Effect of contraction frequency on the contractile and noncontractile phases of muscle venous blood flow

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The purpose of this study was to test the hypothesis that increasing muscle contraction frequency, which alters the duty cycle and metabolic rate, would increase the contribution of the contractile phase to mean venous blood flow in isolated skeletal muscle during rhythmic contractions. Canine gastrocnemius muscle (n = 5) was isolated, and 3-min stimulation periods of isometric, tetanic contractions were elicited sequentially at rates of 0.25, 0.33, and 0.5 contractions/s. The O2 uptake, tension-time integral, and mean venous blood flow increased significantly (P < 0.05) with each contraction frequency. Venous blood flow during both the contractile (106 ± 6, 139 ± 8, and 145 ± 8 ml·100 g–1·min–1) and noncontractile phases (64 ± 3, 78 ± 4, and 91 ± 5 ml·100 g–1·min–1) increased with contraction frequency. Although developed force and duration of the contractile phase were never significantly different for a single contraction during the three contraction frequencies, the amount of blood expelled from the muscle during an individual contraction increased significantly with contraction frequency (0.24 ± 0.03, 0.32 ± 0.02, and 0.36 ± 0.03 ml·N–1·min–1, respectively). This increased blood expulsion per contraction, coupled with the decreased time in the noncontractile phase as contraction frequency increased, resulted in the contractile phase contribution to mean venous blood flow becoming significantly greater (21 ± 4, 30 ± 4, and 38 ± 6%) as contraction frequency increased. These results demonstrate that the percent contribution of the muscle contractile phase to mean venous blood flow becomes significantly greater as contraction frequency (and thereby duty cycle and metabolic rate) increases and that this is in part due to increased blood expulsion per contraction.

From the muscle into the venous circulation. In the noncontractile phase (i.e., when force is not being produced) of the muscle duty cycle, arterial flow is restored as compression on the vessels within the muscle is removed, and venous flow is reduced compared with that seen during the contractile phase. During the contractile phase, the amount of blood expelled from the muscle depends on the force of the contraction and the volume of blood within the muscle at the start of the contraction. In contrast, blood flow through the muscle during the noncontractile phase is determined by the vascular pressure difference across the muscle and the resistance within the muscle to blood flow. When the arterial and venous pressures remain relatively stable during muscle contractile work, the overall mean muscle blood flow will be determined in large part by the degree of vasodilation within the muscle (10), which in turn is regulated by numerous factors (see Ref. 18). In addition, if the time spent in the contractile phase during rhythmic contractions is high, mean blood flow may be impeded by the long periods of vascular compression (25). Therefore, the mean muscle blood flow is determined by a complex interaction of factors that are strongly influenced by the metabolic rate and the time the muscle is in the contractile and noncontractile phases (5, 11).

Although the pulsatile nature of muscle blood flow has been known to significantly influence mean blood flow, there have been few studies in which the percent contribution of the two muscle phases to total muscle blood flow have been carefully examined when duty cycle and metabolic rate are varied. In the present study, we tested the hypothesis that increasing muscle contraction frequency, which alters the duty cycle and metabolic rate, would increase the contribution of the contractile phase to mean venous blood flow in isolated skeletal muscle during rhythmic contractions.

METHODS

Five adult mongrel dogs of either sex with a weight range of 12–19 kg were anesthetized with 30 mg/kg pentobarbital

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sodium. Maintenance doses were given as required. The dogs were intubated with cuffed endotracheal tubes, and ventilation was maintained with a Harvard 613 ventilator at a rate that achieved normal values of arterial PO₂ and PCO₂. Esophageal temperature was maintained near 37°C by the use of heating pads. The animals were given heparin at a dosage of 1,500 U/kg after the surgery.

Surgical preparation. The left gastrocnemius-flexor digitorum superficialis muscle complex (for convenience referred to as gastrocnemius) was isolated as described previously (13). Briefly, the muscle was isolated from nearby muscle groups, and all vessels draining into the popliteal vein except for those from the gastrocnemius were ligated to isolate the venous outflow from the gastrocnemius. The arterial circulation to the gastrocnemius was isolated by ligating all vessels from the femoral and popliteal artery that did not enter the gastrocnemius. The left popliteal vein was cannulated, and the venous outflow from the isolated muscle was returned to the animal via a jugular catheter.

The right femoral artery was catheterized for arterial blood sampling. This catheter was connected to the left femoral artery via a Sigmamotor pump to control perfusion pressure. All experimental conditions were conducted with perfusion pressure controlled by the pump. A pressure transducer in this line at the head of the muscle constantly monitored perfusion pressure. A carotid artery was also catheterized to monitor systemic blood pressure. The left sciatic nerve, which innervates the gastrocnemius, was doubly ligated and cut between ties. To prevent cooling and drying, all exposed tissues were covered with saline-soaked gauze and with a sheet of Saran. After the muscle was surgically isolated, the Achilles tendon was attached to an isometric myograph to measure force development.

The hindlimb was fixed at the knee and ankle and attached to the myograph with struts to minimize movement. Known weights were used at the end of each experiment to calibrate the force myograph. The isometric force developed by each muscle was normalized to the weight of that muscle. Before each contraction period, the resting muscle was passively stretched until the force produced by a single twitch contraction was maximal.

Experimental protocol. Rhythmic, isometric muscle contractions (tetanic) were elicited by stimulation of the sciatic nerve with square wave impulses (6–8 V) of 0.2-msec duration at a rate of 50 impulses/sec, with this train of impulses lasting 0.2 s (10 impulses during each contraction). Each muscle was stimulated to contract in a sequential manner for 3 min at each of three stimulation frequencies: 1 contraction every 4 (0.25 contractions/second), 3 (0.33 contractions/second), and 2 (0.5 contractions/second) s. Muscle perfusion pressure for these three stimulation patterns was kept at ~130 mmHg. We have previously demonstrated (8), using muscle contractions similar to those used in this investigation, that a steady-state blood flow and O₂ uptake are achieved by the end of 2 min.

Measurements. Arterial blood samples from the arterial line entering the muscle and venous samples from the left popliteal vein (as close to the gastrocnemius as possible) were drawn anaerobically at the end of each rest period and at the end of each 3-min contraction period. These samples were kept on ice for the brief time before measurement. A 6-mm bore, in-line ultrasonic flowmeter (100-Hz frequency response; Transonic Systems T108, Ithaca, NY) inserted in the venous outflow line provided rapid (50 Hz) venous blood flow measurements. The flow probe was calibrated within each experiment by comparing the recording of mean venous flow measured with the flow probe to venous blood flow measurements made simultaneously by timed blood collections into a graduated cylinder. A Biopac Systems MP100WSW (Santa Barbara, CA) analog-to-digital converter was used to transform the analog force and venous flow signals, and the digital data were collected and analyzed with AcqKnowledge III 3.2.6 software (Biopac Systems).

Muscle force development was determined from the computer acquisition system and reported as Newtons per 100 g of muscle mass. The time during which muscle force development was produced (contractile phase) and the time during which no force development occurred (noncontractile phase) were measured for the last 10 contractions at each stimulation frequency. Muscle blood flow for the contractile and noncontractile phases was calculated by averaging the appropriate blood flow waveform integrals for each of these 10 duty cycles. Mean venous muscle blood flow was measured as the venous outflow over the last 30 s of each contraction period (steady state of blood flow is achieved well before this time), except for the 0.25-Hz treatment in which the last 32 s were used (so that 8 complete contractile cycles could be counted). The mean muscle vascular resistance (not taking into account the blood flow changes within a contraction cycle) was calculated for each stimulation condition by dividing the mean arterial muscle perfusion pressure by the mean venous steady-state blood flow. Muscle fatigue was calculated as the decline in muscle force development with time and reported as the force development at that time as a percentage of the initial force development for that condition. The muscle was removed and weighed at the end of each experiment.

Blood PO₂, PCO₂, and pH were measured within 5–8 min with a blood-gas analyzer (IL model 813) at 37°C, and hemoglobin concentration, % O₂ saturation, and O₂ concentration were measured with an IL 282 CO-oximeter. These instruments were calibrated before each experiment and regularly throughout each experiment.

Statistics. Repeated-measures analysis of variance was used for the statistical analysis. When significance was found for a variable, Duncan’s multiple-range test was used to determine which contraction frequencies were significantly different from each other. Values are expressed as means ± SE. In all statistical analyses, the 0.05 level of significance was used.

RESULTS

Mean weight of the gastrocnemius muscles (n = 5) removed after the end of the experiment was 63 ± 5 g.

The arterial PO₂ (95 ± 7 Torr), arterial PCO₂ (45 ± 2 Torr), arterial pH (7.36 ± 0.02), arterial HCO₃⁻ concentration (26 ± 2 mM), arterial Hb concentration (15.1 ± 0.6 g/100 ml), and arterial O₂ concentration (17.1 ± 0.8 ml/100 ml) were similar among experiments and did not change through the three work periods (0.25, 0.33, and 0.5 contractions/second). Perfusion pressure to the muscle was kept constant at 130 ± 8 mmHg for the three contraction periods.

Peak muscle force development was not significantly different for the three contraction frequencies: 450 ± 80, 440 ± 71, and 409 ± 68 N/100 g, respectively. Figure 1 shows the developed force and simultaneous venous blood flow measurement for two contractions in one muscle at the 0.33-Hz contraction frequency. The time that the muscle produced force (contractile phase)
was not significantly different among the three contraction frequencies (0.56 ± 0.02, 0.55 ± 0.02, and 0.53 ± 0.02 s). The time in the noncontractile phase decreased significantly with increasing contraction frequency, from 3.44 ± 0.04 to 2.45 ± 0.03 to 1.46 ± 0.03 s. Therefore, the ratio of the time the muscle spent in the contractile phase to time spent in the noncontractile phase (duty cycle) increased significantly with increasing contraction frequency (0.17 ± 0.01, 0.22 ± 0.01, and 0.37 ± 0.03, respectively).

O$_2$ uptake and the work performed by the muscle (tension-time index) over the 3-min contractile period increased proportionally with the increase in contraction frequency. Mean venous muscle blood flow increased significantly in a linear fashion with increasing contraction frequency (71 ± 6, 84 ± 9, and 107 ± 11 ml·100 g$^{-1}$·min$^{-1}$ during 0.25, 0.33, and 0.5 contractions/s, respectively). As expected with a significant increase in mean blood flow and constant perfusion pressure, mean muscle vascular resistance fell significantly ($P < 0.05$) with increasing contraction frequency (1.83 ± 0.05, 1.55 ± 0.04, and 1.22 ± 0.04 mmHg·ml$^{-1}$·min$^{-1}$·100 g$^{-1}$, respectively). Figure 2 illustrates that the blood flow during the contractile (106 ± 6, 139 ± 8, and 145 ± 8 ml·100 g$^{-1}$·min$^{-1}$) and noncontractile phases (64 ± 3, 78 ± 4, and 91 ± 5 ml·100 g$^{-1}$·min$^{-1}$) increased (all significantly different, except for the contractile phase in the 0.33 and 0.5 contractions/s) with increasing contraction frequency.

Although there was no significant difference in the developed force or time in the contractile phase for an individual contraction among the three contraction frequencies. Figure 3 illustrates that the blood flow expelled from the muscle per unit of contractile force increased significantly with increasing contraction frequency (0.24 ± 0.03, 0.32 ± 0.02, and 0.36 ± 0.03 ml·N$^{-1}$·min$^{-1}$, respectively). The result of both the reduced time in the noncontractile phase and the greater blood expulsion per contraction (Fig. 3) resulted in the contribution of the contractile phase to mean blood flow becoming significantly greater (21 ± 4, 30 ± 4, and 38 ± 6%, respectively; Fig. 4) as contraction frequency increased.

Fig. 1. Developed force and simultaneous venous blood flow measurement for 2 consecutive contractions in 1 muscle at the 0.33-Hz stimulation frequency.

Fig. 2. Values of muscle venous blood flow during the contractile and noncontractile phases of the muscle duty cycle. All blood flow values within a condition (contractile, noncontractile) were significantly greater ($P < 0.01$) as contraction frequency increased except for blood flow during the 0.33 and 0.5 contractions/s conditions in the contractile phase. Values are means ± SE; $n = 5$.

Fig. 3. Blood flow expelled from the muscle during the contractile phase as a function of force produced by the muscle for each of the 3 contraction frequencies. This value increased significantly ($P < 0.01$) as contraction frequency increased. Values are means ± SE; $n = 5$. 

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DISCUSSION

The results of the present study demonstrate that the contribution of the contractile phase to mean blood flow in rhythmically contracting skeletal muscle became larger as the contraction frequency (and thereby duty cycle and metabolic rate) increased and that this was in part a result of an increase in the amount of blood expelled for a given force development during the contractile phase and not solely owing to the reduced time in the noncontractile phase.

Muscle blood flow has been characterized as being pulsatile during rhythmic exercise (6), such that the mean blood flow is a summation of the flows that occur during the contraction and noncontraction periods (see Fig. 1). When the vasculature of the muscle is forcefully compressed during the contractile phase of the duty cycle, arterial inflow is inhibited and blood is rapidly expelled from the muscle into the extramuscular venous circulation. On muscle relaxation, arterial flow into the muscle is restored and venous flow returns to steady-state values. At the onset of steady-state contractions, before neural, metabolic, endothelial, and myogenic factors are capable of initiating substantial vasodilation, the initial contractile expulsion of blood from the muscle has been suggested to contribute significantly to the immediate increase in muscle blood flow (3, 23, 26). However, during steady-state rhythmic contractions, after the vasodilatory processes have been successfully activated, it is controversial whether the contractile ejection of blood flow (“muscle pump” effect) is a determinant of steady-state muscle flow (see Ref. 19). Although evidence from some studies (1, 16, 17, 24) has suggested that the action of the muscle pump may partially determine the overall muscle blood flow response during rhythmic contractions, other studies have provided results that demonstrate little or no importance of the muscle pump in helping set steady-state blood flow (4, 10, 19, 22).

Irrespective of whether the “muscle pump” effect is a significant determinant of the overall blood flow response during steady-state rhythmic contractions, it is clear that the contraction and relaxation phases of a single contractile cycle result in very different temporal blood flow profiles during steady-state contractions (see Fig. 1), with each phase contributing quite differently to the overall steady-state blood flow response. It would be expected that the contribution of the contractile phase to overall mean blood flow during rhythmic contractions may depend on the strength of the contraction, the volume of blood contained in the muscle at the onset of contraction, and the ratio of the time in which the muscle is in the contractile and noncontractile phases (duty cycle). The intent of the present study was not to study whether the “muscle pump” per se is an important determinant of steady-state muscle blood flow but to characterize in an isolated muscle model the overall contribution of the contractile and noncontractile phases to the mean venous muscle blood flow at different contraction frequencies. By using an isolated muscle model in which all fibers were simultaneously activated, we were able to avoid issues related to differences in fiber-type recruitment patterns and blood flow heterogeneity that can confound interpretation of other muscle model systems as contraction frequency is varied. In addition, with all fibers activated maximally at each contraction frequency, work output and metabolic rate increased in conjunction with contraction frequency and duty cycle. This is in contrast to studies that have examined the effect of differences in contraction frequency, at similar power outputs, on metabolic and blood flow responses (5, 11, 12).

As has been demonstrated (2, 4, 9, 15, 20, 21), arterial blood inflow into contracting muscle is significantly diminished during the contractile phase owing to vascular compression, and muscle perfusion is reestablished during the noncontractile phase. If the duration of the contractile phase of the duty cycle is large, then this can have detrimental effects on overall muscle perfusion and result in inadequate delivery of O2 and diminished muscle contractility (fatigue). In addition, if the frequency (and not necessarily duration) of contractions becomes so great that blood flow and filling between contractions is of a short duration, then the mean muscle blood flow can again be compromised and contractility may be attenuated (5, 11, 25). In the present study, the frequency and duration of contractions was below the threshold for any negative effect of this impediment of blood flow to occur, and muscle contractility was not affected, as demonstrated by the maintenance of steady-state force development at all contraction frequencies.

An important component of the present study was that muscle work and O2 uptake rose proportionally with contraction frequency. Therefore, the changes demonstrated in the contribution of the contractile and noncontractile phases to muscle blood flow were influenced by both the increased metabolic rate and duty cycle. Mean venous muscle blood flow rose proportionally with increased contraction frequency, demonstrating the tight coupling of O2 supply to increased metabolic demand (14). The factors that are known to couple muscle blood flow to the metabolic rate during steady-state rhythmic contractions are numerous and complex.
increase in blood muscle vasodilation (as demonstrated by the decreased mean muscle vascular resistance) that resulted in the increase in blood flow. In the noncontractile phase of the duty cycle, the greater vasodilation with increased contraction frequency and metabolic rate resulted in a higher blood flow during that time period, as shown in Fig. 2. However, it is important to take into account that the time period of the noncontractile phase of the muscle duty cycle was progressively reduced as contraction frequency increased, from $3.44 \pm 0.04$ to $2.45 \pm 0.03$ to $1.46 \pm 0.03$ s for 0.25, 0.33, and 0.5 contractions/s, respectively. The overall effect was that even though the blood flow during the noncontractile time period was elevated (see Fig. 2), the reduced time of the noncontractile phase blunted the relative importance of this increase. In contrast, even though the time that the muscle spent in the contractile phase of an individual contraction remained constant (~0.5 s) among the three different contraction frequencies, the total overall time (per minute) that the muscle spent in the contraction phase increased as contraction frequency increased (doubling as the frequency of contractions increased from 1 every 4 s to 1 every 2 s). The decreased overall time spent in the noncontractile phase coupled with the increased time spent in the contractile phase was an important factor in the increased percent contribution of the contractile phase to overall mean blood flow, as shown in Fig. 4.

However, one of the important observations of the present study, as shown in Fig. 2, was that the amount of blood pumped from the muscle during the muscle contractile phase rose as the contraction frequency increased. As the force and duration of each individual contraction remained constant throughout all three contraction frequencies, the amount of blood expelled from the muscle during the contractile phase for a given force development (Fig. 3) rose significantly with increased contraction frequency and metabolic rate. This interesting observation suggests that the volume of blood contained in the muscle was greater with increasing contraction frequency. A greater volume of blood contained in the muscle allowed for a greater ejection of blood for a given contraction. This expansion of the muscle blood volume as contraction frequency increased was a result of the muscle vascular bed being expanded by vasodilatory processes that occurred with increased metabolic rate, and occurred even though the time between contractions (“filling” time) decreased. In effect, some other factor regulates arterial inflow and the muscle simply expels whatever blood is contained in the muscle vasculature. Therefore, the increased time that the muscle spent in the contractile phase (per minute), together with the greater amount of blood expelled per contraction, resulted in the total percent contribution of the contractile phase blood expulsion becoming a significantly larger portion of the overall mean muscle blood flow (Fig. 4) as contraction frequency was increased.

In conclusion, these results demonstrate that, during rhythmic tetanic contractions, the contribution of the contractile phase of the muscle duty cycle becomes a significantly larger percentage of the mean muscle blood flow response as contraction frequency (and thereby metabolic rate) increases, and that this is in part due to a significantly greater amount of blood expelled for a given force development during the contractile phase.

DISCLOSURES

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