

THE EFFECTS OF AGING ON CAPILLARY HEMODYNAMICS IN
CONTRACTING RAT SPINOTRAPEZIUS MUSCLE

by

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Abstract

Advancing age alters the structural and functional determinants of convective and diffusive muscle oxygen (O_2) flux. However, capillary red blood cell (RBC) hemodynamics have not been investigated during contractions in muscles of old animals.

PURPOSE: To test the hypothesis that aging induces significant alterations in capillary hemodynamics during electrically-induced contractions in the spinotrapezius muscle of old Fischer 344 x Brown Norway rats when compared to younger counterparts.

METHODS: The spinotrapezius muscle was observed via intravital microscopy in 8 old (O: 26-30 months) and 5 young (Y: 6-8 months) animals. Wire electrodes elicited 1 Hz (6-8 volts) contractions for 3 minutes. RBC flux (F_{RBC}), velocity (V_{RBC}), capillary hematocrit (H_{CAP}), and total microvascular O_2 delivery ($\dot{Q}O_{2m}$) were measured both at rest and during the steady-state of muscle contractions. **RESULTS:** At rest F_{RBC} and V_{RBC} were elevated in O compared to Y rats, while there was no difference in H_{CAP} or $\dot{Q}O_{2m}$ between groups. During the contracting steady-state, ΔF_{RBC} (Y: 28.8 ± 7.7 , O: -2.9 ± 1.4 cells/s), ΔV_{RBC} (Y: 253 ± 68 , O: -4 ± 15 $\mu\text{m/s}$), ΔH_{CAP} (Y: 0.02 ± 0.02 , O: -0.03 ± 0.01 cells/ μm), and $\Delta \dot{Q}O_{2m}$ (Y: 892 ± 255 , O: -24 ± 30 cells/s/mm) cells/s/mm were all lower ($P < 0.05$) in O compared to Y rats. **CONCLUSION:** These results indicate that despite maintained total convective and diffusive O_2 transport at rest, advancing age results in significant alterations in capillary hemodynamics during electrically-induced contractions. These alterations likely contribute to the mechanisms responsible for the reduced exercise capacity commonly found in elderly populations.

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CHAPTER 1 - Introduction

Following maturation, advancing age is accompanied by both peripheral and central structural and functional alterations within the oxygen (O₂) transport pathway that lead to a decline in maximal exercise tolerance and muscle function. For example, the senescent myocardium may exhibit a reduced capacity to elevate cardiac output to meet the metabolic demands of exercise (Lakatta, 1990; Folkow & Svanborg, 1993) and skeletal muscle perfusion may be compromised (Wahren *et al.*, 1974; Irion *et al.*, 1987). Recently, additional focus has been placed on a reduced ability of the aging skeletal muscle microcirculation to extract O₂ despite maintained convective O₂ delivery (McGuire *et al.*, 2001; Hepple *et al.*, 2003), thereby implicating an important peripheral component in the exercise intolerance of elderly individuals.

Within aging skeletal muscle there is a reduction in the number of feed arteries (Behnke *et al.*, 2006) and capillaries as well as mitochondrial volume density (Conley *et al.*, 2000). In addition, aging affects the myogenic control of vascular conductance (Musch *et al.*, 2004; Behnke *et al.*, 2006), reduces the bioavailability of nitric oxide (NO), and attenuates endothelium-dependent vasodilation (Muller-Delp *et al.*, 2002b; Muller-Delp, 2006) resulting in a reduced ability to regulate vascular tone. These alterations are likely to contribute to the age-induced decrease in blood flow observed during dynamic leg exercise (Proctor *et al.*, 1998) and electrically-induced muscle contractions (Hammer & Boegehold, 2005), which may be accompanied by a redistribution of flow among different fiber types (Musch *et al.*, 2004). Impaired active peripheral circulatory control results in temporal reduction of muscle O₂ delivery relative

to the O₂ demands of exercise (Behnke *et al.*, 2005). Until recently, capillary rarefaction was held responsible for the inability of aged muscles to extract O₂ and sustain the transcapillary O₂ fluxes found in their younger counterparts. However, recent reports of maintained or increased capillarity and total capillary-myocyte interface relative to mitochondrial oxidative capacity do not support the presence of a capillary structural limitation to O₂ flux in aged muscle (Hepple & Vogell, 2004; Mathieu-Costello *et al.*, 2005). Given that the number of red blood cells (RBCs) along the capillary length at any given time serves as the primary determinant of tissue O₂ diffusing capacity (Groebe & Thews, 1990), alterations in microcirculatory hemodynamics and RBC distribution constitute a likely mechanism for the reduced O₂ flux and, as such, may contribute to the exercise intolerance of the elderly.

To date, few studies have examined the effects of aging on microcirculatory hemodynamics, in part, because aging results in the proliferation of collagenous overgrowth and fascial attachments making optical clarity of the peripheral microcirculation difficult at best (Tymel *et al.*, 1992; Russell *et al.*, 2003). Recently, our laboratory has utilized a modified form of the rat spinotrapezius muscle preparation for observation of microvascular function via video microscopy in health (Kindig *et al.*, 2002) disease (Kindig *et al.*, 1999; Padilla *et al.*, 2006) and aging (Russell *et al.*, 2003) research. The spinotrapezius represents an excellent muscle for microscopic analysis due to its mixed fiber type composition (Delp & Duan, 1996) and oxidative capacity similar to that of the human quadriceps (Leek *et al.*, 2001). Resting measurements have demonstrated that aging reduces the lineal density of RBC-perfused capillaries, and increases RBC velocity (V_{RBC}) and flux (F_{RBC}) within the continuously perfused

microvessels (see also (Tymk *et al.*, 1992)) such that overall F_{RBC} (i.e. microvascular O_2 delivery, $\dot{Q}_{O_{2m}}$) is maintained (Russell *et al.*, 2003). Additionally, measurements of arteriolar blood flow in the aged spinotrapezius during electrically-induced contractions have revealed that contractions up to 2 Hz may not elicit increases in arteriolar flow in contrast to the several-fold increase this contraction protocol induces in young muscles (Hammer & Boegehold, 2005). However, to our knowledge, analysis of the effects of aging on peripheral capillary hemodynamics during contractions are completely absent from the literature.

Therefore, the present investigation analyzed the spinotrapezius muscle of young (3-4 month old) and old (26-30 month old) Fischer 344 x Brown Norway rats to determine the effects of aging on capillary hemodynamics during electrically-induced contractions. We tested the following original hypotheses: 1) Aged rats would exhibit a significantly attenuated increase in F_{RBC} and V_{RBC} in response to contractions, and 2) the attenuated F_{RBC} and V_{RBC} will be associated with a blunted increase in capillary hematocrit (H_{CAP}) and, by implication, reduce total O_2 extraction in aged vs. young muscles.

CHAPTER 2 - Review of Literature

Effects of age on functional capacity and muscle fatigue

Beyond physical maturation, aging is a process that is marked by a decline in exercise tolerance and the capacity to perform physical work. Specifically, aged individuals show a reduction in maximal O₂ uptake ($\dot{V}O_{2\max}$) (Ogawa *et al.*, 1992) and a decreased power output and increased rate of muscle fatigue during repeated quadriceps contractions (Petrella *et al.*, 2005). Aging-associated limitations in functional capacity and exercise tolerance have important implications for elderly populations where exercise may be a beneficial therapeutic intervention aimed at improving quality of life.

Alterations in functional capacity may be due to varying combinations of aging-induced derangements in the structure and/or function of both central and peripheral components of the O₂ transport pathway. An elegantly-designed longitudinal study by McGuire and colleagues (McGuire *et al.*, 2001) found that stroke volume is increased with age, thereby compensating for the aging-associated decline in maximal heart rate (HR) thereby maintaining maximal cardiac output. This important finding has shifted the focus of the search for the primary mechanistic basis of aging-induced decrements in exercise tolerance away from central adaptations towards aging-induced alterations in the periphery.

Aging-induced alterations in exercise hyperemia

It is generally accepted that, in healthy young individuals, muscle blood flow (\dot{Q}_m) to the submaximally exercising muscles is increased in such a fashion that the O₂

delivery does not limit the $\dot{V}O_2$ of the exercising muscle ($\dot{V}O_{2m}$) either in the transition from rest to exercise or during the steady-state (Barstow *et al.*, 1990; Grassi, 2000). However, many studies have investigated the possibility of a reduction in \dot{Q}_m to active muscle with advancing age, which would act to limit exercise tolerance. For example, several studies have reported \dot{Q}_m reductions when comparing old rats to their younger counterparts during electrical stimulation of skeletal muscle (Irion *et al.*, 1987; Hammer & Boegehold, 2005). Similarly, in humans, \dot{Q}_m to the active leg muscles is reduced during dynamic exercise in older men (Wahren *et al.*, 1974; Proctor *et al.*, 1998) and women (Proctor *et al.*, 2003a). Recently, Donato *et al.* (Donato *et al.*, 2006) demonstrated that the difference in the leg \dot{Q}_m response to exercise found between young and old subjects was a product of the response to the exercise stimulus itself, as opposed to variations in resting \dot{Q}_m between age groups. Collectively, the results of the aforementioned studies are in conflict with other reports where no difference in \dot{Q}_m was found between young and old subjects during forearm exercise (Jasperse *et al.*, 1994) or dynamic leg exercise (Olive *et al.*, 2002; Proctor *et al.*, 2003b). Specifically, Olive *et al.* (Olive *et al.*, 2002) demonstrated that exercising \dot{Q}_m is a function of physical activity patterns but not age differences.

Despite contrasting reports concerning bulk blood flow in aged individuals, circulatory maladaptations may be occurring within the aged peripheral circulation that would reduce exercise capacity in the face of maintained bulk \dot{Q}_m . For example, using radiolabeled microspheres, Musch *et al.* (Musch *et al.*, 2004) reported no difference between young and old rats total limb \dot{Q}_m in response to treadmill running exercise.

However, they were able to demonstrate that aging elicited a redistribution of \dot{Q}_m away from highly oxidative muscles towards highly glycolytic muscles. It is likely that the reduced \dot{Q}_m to the oxidative fibers seen during submaximal exercise is closely linked to the earlier onset of fatigue that is witnessed in the aged population via mismatching of the $\dot{Q}_m/\dot{V}O_{2m}$ ratio. In addition to \dot{Q}_m redistribution, middle-aged men may tend to elicit a higher mean arterial pressure (MAP) and lower vascular conductance ($\dot{Q}_m = \text{MAP} \times \text{vascular conductance}$) during exercise when compared with younger men (Magnusson *et al.*, 1994; Lawrenson *et al.*, 2003; Poole *et al.*, 2003). The increase in perfusion pressure most likely reflects a heightened state of vasoconstriction (or reduced vasodilation) of the vessels supplying the working muscles (Proctor & Parker, 2006). Overall, the redistribution of \dot{Q}_m away from the highly oxidative fibers as well as a lower vascular conductance and increased driving pressure most likely translates into alterations that impair skeletal muscle microvascular function during exercise in old subjects.

Aging-induced alterations within the microvasculature

Although central (Ogawa *et al.*, 1992) and peripheral (Musch *et al.*, 2004) derangements to \dot{Q}_m certainly exist in aged individuals, the effect that these perturbations have on capillary-tissue O_2 exchange is speculative at best without direct functional assessment of the peripheral microcirculation. Elegant modeling studies by Groebe and Thews (Groebe & Thews, 1986) and Friederspiel and Popel (Federspiel & Popel, 1986) have suggested that the total number of red blood cells that lie adjacent to a muscle fiber is the primary determinant of the diffusion capacity of O_2 (DO_2). In turn, the DO_2 is a principle determinant of skeletal muscle O_2 extraction. Alterations in either

microcirculatory structural or functional capacity could account for limitations to DO_2 with aging. However, reports by Hepple et al. (Hepple & Vogell, 2004) and Mathieu-Costello et al. (Mathieu-Costello *et al.*, 2005) have demonstrated that capillarity and, more specifically, the capillary-fiber-interface is either maintained or improved in aged animals relative to the fiber oxidative capacity, thereby implicating derangements in microvascular function.

Technological and ethical limitations have prevented direct observation of the peripheral i.e, muscle microcirculation in humans. Therefore, a rat model has commonly been employed to examine functional microcirculatory indices of O_2 delivery including F_{RBC} , V_{RBC} , capillary hematocrit (H_{cap}), capillary lineal density (number of capillaries per muscle width), countercurrent flow, and the partial pressure of O_2 (PO_2) in the microcirculation (Kindig *et al.*, 1999; Kindig *et al.*, 2002; Richardson *et al.*, 2003; Russell *et al.*, 2003; Behnke *et al.*, 2005; Padilla *et al.*, 2006). In the rat, one of the muscles commonly used to examine the microcirculation is the spinotrapezius muscle (Kindig *et al.*, 1998; Kindig *et al.*, 1999; Kindig & Poole, 1999, , 2001; Kindig *et al.*, 2002; Russell *et al.*, 2003). The spinotrapezius muscle is located on the superficial dorsal region of the rat having its origin on the vertebral column and insertion on the scapula. The muscle is ideal for microcirculatory observation due to its mixed fiber type composition (Delp & Duan, 1996) and oxidative capacity similar to that of human quadriceps muscle (Leek *et al.*, 2001). Additionally, it can be readily exteriorized, there is an absence of fascia and large vessels obscuring the microvascular field, and it is very thin, making it suitable for transmission light microscopy. Using intravital microscopy, Russell et al. (Russell *et al.*, 2003) was able to demonstrate that differences exist in

capillary hemodynamics at rest between young and old rats. Specifically, in old rats the lineal density of capillaries sustaining RBC perfusion was decreased, while V_{RBC} and F_{RBC} were increased within the flowing capillaries. In addition, the number of capillaries demonstrating countercurrent flow, which provides for a higher average PO_2 surrounding the myocyte (Kobayashi *et al.*, 1990), was reduced. These alterations were such that convective $\dot{Q}O_{2m}$ (lineal density of flowing capillaries x mean F_{RBC}) at rest was not affected. The increased V_{RBC} in aged rats supported the results of a previous investigation that reported an increased V_{RBC} within the extensor digitorum longus muscle of old rats at rest (Tymk *et al.*, 1992).

In contrast to resting microvascular flow measurements in aged skeletal muscle, measurements in contracting aged muscle have remained elusive primarily due to reductions in visual clarity of the muscle when viewed under magnification. Despite these limitations, Hammer and Boegehold (Hammer & Boegehold, 2005) utilized the rat spinotrapezius muscle and made measurements of arteriolar flow in young and old animals in response to 3 minutes of electrically-induced contractions of varying frequencies (.5, 1, and 2 Hz). Their results demonstrated that up to 2 Hz contractions, aged rats elicited no consistent increase in arteriolar V_{RBC} while consistent increases were observed in their younger counterparts. However, a significant limitation to the study was that, due to contraction-mediated disruption of the microvascular field, the \dot{Q}_m response was limited to assessment during the immediate post-contraction period. More importantly, functional determination of RBC hemodynamics in the skeletal muscle microcirculation, the site of peripheral gas exchange, is still lacking. The alterations in microcirculatory structure and function that exist in old rats at rest, and likely persist

during contractions, may reflect alterations in peripheral vascular control (see Discussion) which impair O₂ delivery and exchange during exercise, despite maintenance of these variables under resting conditions.

Summary

Aged individuals demonstrate reductions in $\dot{V}O_{2\max}$ and impairments in muscle function. These maladaptations are consequent to a reduced \dot{Q}_m , altered peripheral \dot{Q}_m distribution, and/or variation in the control of vascular conductance. While the effect that aging-induced alterations in peripheral O₂ delivery have on skeletal muscle capillary RBC hemodynamics has been described at rest, to date, it is currently unknown if RBC capillary hemodynamics are altered in aged muscle during contractions. Analyzing peripheral microcirculatory alterations during contractions is important because it has direct implications for peripheral gas exchange during exercise and, therefore, the ability of aged individuals to sustain dynamic exercise.

CHAPTER 3 - Methods

Animals

A total of 13 animals, 8 old (26-30 months) and 5 young (6-8 months) male Fischer 344 x Brown Norway F1 hybrid (F344xBN) rats were used in the present investigation. These ages were chosen because they represent young adult (6-8 months) and senescent (26-30 months) rats in accordance with the lifespan of the F344xBN strain. Additionally, the F344xBN represents an ideal model to investigate the effects of healthy aging in that they are the result of a highly judicious breeding process designed to minimize the incidence of many typical aging-induced pathologies that might confound data interpretation. All rats were maintained on a 12:12 hour light-dark cycle and provided food and water *ad libitum*.

Animals were initially anesthetized with pentobarbital sodium (30-50 mg/kg i.p. to effect), with supplementation as necessary throughout the duration of the protocol, and placed on a heating pad. Core temperature was measured via a rectal probe and maintained at ~37° C. A catheter (Intramedic polyethylene-50 connected to polyethylene-10 tubing, Clay Adams Brand, Sparks, MD) was placed in the aortic arch via the right carotid artery for continuous monitoring of MAP and HR throughout the experiment. Upon completion of each experiment, animals were euthanized via pentobarbital overdose. All experimental procedures were approved by the Kansas State University Institutional Animal Care and Use Committee.

Muscle preparation

The rat spinotrapezius muscle is a postural muscle, originating in the lower-thoracic and upper-lumbar region of the vertebral column and inserting on the scapula, which also functions to stabilize the scapula, for example, during downhill treadmill running (Kano *et al.*, 2004). The left spinotrapezius muscle was exposed and exteriorized as described previously (Poole *et al.*, 1997; Kindig *et al.*, 2002; Russell *et al.*, 2003), in a manner which preserves the vascular network and neural connections supplying the muscle (Bailey *et al.*, 2000). Briefly, the caudal end of the muscle was isolated from its insertion and sutured at 5 equidistant points to a thin wire manifold and attached to a stretching and swivel apparatus. Thin stainless steel wire electrodes were sutured to both the peripheral caudal end and near the motor point on the more proximal ventral surface of the muscle, in a manner that elicited optimal muscle fiber shortening. The manifold was secured to the platform with the ventral aspect of the muscle reflected upwards for microscopic observation. Sarcomere length was initially set at $\sim 2.7 \mu\text{m}$, which prevents stretch-induced flow attenuations and elicits normal physiological capillary flow (Kindig & Poole, 1999). Throughout the experiment, the spinotrapezius muscle was kept moist by constantly superfusing it with a Krebs-Henseleit bicarbonate-buffered solution equilibrated with 95% N₂-5% CO₂, and exposed surrounding tissue was covered with saran wrap (Dow Brands, Indianapolis, IN).

Experimental protocol

A microcirculatory field from the mid-caudal region of the spinotrapezius that provided optimal clarity for the visualization of a minimum of 5-6 capillaries was selected randomly for analysis. Images were obtained with an intravital microscope

(Nikon Eclipse E600-Fn, Tokyo, Japan) equipped with a non-contact immersion lens (x40, numerical aperture 0.8) under a final magnification of x1,184. Images were transmitted in real time to a high-resolution color monitor (Sony Trinitron, PVM-1954Q, Ichinomiya, Japan) and recorded (JVC S-VHS Master XG) for future off-line analysis via videocassette recorder (JVC BR-S822U, Elmwood Park, NJ). Resting data was obtained for 60 seconds, after which electrical stimulation (1 Hz twitch contractions, 2 ms duration, 6-8 volts) elicited muscle contractions for 180 seconds. These stimulation parameters were selected based on the fact that in young healthy rats, utilizing the same preparation, they are known to induce significant increases in microcirculatory hemodynamic and metabolic variables (i.e. 2-3 fold increases F_{RBC} and >4 fold increase in $\dot{V}O_{2m}$; Behnke *et al.*, 2002; Kindig *et al.*, 2002). Following the experimental protocol, 100 μ l blood samples were drawn for measurement of arterial blood gases, systemic hematocrit, pH, and blood lactate concentrations. Both the right and left spinotrapezius muscles were carefully dissected, weighed, and frozen.

Off-line analysis

Capillary diameter (d_C) was measured at rest using precision calipers at two different sites along each capillary where the endothelium was clearly visible. Sarcomere length was determined in each muscle fiber where 11 consecutive A-bands could be clearly distinguished. Capillary flow was observed in real-time and using frame-by-frame analysis (30 frames/s). The percentage of vessels sustaining continuous RBC perfusion was determined as: (number of continuously RBC-perfused vessels \div total number of vessels in a given muscle region) \times 100. F_{RBC} was measured as the number of RBCs passing an arbitrary point per second (cells/s), and V_{RBC} was measured as RBC

speed along the visible capillary length (μ/s). Both F_{RBC} and V_{RBC} were measured in all clearly visible capillaries, within the same frames (where possible), at rest and between 150-180s of contractions. This time-frame (i.e., 150-180s) was assumed to be the contracting steady-state based on the observation that in aged contracting spinotrapezius muscle, the microvascular PO_2 does not change over an identical time period (Behnke *et al.*, 2005). Where poor visual clarity did not allow F_{RBC} and V_{RBC} determination within the same frames, the hemodynamics were measured in as close temporal proximity as possible. In all capillaries where F_{RBC} and V_{RBC} were measured, capillary tube hematocrit (H_{CAP}) was calculated as $H_{CAP} = (RBC_{VOLUME} \times F_{RBC}) \div [\pi \times (d_c/2)^2 \times V_{RBC}]$, where RBC_{VOLUME} was assumed to be $61 \mu m^3$ (Altman & Dittmer, 1974), and capillary cross-sectional shape was assumed to be circular *in vivo* (Mathieu-Costello *et al.*, 1998). In some cases (particularly in aged muscles), poor clarity dictated measurement of V_{RBC} and H_{CAP} and the equation was rearranged to solve for F_{RBC} (i.e. $F_{RBC} = ([\pi \times (d_c/2)^2] \times V_{RBC} \times H_{CAP}) \div RBC_{VOLUME}$). $\dot{Q}O_{2m}$ to the whole muscle region at rest and the steady-state of contractions was calculated as the product of the lineal density of flowing capillaries x mean capillary F_{RBC} .

Statistical analysis

All data are presented as mean \pm SE. Means were compared with paired and unpaired Student's *t*-tests. The level of significance was set at $P \leq 0.05$.

CHAPTER 4 - Results

MAP was not different between young (Y) and old (O) rats at rest (Y: 85 ± 4 , O: 104 ± 7 mmHg, $P > 0.05$) or during contractions (Y: 94 ± 2 , O: 97 ± 11 mmHg, $P > 0.05$), and did not change from rest to contractions in either group.

Structural variables and capillary flow

Structural and general capillary flow data are presented in Table 4-1. The lineal density of capillaries was reduced ($P < 0.05$) while the percentage of capillaries supporting flow was similar ($P > 0.05$) in O compared to Y rats. As a result, there was an aging-induced reduction ($P < 0.05$) in the lineal density of capillaries supporting RBC flow at rest and during contractions. The percentage of capillaries exhibiting countercurrent flow was similar between groups ($P > 0.05$).

RBC hemodynamics

F_{RBC} data is presented in Figure 4-1 for Y and O rats at rest and during contractions. During contractions, F_{RBC} increased ($P < 0.05$) from resting values in Y but not in O ($P > 0.05$). Thus, from rest to contractions, the ΔF_{RBC} (Y: 28.8 ± 7.7 , O: -2.9 ± 1.4 cells/s, $P < 0.05$) was lower in O rats. V_{RBC} data is presented in Figure 4-2 and, similar to F_{RBC} , at rest V_{RBC} was elevated in O rats while it was increased during contractions in Y ($P < 0.05$) but not O ($P > 0.05$) animals. Thus, from rest to contractions, the ΔV_{RBC} (Y: 253 ± 68 , O: -4 ± 15 $\mu\text{m/s}$, $P < 0.05$) was lower in O rats. H_{CAP} (Figure 4-3) was similar ($P > 0.05$) between Y and O rats at rest and contractions elicited no changes ($P > 0.05$) from resting values in either group. However, the

contracting H_{CAP} was lower in O (0.19 ± 0.02 cells/ μm) compared to Y (Y: 0.25 ± 0.02 cells/ μm $P < 0.05$), and the transition to the contracting steady-state resulted in a lower ΔH_{CAP} in the O (-0.03 ± 0.01 cells/ μm) versus Y (0.02 ± 0.02 cells/ μm , $P < 0.05$) rats.

Contractions resulted in an increase in $\dot{Q}O_{2m}$ in Y (1615 ± 402 cells/s/mm, $P < 0.05$) with no change from resting values in O (780 ± 121 cells/s/mm, $P > 0.05$) rats (Figure 4-4). Therefore, the $\Delta \dot{Q}O_{2m}$ from rest to contractions that was evident in the Y (892 ± 255 cells/s/mm, $P < 0.05$) did not occur in the O (-24.2 ± 30 cells/s/mm) rats.

RBC flux-velocity relationship

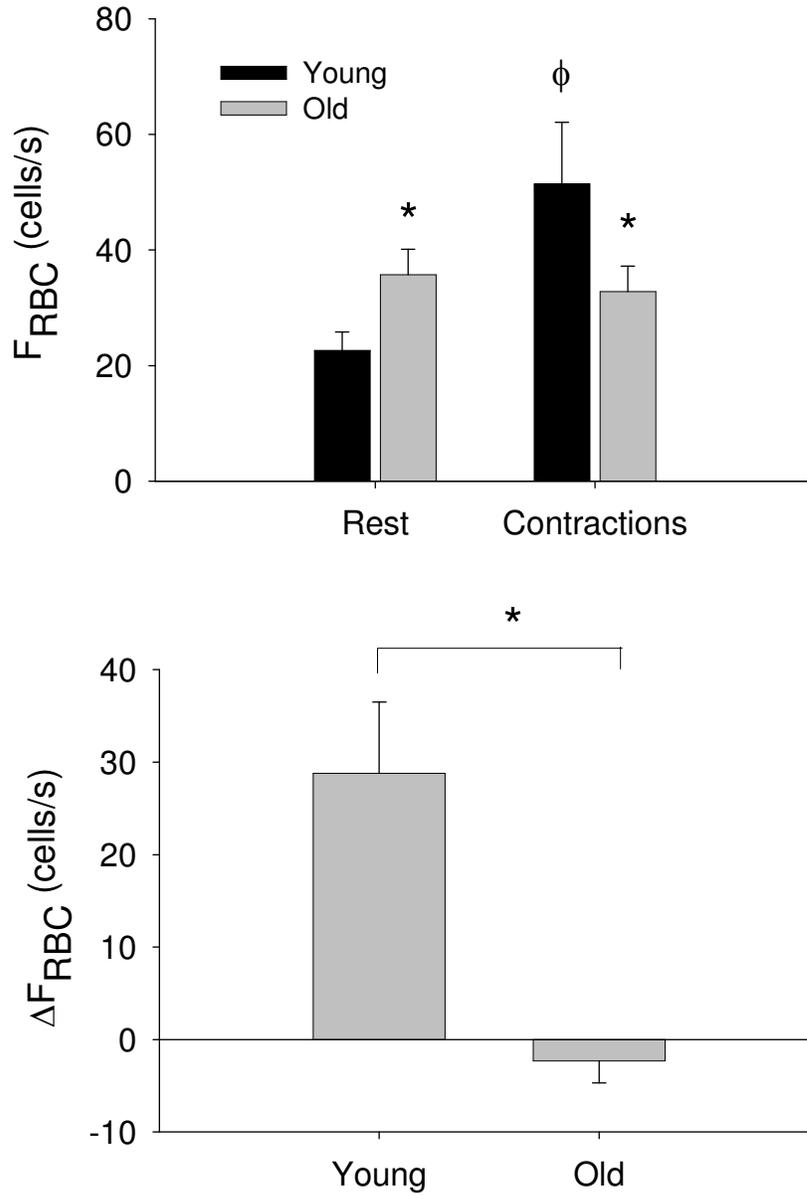
In both age groups, there was a significant correlation ($P < 0.05$ for both) between F_{RBC} and V_{RBC} as determined from mean values for rest and contractions from each muscle (Figure 4-5). The slope of this relationship dictates the capillary hematocrit and, accordingly, the steeper slope in the O compared to Y muscles reflects the reduced H_{CAP} in O rats during contractions.

Table 4-1: Structural variables and capillary flow

	<i>Young</i>		<i>Old</i>	
	<u>Rest</u>	<u>Contractions</u>	<u>Rest</u>	<u>Contractions</u>
Sarcomere length, μm	2.7 ± 0.1	<i>N/A</i>	2.7 ± 0.1	<i>N/A</i>
Capillary diameter, μm	5.5 ± 0.3	<i>N/A</i>	6.1 ± 0.2	<i>N/A</i>
Countercurrent flow, %	21 ± 3	<i>N/A</i>	16 ± 3	<i>N/A</i>
Lineal density, capillaries/mm	32 ± 2	<i>N/A</i>	$24 \pm 1^*$	<i>N/A</i>
Flowing capillaries, %	96 ± 2	96 ± 2	93 ± 2	97 ± 2
Flowing density, capillaries/mm	31 ± 3	30 ± 1	$22 \pm 1^*$	$24 \pm 1^*$

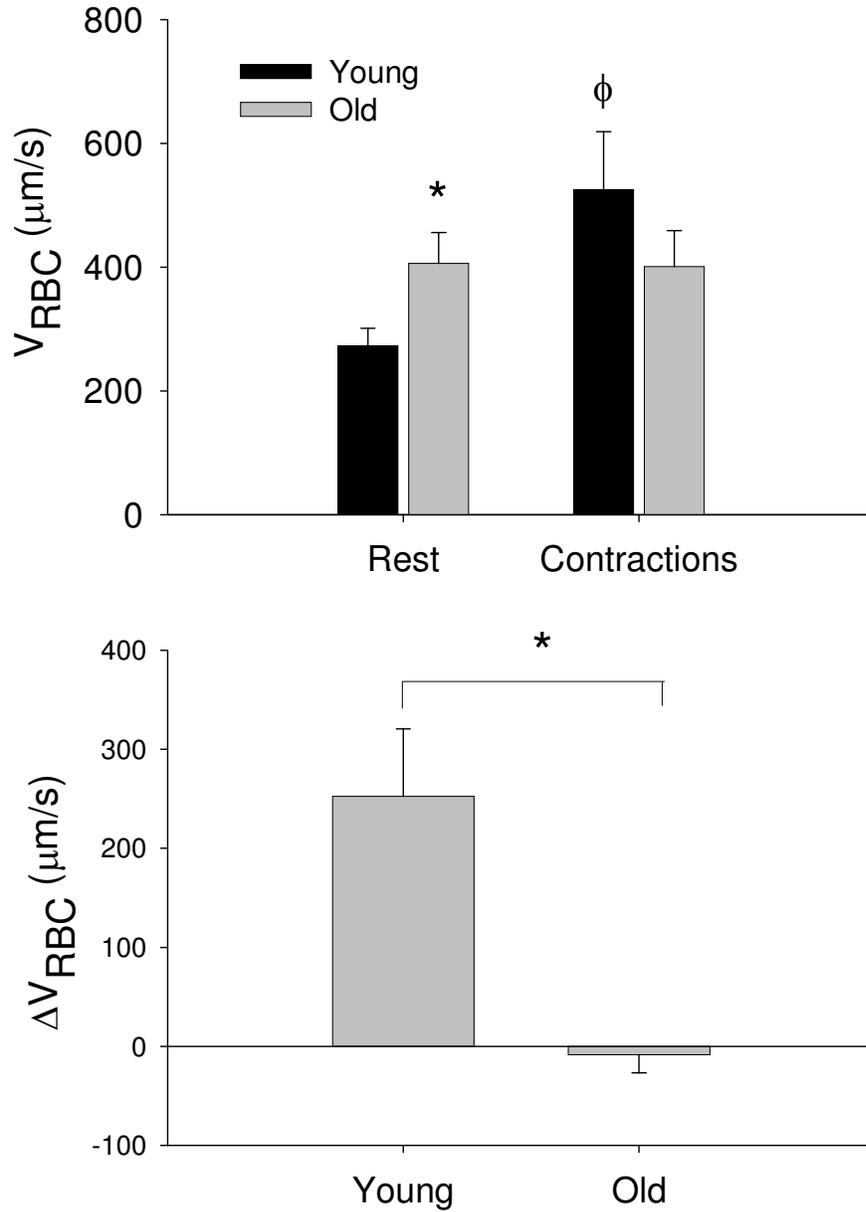
Values are means \pm SE. Lineal density, lineal density of total capillaries; flowing density, lineal density of flowing capillaries. * $P < 0.05$ compared to young.

Figure 4-1: RBC flux



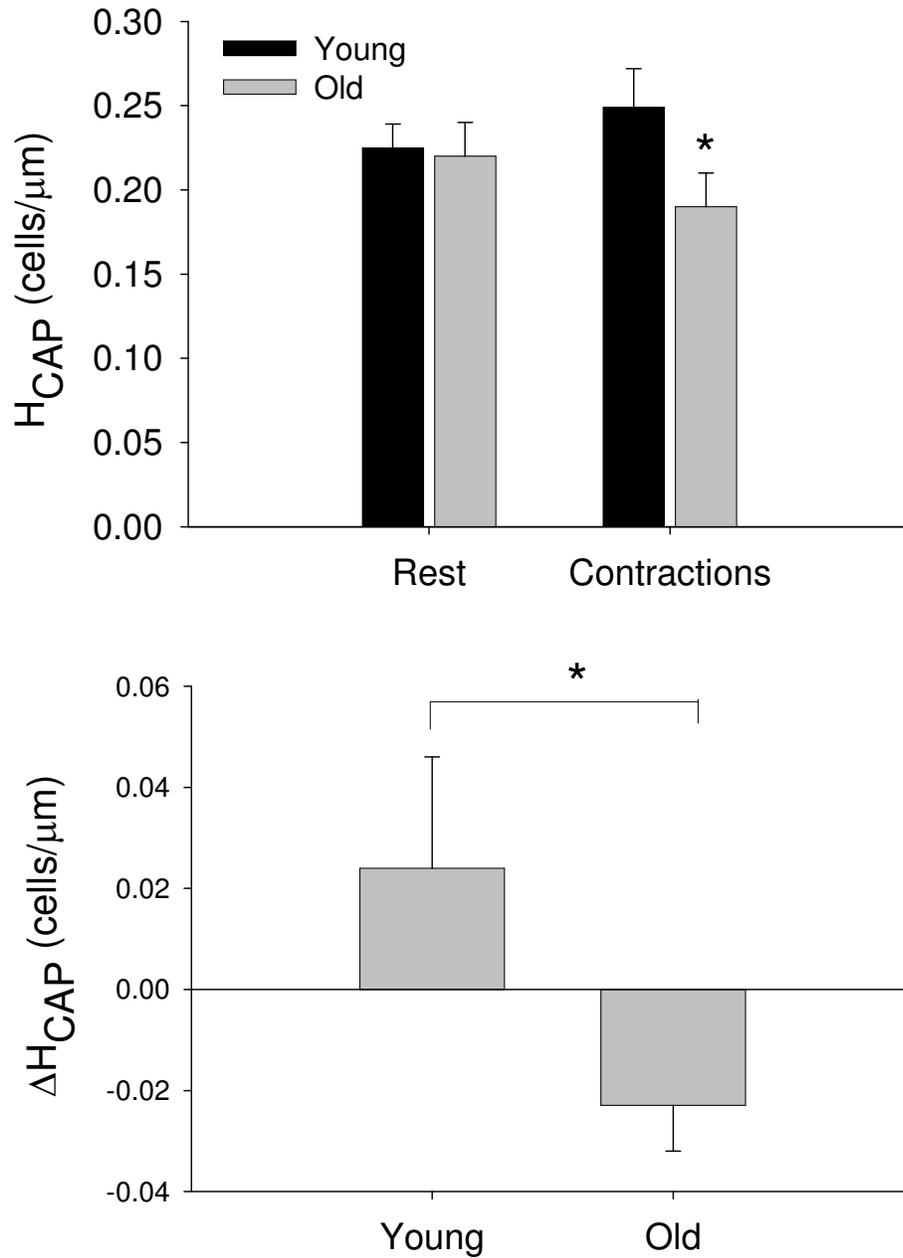
Top Panel: Capillary red blood cell flux (F_{RBC}) measured at rest and during the contracting steady-state in Y and O animals. *Bottom Panel:* The change in F_{RBC} from rest to contractions for both age groups. * $P < 0.05$ compared to Y, $\phi P < 0.05$ compared to rest.

Figure 4-2: RBC velocity



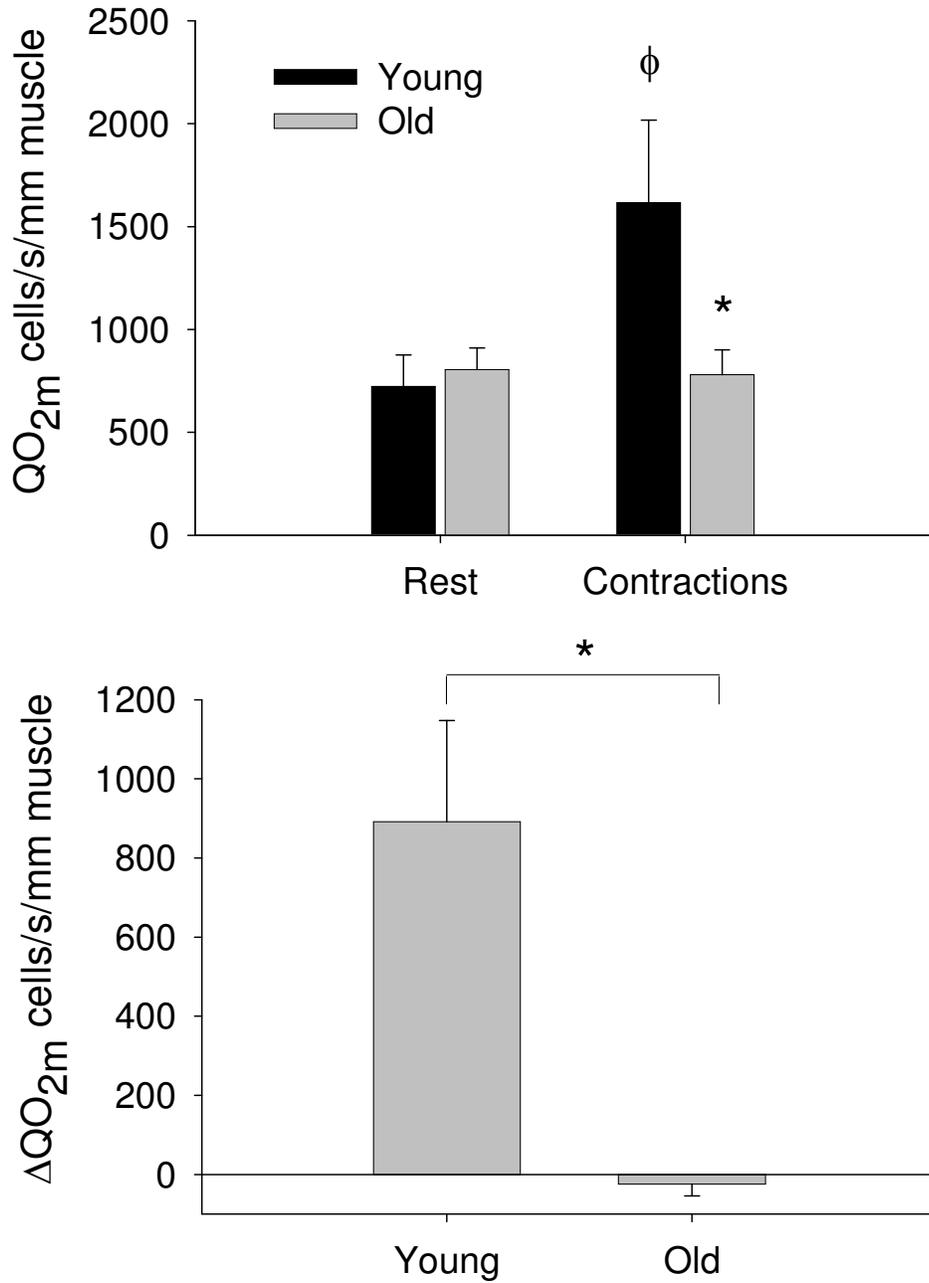
Top Panel: Capillary red blood cell velocity (V_{RBC}) measured at rest and during the contracting steady-state in Y and O animals. *Bottom Panel:* The change in V_{RBC} from rest to contractions for both age groups. * $P < 0.05$ compared to Y, $\phi P < 0.05$ compared to rest.

Figure 4-3: Capillary hematocrit



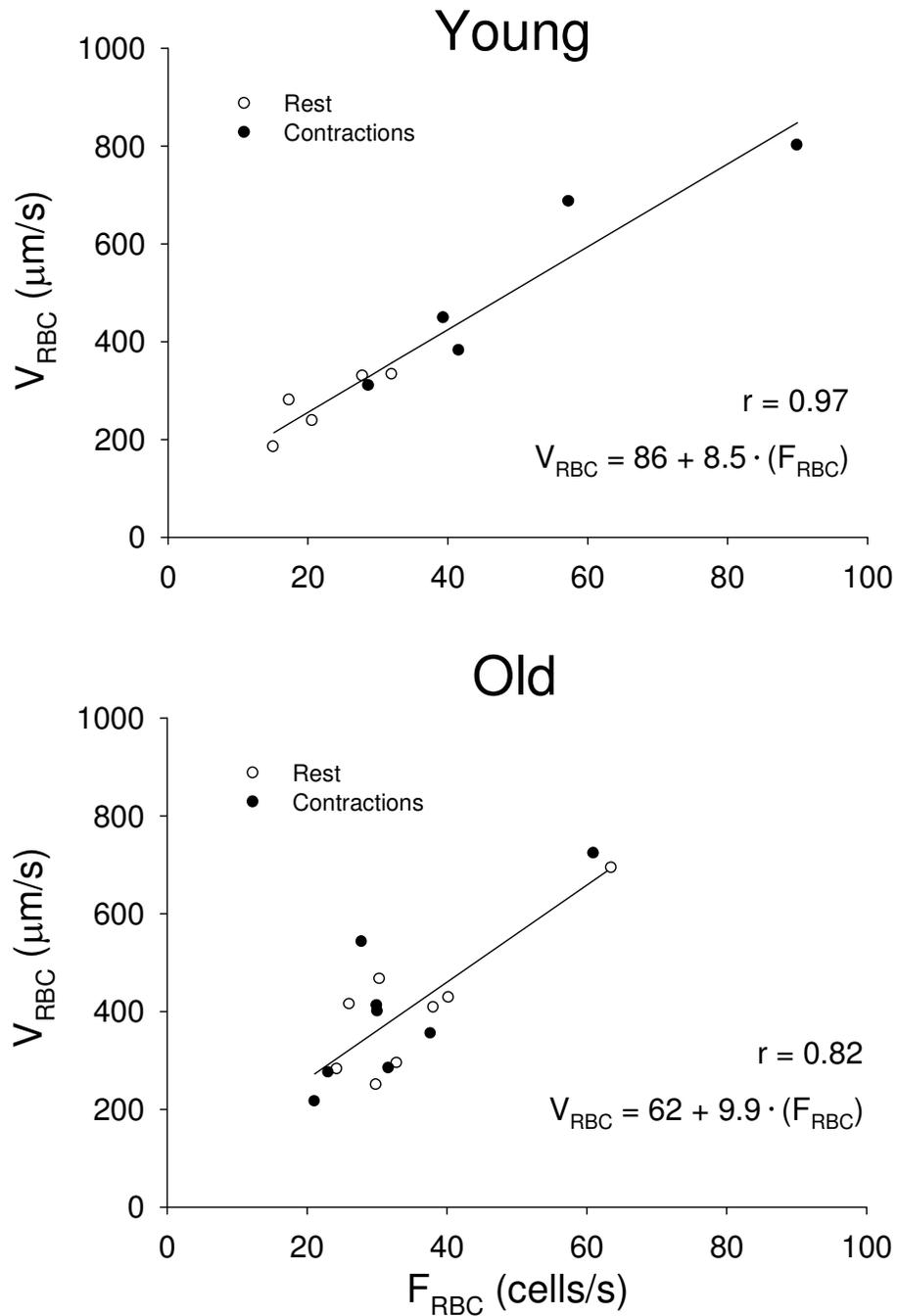
Top Panel: Capillary hematocrit (H_{CAP}) measured at rest and during contractions in Y and O animals. *Bottom Panel:* The change in H_{CAP} from rest to contractions for both age groups. * $P < 0.05$ compared to Y.

Figure 4-4: Microvascular O₂ delivery



Top Panel: Microvascular O₂ delivery ($\dot{Q}O_{2m}$) measured at rest and during contractions in Y and O animals. Bottom Panel: The change in $\dot{Q}O_{2m}$ from rest to contractions for both age groups. * $P < 0.05$ compared to Y, $\phi P < 0.05$ compared to rest.

Figure 4-5: RBC flux-velocity relationship



Relationship between red blood cell velocity (V_{RBC}) and flux (F_{RBC}) in Y (top panel) and O (bottom panel) muscles. Open symbols: rest; closed symbols: contractions.

CHAPTER 5 - Discussion

The present investigation is the first to examine the effects of aging on skeletal muscle capillary hemodynamics during contractions. As demonstrated previously at rest, old muscles exhibit a reduced lineal density of RBC-flowing capillaries and within these capillaries there is an elevated V_{RBC} and F_{RBC} such that overall blood and O_2 supply is not different from their young counterparts (Russell *et al.*, 2003). The principal novel finding of the present investigation is that it is possible to actively contract the spinotrapezius muscle of aged animals without further increasing V_{RBC} or F_{RBC} . Furthermore, the increased H_{CAP} normally observed in contracting muscles of young animals was absent in old rats. Thus, compared with young rats, for an equivalent contractile stimulus, muscles from aged rats exhibit lower convective and diffusive capillary O_2 delivery and any resultant attenuation in microcirculatory blood-muscle O_2 flux is likely to contribute to the exercise intolerance evident in elderly populations.

Comparisons to previous research

In concert with the bulk of intravital microscopy studies, the present data indicates that the vast majority (i.e. > 80%) of capillaries sustains RBC flow at rest, and this value was not different for young and old rats (Y: 96%, O: 93%). As demonstrated previously (Russell *et al.*, 2003), the lineal density of total capillaries and the lineal density of flowing capillaries was reduced in old rats.

Rest: The resting values for F_{RBC} and V_{RBC} reported herein for young rats are similar to previously published values. In addition, and consistent with the findings of

Russell et al. (Russell *et al.*, 2003) for old rats, F_{RBC} (35.7 cells/s) and V_{RBC} (406 $\mu\text{m/s}$) were both considerably higher than the established values for young rats (Sarelius & Duling, 1982; Kindig & Poole, 1998; Kindig *et al.*, 2002; Russell *et al.*, 2003). Indeed, the present V_{RBC} value is even higher than reported by Russell et al. (Russell *et al.*, 2003) which may be the consequence of the slightly older age range (i.e., up to 30 vs. 28 months) in the rats in the present investigation. The H_{CAP} of 0.23 and 0.22 cells/ μm for young and old rats, respectively, are close to our values (Y: 27, O: 29 cells/ μm) established for the F344xBN strain (Russell *et al.*, 2003).

Contractions: During contractions, the young rats exhibited an increase in F_{RBC} and V_{RBC} . In addition, the ΔH_{CAP} from rest to contractions was elevated in young compared to old rats. However, the difference was the result of a moderate increase in H_{CAP} in the young combined with a small decrease in the old.

As mentioned previously, to our knowledge the literature contains no previous reports of capillary hemodynamics in aged muscle during contractions. Notwithstanding this fact, our data are consistent with those of Hammer and Boegehold (Hammer & Boegehold, 2005) who found no increase in rat spinotrapezius arteriolar \dot{Q}_m in response to electrically-induced muscle contractions utilizing stimulation parameters similar to our own. In that investigation, 1 Hz contractions elicited no consistent increases in arteriolar diameter or V_{RBC} in 24 month-old male Sprague-Dawley rats. In contrast, some studies have found no difference between \dot{Q}_m to exercising limbs in young and old subjects (Jasperse *et al.*, 1994; Olive *et al.*, 2002; Proctor *et al.*, 2003b). However, the current literature is certainly not definitive on this issue (Wahren *et al.*, 1974; Proctor *et al.*, 1998; Proctor *et al.*, 2003a) with differences possibly related to the activity level of the

population studied, the function of the active muscle(s), and/or the intensity of the exercise. More importantly, recent evidence has established that large artery and limb blood flow may not be an appropriate analog of regional microvascular flow (Musch *et al.*, 2004; Harper *et al.*, 2006) which may explain the disparity between studies that have measured bulk arterial flow as distinct from microcirculatory flow (present results; Hammer & Boegehold, 2005).

Implications for blood-muscle O₂ transfer

The O₂ extraction analysis initially developed by Piiper and Scheid (Piiper & Scheid, 1981), and adapted for skeletal muscle by Wagner and colleagues (Roca *et al.*, 1992), can be used to predict the effect of aging on fractional O₂ extraction within the microcirculation. Specifically, blood-muscle O₂ flux is primarily determined by the relationship between DO₂ and \dot{Q}_m such that $\dot{V}O_{2m} = \dot{Q}_m (1 - e^{-DO_2/\beta Q_m})$, and therefore, $\dot{V}O_{2m}/\dot{Q}_m = O_2 \text{ extraction} = 1 - e^{-DO_2/\beta Q_m}$, where DO₂ is the diffusing capacity for O₂ and β is the slope of the O₂ dissociation curve in the physiologically relevant range. In the microcirculation, F_{RBC} is the instantaneous measurement of \dot{Q}_m , therefore, O₂ extraction = $1 - e^{-DO_2/\beta F_{RBC}}$. Substantial evidence supports that the principal site of O₂ diffusion resistance lies between the RBC and the immediate subsarcolemmal space (Gayeski & Honig, 1983; Hepple *et al.*, 2000), therefore, DO₂ will be primarily determined by the total capillary surface area available for O₂ exchange (Mathieu-Costello *et al.*, 1991) and the number of RBCs present along the capillary length (Groebe & Thews, 1990). At rest, the similar H_{CAP} present between young and old rats suggests that the DO₂ per capillary is maintained with aging. However, the elevated F_{RBC} at rest reduces the DO₂/ βF_{RBC} ratio

within each capillary, and therefore diminishes the reserve capacity to increase O_2 extraction. More importantly, the aging-induced reduction in the lineal density of flowing capillaries would reduce the mean DO_2 per muscle area or volume. This has important implications for exercise in that, if >90% of capillaries are supporting RBC flow at rest, the capacity for DO_2 to increase during exercise is limited to increases in H_{CAP} (i.e. longitudinal recruitment of capillary surface area along already-flowing capillaries). Our data indicate that mild-moderate contractions of aged muscle are accompanied by a significantly lower H_{CAP} (and therefore DO_2) compared to young muscle. This scenario would be expected to compromise the potential for elevating blood-myocyte O_2 flux and, therefore, muscle oxidative function.

Previously, we hypothesized that if the elevated V_{RBC} evident in old muscles at rest lead to a correspondingly faster V_{RBC} during high intensity exercise, the resultant shortening of RBC transit time might necessitate a reduction of intracellular PO_2 to achieve the required level of blood-muscle O_2 flux (Poole *et al.*, 2006). Because the contractions implemented in the present investigation did not elicit any increase in either perfusive or diffusive tissue O_2 transport, this mechanism (i.e., reduced intracellular PO_2) will have been solely responsible for augmenting blood-muscle O_2 flux via greater fractional O_2 extraction. Such a mechanism at both moderate and high contraction intensities may be responsible, in part, for the slowed $\dot{V}O_2$ kinetics (Babcock *et al.*, 1994; Chilibeck *et al.*, 1995) and increased O_2 deficit evident in elderly populations.

Mechanisms of microcirculatory dysfunction

Following the onset of contractions, the increase in \dot{Q}_m occurs in a distinct biphasic response (Kindig *et al.*, 2002). The initial, rapid phase of the increase (phase I) is generally attributed to muscle pump combined with rapid vasodilation that may potentially be mediated, in part, by compressive forces acting on the vasculature in addition to K^+ released from the contracting myocytes accumulating in the muscle interstitium (Bacchus *et al.*, 1981; Tschakovsky *et al.*, 2004; Clifford, 2007). Phase I lasts 15-20 seconds and is followed by a more pronounced phase II which is attributed primarily to endothelial-mediated vasodilatory mechanisms that include NO, prostacyclin, endothelial derived hyperpolarizing factor, primarily reflecting metabolic regulation/feedback control. The present observation that contractions resulted in no discernible alteration in capillary hemodynamics in aged muscle requires that aging impacts significantly both the muscle pump effect and arteriolar dilation.

The absence of any increase in F_{RBC} (Figure 5-1) requires that the muscle pump is ineffectual in the spinotrapezius muscle of old rats. It may be argued that this muscle might not be expected to evoke a muscle pump effect due to the absence of a substantial muscle belly and the non-physiological muscle fiber recruitment pattern elicited by electrical stimulation. However, Kindig *et al.* (Kindig *et al.*, 2002) observed that a contraction stimulus identical to the one utilized herein results in a biphasic increase in F_{RBC} in young rats, with the instantaneous (i.e. within ~1s) elevation in F_{RBC} normally attributed, in part, to a muscle pumping action (Tschakovsky *et al.*, 1996).

Healthy aging results in a decreased compliance of the venous vessels. It is possible that at mild-moderate contraction intensities, aged skeletal muscle is unable to

produce the force required to compress the stiffened capacitance vessels and is, therefore, unable to produce negative venular pressures upon muscle relaxation. Additionally, the chronic high microvascular flow (increased F_{RBC} and V_{RBC}) at rest in aged rats may offset the muscle pump effect by rapidly refilling the venules and minimizing any transient gain in the arteriolar-venule pressure gradient. This scenario was originally suggested by Tschakovsky and Sheriff (Tschakovsky & Sheriff, 2004) as a possible explanation for the fact that the muscle pump did not further increase the exercise hyperemic response in the dog hindlimb when maximal pharmacological vasodilation was induced prior to the onset of conscious treadmill exercise (Hamann *et al.*, 2003). Old rat muscles might be subjected to both of these effects.

During exercise, skeletal muscle vascular tone is controlled by both feed-forward (e.g., conducted, shear-stress induced), and feedback (e.g., metabolic regulation) mechanisms both of which may become impaired to different degrees with advancing age. For example, aging is associated with reductions in functional sympatholysis (Dinenno *et al.*, 2005; Parker *et al.*, 2007), myogenic control (Muller-Delp *et al.*, 2002a), and contraction-induced rapid vessel relaxation (Carlson *et al.*, 2008). Moreover, endothelium-dependent vasodilation is impaired (Muller-Delp *et al.*, 2002b), with the dysfunction likely occurring preferentially in arterioles supplying oxidative but not glycolytic muscles (Woodman *et al.*, 2002). While several discrete signaling pathways contribute to the endothelium-dependent regulation of vascular tone, NO appears to play a principal role, particularly in oxidative muscles (Hirai *et al.*, 1994). It is well-established that the NO-mediated dilation is reduced in aged vessels when compared to younger counterparts. For example, aging reduces NO-mediated vasodilation in response

to contractions in the skeletal muscles of humans (Taddei *et al.*, 2000; Schrage *et al.*, 2007) and rats (Muller-Delp *et al.*, 2002b; Woodman *et al.*, 2002; Spier *et al.*, 2004). However, what remains unclear is whether or not at rest the aged vasculature has already utilized some of its dilatory capacity in order to accommodate the same bulk flow through fewer resistance vessels i.e., greater flow, and therefore dilation, per vessel (Behnke *et al.*, 2006). This may represent a reduction in the reserve capacity of one or more vasoregulatory pathways (i.e. endothelium-independent dilation) in preference to dilatory function *per se* as reported by others (Muller-Delp *et al.*, 2002b; Woodman *et al.*, 2002). The combination of these impaired vasoregulatory mechanisms is likely to require higher strength contractions to increase arteriolar flow above resting values and, importantly, will significantly compromise the reserve capacity to increase arteriolar flow during maximal exercise.

Methodological considerations

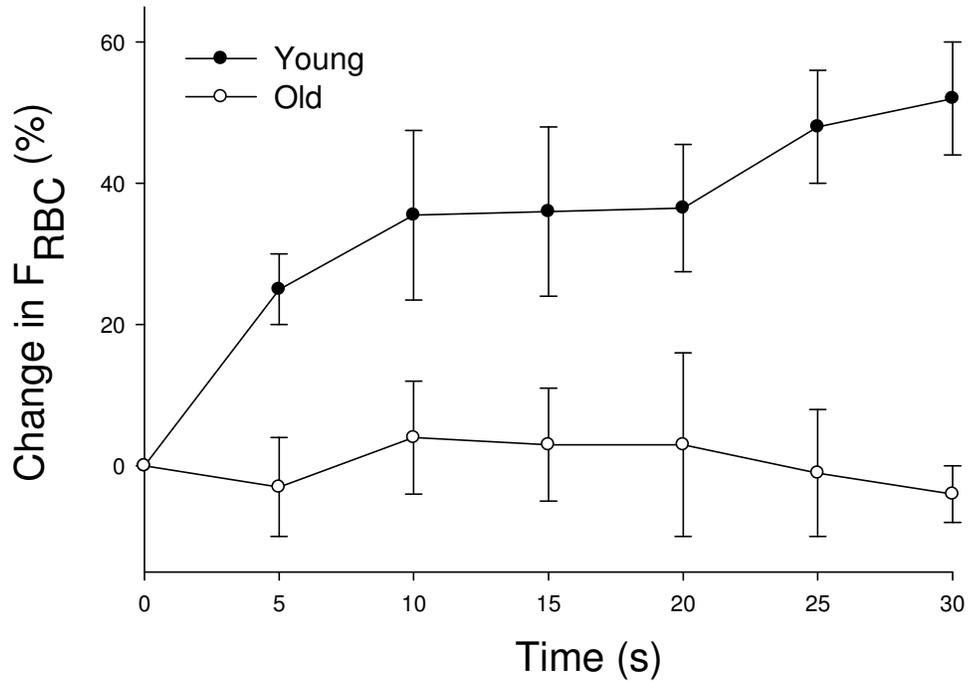
The stimulation parameters utilized herein are identical to those used previously in rats and allow for the restoration of visual clarity in ~15 frames between contractions - a necessary condition for determination of capillary hemodynamics. Higher-intensity maximal-strength tetanic contractions would limit hemodynamic assessment to the immediate post-contraction recovery period. Recovery of hemodynamic variables is sufficiently fast that such procedures would obviate measurement of true contracting values if, of course, they were different from rest (Ferreira *et al.*, 2006). Whereas we would predict that higher-intensity contractions would result in increases in capillary hemodynamics in old muscles, albeit to a lesser extent than that of younger muscles, we do not, at present, have the technical ability to conduct these studies. Further

methodological considerations regarding the rat spinotrapezius preparation have been discussed in detail previously (Bailey *et al.*, 2000; Kindig *et al.*, 2002).

Conclusion

This study is the first to demonstrate that old age results in significant alterations in capillary hemodynamics in the rat spinotrapezius muscle during the mild-moderate metabolic stresses associated with submaximal electrically-induced 1 Hz twitch contractions. Specifically, from rest to the contracting steady-state, when utilizing stimulation parameters that result in increased F_{RBC} , V_{RBC} , H_{CAP} and $\dot{Q}O_{2m}$ in the spinotrapezius muscle of young animals, the aged rat spinotrapezius evidences no significant increases in these variables. The attenuation of increases in both conductive and diffusive measures of O_2 transport in aged muscle capillaries has significant implications for blood-muscle O_2 delivery, and therefore, the ability for elderly individuals to sustain muscle oxidative function during dynamic exercise.

Figure 5-1: RBC flux dynamics



The percent change in red blood cell flux (F_{RBC}) upon the initiation of contractions in 5 randomly selected capillaries from old muscles. Young data is the average F_{RBC} response from previously published young healthy controls (Kindig *et al.*, 2002; Richardson *et al.*, 2003).

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