Chronic femoral artery ligation exaggerates the pressor and sympathetic nerve responses during dynamic skeletal muscle stretch in decerebrate rats

by

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Abstract

Mechanical and metabolic signals arising during skeletal muscle contraction reflexly increase sympathetic nerve activity and blood pressure (i.e., the exercise pressor reflex). In a rat model of simulated peripheral artery disease (PAD) in which a femoral artery is chronically (~72 hours) ligated, the mechanically-sensitive component of the exercise pressor reflex during 1 Hz dynamic contraction is exaggerated compared to that found in normal rats. Whether this is due to an enhanced acute sensitization of mechanoreceptors by metabolites produced during contraction or involves a chronic sensitization of mechanoreceptors is unknown. To investigate this issue, in decerebrate, unanesthetized rats we tested the hypothesis that the increases in mean arterial blood pressure (MAP) and renal sympathetic nerve activity (RSNA) during 1 Hz dynamic stretch are larger when evoked from a previously "ligated" hindlimb compared to those evoked from the contralateral "freely perfused" hindlimb. Dynamic stretch provided a mechanical stimulus in the absence of contraction-induced metabolite production that replicated closely the pattern of the mechanical stimulus present during dynamic contraction. We found that the increases in MAP (freely perfused: 14±1, ligated: 23±3 mmHg, $p=0.02$) and RSNA were significantly greater during dynamic stretch of the ligated hindlimb compared to the increases during dynamic stretch of the freely perfused hindlimb. These findings suggest that the exaggerated mechanically-sensitive component of the exercise pressor reflex found during dynamic muscle contraction in this rat model of simulated PAD involves a chronic sensitizing effect of ligation on muscle mechanoreceptors and cannot be attributed solely to acute contraction-induced metabolite sensitization.

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Acknowledgements

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Preface

Peripheral artery disease (PAD), a cardiovascular disease characterized by atherosclerotic plaque buildup and progressive narrowing of arteries in the lower extremities, affects an estimated 8.5 million Americans and 200 million individuals worldwide (3, 23). The prevalence of PAD has been shown to increase exponentially across age groups - approximately doubling each decade for most ethnic groups (3). Environmental irritants (i.e. cigarette smoking), cardiometabolic risk factors such as physical inactivity, hypertension, hyperlipidemia, and diabetes, as well as genetic factors are all implicated in contributing to the development of PAD (41). The most common symptom leading to a diagnosis of PAD is exertional pain that subsides with rest (termed intermittent claudication), but this symptom is present in only a small portion of PAD patients which contributes to an underdiagnosis of this disease in the primary care setting (41). Regardless of whether an individual with PAD presents with intermittent claudication they are still subject to an increased risk of myocardial infarction, stroke, and all-cause mortality (19, 41). Compared to age-matched healthy subjects, exercise in PAD patients has been shown to elicit exaggerated increases in sympathetic nerve activity (SNA) and blood pressure (5, 7, 8, 15, 28). In PAD patients, large increases in blood pressure during treadmill walking have been linked to an increased risk of a major ischemic event and all-cause mortality (14). Exaggerated increases in SNA during exercise contribute to exercise intolerance, reduced quality of life, and ultimately increased costs of healthcare. Therefore, investigation into the underlying mechanisms contributing to the exaggerated SNA and blood pressure responses during exercise in PAD patients may importantly lead to the development of novel treatment options capable of improving quality of life and reducing risks and costs associated with PAD.

Chapter 1 - Introduction

Increases in sympathetic nerve activity, blood pressure, and heart rate that occur during exercise are caused, in part, by a neural feedback mechanism termed the exercise pressor reflex (4, 16, 20). This reflex is activated when mechanical and metabolic signals originating within contracting skeletal muscles stimulate thinly myelinated group III and unmyelinated group IV muscle afferents (34, 36, 44). The exercise pressor reflex is exaggerated in patients with atherosclerotic peripheral artery disease (PAD; 31, 39, 40) which contributes to the larger increase in blood pressure found during treadmill walking in this patient population compared to that found in aged-matched healthy controls (5, 7). The exercise pressor reflex is also exaggerated in a rat model of simulated PAD in which a femoral artery is ligated ~24-72 hours before the experiment (51). The rat model of simulated PAD has been used extensively in recent years to investigate the mechanisms that may contribute to the exaggerated exercise pressor reflex found in PAD patients (27, 48, 49, 54, 57).

Copp et al. (12) found recently that the mechanically-sensitive component of the exercise pressor reflex was exaggerated in rats with a ligated femoral artery compared to that found in rats with freely perfused femoral arteries. Specifically, GsMTx4, a relatively selective antagonist for mechano-gated piezo channels (2, 6), reduced the pressor and renal sympathetic nerve responses during dynamic hindlimb muscle contraction to a greater extent in "ligated" versus "freely perfused" rats (12). Femoral artery ligation did not, however, increase piezo1 or piezo2 channel protein expression in L_4 and L_5 dorsal root ganglia (DRG) tissue which suggested that an acute and/or chronic sensitization of mechanoreceptors accounted for the exaggerated mechanically-sensitive component of the exercise pressor reflex rather than increased mechanoreceptor expression. A method of investigating whether ligation results in chronic mechanoreceptor

sensitization is to perform a muscle/tendon stretch maneuver thereby stimulating mechanoreceptors in the absence of contraction-induced metabolite production (46). Previous studies have utilized only static muscle stretch protocols to investigate the effect of chronic femoral artery ligation on reflex cardiovascular control and while static stretch provides valuable insight into the mechanoreceptor stimulation present during static contraction (46), its ability to provide insight into mechanoreceptor stimulation present during dynamic contraction may be limited. Pertinent to this issue, Daniels et al. (13) performed a 1 Hz dynamic hindlimb muscle stretch protocol in the cat to replicate the repetitive mechanical stimuli present during 1 Hz dynamic muscle contraction. In that investigation, however, direct comparisons between dynamic stretch and dynamic contraction were not made and sympathetic nerve activity was not measured. A characteristic feature of the increase in sympathetic nerve activity during dynamic contractions is a bursting pattern that is synchronized with muscle tension development which has been interpreted to suggest that the contractions provided a robust mechanical stimulus (11, 12, 55). The degree to which 1 Hz dynamic stretch replicates that RSNA bursting pattern is unknown.

The findings summarized above prompted us to test the hypothesis that 1 Hz dynamic muscle stretch evoked increases in blood pressure and RSNA that were quantitatively and qualitatively similar to those evoked during 1 Hz dynamic contraction. We then used the dynamic stretch protocol to gain mechanistic insight into the exaggerated mechanically-sensitive component of the exercise pressor reflex that was found during dynamic contractions in rats with a ligated femoral artery (12). Specifically, in decerebrate, unanesthetized rats we tested the hypothesis that the increase in blood pressure and RSNA would be greater during dynamic

stretch of the hindlimb muscles in which the femoral artery was ligated for 72 hours compared to the increases during stretch of the contralateral freely perfused hindlimb muscles.

Chapter 2 - Methods

All experimental procedures were approved by the Institutional Animal Care and Use Committee of Kansas State University and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Experiments were performed on young adult (~12 weeks old) male Sprague-Dawley rats (n=29, average body weight: 394±10 g), Charles River Laboratories). The rats were housed two per cage in temperature (maintained at ~22 °C) and light (12/12 hour light/dark cycle running from 7 am to 7 pm) controlled accredited facilities with standard rat chow and water provided *ad libitum*. At the end of each experiment, the decerebrated rats (see below) were euthanized with an intravenous injection of saturated (>3 mg/kg) potassium chloride.

Surgical Procedures. 13 of the 29 rats in this study had their left femoral artery ligated ~72 hours prior to the experimental protocol. Specifically, the rats were anesthetized with ~3% isoflurane (balance O_2) after which their left femoral artery was surgically exposed and tightly ligated with 5-0 silk suture ~3-5 mm distal to the inguinal ligament. In three of these 13 rats, the right femoral artery was also surgically exposed and isolated and a sham ligation procedure was completed in which a 5-0 suture was passed under the femoral artery but not tied. The incisions were closed and rats were administered meloxicam (1-2 mg/kg s.c.) as an analgesic. The experimental protocol was initiated ~72 hours following the survival surgery.

On the day of the experiment, all rats were anesthetized with ~3% isoflurane (balance O_2). A sufficient depth of anesthesia was confirmed by the absence of toe-pinch and blink reflexes. The trachea was cannulated and the lungs were mechanically ventilated (2% isoflurane balance O_2) with the gaseous anesthetic. The right jugular vein and both common carotid arteries were cannulated with PE-50 catheters to allow for the administration of drugs/fluids, the

measurement of arterial blood pressure (Physiological Pressure Transducer, AD Instruments), and sampling of arterial blood gasses. In all rats, the left calcaneus bone was severed and the triceps surae (gastrocnemius, soleus, and plantaris) muscles were exposed. In the 13 rats in which the left femoral artery was previously ligated, the right calcaneus bone was also severed and the triceps surae muscles exposed. The triceps surae muscles were then connected by string to a force transducer (Grass FT03) attached to a rack and pinion. A retroperitoneal approach was used to expose bundles of the renal sympathetic nerve, which were then glued (Kwik-Sil; World Precision Instruments) onto a pair of thin stainless-steel recording electrodes connected to a high impedance probe (Grass model HZP) and amplifier (Grass P511). Multi-unit signals from the renal sympathetic nerve fibers were filtered at high and low frequencies (1 KHz and 100 Hz, respectively). At the end of all experiments in which RSNA was measured, post-ganglionic sympathetic nerve activity was abolished with administration of hexamethonium bromide (20 mg/kg i.v.) to allow for the quantification of background noise. In eight rats with freely perfused femoral arteries, the left sciatic nerve was exposed and placed on shielded stimulating electrodes in order to electrically induce hindlimb skeletal muscle contraction.

Upon completion of the initial surgical procedures, all rats were placed in a Kopf stereotaxic frame and spinal unit with clamps around the pelvis and rostral lumbar vertebrae. A precollicular decerebration was then performed because inhalant anesthesia has been shown to depress the exercise pressor reflex in rats (45). Specifically, after the administration of dexamethasone (0.2 mg i.v.) to reduce brainstem edema, all neural tissue rostral to the superior colliculus was aspirated. Once the decerebration was complete, anesthesia was terminated and the lungs were mechanically ventilated with room air. All rats were then given at least 60 minutes to recover before the initiation of any experimental protocol. Core temperature was

measured by a rectal probe and maintained at ~37-38°C by an automated heating system (Harvard Apparatus). Arterial pH and blood gases were analyzed (Radiometer ABL80 Flex) periodically throughout each experiment from small blood samples (~75 µl) taken from a carotid artery catheter and were maintained within normal physiological ranges (pH: 7.35-7.45; pCO_2: 38-40 mmHg; PO_2: ~100 mmHg) by administering sodium bicarbonate and/or adjusting ventilation as necessary.

Experimental protocol. We first compared the increases in blood pressure, RSNA, and HR that occurred during 30 seconds of 1 Hz dynamic stretch and 1 Hz dynamic contraction in rats with freely perfused femoral arteries. In each of these experiments, we performed either dynamic stretch (n=8), dynamic contraction (n=7), or both dynamic stretch and dynamic contraction (n=1). Prior to each maneuver, baseline muscle tension was set at ~100 g and baseline data were collected for 30 seconds. To evoke dynamic triceps surae muscle stretch, the rack and pinon was manually turned back and forth by an experienced investigator in a rhythmic manner for 30 seconds at a 1 Hz cadence which was set by a metronome. Care was taken to develop similar muscle tension during each stretch. In 2 rats in which we performed dynamic stretch we subsequently cut the left sciatic, femoral, and obturator nerves and then repeated the dynamic stretch protocol. Severing the nerves abolished the pressor response during dynamic stretch in both instances which indicated that the pressor response observed during dynamic stretch when the nerves were intact was entirely reflex in origin. To evoke dynamic hindlimb muscle contraction, the left sciatic nerve was stimulated (40 Hz, 500 ms train duration, 0.01 ms pulse duration, ≤2x motor threshold) with shielded stainless-steel electrodes for 30 s. To ensure that the increase in blood pressure and RSNA during contraction was not due to the electrical activation of the axons of the thin fiber muscle afferents in the sciatic nerve we administered the

paralytic pancuronium bromide (1 mg/kg i.v.) and the sciatic nerve was stimulated for 30 seconds with the same parameters as those used to elicit contraction. No increase in blood pressure or RSNA was observed during the stimulation period following the administration of pancuronium bromide.

In 10 rats in which the left femoral artery was ligated ~72 hours before the experiment we compared the increases in blood pressure, RSNA, and HR that occurred during 30 seconds of dynamic stretch of the muscles of the freely perfused versus the contralateral ligated hindlimb. The dynamic stretch protocols were performed exactly as described above, in random order for the different hindlimbs, and were separated by ~5 minutes. In three additional rats, we compared the pressor response to dynamic stretch between the hindlimb in which the femoral artery was previously ligated and the contralateral hindlimb in which the femoral artery was subjected to a sham ligation procedure. The dynamic stretch maneuvers in these experiments were performed as described above and in random order.

In subsets of freely perfused (n=5) and ligated hindlimbs (n=8) from the above experiments in which we performed dynamic stretch we also performed 30 seconds of static stretch so that we could compare the increases in blood pressure and RSNA between the different stretch maneuvers that were matched for peak tension development. To evoke static stretch, baseline muscle tension was set at ~100 g and baseline data were collected for 30 seconds. The rack and pinion was then rapidly turned and held constant 30 seconds.

Data analysis. Data were collected with a PowerLab and LabChart data acquisition system (AD Instruments). Arterial blood pressure, muscle tension, and RSNA were measured, and mean arterial pressure (MAP) and heart rate (HR) were calculated and displayed in real time and recorded for offline analysis. The original RSNA data were rectified and corrected for the

background noise following administration of hexamethonium bromide. Baseline MAP, HR, and RSNA were determined from the 30 s baseline periods that preceded each maneuver. Baseline RSNA signal-to-noise ratios were calculated using the baseline RSNA prior to each maneuver and the noise recorded following hexamethonium bromide injection (37). In our first set of experiments, there was no difference ($p=0.86$) in the baseline RSNA signal-to-noise ratio between dynamic stretch (1.7±0.5, n=8), and dynamic contraction (1.8±0.4, n=8). Moreover, there was no difference ($p=0.90$) in the baseline RSNA signal-to-noise ratio calculated prior to stretch of the freely perfused (2.4±0.8) and the ligated (2.5±0.8) hindlimb. The peak increase in MAP (peak Δ MAP) and HR (peak Δ HR) during dynamic contraction, dynamic stretch, and static stretch were calculated as the difference between the peak values wherever they occurred during the maneuvers and their corresponding baseline value. Time courses of the increase in MAP and RSNA were plotted as their change from baseline (expressed in absolute terms for MAP and relative to baseline for RSNA which was set to 0%). The Δ tension-time index (Δ TTI) was calculated for each maneuver by integrating the area under the tension signal during the maneuver and subtracting the integrated area during the baseline period. The onset latency of the increase in RSNA during contraction and stretch was defined and identified as the time from the beginning of the maneuver to the first obvious increase in RSNA above baseline.

Data are expressed as mean±SEM. Data were compared with paired or unpaired Student's t-tests or regular or repeated measures ANOVAs with Holm-Sidak post hoc tests as appropriate. Statistical significance was defined as $p<0.05$.

Chapter 3 - Results

Dynamic contraction vs. dynamic stretch in freely perfused rats. There were no differences in the increase in MAP, RSNA (Figure 1), or HR (peak Δ HR contraction: 17±3, stretch: 17±3 bpm, p=0.96) between dynamic contraction and dynamic stretch of the hindlimb muscles of freely perfused rats. There was no difference in the average peak Δ tension or the Δ TTI between the dynamic stretch and contraction maneuvers (Figure 1). The increase in RSNA during contraction and stretch was characterized by the development of distinct RSNA bursts that appeared in a similar frequency to the 1 Hz muscle tension development of each maneuver (Figure 2). The onset latency of the increase in RSNA was significantly longer ($p<0.001$) during dynamic stretch (1028±154 ms, range: 555-1894 ms) compared to dynamic contraction (271±43 ms, range: 133-498 ms). As evident by the original tracings from six different experiments shown in Figure 2, the prolonged onset latency during dynamic stretch resulted in an RNSA bursting pattern that, in general, appeared "right shifted" in relation to muscle tension development. In contrast, the short onset latency present during dynamic contraction resulted in an RSNA bursting pattern that appeared in relative synchrony with muscle tension development.

Dynamic stretch in freely perfused vs. ligated hindlimbs. In 10 rats, we compared the increase in MAP, RSNA, and HR during dynamic muscle stretch between a hindlimb in which the femoral artery was freely perfused and the contralateral hindlimb in which the femoral artery was ligated ~72 hours before the experiment. The increase in MAP and RSNA (Figures 3 and 4), but not HR (peak Δ HR freely perfused: 13±3, ligated: 18±3 bpm, p=0.24) was greater during dynamic stretch of the ligated hindlimb compared to the freely perfused hindlimb. The onset latency of the increase in RSNA above baseline was not different between hindlimbs (freely perfused: 995±149, ligated: 1150±244 ms, p=0.28). There was no difference in the average peak

Δ tension or the Δ TTI of the dynamic stretches when compared between the freely perfused and ligated hindlimb. In three additional rats, we compared the pressor response during dynamic stretch between a ligated hindlimb and the contralateral sham-operated hindlimb to confirm that our findings were not due to the general invasiveness of the surgical procedure. The peak Δ MAP was greater during dynamic stretch of the ligated hindlimb (28±9 mmHg) compared to the contralateral sham-operated hindlimb (16±5 mmHg, p=0.049). There was no difference in the Δ TTI during stretch between the sham (6.8±0.3 kg·s) and ligated hindlimbs (6.5±0.6 kg·s, p=0.54).

Dynamic stretch vs. static stretch in freely perfused and ligated hindlimbs. In a subset of five rats from the experiments above in which we dynamically stretched a freely perfused hindlimb we also performed static stretch. There was no difference in the increase in MAP, RSNA (Figure 5) or HR (static: 14±3, dynamic: 20± 4 bpm, p=0.10) during the different stretch maneuvers in the freely perfused hindlimb. The peak tension developed during static stretch was not different from the average peak tension developed during dynamic stretch. As expected, the Δ TTI was significantly lower during dynamic stretch compared to static stretch due to the contrasting tension profiles of these maneuvers. In a subset of eight rats from the experiments above in which we dynamically stretched the ligated hindlimb we also performed static stretch. In these eight rats, the increase in MAP and RSNA (Figure 6), but not HR (static: 9±3, dynamic: 18±3 bpm, p=0.08), was significantly greater during dynamic versus static stretch of the ligated hindlimb. The peak tension developed during static stretch was not different from the average peak tension developed during dynamic stretch. The Δ TTI was significantly lower during dynamic stretch compared to static stretch. When compared across the different subsets of rats in which we statically stretched the hindlimb muscles, there was no difference in the peak increase in MAP during static stretch between a freely perfused (18±4 mmHg) and a ligated

(16±4 mmHg, p=0.81) hindlimb. The Δ TTI of the static stretches were not different between the freely perfused (14±1 kg·s) and the ligated (14±1 kg·s, p=0.99) hindlimb.

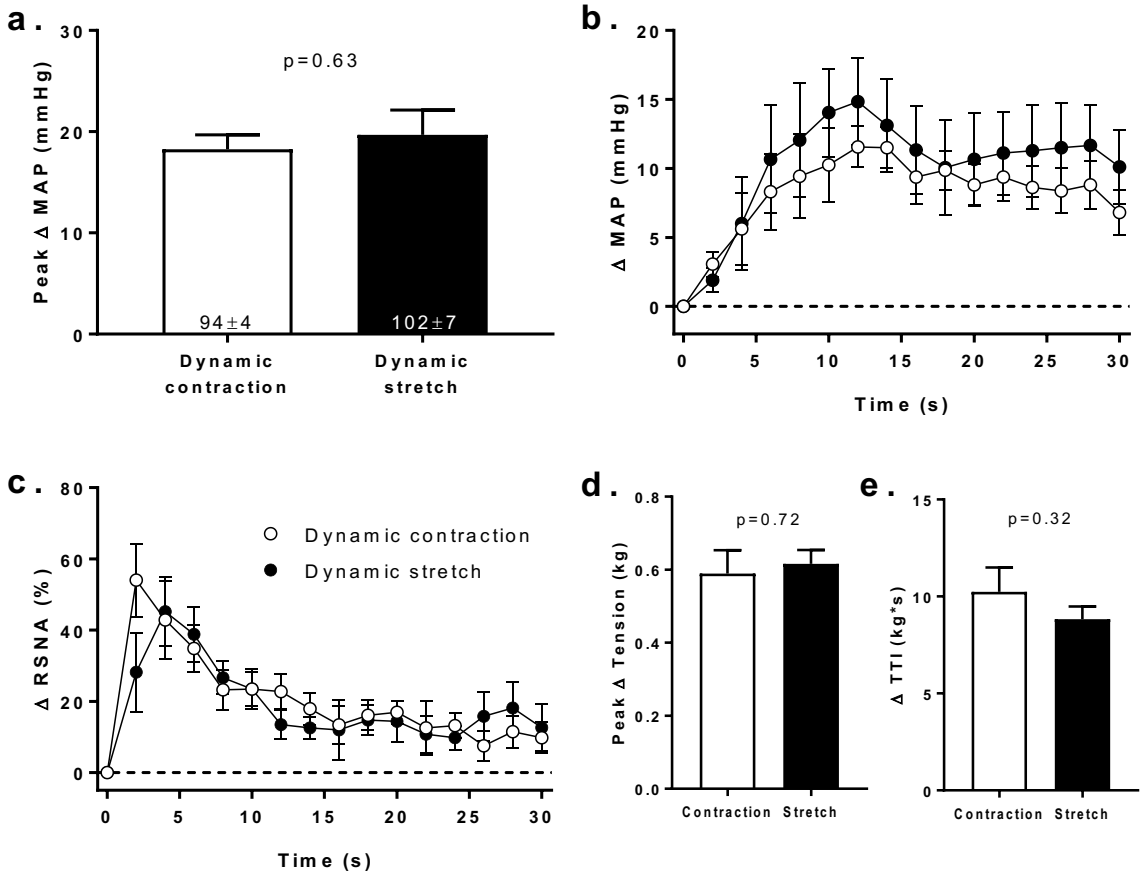

Figure 1. Dynamic contraction vs. dynamic stretch of freely perfused rat hindlimbs.

Comparison of the effects of dynamic stretch (n=9 for MAP, n=8 for RSNA) and dynamic contraction (n=8) of a freely perfused hindlimb on changes in MAP (a, b) and RSNA (c). Panel "d" shows the average peak Δ tension of the contractions and stretches. TTI=tension-time index (e). Baseline MAP is shown within mean bars and was not significantly different (p=0.39). Moreover, absolute peak MAP was not different between contraction (113±4 mmHg) and stretch (122±7 mmHg, p=0.32).

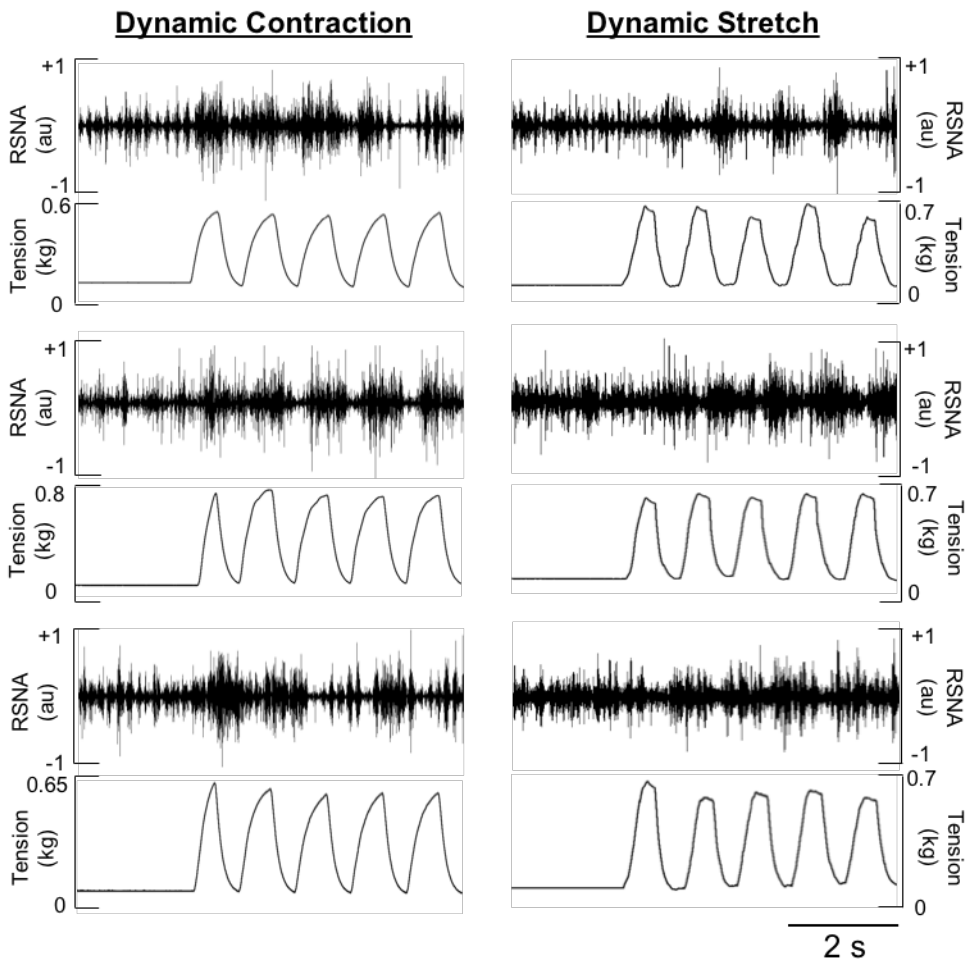

Figure 2. RSNA bursts during dynamic contraction and dynamic stretch.

Examples of the distinct RSNA bursts that occur with each tension development during 1 Hz dynamic contraction (n=3, left panels) and stretch (n=3, right panels) which are evidence of the robust mechanical stimulus produced by these maneuvers. Note also the prolonged onset latency of the increase in RSNA during stretch compared to contraction.

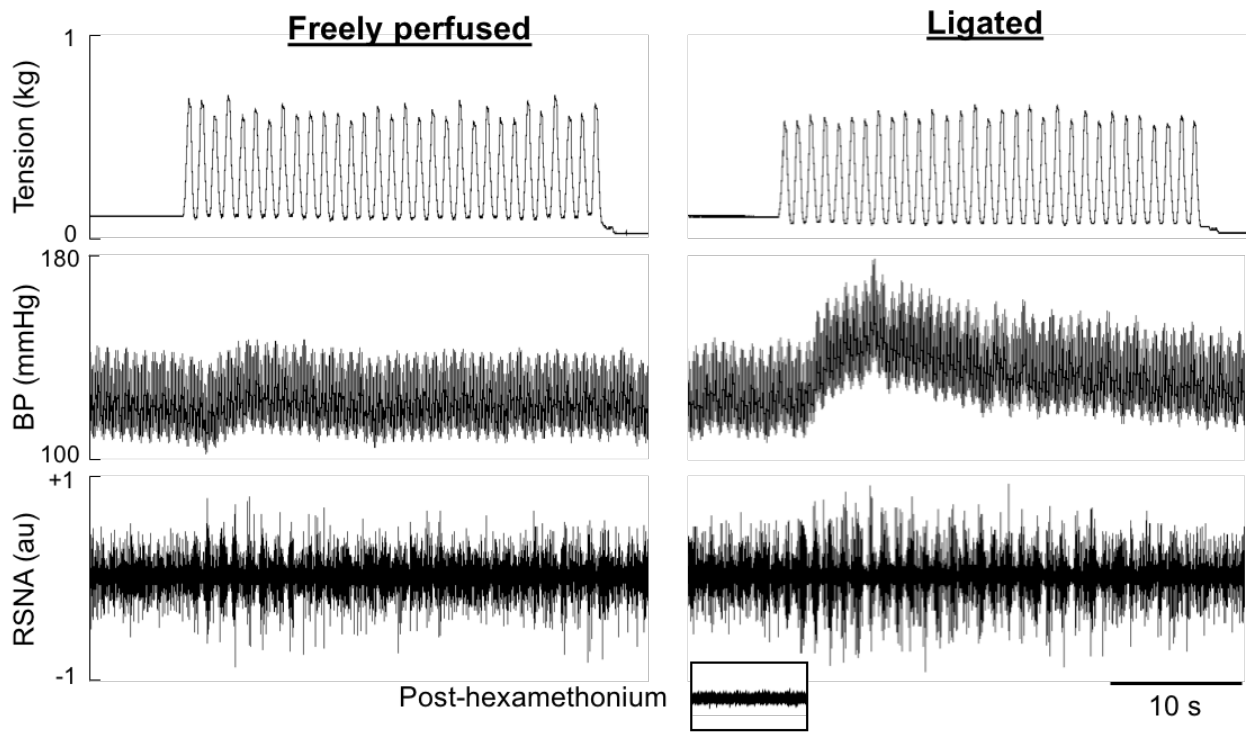

Figure 3. Original data for dynamic stretch of a freely perfused vs. ligated hindlimb.

An example of original data from one rat showing the increase in blood pressure (BP) and renal sympathetic nerve activity (RSNA) during ~30 seconds of dynamic stretch of the muscles of the hindlimb in which the femoral artery was freely perfused and the contralateral hindlimb in which the femoral artery was previously ligated. The inset shows an ~10 second period of the background noise recorded following the injection of hexamethonium bromide.

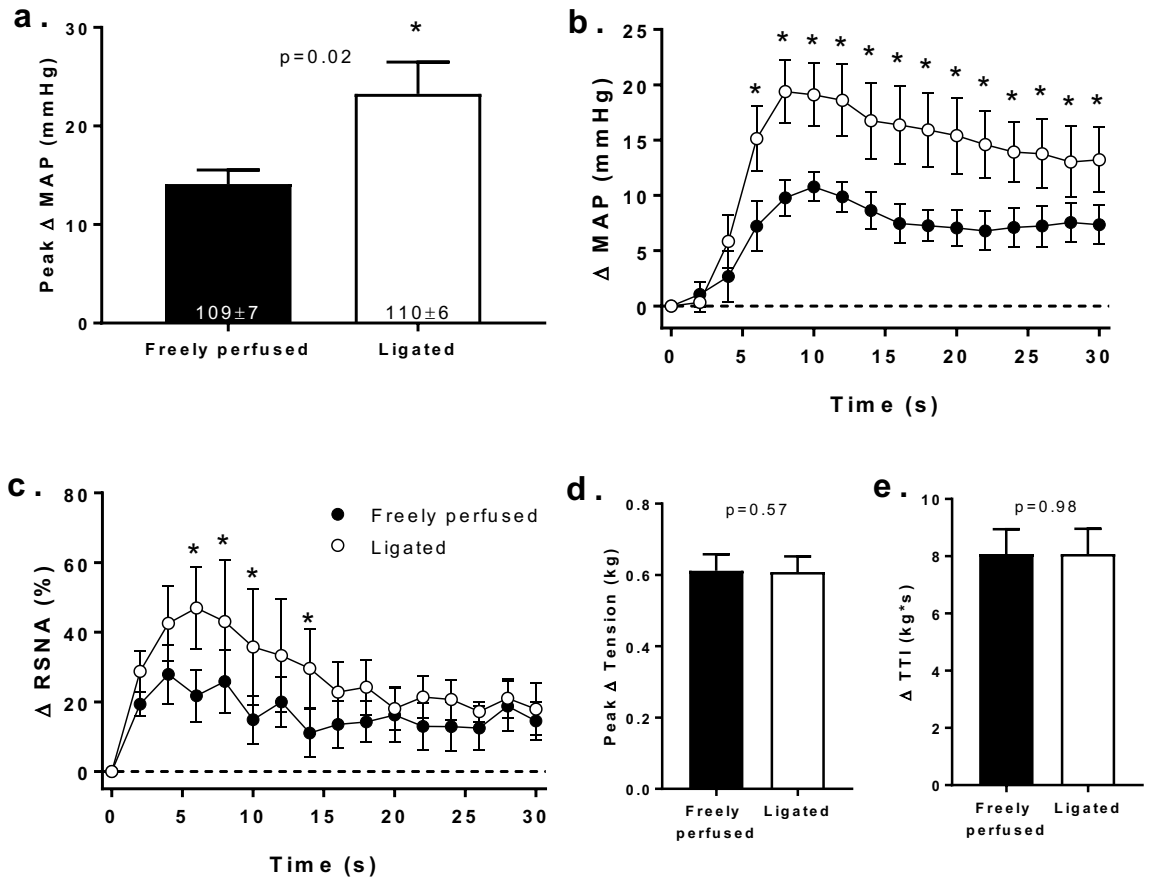

Figure 4. Dynamic stretch of freely perfused compared to ligated rat hindlimbs.

Comparison of the effect of ligation on the increases in MAP (a, b) and RSNA (c) during dynamic stretch (n=10). Panel "d" shows the average peak Δ tensions during dynamic stretch of the freely perfused and the ligated hindlimb. TTI=tension time index (e). The asterisks indicate a statistically significant difference ($p<0.05$) versus freely perfused.

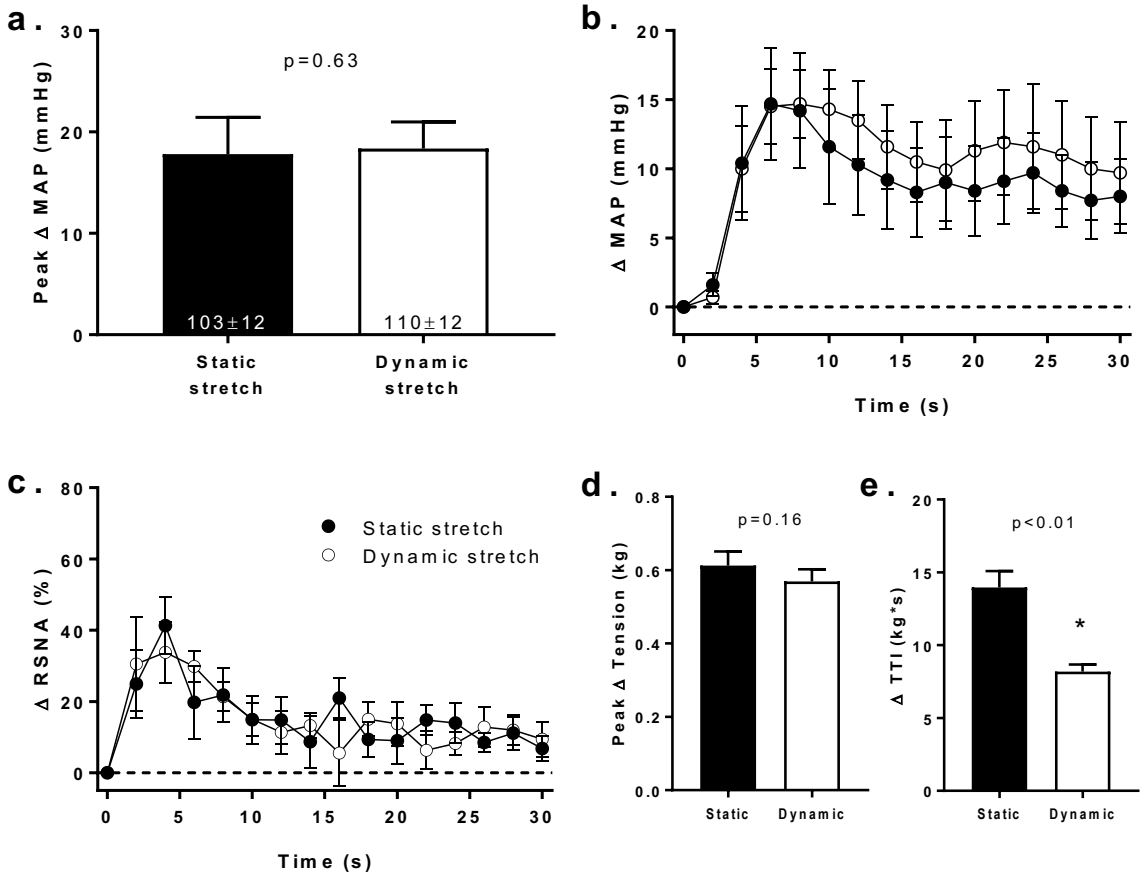

Figure 5. Static stretch vs. dynamic stretch of freely perfused rat hindlimbs.

Comparison of the effects of static and dynamic stretch on changes in MAP (a, b) and RSNA (c) in five rats with freely perfused hindlimbs. Panel "d" shows the peak Δ tension during static stretch and the average of the peak Δ tensions during dynamic stretch. TTI=tension-time index (e.). Data within bar graphs represent the corresponding baseline MAP. The asterisk indicates a statistically significant difference versus static stretch.

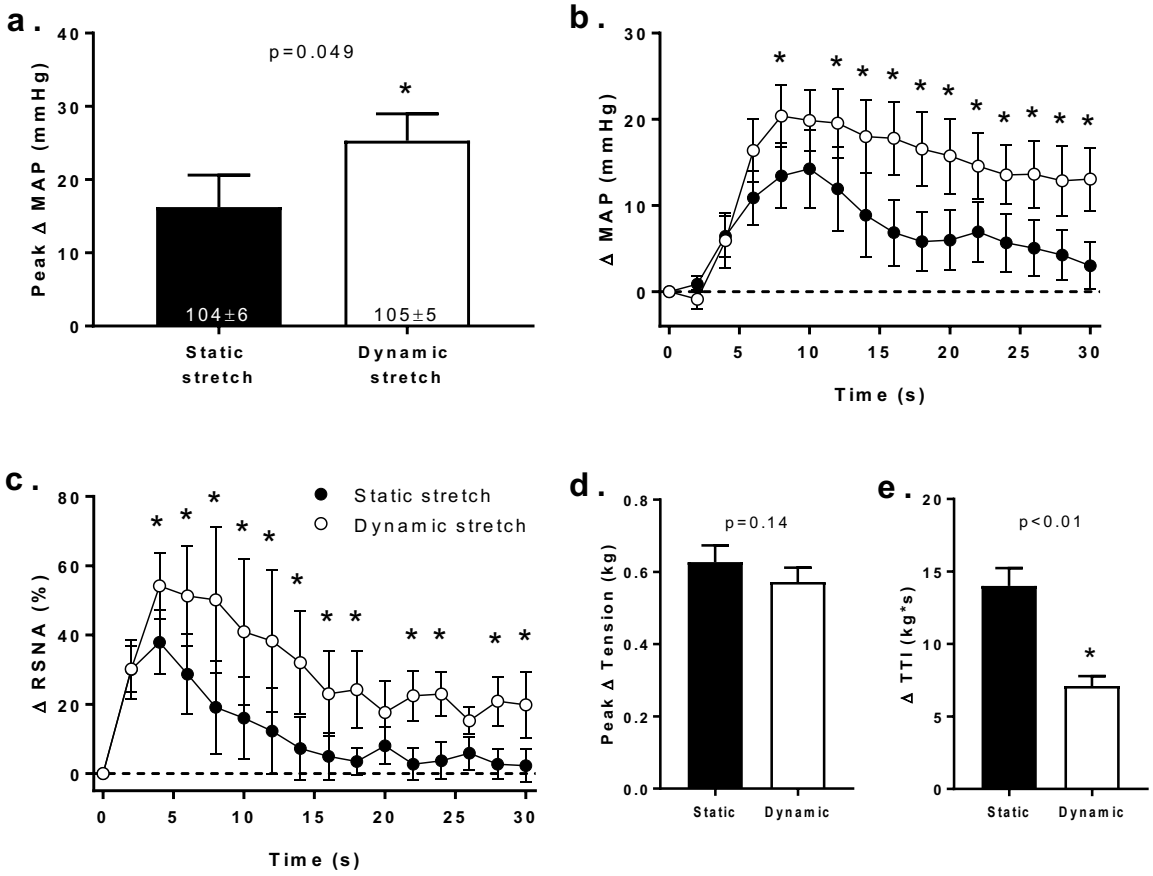

Figure 6. Static stretch vs. dynamic stretch of ligated rat hindlimbs.

Comparison of the effects of static and dynamic stretch on changes in MAP (a, b) and RSNA (c) in eight rats with hindlimbs with a ligated femoral artery. Panel "d" shows the peak Δ tension during static stretch and the average of the peak Δ tensions during dynamic stretch. TTI=tension-time index (e). Data within bar graphs represent the corresponding baseline MAP. The asterisks indicate a statistically significant difference ($p<0.05$) versus static stretch.

Chapter 4 - Discussion

The mechanically-sensitive component of the exercise pressor reflex during dynamic muscle contractions was found recently to be exaggerated when evoked from a previously ligated hindlimb compared to the reflex evoked from a freely perfused hindlimb (12). That investigation was designed specifically to gain translational mechanistic insights into the larger increases in blood pressure found in PAD patients versus aged-matched healthy counterparts during low-intensity rhythmic plantar flexion exercise (31, 39, 40), and low-intensity treadmill walking (5, 7). To investigate the mechanisms contributing to the exaggerated mechanically-sensitive component of the exercise pressor reflex in ligated rats we used a 1 Hz dynamic stretch protocol to produce repetitive mechanical stimuli that were independent of metabolites produced during dynamic skeletal muscle contraction. We found that the increases in blood pressure and RSNA during dynamic stretch were larger when evoked from the ligated hindlimb compared to the contralateral freely perfused hindlimb. Our finding suggests that femoral artery ligation chronically sensitized the mechanoreceptors that were stimulated during dynamic stretch which likely played a role in the ligated-induced exaggeration of the mechanically-sensitive component of the exercise pressor reflex during dynamic contractions (12).

We first compared the pressor and sympathetic nerve responses during dynamic muscle stretch to those found during dynamic contraction in freely perfused rats to provide insight into the contribution of mechanoreceptors to the responses evoked during dynamic contraction. We found that the magnitude of increase in blood pressure and RSNA were similar between the different maneuvers. In contrast, Stebbins et al. (46) found in cats that static muscle stretch produced 51% of the pressor response produced by static muscle contraction which was interpreted to suggest that approximately half of the pressor response during static contraction

may be attributed to mechanoreceptor stimulation (46). Our present findings may be interpreted to suggest that mechanoreceptors mediated essentially the entirety of the pressor response during dynamic contraction and that metaboreceptors played little role, if any, in the response. That interpretation needs to be made cautiously, however, for several reasons. First, group IV (i.e., primarily metabolically sensitive) muscle afferents have been found to increase their responsiveness during even very low intensity dynamic exercise (1). Second, although static hindlimb muscle stretch in the cat was found to have no impact on muscle effluent venous blood pH, lactate, or potassium (46), we cannot rule out the possibility that dynamic muscle stretch produced some chemical substance that contributed to the evoked pressor response to a small degree (26, 50). Third, muscle mechanoreceptors were stimulated differently during dynamic stretch and contraction in our experiments. Specifically, during stretch the muscles were passively lengthened whereas during contraction the muscles shortened and intramuscular pressure concurrently increased. Notwithstanding those considerations, the distinct RSNA bursts with a short onset latency that were produced during dynamic contractions are consistent with the notion that contractions produced at least a predominately mechanical stimulus and the dynamic stretch protocol replicated closely the frequency and overall magnitude of that stimulus. Thus, the dynamic stretch protocol is a valuable experimental tool that allowed us to gain insight into the muscle mechanoreceptor stimulation present during dynamic contraction in the absence of any contraction-induced metabolite production.

Intermittent and rhythmic skeletal muscle contraction (11, 12, 21, 22, 55) and intermittent skeletal muscle stretch (21, 22) have been found to evoke RSNA bursts that are synchronized with muscle tension development with a short onset latency. Our present finding of a short RSNA onset latency during dynamic contractions agrees with those previous findings. In

contrast to dynamic contraction, the longer RSNA onset latency during dynamic stretch resulted in a "right shifted" RSNA bursting pattern and this was a consistent finding across all dynamic stretch experiments in either freely perfused or ligated hindlimbs. This finding conflicts with previous investigations in decerebrate rats from Koba et al. (21, 22) which show that RSNA increased rapidly at the onset of a one-second stretch. The reason for the discrepancies between our present findings and those of Koba et al. (21, 22) are unknown and await further investigation.

The recent finding that femoral artery ligation did not increase piezo1 or piezo2 channel protein expression in lumbar DRG tissue was interpreted to suggest that mechanoreceptor sensitization accounted for the greater mechanically-sensitive component of the exercise pressor reflex during dynamic contractions in ligated rats versus freely perfused rats (12). That finding could not determine, however, whether the exaggerated role of mechanoreceptors in the ligated rats was due to an enhanced sensitization of mechanoreceptors by metabolites produced acutely during muscle contraction, a more chronic sensitization, or some combination of both. Our present finding that femoral artery ligation exaggerated the pressor and renal sympathetic nerve responses during dynamic stretch suggests that femoral artery ligation resulted in a chronic sensitization of muscle mechanoreceptors. Whether further sensitization occurs acutely by metabolites produced during contraction, and whether that sensitization is enhanced in ligated compared to freely perfused hindlimbs, is unknown but is plausible given that reduced blood flow reserve capacity during exercise likely results in enhanced metabolite production and accumulation. We also cannot rule out the possibility that ligation increased the expression of a class of mechanoreceptor other than piezo. For example, TRPA1 may be mechanically-activated (10) and its expression is increased in rat lumbar DRG tissue after 24 hours of femoral artery

ligation (56). TRPA1 blockade did not reduce the pressor or sympathetic nerve responses during static stretch (56), but whether it is involved in the responses during dynamic stretch is unknown.

The mediator of the chronic sensitization of the mechanoreceptors stimulated during dynamic stretch in the ligated hindlimb in our experiments is unknown. There is evidence to suggest that bradykinin 2 receptors (29) and endoperoxide 4 receptors (38, 57) may have played a role. Pharmacological blockade of those receptors was found to reduce the pressor response during static stretch in ligated rats (29, 57). Whether those findings have implications for our present investigation that utilized dynamic stretch is unclear, however, because femoral artery ligation has been found to augment the pressor response during static stretch in some studies (29, 30, 35, 56, 57) but not in others (24, 25, 43, 52-54). In the present investigation, the pressor response during static stretch was not different between freely perfused and ligated hindlimbs. Moreover, in the freely perfused hindlimb static and dynamic stretch produced similar increases in MAP and RSNA, which is consistent with the previous finding of Daniels et al. (13) from a freely perfused cat hindlimb. In contrast to freely perfused hindlimbs, in ligated hindlimbs we found that the increases in blood pressure and RSNA were greater during dynamic stretch versus static stretch. Considered together, our findings indicate that femoral artery ligation exaggerated the increases in blood pressure and RSNA evoked during dynamic, but not static, hindlimb muscle stretch. The reason for this difference in our experiments is not known. We can only offer our speculation that there may be differences in the proportional contribution of the classes of mechanoreceptors that mediate the pressor response during static and dynamic stretch and that femoral artery ligation impacts the mechanoreceptors stimulated during dynamic stretch more than those stimulated during static stretch.

There are a few experimental considerations that should be noted. First, although dynamic stretch replicates the rhythmicity of muscle mechanoreceptor stimulation present during dynamic muscle contraction, as discussed above, it does not replicate the exact method of mechanoreceptor stimulation present during concentric contractions. Second, in the cat hindlimb, muscle stretch stimulates a somewhat different population of mechanically-sensitive afferents than is stimulated by contraction (18). However, in freely perfused rats 87%, and in ligated rats 100%, of the muscle afferents that were stimulated by stretch were also stimulated by contraction (47). Third, atherosclerosis develops slowly and results in a gradual, progressive narrowing of the arteries in PAD patients (41) whereas we instantaneously occluded a rat femoral artery ~72 hours prior to the experiment. Following ligation and the 72-hour recovery period, hindlimb blood flow remains adequate at rest but blood flow reserve capacity during exercise is markedly reduced which resembles the blood flow patterns at rest and during exercise in PAD patients (9, 17, 32, 33, 42). Thus, we believe that the rat model of femoral artery ligation is a valuable experimental tool which may be used to gain insight in the exercise pressor reflex in PAD patients.

In summary, we demonstrated that in decerebrate, unanesthetized rats the increases in blood pressure and RSNA were greater during dynamic stretch of the muscles of the hindlimb in which the femoral artery was previously ligated compared to the increases during stretch of the muscles of the contralateral freely perfused hindlimb. The dynamic stretch protocol was used to replicate the rhythmic, mechanical stimuli present during dynamic contraction independent of contraction-induced metabolite production. Our findings suggest, therefore, that a chronic sensitization of muscle mechanoreceptors contributed to the exaggerated mechanically-sensitive component of the exercise pressor reflex during dynamic contractions found in ligated rats (12).

Whether an enhanced acute metabolite-induced sensitization of muscle mechanoreceptors during contraction also contributes remains to be investigated. Our findings in this simulated PAD model in which a femoral artery is chronically ligated carry important implications for PAD patients given the technical challenges associated with investigating the mechanisms that contribute to the exaggerated exercise pressor reflex in human subjects.

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