercise was initiated at a speed of ~20 $m \cdot min^{-1}$ and remained steady for ~3 min at which time pre-spheres HR and pressures were recorded. At ~3.5 min of total exercise time blood withdrawal was initiated from the caudal catheter at a rate of 0.25 $ml \cdot min^{-1}$. The carotid catheter was then disconnected from the pressure transducer and ~0.5-0.6 $\cdot 10^6$, 15 µm diameter microspheres (^{46}Sc or ^{85}Sr in random order: Perkin Elmer Life and Analytical Sciences, Waltham, MA) were injected into the aortic arch of the continuously exercising animal for the determination of tissue BF. Upon reconnection of the carotid catheter to the pressure transducer a second MAP reading was immediately recorded post-microspheres. An arterial blood sample (0.2 ml) was then drawn from the carotid artery catheter for the determination of blood gases, hematocrit, pH, and [lactate]. Exercise was terminated and the rat was continuously monitored during a minimum 30 min rest period before the second trial began.

A post-recovery pressure was recorded to establish resting MAP and HR values. The second microsphere injection was performed either at rest (n = 10) or during a second exercise bout (n = 14). In the case of a second exercise bout, the non-isoform specific NOS inhibitor N(G)-nitro-L-arginine-methyl-ester (L-NAME,10 $mg \cdot kg^{-1}$) was infused via the caudal artery catheter for ~10 s. This second exercise condition (ex+L-NAME) was used to evaluate the contribution of NOS-dependent vasodilation to exercise hyperemia. Pressure was recorded every 30 s until the NOS inhibition elicited a persistent rise in MAP at which time the second exercise bout was initiated. The second bout and administration of microspheres were performed identically to the protocol described above. Upon termination of exercise the rat was euthanized with an overdose of pentobarbital (>50 $mg \cdot kg^{-1}$ body weight) via the carotid artery catheter.

Determination of BF and VC

Correct placement of the carotid catheter in the aortic arch was verified by anatomical dissection. After the heart was removed the right ventricle (RV), LV and septum were separated and weighed. Measurement of infarct size in the LV was made via planimetry as described previously (Ferreira, 2006). Hindlimb muscles and muscle portions as well as the lungs, kidneys, and representative organs of the splanchnic region were removed, weighed and placed in counting vials for the determination of radioactivity.

Radioactivity was measured for each tissue on a gamma scintillation counter (Packard Auto Gamma Spectrometer, model 5230). Taking into account the cross-talk fraction between

isotopes, BF to each tissue was determined using the reference sample method described in Musch & Terrell (1992), and tissue BF's were expressed as $ml \cdot min^{-1} \cdot 100\ g^{-1}$ of tissue. The BF results were also normalized to MAP and expressed as VC ($ml \cdot min^{-1} \cdot 100\ g^{-1} \cdot mmHg^{-1}$). Adequate mixing of the microspheres for each BF determination was verified by a <15% difference in BF between the right and left kidneys or right and left hindlimbs.

Statistical analysis

Results were compared within (rest vs. exercise vs. ex+L-NAME) and between (SO vs. FO) groups using mixed 2-way ANOVAs and Student-Newman-Keuls *post hoc* tests where appropriate. Muscle fiber type composition was based on the percentage of type I, type IIa, type IIb, and type IId/x fibers in the individual muscles and muscle parts of the rat hindlimb as reported by Delp & Duan (1996). Significance was set at $P<0.05$, and values are expressed as mean ± SEM.

Chapter 3 - Results

Dietary intake and body mass

2-way ANOVA revealed that pre-diet body mass (SO: 391 ± 24, FO: 370 ± 15 g, $P>0.05$) was not different between groups whereas post-diet body mass was higher in SO rats (SO: 584 ± 32, FO: 513 ± 18 g, $P<0.05$). Diet consumption was not different between groups (SO: 26 ± 2, FO: 28 ± 2 g/day). Post-diet hindlimb muscle mass was not different between groups (SO: 23.5 ± 1.0, FO: 23.0 ± 0.5 g, $P>0.05$) which resulted in a higher post-diet body mass to hindlimb muscle mass ratio in SO rats (SO: 24.8 ± 0.6, FO: 22.3 ± 0.7, $P<0.05$).

Central cardiac and morphological indices of CHF

There was no difference between groups for any of the principal indices of CHF including infarct size, LVEDP, and LV, RV, or lung weight-to-body weight ratio (Table 1).

Effects of FO on exercise performance

Pre-diet O_{2peak} (SO: 79.4 ± 4.0, FO: 84.2 ± 3.8 $ml \cdot kg^{-1} \cdot min^{-1}$) and endurance capacity (SO: 26.5 ± 2.4, FO: 33.8 ± 4.1 min) as well as post-diet O_{2peak} (SO: 62.9 ± 2.5, FO: 63.6 ± 1.8 $ml \cdot kg^{-1} \cdot min^{-1}$) and endurance capacity (SO: 13.8 ± 1.2, FO: 17.5 ± 1.7 min) were not different between groups ($P>0.05$ for all). Moreover, the total work performed during the post-diet endurance capacity tests was not different between groups (SO: 1545 ± 131, FO: 1851 ± 200 J, $P>0.05$).

Effects of FO on HR, MAP, [lactate] and blood gases

HR and MAP at rest, during exercise or during ex+L-NAME were not different between groups (Fig. 1, $P>0.05$ for all). Arterial blood [lactate] was not different between groups at rest (SO: 1.0 ± 0.1, FO: 1.0 ± 0.1 mmol/L, $P>0.05$), during exercise (SO: 3.8 ± 0.4, FO: 4.6 ± 0.5 mmol/L, $P<0.05$) or during ex+L-NAME (SO: 6.5 ± 0.8, FO: 8.1 ± 0.7 mmol/L, $P<0.05$). There

were also no differences in arterial blood pH, PO_2, or PCO_2 between groups at rest, during exercise or during ex+L-NAME (data not shown, $P>0.05$ for all).

Effects of FO on skeletal muscle BF and VC

At rest there were no differences between groups in total hindlimb skeletal muscle BF or VC (Fig. 2) or BF and VC to 27 of 28 individual muscles or muscle portions (exception: anterior portion of the biceps femoris, Tables 2 and 3). During exercise both total hindlimb BF and VC were lower in FO compared to SO (Fig. 2). Specifically, FO resulted in a lower BF in 17, and lower VC in 18, of the 28 individual hindlimb muscles or muscle portions during exercise (Tables 2 and 3). Total hindlimb skeletal muscle BF and VC during ex+L-NAME were not different between groups ($P>0.05$). The absolute and relative reductions in total hindlimb skeletal muscle BF and VC following L-NAME were not different between groups (Fig. 3).

Effects of FO on renal and splanchnic BF and VC at rest and during exercise

The majority of renal and splanchnic organ BF and VC were not different between groups. The exception was a higher adrenal BF and VC at rest in FO compared to SO (Table 4).

Chapter 4 - Discussion

The results presented herein provide novel insight regarding the effects of dietary FO supplementation on exercising skeletal muscle vascular control in a rat model of CHF. The primary findings are 1) FO supplementation resulted in lower BF and VC when compared to SO 2) changes in BF and VC after NOS inhibition via L-NAME did not differ between SO and FO 3) exercise performance and arterial blood [lactate] were not different despite the difference in skeletal muscle BF during submaximal treadmill exercise.

Effects of FO on exercising skeletal muscle BF and VC

Previously, we found PUFA supplementation produced a higher exercising skeletal muscle BF and VC for healthy rats in the absence of changes in MAP or HR (Stebbins, 2010). This was subsequent to evidence demonstrating PUFAs effectiveness in augmenting exercising forearm BF and VC in healthy humans (Walser, 2006). The current data suggests that this higher skeletal muscle BF and VC seen in healthy animals cannot be extended to animals with CHF. On the contrary, in the condition of CHF, PUFA supplementation appears to reduce skeletal muscle BF and VC during submaximal treadmill exercise when compared to SO supplemented controls. The greater exercising skeletal muscle BF seen previously in the healthy rats was attributed to a decrease in vascular resistance given that perfusion pressure (MAP) and HR were unchanged. Consistent with this interpretation we posit that a lack of change in MAP or HR for the CHF rats indicates the lower BF with FO is driven by increases in contracting skeletal muscle vascular resistance. However, the increase in vascular resistance is not due to an impaired vascular function per se. Considering that the body mass to hindlimb muscle mass ratio is lower in FO it is clear that less work is required of the active hindlimb skeletal muscle to achieve the same running speed relative to the heavier SO counterparts. Therefore, with the same muscle mass performing less work it is expected that BF would be lower in FO compared to SO as seen herein. This contention that the adequacy of the exercise induced hyperemic response was similar between groups is supported by no differences in either arterial blood [lactate] during exercise or endurance capacity post-diet. Thus, rather than representing a detrimental decrement in vascular function, the magnitude of the BF and VC response in FO appears to be consistent with SO in attempting to match the energetic demand of the exercising hindlimb skeletal muscle.

It is also noteworthy that FO has been shown to reduce HR and oxygen consumption during steady state submaximal cycle exercise (Peoples, 2008). Although we do not have data for submaximal VO_2, a reduction in oxygen consumption cannot be ruled out to partially explain the decreased BF and VC seen in the current study.

Effect of FO on NOS-derived NO-bioavailability

In the current study NOS-derived NO-bioavailability was not augmented as evidenced by the similar BF and VC between groups during exercise with L-NAME administration. This refutes a role for PUFAs in ameliorating vascular dysfunction via increased NO-bioavailability. In contrast, Tagawa and colleagues (1999) were able to demonstrate that PUFA supplementation can augment forearm vasodilation in patients with coronary artery disease of non-ischemic origin and that inhibition of NO synthesis abolished these improvements. Further work indicated that the mechanism for PUFA induced increases in NO-bioavailability was likely due to elevated eNOS expression (Stebbins, 2008; Okuda, 1997).

These divergent findings may be explained by the differences in cardiovascular disease of ischemic versus non-ischemic origin. Specifically, ischemic insult elicits profound acute inflammation and a complex cardiac remodeling process involving a myriad of signaling molecules. This complexity of dysfunction may explain why the mechanism for upregulation of eNOS expression in cultured human endothelial cells via PUFAs fails to improve NO bioavailability under CHF conditions in vivo. Importantly, the hallmark characteristics of CHF include not only marked reductions in NO bioavailability, but also the presence of a pronounced oxidative stress. This is due partly to NOS uncoupling which results in fundamental alterations to the delicate nitroso-redox balance. In CHF tetrohydrobiopterin (BH_4), the obligatory cofactor for NOS function, is less abundant due to its reduced conversion from dihydrobiopterin (BH_2; Alkaitis, 2012). In the absence of this cofactor, which participates in arginine oxidation, NOS will produce a greater abundance of the free radical superoxide (O_2^-; Xia, 1998). Given this occurence, upregulating eNOS expression, but failing to correct the low BH_4-derived uncoupling may provide for an increase in the production of O_2^- relative to NO. To summarize, while there was no effect of FO supplementation on NO-mediated vasodilation the potential uncoupling of NOS from NO production precludes determination of a FO effect on altering eNOS expression based on the NOS inhibition data (Bauersachs, 1999).

Effects of FO on exercise performance

Contrary to our hypothesis FO did not attenuate CHF-induced exercise intolerance as indicated by measurements of endurance capacity and O_{2peak}. Crucially, the calculation of total work performed during the endurance capacity protocol was similar between groups despite the higher body mass to hindlimb muscle mass in SO compared to FO. As discussed previously, the similar exercise performance despite a lower BF indicates that the sufficiency of the hyperemic response to exercise was not significantly affected by FO. This notion is reinforced by the similar arterial blood [lactate] between groups suggesting that the metabolic perturbations of the active skeletal muscle were not negatively impacted by the lower BF.

An additional consideration beyond vascular control is the multi-system dysfunction present in CHF which collectively impacts exercise performance (Piepoli, 2010(1), Piepoli 2010(2)). Importantly, recent evidence suggests marked decrements in skeletal muscle function including decreased capillary RBC flux, decreased mitochondrial volume and function, and increased myocyte apoptosis (Adams, 1999; Delp, 1997; Poole, 2012; Richardson, 2003). These alterations at the capillary-myocyte interface are likely important determinants of O_{2peak} and exercise capacity.

CHF also impairs the redistribution of BF during exercise in a fiber-type dependent manner with attenuation of exercise hyperemia directly dependent upon the percentage of oxidative fibers (type I and IIa) found in the muscle or muscle portions. Thus, despite overall decrements BF and VC, exercise performance may not have been significantly compromised since the reductions in individual muscles and muscle portions were not driven by fiber type. This indicates that while bulk hindlimb BF and VC were lower a similar distribution of the available O_2 supply was evident between diets.

Experimental considerations

Since epidemiological studies first highlighted the relationship between PUFAs and cardiovascular health, the utilization of FO as a dietary vehicle for PUFA administration has been an investigation of potential clinical significance. The important aspect of the feeding methods was that in the current study consumption was monitored and did not differ between rats, ensuring that the delivery of PUFAs was consistent, as well as being relevant to conventional administration outside of the laboratory setting.

The strength of the current study lies in the ability to determine vascular control in a model of severe, compensated CHF which is difficult to achieve in human studies. The coronary artery ligation technique in the rat allows for the development of CHF severity similar to patients classified as stage IV in the NYHA guidelines as evidenced by the elevated LV, RV and lung-to-body weight ratios in MI compared to sham animals (Musch, 1992; Hirai, 1995). It is important to note that the animals in the current studies reflected severe myocardial damage and tremendous functional decrements. Within this model, the radiolabelled microsphere technique allows investigation of inter- and intramuscular BF not currently explored in CHF with PUFA supplementation. Measurements of this type are particularly insightful given the fiber type specific changes in vascular control evident in CHF.

Conclusions

The current study provides the first data regarding the effects of a PUFA rich diet on vascular control in a rat model of CHF. The main novel findings were 1) FO supplementation resulted in lower BF and VC when compared to SO 2) changes in BF and VC after NOS inhibition via L-NAME did not differ between SO and FO 3) exercise performance and blood [lactate] were not different despite the difference in skeletal muscle BF during submaximal treadmill exercise. These results suggest that FO supplementation does not augment vascular control or exercise capacity in a rat model of CHF.

Table 1. Morphological and hemodynamic characteristics of SO and FO CHF rats.

	SO	**FO**
LVEDP, mmHg	14 ± 2	11 ± 1
LV dp/dt, mmHg/s	7000 ± 376	7000 ± 277
LV/body mass, mg/g	2.10 ± 0.14	2.18 ± 0.06
RV/body mass, mg/g	0.68 ± 0.10	0.60 ± 0.02
Lung/body mass, mg/g	3.98 ± 0.39	4.28 ± 0.43
Infarct size, %	32 ± 3	31 ± 1

Data are mean ± SEM. LVEDP, left ventricular end diastolic pressure; LV dp/dt, left ventricular dpressure/dtime RV, right ventricle; LV, left ventricle. SO; *n*=10, FO; *n*=15

Table 2. Effects of FO supplementation on hindlimb muscle BF at rest and during exercise pre- and post-LNAME (ml · min^{-1} · 100 g^{-1}).

	SO			FO		
	Rest	**Exercise**	**Ex+LNAME**	**Rest**	**Exercise**	**Ex+LNAME**
Ankle extensors						
Soleus (9%)	49 ± 22	275 ± 40	274 ± 46	46 ± 9	267 ± 17	237 ± 30
Plantaris (80%)	10 ± 3	245 ± 32	210 ± 10	5 ± 1	201 ± 12	169 ± 17
Gastrocnemius, red (14%)	16 ± 8	411 ± 43	451 ± 22	14 ± 3	306 ± 24*	285 ± 46
Gastrocnemius, white (100%)	6 ± 2	61 ± 5	77 ± 11	8 ± 1	40 ± 5*	34 ± 3*
Gastrocnemius, mixed (91%)	10 ± 1	166 ± 16	178 ± 5	7 ± 1	133 ± 8*	116 ± 14
Tibialis posterior (73%)	14 ± 5	143 ± 11	106 ± 7†	9 ± 2	109 ± 9*	80 ± 15
Flexor digitorum longus (68%)	3 ± 1	104 ± 21	117 ± 34	7 ± 2	71 ± 12	87 ± 13
Flexor halicus longus (71%)	7 ± 1	92 ± 13	81 ± 8	6 ± 1	70 ± 5	71 ± 11
Ankle flexors						
Tibialis anterior, red (63%)	11 ± 5	342 ± 24	263 ± 4†	4 ± 2	279 ± 19*	228 ± 30
Tibialis anterior, white (80%)	12 ± 3	117 ± 9	106 ± 7	10 ± 1	88 ± 6*	76 ± 11
Extensor digitorum longus (76%)	12 ± 2	69 ± 8	40 ± 2†	12 ± 4	46 ± 3*	49 ± 12
Peroneals (67%)	18 ± 7	143 ± 13	104 ± 13†	8 ± 1	104 ± 4*	84 ± 15
Knee extensors						
Vastus intermedius (4%)	51 ± 19	355 ± 66	314 ± 77	56 ± 27	348 ± 15	310 ± 37
Vastus medialis (82%)	13 ± 4	181 ± 24	138 ± 12	13 ± 5	158 ± 13	109 ± 10
Vastus lateralis, red (35%)	29 ± 11	357 ± 50	342 ± 19	28 ± 13	303 ± 32	267 ± 41
Vastus lateralis, white (100%)	6 ± 2	34 ± 5	31 ± 3	8 ± 2	24 ± 3*	24 ± 4
Vastus lateralis, mixed (89%)	12 ± 3	170 ± 17	190 ± 9	9 ± 3	129 ± 10*	109 ± 19
Rectus femoris, red (66%)	13 ± 3	253 ± 27	220 ± 14	5 ± 1	229 ± 15	159 ± 21
Rectus femoris, white (100%)	9 ± 1	112 ± 12	95 ± 8†	6 ± 1	97 ± 5	69 ± 6†
Knee flexors						
Biceps femoris anterior (100%)	13 ± 7	59 ± 8	64 ± 4†	5 ± 1	25 ± 3*	28 ± 6
Biceps femoris posterior (92%)	11 ± 3	99 ± 13	78 ± 6	5 ± 0	67 ± 4*	59 ± 6
Semitendinosus (83%)	12 ± 3	64 ± 10	44 ± 7	7 ± 1	41 ± 5*	40 ± 9
Semimembranosus, red (72%)	15 ± 6	137 ± 13	143 ± 9	8 ± 2	105 ± 9*	102 ± 17
Semimembranosus, white (100%)	9 ± 1	37 ± 5	43 ± 6	7 ± 0	30 ± 3	35 ± 10
Thigh adductors						
Adductor longus (5%)	151 ± 8	315 ± 60	201 ± 55	112 ± 27	266 ± 24	197 ± 32
Adductor magnus & brevis (89%)	13 ± 2	94 ± 9	77 ± 5†	5 ± 1	63 ± 4*	56 ± 5
Gracilis (77%)	17 ± 5	46 ± 5	42 ± 8	11 ± 2	32 ± 4*	34 ± 4
Pectineus (69%)	18 ± 4	59 ± 8	44 ± 12	14 ± 3	37 ± 5*	36 ± 10

Data are mean ± SEM. Values in parentheses indicate % type IIb + d/x according to Delp & Duan (1996). Rest: SO, *n*=4; FO, *n*=6; Exercise: SO, *n*=10; FO, *n*=15; Exercise+LNAME: SO, *n*=6, FO, *n*=9. **P*<0.05 vs. SO. †*P*<0.05 vs. exercise.

Table 3. Effects of FO supplementation on hindlimb muscle VC at rest and during exercise pre- and post-LNAME (ml · min^{-1} · 100 g^{-1} · mmHg^{-1}).

	SO			FO		
	Rest	**Exercise**	**Ex+LNAME**	**Rest**	**Exercise**	**Ex+LNAME**
Ankle extensors						
Soleus (9%)	0.40 ± 0.18	2.14 ± 0.30	1.90 ± 0.36	0.38 ± 0.08	2.05 ± 0.15	1.69 ± 0.23
Plantaris (80%)	0.08 ± 0.03	1.96 ± 0.30	1.44 ± 0.10	0.04 ± 0.01	1.54 ± 0.11	1.19 ± 0.11
Gastrocnemius, red (14%)	0.13 ± 0.06	3.23 ± 0.34	3.09 ± 0.17	0.12 ± 0.02	2.33 ± 0.18*	1.98 ± 0.28
Gastrocnemius, white (100%)	0.05 ± 0.01	0.47 ± 0.04	0.52 ± 0.07	0.07 ± 0.01	0.31 ± 0.04*	0.24 ± 0.02*
Gastrocnemius, mixed (91%)	0.08 ± 0.01	1.30 ± 0.12	1.22 ± 0.04	0.06 ± 0.01	1.02 ± 0.08*	0.82 ± 0.09
Tibialis posterior (73%)	0.11 ± 0.04	1.12 ± 0.08	0.73 ± 0.06†	0.07 ± 0.02	0.84 ± 0.08*	0.57 ± 0.11
Flexor digitorum longus (68%)	0.03 ± 0.01	0.80 ± 0.15	0.80 ± 0.23	0.06 ± 0.02	0.55 ± 0.10	0.62 ± 0.10
Flexor halicus longus (71%)	0.05 ± 0.01	0.72 ± 0.10	0.55 ± 0.05	0.05 ± 0.01	0.54 ± 0.04*	0.50 ± 0.08
Ankle flexors						
Tibialis anterior, red (63%)	0.09 ± 0.04	1.80 ± 0.17	1.80 ± 0.04†	0.03 ± 0.01	2.14 ± 0.16*	1.60 ± 0.21
Tibialis anterior, white (80%)	0.10 ± 0.03	0.91 ± 0.06	0.72 ± 0.05†	0.08 ± 0.01	0.67± 0.06*	0.53 ± 0.08
Extensor digitorum longus (76%)	0.10 ± 0.02	0.53 ± 0.06	0.28 ± 0.02†	0.10 ± 0.03	0.35 ± 0.02*	0.35 ± 0.09
Peroneals (67%)	0.15 ± 0.06	1.11 ± 0.10	0.72 ± 0.11†	0.06 ± 0.01	0.80 ± 0.04*	0.60 ± 0.11
Knee extensors						
Vastus intermedius (4%)	0.41 ± 0.15	2.80 ± 0.52	2.16 ± 0.56	0.45 ± 0.22	2.65 ± 0.12	2.18 ± 0.25
Vastus medialis (82%)	0.10 ± 0.03	1.42 ± 0.18	0.95 ± 0.09	0.10 ± 0.04	1.23 ± 0.12	0.77 ± 0.08
Vastus lateralis, red (35%)	0.23 ± 0.09	2.77 ± 0.37	2.34 ± 0.14	0.23 ± 0.11	2.29 ± 0.23	1.85 ± 0.24
Vastus lateralis, white (100%)	0.05 ± 0.01	0.26 ± 0.04	0.21 ± 0.02†	0.06 ± 0.01	0.19 ± 0.02*	0.17 ± 0.03
Vastus lateralis, mixed (89%)	0.10 ± 0.03	1.32 ± 0.13	1.30 ± 0.04	0.08 ± 0.02	0.98 ± 0.07*	0.75 ± 0.11
Rectus femoris, red (66%)	0.10 ± 0.02	2.00 ± 0.22	1.51 ± 0.13	0.04 ± 0.01	1.75 ± 0.12	1.11 ± 0.13†
Rectus femoris, white (100%)	0.07 ± 0.01	0.88 ± 0.09	0.65 ± 0.07†	0.05 ± 0.01	0.74 ± 0.05	0.48 ± 0.03†
Knee flexors						
Biceps femoris anterior (100%)	0.10 ± 0.06	0.45 ± 0.06	0.44 ± 0.03†	0.04 ± 0.01†	0.19 ± 0.02*	0.20 ± 0.04
Biceps femoris posterior (92%)	0.09 ± 0.02	0.77 ± 0.09	0.53 ± 0.05	0.04 ± 0.00	0.52 ± 0.04*	0.42 ± 0.05
Semitendinosus (83%)	0.10 ± 0.03	0.49 ± 0.07	0.31 ± 0.05	0.05 ± 0.01	0.32 ± 0.04*	0.29 ± 0.07
Semimembranosus, red (72%)	0.12 ± 0.05	1.07 ± 0.09	0.97 ± 0.06	0.07 ± 0.01	0.81 ± 0.08*	0.73 ± 0.13
Semimembranosus, white (100%)	0.07 ± 0.01	0.29 ± 0.04	0.29 ± 0.04	0.06 ± 0.00	0.23 ± 0.03	0.25 ± 0.07
Thigh adductors						
Adductor longus (5%)	1.22 ± 0.05	2.49 ± 0.47	1.39 ± 0.40†	0.91 ± 0.23	2.05 ± 0.19	1.38 ± 0.23
Adductor magnus & brevis (89%)	0.11 ± 0.02	0.74 ± 0.07	0.53 ± 0.04†	0.04 ± 0.01	0.48 ± 0.03*	0.40 ± 0.04
Gracilis (77%)	0.14 ± 0.04	0.36 ± 0.04	0.29 ± 0.06	0.09 ± 0.02	0.25 ± 0.03*	0.24 ± 0.03
Pectineus (69%)	0.14 ± 0.03	0.46 ± 0.06	0.31 ± 0.09	0.11 ± 0.03	0.29 ± 0.04*	0.26 ± 0.07

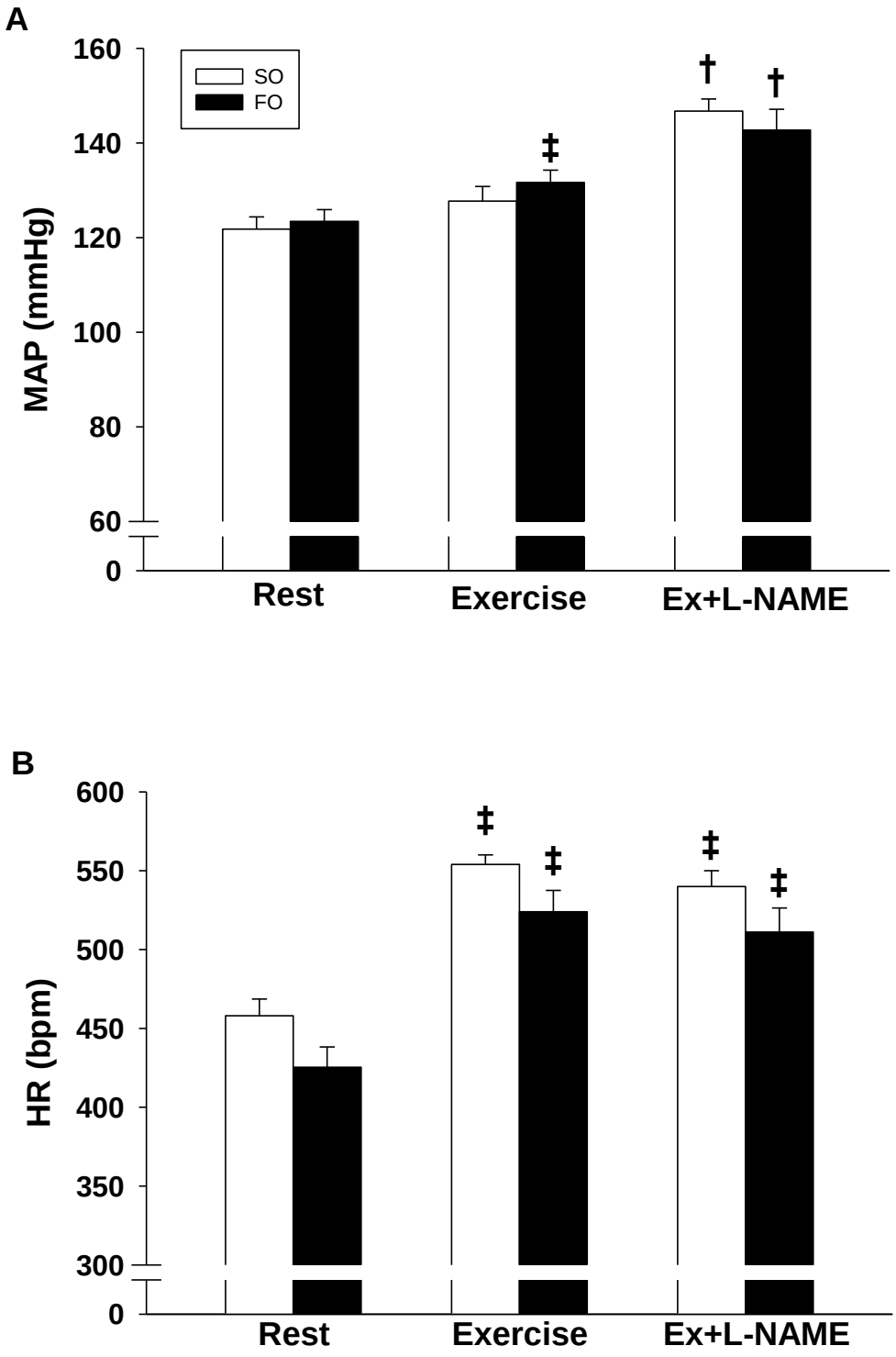
Data are mean ± SEM. Values in parentheses indicate % type IIb + d/x according to Delp & Duan (1996). Rest: SO, *n*=4; FO, *n*=6; Exercise: SO, *n*=10; FO, *n*=15; Ex+LNAME: SO, *n*=6, FO, *n*=9.
**P*<0.05 vs. SO. †*P*<0.05 vs. exercise.

Table 4. Effects of FO supplementation on kidney and splanchnic region organ BF (ml · min^{-1} · 100 g^{-1}) and VC (ml · min^{-1} · 100 g^{-1} · $mmHg^{-1}$) at rest and during exercise pre- and post-LNAME.

	SO			FO		
	Rest	**Exercise**	**Ex+LNAME**	**Rest**	**Exercise**	**Ex+LNAME**
BF						
Kidney	688 ± 86	395 ± 51	236 ± 35	625 ± 55	368 ± 35	215 ± 20†
Stomach	77 ± 19	47 ± 9	26 ± 4	74 ± 16	69 ± 34	15 ± 2
Adrenals	472 ± 172	346 ± 55	101 ± 16†	785 ± 144*	334 ± 32	71 ± 7†
Spleen	524 ± 64	58 ± 11	51 ± 7	483 ± 150	55 ± 7	21 ± 6
Pancreas	172 ± 46	111 ± 21	61 ± 11	109 ± 22	80 ± 9	27 ± 4†
Sm. Intestine	357 ± 65	258 ± 44	159 ± 13	351 ± 60	211 ± 16	108 ± 16†
Lg. Intestine	82 ± 7	81 ± 17	45 ± 8	101 ± 18	72 ± 8	29 ± 4†
Liver**	30 ± 9	32 ± 4	17 ± 4	45 ± 9	34 ± 3	11 ± 2†
VC						
Kidney	5.61 ± 0.77	3.11 ± 0.40	1.63 ± 0.26†	5.09 ± 0.46	2.87 ± 0.29	1.52 ± 0.14†
Stomach	0.63 ± 0.15	0.37 ± 0.07	0.18 ± 0.03	0.61 ± 0.13	0.54 ± 0.27	0.11 ± 0.01
Adrenals	3.78 ± 1.36	2.71 ± 0.41	0.70 ± 0.13†	6.45 ± 1.25*	2.62 ± 0.29	0.51 ± 0.06†
Spleen	4.26 ± 0.58	0.46 ± 0.09	0.35 ± 0.05	3.95 ± 1.17	0.43 ± 0.05	0.14 ± 0.04
Pancreas	1.39 ± 0.37	0.87 ± 0.17	0.42 ± 0.07†	0.89 ± 0.18	0.63 ± 0.08	0.19 ± 0.03†
Sm. Intestine	2.93 ± 0.57	2.04 ± 0.36	1.09 ± 0.12	2.89 ± 0.51	1.65 ± 0.14	0.75 ± 0.10†
Lg. Intestine	0.67 ± 0.06	0.63 ± 0.13	0.31 ± 0.06	0.84 ± 0.16	0.57 ± 0.07	0.20 ± 0.03†
Liver**	0.24 ± 0.07	0.26 ± 0.03	0.12 ± 0.03	0.37 ± 0.08	0.45 ± 0.18	0.08 ± 0.02†

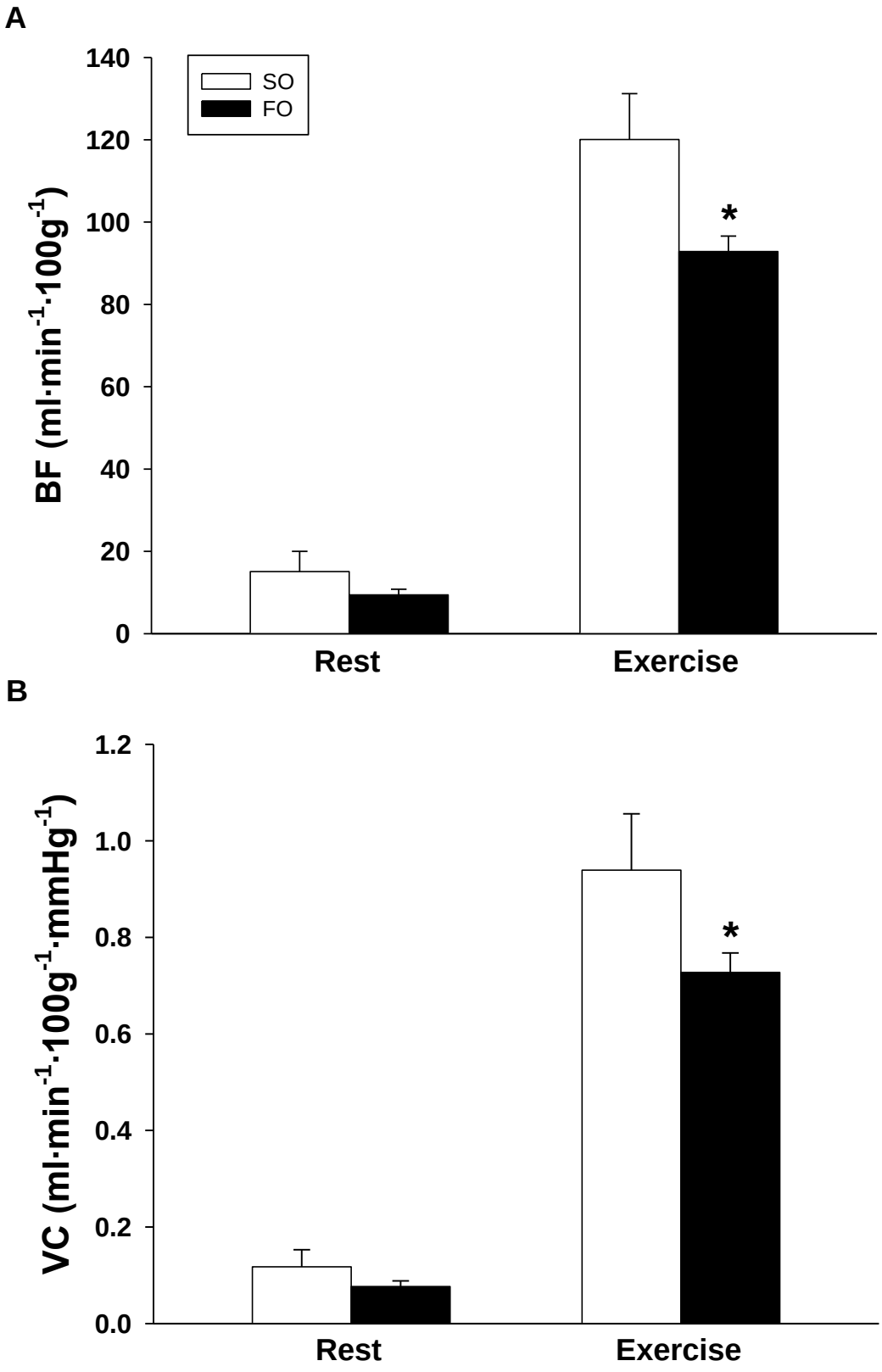
Data are mean ± SEM. Rest: SO, *n*=4; FO, *n*=6; Exercise: SO, *n*=10; FO, *n*=15; Exercise+LNAME: SO, *n*=6, FO, *n*=9. *P<0.05 vs. SO. †P<0.05 vs. exercise. **Indicates arterial, not portal, BF and VC.

Figure 1



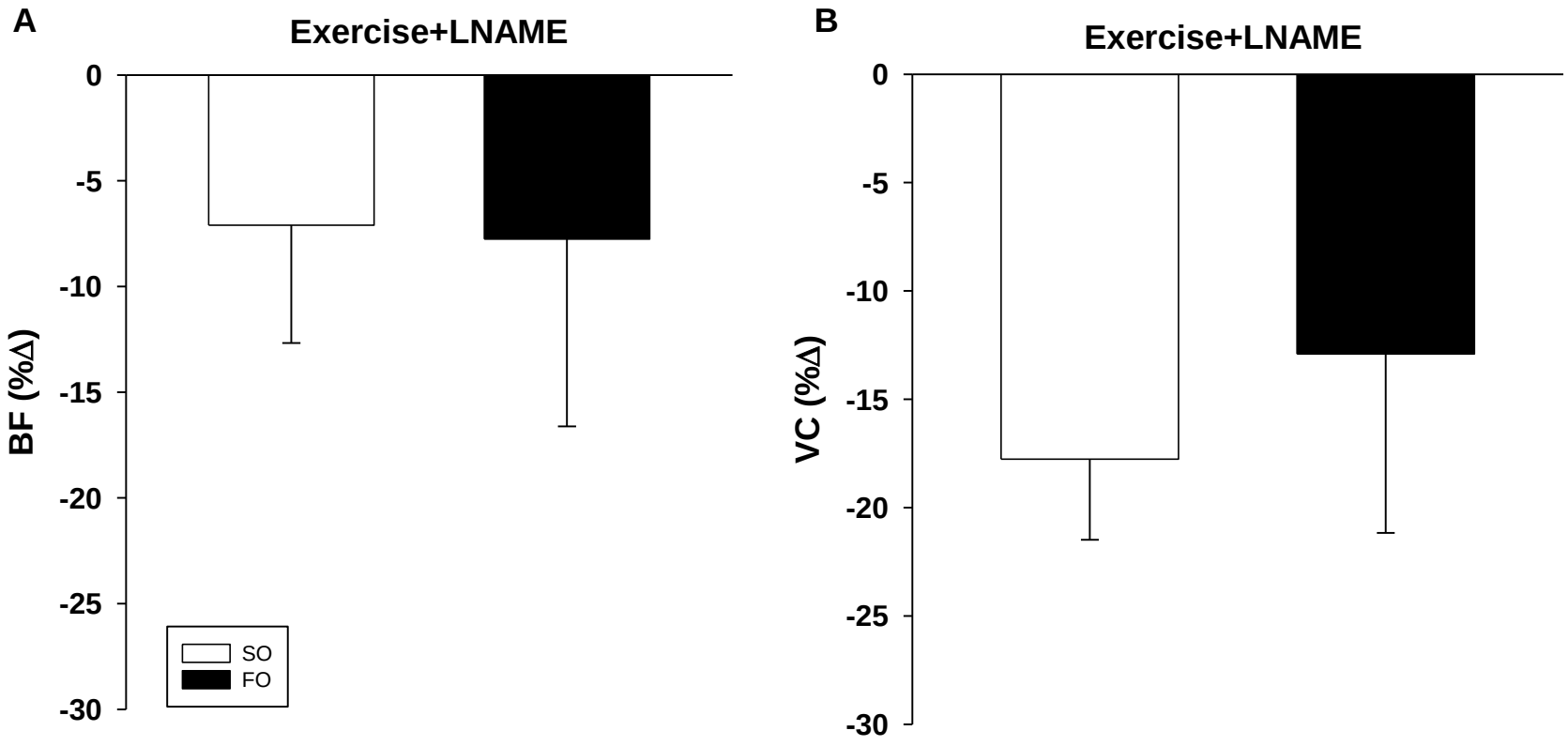
FO supplementation had no effect on MAP or HR at rest, during exercise or ex+L-NAME compared to SO. †P<0.05 vs. rest and exercise. ‡P<0.05 vs. rest.

Figure 2



A. Effect of FO supplementation on hindlimb skeletal muscle BF at rest and during submaximal exercise. B. Effect of FO supplementation on hindlimb skeletal muscle VC at rest and during submaximal exercise. *P<0.05 vs. SO.

Figure 3



A. Percent change of hindlimb muscle BF during submaximal exercise.
B. Percent change of hindlimb muscle VC during submaximal exercise. *P<0.05 vs. SO.

Chapter 5 - References

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