Effect of contraction frequency on leg blood flow during knee extension exercise in humans

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Hoelting, Brian D., Barry W. Scheuermann, and Thomas J. Barstow. Effect of contraction frequency on leg blood flow during knee extension exercise in humans. *J Appl Physiol* 91: 671–679, 2001.—Previous studies in isolated muscle preparations have shown that muscle blood flow becomes compromised at higher contraction frequencies. The purpose of this study was to examine the effect of increases in contraction frequency and muscle tension on mean blood flow (MBF) during voluntary exercise in humans. Nine male subjects [23.6 ± 3.7 (SD) yr] performed incremental knee extension exercise to exhaustion in the supine position at three contraction frequencies [40, 60, and 80 contractions/min (cpm)]. Mean blood velocity of the femoral artery was determined beat by beat using Doppler ultrasound. MBF was calculated by using the diameter of the femoral artery determined at rest using echo Doppler ultrasound. The work rate (WR) achieved at exhaustion was decreased (P < 0.05) as contraction frequency increased (40 cpm, 16.2 ± 1.4 W; 60 cpm, 14.8 ± 1.4 W; 80 cpm, 13.2 ± 1.3 W). MBF was similar across the contraction frequencies at rest and during the first WR stage but was higher (P < 0.05) at 40 than 80 cpm at exercise intensities >5 W. MBF was similar among contraction frequencies at exhaustion. In humans performing knee extension exercise in the supine position, muscle contraction frequency and/or muscle tension development may appreciably affect both the MBF and the amplitude of the contraction-to-contraction oscillations in muscle blood flow.

THE ABILITY OF SKELETAL MUSCLE to sustain repeated contractions during rhythmic exercise is dependent on an adequate blood supply. A failure to increase blood flow in proportion to the metabolic demands of exercising muscle may result in early fatigue. Studies in humans performing single-leg knee extension exercise have shown that, when a small muscle mass is recruited, such as the quadriceps (~2.5 kg), leg blood flow increases in proportion to increases in power output without any evidence of a blood flow limitation at higher work rates (1, 24). However, previous studies using isometric (static) knee extension exercise have indicated that, when the quadriceps contract at 10% maximal voluntary contraction (MVC), intramuscular pressures are increased sufficiently to occlude blood flow in some regions of the contracting muscle (10, 33, 36). As the percentage of MVC was increased further, blood flow during contraction became progressively compromised because of further increases in intramuscular pressure.

The growing application of nuclear magnetic resonance spectroscopy to examine mechanisms controlling cellular respiration has led researchers to utilize knee extension exercise. With the exception of Richardson et al. (26), who used a large bore magnet, most knee extension exercise in a magnetic resonance spectroscopy magnet is characterized by a limited range of motion because of the physical constraints of the magnet (for example, Ref. 28). Unlike the cyclic motion of the Krogh ergometer typically used to examine muscle blood flow during knee extension exercise (1), the exercise mode in a magnet results in greater muscle tension development for the same power output over the entire range of motion, which may lead to greater impedance to blood flow. In addition to this mode-specific exercise, subjects are required to exercise while lying supine, which has been shown to affect the non-steady-state blood flow response (17) and, consequently, affect the metabolic response to the exercise (37).

It is readily apparent from the studies of Walloe and Wesche (35) that large oscillations in blood flow occur consequent to the mechanical interference caused by intermittent muscular contractions, which are followed by a transient hyperemia before the onset of the next muscular contraction. Studies in isolated muscle preparations have shown that the time available for flow between rhythmic contractions (relaxation duration) also affects the mean blood flow (MBF) response (9). There is evidence (2, 4, 13) in the diaphragm that a critical duty cycle (i.e., ratio of contraction time to total cycle duration) of ~20–50% exists, above which blood flow may become mechanically impeded. Additionally, Dodd et al. (6) examined blood flow in a dog hind-leg preparation and concluded that muscle tension development and contraction duty cycle were the primary determinants regulating blood flow. Previous exercise studies in humans (32, 35) have used contraction-relaxation phases that are relatively longer than those observed during natural locomotor activities, such as

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running or cycling, where contraction frequencies may be as high as 80 contractions/min (cpm). Interestingly, Kagaya (14) observed a plateau in MBF in the calf muscle of humans as running velocity increased from 60 to 220 m/min, suggesting that blood flow may have been impeded as contraction frequency (i.e., strides per minute) increased. However, blood flow was determined using strain-gauge plethysmography, which precluded obtaining blood flow measurements during exercise.

Therefore, the purpose of the present investigation was to characterize the parameters of rhythmic muscle contraction (frequency, relaxation duration, tension), which may affect the mean and instantaneous leg blood flow during exercise in humans. We tested the hypothesis that, at the same power output, increases in blood flow during exercise in humans. We tested the hypothesis that, at the same power output, increases in blood flow during exercise in humans. We tested the hypothesis that, at the same power output, increases in blood flow during exercise in humans. We tested the hypothesis that, at the same power output, increases in blood flow during exercise in humans. Additionally, the relationship between exercise intensity and blood flow has been impeded as contraction frequency (i.e., strides per minute) increased. However, blood flow was determined using strain-gauge plethysmography, which precluded obtaining blood flow measurements during exercise.

All exercise tests were performed at least 2 h postprandial. The subjects were asked to abstain from alcohol and strenuous exercise for 24 h before each test. Before the study, each subject was familiarized with the testing procedures and the exercise protocol by performing five to seven practice sessions. As part of this, each subject learned to keep the concentric and eccentric portions of the kick motion constant for all three kicking rates, so that the duty cycle and duration of relaxation between contractions varied directly with kicking frequency. After the practice sessions, each subject performed an incremental exercise test on 3 separate days, with at least 48 h allowed between tests for recovery. The order of the exercise trials was randomly applied. The subjects rested in the supine position for 30 min before beginning the exercise bout. The incremental exercise test consisted of 2 min of rest followed by 2-min stages in which the work rate was progressively increased until the subject could no longer maintain the required contraction frequency. The exercise test was followed by 8 min of recovery. For each subject, the 2-min increments in work rate were carefully matched so that the rate of work performed at each stage (i.e., power) was similar across the contraction frequencies. Room temperature was maintained between 19 and 22°C.

Mean blood velocity measurements. Continuous measurement of blood velocity in the right femoral artery was obtained with a Doppler ultrasound velocimetry system (model 500-V, Multigon Industries) operating in pulsed mode. The pulsed-wave Doppler transducer, with an operating frequency of 4 MHz and fixed transducer crystal and sound beam angle of 45° relative to the skin, was placed flat on the thigh 2–3 cm below the inguinal ligament, above and parallel to the common femoral artery. This position was selected to minimize turbulent flow arising from the bifurcation of the common femoral artery into the superficial and profundus branches. The gate was set at full width to ensure complete femoral artery insonation. The frequency spectrum of Doppler audio signals was converted to an instantaneous mean blood velocity by using a quadrature audio demodulator that was calibrated according to the specifications of the manufacturer (Hokanson). Software developed in our laboratory was used to calculate three indexes of femoral artery blood velocity averaged over one cardiac cycle between the R-wave-R-wave interval: 1) net mean blood velocity, calculated as the integrated area under the average velocity profile; 2) antegrade blood velocity (i.e., caudal direction velocities); and 3) retrograde blood velocity (i.e., rostral direction velocities). The blood velocities were expressed per minute (i.e., cm/min) by multiplying the cardiac cycle-by-cycle values by the corresponding heart rate (HR).

Femoral artery diameter measurement. On a separate day, the femoral artery cross-sectional area (CSA) was determined by using a duplex Doppler computed sonography system (model 128XP, Acuson) in two-dimensional echo mode. The vessel diameter was determined during supine rest over 10–15 cardiac cycles at peak systole and end diastole from a cross-sectional view of the artery at the level used to measure blood velocity. The measurement of arterial diameter was performed on cardiac cycles that provided optimal resolution of the arterial borders. The mean of the peak systolic and end-diastolic diameters over 10–15 beats was used to calculate an average diameter (D) using the estimate, D = (systole/3) + 2·(diastole/3) (22). The average diameter measurements were used for computing the CSA (CSA = πD²) of the artery, which in turn was multiplied by the appropriate blood velocity (cm/min) to obtain the relevant flows (ml/min): net MBF (MBFₙₑₓₑₚₑ), antegrade MBF (MBFₐₑₚₑ), and retrograde MBF (MBFᵣₑₚₑ).

Subjects. Nine healthy male volunteers participated in the study. The average physical characteristics of the subjects were 23.6 ± 3.7 yr (range: 20–45 yr) of age, 177.0 ± 2.1 cm (168–188 cm) in height, 77.0 ± 3.2 kg (68–93 kg) in weight, and 3.43 ± 0.57 l/min (2.5–4.4 l/min) peak O₂ uptake. Subjects were informed of all possible risks and discomforts associated with the experiment protocol before providing written consent. This study was approved by the Human Subjects Committee at Kansas State University where all exercise tests were conducted.

Experimental design. For this experiment, subjects performed incremental one-leg knee extension exercise in the supine position. Each incremental test was performed at a contraction frequency of 80 cpm (fast), 60 cpm (medium), or 40 cpm (slow). A metronome and verbal encouragement were used to assist the subjects in maintaining the appropriate kicks. The leg ergometer used in this study was specifically designed for use in a magnetic resonance scanner; therefore, the distance traveled by the lower limb during exercise tests were conducted.

The subjects were asked to abstain from alcohol and strenuous exercise for 24 h before each test. Before the study, each subject was familiarized with the testing procedures and the exercise protocol by performing five to seven practice sessions. As part of this, each subject learned to keep the concentric and eccentric portions of the kick motion constant for all three kicking rates, so that the duty cycle and duration of relaxation between contractions varied directly with kicking frequency. After the practice sessions, each subject performed an incremental exercise test on 3 separate days, with at least 48 h allowed between tests for recovery. The order of the exercise trials was randomly applied. The subjects rested in the supine position for 30 min before beginning the exercise bout. The incremental exercise test consisted of 2 min of rest followed by 2-min stages in which the work rate was progressively increased until the subject could no longer maintain the required contraction frequency. The exercise test was followed by 8 min of recovery. For each subject, the 2-min increments in work rate were carefully matched so that the rate of work performed at each stage (i.e., power) was similar across the contraction frequencies. Room temperature was maintained between 19 and 22°C.

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Statistical analysis. During the incremental tests, each subject fatigued at different stages of the tests, and, therefore, comparisons of the exercise responses were made up to the last power output completed by all subjects (i.e., the first four exercise stages) and at exhaustion. A two-way repeated-measures ANOVA design was used to test for differences for the cardiovascular and leg ergometer variables of interest with power output and kicking frequency as the main effects. All values are presented as means ± SE, unless indicated otherwise. Statistical significance was accepted at P < 0.05.

RESULTS

Ergometer data. The group mean values for the average ergometer response over the entire exercise test (excluding the last 30 s of exercise) are listed in Table 1. The slope of the power output-time relationship for each kicking frequency is presented in Fig. 1. Power output increased linearly for all contraction frequencies with no significant differences among increases for each of the kicking protocols. Table 2 shows that end-exercise power output was lower as contraction frequency increased, such that 40, 60, and 80 cpm elicited peak power outputs of 16.2 ± 1.4, 14.8 ± 1.4, and 13.2 ± 1.3 W, respectively (P < 0.05).

As was our intent in the protocol, at all submaximal exercise intensities, no significant difference for either the upstroke or downstroke duration for all three contraction frequencies was observed. Because the contraction duration was similar (~0.16 s) for each kicking frequency, the relaxation duration and duty cycle were varied, consistent with the experimental design. However, by the end of exercise when muscle tension development was highest and fatigue was increasing, upstroke duration became significantly longer for both the 40- and 60-cpm protocols (Table 2). In addition, end-exercise downstroke duration for 40 cpm was significantly shorter because of the high pressures pulling the leg back into the starting position. There were no significant changes in relaxation duration or contraction frequency throughout the range of exercise intensities but rather exhibited an attenuated rise at the higher power outputs. Figure 3A illustrates that, beyond a power output of 5 W, MBF
\text{NET} was higher (P < 0.05) for 40 than for 80 cpm. At a power output of 9 W, MBF\text{NET} was also higher (P < 0.05) during exercise at 40 than 60 cpm. However, at exhaustion, no difference in MBF\text{NET} was observed among the kicking frequencies because of large intersubject variability in both the peak power output achieved and the MBF\text{NET} response (Table 2).

Net blood flow (MBF\text{NET}). The cardiac cycle-by-cycle blood flow for one subject performing incremental exercise at 40, 60, and 80 cpm is shown in Fig. 2. At rest, MBF\text{NET} was similar among kicking frequencies (369.2 ± 148.6, 424.3 ± 206.4, and 322.2 ± 135.8 ml/min for 40, 60, and 80 cpm, respectively). With exercise, MBF\text{NET} increased (P < 0.05) from rest for all kicking frequencies. However, for all three kicking frequencies, MBF\text{NET} did not increase linearly throughout the range of exercise intensities but rather exhibited an attenuated rise at the higher power outputs. Figure 3A illustrates that, beyond a power output of 5 W, MBF\text{NET} was higher (P < 0.05) for 40 than for 80 cpm. At a power output of 9 W, MBF\text{NET} was also higher (P < 0.05) during exercise at 40 than 60 cpm. However, at exhaustion, no difference in MBF\text{NET} was observed among the kicking frequencies because of large intersubject variability in both the peak power output achieved and the MBF\text{NET} response (Table 2).

Table 1. Mean group responses for ergometer variables during submaximal knee extension exercise

<table>
<thead>
<tr>
<th>Variable</th>
<th>40</th>
<th>60</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upstroke duration, s</td>
<td>0.16 ± 0.01</td>
<td>0.16 ± 0.01</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>Downstroke duration, s</td>
<td>0.11 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>Relaxation duration, s</td>
<td>1.30 ± 0.01</td>
<td>0.84 ± 0.02</td>
<td>0.59 ± 0.02</td>
</tr>
<tr>
<td>Duty cycle</td>
<td>0.11 ± 0.01</td>
<td>0.16 ± 0.01</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td>Kicking frequency, cpm</td>
<td>41.3 ± 0.3</td>
<td>60.1 ± 0.9</td>
<td>79.8 ± 0.9</td>
</tr>
</tbody>
</table>

Values are means ± SE. cpm, Contractions per minute. *Significantly different from 80 cpm, P < 0.05. †Significantly different from 60 cpm, P < 0.05.

Table 2. Group mean responses for mean blood flow and ergometer responses at end exercise

<table>
<thead>
<tr>
<th>Variable</th>
<th>40</th>
<th>60</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBF\text{NET}, l/min</td>
<td>2.16 ± 0.25</td>
<td>1.65 ± 0.24</td>
<td>1.60 ± 0.27</td>
</tr>
<tr>
<td>MBF\text{ANT}, l/min</td>
<td>2.30 ± 0.27</td>
<td>1.92 ± 0.25</td>
<td>1.88 ± 0.30</td>
</tr>
<tr>
<td>MBF\text{RET}, l/min</td>
<td>0.144 ± 0.03†</td>
<td>0.268 ± 0.06†</td>
<td>0.279 ± 0.05†</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>109.2 ± 5.4</td>
<td>110.3 ± 6.6</td>
<td>114.7 ± 6.4</td>
</tr>
<tr>
<td>Kicking frequency, cpm</td>
<td>41.4 ± 0.27†</td>
<td>59.16 ± 1.6†</td>
<td>79.85 ± 1.5†</td>
</tr>
<tr>
<td>Upstroke duration, s</td>
<td>0.20 ± 0.02†</td>
<td>0.20 ± 0.02†</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>Downstroke duration, s</td>
<td>0.09 ± 0.01†</td>
<td>0.11 ± 0.01</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>Relaxation duration, s</td>
<td>1.26 ± 0.02†</td>
<td>0.829 ± 0.04†</td>
<td>0.583 ± 0.01†</td>
</tr>
<tr>
<td>Duty cycle</td>
<td>0.14 ± 0.02†</td>
<td>0.19 ± 0.02†</td>
<td>0.23 ± 0.01†</td>
</tr>
<tr>
<td>Power output, W</td>
<td>16.2 ± 1.4†</td>
<td>14.8 ± 1.4†</td>
<td>13.2 ± 1.3†</td>
</tr>
</tbody>
</table>

Values are means ± SE. MBF\text{NET}, MBF\text{ANT}, MBF\text{RET} net, ante-grade, and retrograde mean blood flow, respectively. *Significantly different from 80 cpm, P < 0.05. †Significantly different from 60 cpm, P < 0.05.
Antegrade blood flow (MBF\textsubscript{ANT}). Figure 3B shows that MBF\textsubscript{ANT} increased from resting levels for all kicking frequencies in a pattern similar to that for MBF\textsubscript{NET}. Only at a power output of 9 W was there a difference ($P < 0.05$) between 40 and 80 cpm. End-exercise values for MBF\textsubscript{ANT} were not significantly different among exercise protocols.

Retrograde blood flow (MBF\textsubscript{RET}). At rest, ~40% of the forward blood flow in the femoral artery (MBF\textsubscript{RET}) “rebounded” during each cardiac cycle, resulting in significant retrograde blood flow (MBF\textsubscript{RET}) within each cardiac cycle (Fig. 3C). Resting MBF\textsubscript{RET} was not significantly different among kicking protocols. At the lightest work intensity (3 W), there was a significant reduction in MBF\textsubscript{RET} compared with rest for all of the kicking frequencies. Above 3 W, MBF\textsubscript{RET} increased ($P < 0.05$) for both 60 and 80 cpm but remained constant for the 40-cpm trial. Figure 3C shows that, at >5 W, MBF\textsubscript{RET} was less ($P < 0.05$) in 40 compared with 80 cpm and that, at >7 W, MBF\textsubscript{RET} was also less ($P < 0.05$) in 40 than 60 cpm.

HR and blood pressure. HR increased from resting values to similar peak values at exhaustion (Table 2). For all submaximal work rates, HR at 80 cpm was greater than at 40 cpm ($P < 0.05$), although this difference was ~10 beats/min on average. At a power output >5 W, HR was higher ($P < 0.05$) during exercise at 60 than at 40 cpm.

Because of difficulties with the blood pressure measurements during exercise, mean arterial pressure (MAP) was obtained in only five of the subjects during both 40 and 80 cpm. MAP increased ($P < 0.05$) from resting values (80.8 ± 5.2 and 88.3 ± 4.8 mmHg for 40 and 80 cpm, respectively) to end exercise (108.9 ± 26.4 mmHg).
was our ability to alter the relaxation duration and resulting duty cycle for each of the kicking protocols. This was successfully accomplished in each subject by maintenance of a constant contraction duration while kicking frequency was varied. Consistent with our hypothesis, we found that, at the same power output, an increase in kicking frequency was associated with an attenuated increase in the MBF response. Moreover, blood flow for each of the kicking frequencies did not rise linearly as a function of power output but rather showed a blunted response at higher power outputs.

Limitations. One assumption that we made in the present experiment was that resting femoral artery diameters were representative of exercise values. This assumption was based on preliminary studies performed in our laboratory (12) that showed that the femoral artery diameter did not significantly change from rest to exercise during knee extension exercise identical to that performed in the present study. The observation of a constant femoral artery diameter for rest and upright knee extension exercise was also reported by Rådegran (22). In contrast, MacDonald et al. (17) have shown an increase in femoral artery diameter during the rest-to-exercise transition when knee extension exercise was performed in the supine but not the upright position. If our assumption that femoral artery diameter remained unchanged from rest values was incorrect, then, based on the change in femoral artery diameter reported by MacDonald et al., we may have underestimated MBF by up to 16% in the present study. However, we are not aware of any reports that would suggest that a significant difference in conduit artery diameter may be expected simply by changing kicking frequency while maintaining the same power output and, therefore, metabolic requirements. Indeed, the difference in MBF between 40 and 80 cpm cannot be explained by assuming that an increase in arterial diameter occurred during exercise at 80 but not 40 cpm. The diameter of the femoral artery will be determined, in part, by the distending pressure. An increase in distending pressure, determined as the MAP, is associated with an increase in the femoral artery diameter (30). Blood pressure was recorded noninvasively by means of arterial tonometry in the present study, but data for only five subjects were reliable for 40 and 80 cpm because of the sensitivity of the blood pressure monitoring system to slight movements of the arm during exercise. Results from these five subjects did indicate a significant increase in MAP with increasing power output, but no difference was observed among contraction frequencies. This suggests that femoral artery distending pressure, and thus diameter, was not significantly different among kicking conditions.

The response of MBF\textsubscript{NET} during incremental knee extension exercise. At rest, MBF\textsubscript{NET} was \(\sim 370\) ml/min, similar to values previously reported by others using Doppler (22, 23, 35) and thermodilution techniques (1). With exercise, MBF\textsubscript{NET} increased to over three times the resting value by the end of the lightest workload (\(\sim 3\) W). Surprisingly, MBF\textsubscript{NET} did not increase lin-

and 115.7 ± 12.3 mmHg for 40 and 80 cpm, respectively); no difference was observed between 40 and 80 cpm.

DISCUSSION

The present study was designed to quantify the effect of contraction frequency and muscle tension on leg blood flow in humans during exercise in the supine position. Essential to the interpretation of our findings
early with further increases in power output as expected; we have confirmed this observation in a single subject using the same exercise protocol but using the thermodilution technique developed by Andersen and Saltin (1) to measure blood flow (Fig. 4). This finding is in disagreement with earlier reports, which showed a linear rise in leg blood flow with increases in power output to maximal exercise (1, 25, 29). This discrepancy may be explained, in part, by differences in the exercise protocols and/or knee extension ergometer used. Knee extension exercise has typically been performed using the Krogh style of ergometer in which the knee is able to move through a full range of motion (i.e., 90–170°) (1, 25, 29). In the present study, the range of motion for the lower limb was ~25% of that of the Krogh ergometer (~90–110°). This implies that, to generate the same power output (at a given contraction frequency), the muscle tension would have to be on the order of four times greater on the present ergometer compared with the muscle tension developed on the Krogh-style ergometer.

Several studies using isometric (10, 36) and dynamic exercise (31, 35) have demonstrated that contraction of the quadriceps muscles requiring 10% of a MVC can mechanically impede blood flow in the femoral artery. Although we did not measure MVC in all nine subjects, a preliminary examination of two subjects suggests that we exceeded 10% of their MVC early during the exercise bout and may have actually exceeded 50% of their MVC by the end of exercise. This suggests that blood supply to the working muscle may have been compromised at a relatively low power output, even though the exercise was intermittent, and, as a consequence, MBF_{NET} did not increase linearly with further linear increases in power output.

In addition to the greater range of motion and associated lower muscle tension on the Krogh ergometer, studies examining muscle blood flow responses during knee extension exercise, for the most part, have performed the exercise with the subject seated in the upright position (1, 16, 18, 22). Earlier work (7, 8, 17, 34) has shown that leg muscle blood flow was greater in the upright position compared with the supine position during exercise, due in part to increases in perfusion pressure related to the effect of gravity. In our study, the subjects exercised supine, so that the contracting muscles were above the level of the heart. In this case, the hydrostatic pressure gradient was absent or slightly negative. Therefore, in the present study, higher muscle tensions and lower arterial pressures may have attenuated the blood flow response as work rate was increased.

Kagaya (14), using plethysmography to examine blood flow changes in humans during normal activities such as walking or running at a variety of speeds, showed a blunted blood flow response as running velocity increased beyond 80 strides/min (~6.5 mph). However, the mechanism for the attenuated blood flow response was not determined. Based on the present findings, one explanation may be that the increase in contraction frequency with running velocity resulted in a decrease in the time for relaxation between muscle contractions, which limited blood flow (discussed below). Alternatively, an increase in muscle tension could have accompanied the increase in running velocity, which then may have limited blood flow. Irrespective, the increase in blood flow in these previous studies did not match the increase in metabolic demand as expected.

The effect of contraction frequency on muscle blood flow. In partial agreement with our hypothesis, the increase in MBF_{NET} was greater during 40 than 80 cm/s at power outputs >5 W, although no difference was observed among contraction frequencies at exhaustion. Previous studies have demonstrated that the net blood flow per contraction is dependent on both the relaxation and contraction duration (4, 11, 35). In the present study, the highest MBF_{NET} was observed at the lowest contraction frequency, which was also associated with the longest duration between muscle contractions. At higher frequencies (80 cm/s), blood flow was lower than at 40 cm/s and did not increase further with increasing power outputs >5 W, possibly because of the decrease in relaxation duration. The effect of reduced relaxation duration on blood flow is further depicted in Fig. 5. When muscle blood flow was expressed as the amount of flow per contraction (i.e., ml/contraction), MBF_{NET} during 80 cm/s was less than one-half of that for 40 cm/s. Interestingly, this difference in MBF_{NET} was associated with a lower MBF_{ANT} during 80 compared with 40 cm/s because the amount of retrograde blood flow per contraction was similar across contraction frequencies (Fig. 5). This observation is consistent with the hypothesis that the time allowed between contractions may determine, in part, the overall perfusion of the exercising muscle.

The effect of high-contraction frequency on blood flow during knee extension exercise has not been pre-
Previously examined in humans. However, a number of studies have examined the effect of contraction frequency on blood flow using longer contraction and/or relaxation durations (32, 35). For example, Walloe and Wesche (35) reported that, at a constant muscle tension (30% MVC), the time-averaged velocities were similar during exercise bouts that involved contracting the knee extensor muscles for either 4 or 2 s with duration of relaxation ranging from 2 to 4 s. Similarly, Shoemaker and colleagues (32) reported that MBF was similar during forearm exercise using contraction-to-relaxation durations of either 1 s or 2 s. The results of these studies suggest that blood flow is independent of the time of relaxation, provided that the duration between muscle contractions is sufficiently long. However, the results of the present study suggest that muscle blood flow may be significantly compromised by contraction frequency if the time spent between contractions is reduced enough. During exercise at 40 cpm, the relaxation duration was ~1.3 s. In comparison, the relaxation duration during exercise at 80 cpm (~0.6 s) was associated with a lower MBF with incremental exercise. Thus, in addition to the nonlinearities of the MBF, these observations are consistent with a critical relaxation duration being reached at higher contraction frequencies, which may have further impeded arterial inflow to the exercising muscle.

One of the unique features of the present study was our ability to partition femoral artery blood flow into antegrade (MBF<sub>ANT</sub>) and retrograde (MBF<sub>RET</sub>) flow within each cardiac cycle. An interesting finding in the present study was the response of MBF<sub>RET</sub> as a function of contraction frequency and muscle tension development. At rest when the effects of muscle contraction did not mechanically impede muscle blood flow, there was the equivalent of >200 ml/min of retrograde blood flow. Retrograde blood flow is thought to be due to the reflection of the pressure and flow waves returning from downstream bifurcations and sites of increased vascular tone (5, 20). The results of a recent study of quadriplegic patients (19) showed that the loss of vasomotor tone in the capillary beds of the thigh muscles was associated with the disappearance of retrograde blood flow in the femoral artery, consistent with the

Fig. 5. The group MBF<sub>NET</sub> (A), MBF<sub>ANT</sub> (B), and MBF<sub>RET</sub> (C) blood flow response expressed in milliliters per contraction during incremental knee extension exercise to exhaustion for 40, 60, and 80 cpm. *Significantly different from corresponding value at 80 cpm, P < 0.05. †Significantly different from corresponding value at 60 cpm, P < 0.05. Note that statistics were only performed on the first 4 power outputs and at exhaustion, where all 9 subjects contributed data to the group mean.

Fig. 6. MBF<sub>RET</sub> expressed in both flow per unit of time (ml/min; A) and the amount of blood flow per muscular contraction-relaxation cycle (ml/contraction; B) as a function of tension developed by the muscle (i.e., same psi) for each of the exercise bouts.
contention that downstream impedance determines, at least in part, the magnitude of retrograde blood flow. In the present study, MBF\textsubscript{RET} was reduced at the lightest workload by \sim 65%, which probably reflects reduced arterial tone within the contracting muscles. After the initial decrease, MBF\textsubscript{RET} (ml/min) remained relatively constant during exercise at 40 cpm but progressively increased during both 60- and 80-cpm exercise (Fig. 6A). However, when expressed relative to the developed muscle tension, MBF\textsubscript{RET} obtained similar values at each kicking frequency, at least up to 8 psi (Fig. 6B). These observations suggest that retrograde blood flow during exercise is primarily due to contraction-induced impedance to blood flow rather than to changes in downstream impedance because of changes in vasomotor tone. It should be noted, however, that, during exercise, MBF\textsubscript{RET} represents only 10–15\% of the antegrade blood flow response. Thus the attenuation in MBF\textsubscript{NET} as power output increased for all three kicking conditions was not the sole result of a significant increase in retrograde blood flow but rather was due primarily to a progressive attenuation in antegrade blood flow.

The effect of kicking frequency on end-exercise blood flow responses. Previous studies have shown that increases in contraction frequency are associated with a decrease in the time to exhaustion or the total number of contractions completed during an exercise bout (3, 27). In the present study, power output was matched at each submaximal exercise stage for all three kicking frequencies. The highest kicking frequency was associated with a lower end-exercise power output, consistent with the findings of earlier studies. Although the difference in end-exercise MBF\textsubscript{NET} did not reach statistical significance because of large inter- and intra-subject variability, MBF\textsubscript{NET} was \sim 560 ml/min lower during exercise at 80 compared with 40 cpm, suggesting that MBF\textsubscript{NET} may have been limited at the higher contraction frequencies and, as a consequence, may have contributed to the earlier fatigue.

The subjects were able to maintain the appropriate kicking frequencies throughout the test, despite the increases in workload. On further evaluation of the raw signals collected from the exercise ergometer, we found that there were intermittent increases in the upstroke duration toward the end of exercise, suggesting that subjects were fatiguing. Even though the upstroke duration was longer at the end of exercise, contraction frequency remained constant throughout the exercise bout, resulting in significant increases in the duty cycle by the end of exercise. Interestingly, the longer duty cycle at end exercise was only observed in the 40- and 60-cpm protocols. This may be explained in part by the higher muscle tension required to achieve the same power output at these lower kicking frequencies. The force-velocity curve predicts that the maximum possible contraction velocity will decline as the force generated by the muscle increases. These slight increases in duty cycle at the higher workloads may have had an adverse effect on blood flow and contributed to the variability in the end-exercise blood flow response.

The response of the individual subject (Fig. 2) clearly demonstrates the large fluctuations or swings in arterial flow that occur during muscular contractions. Similar findings have been reported by others using Doppler ultrasound (22, 23, 35). In addition, researchers using thermodilution techniques to follow blood flow have mentioned similar oscillations on the venous side (1, 21). It is unclear how these oscillations in flow at the level of the conduit artery might affect blood flow in the capillary network supplying the muscle. If these oscillations in blood flow persist within the capillaries, they may present a profound challenge to oxygen extraction by the muscle. Earlier work from Klitzman and Duling (15) suggested that the exercise hyperemia that follows each muscle contraction must increase red blood cell velocity and shorten transit time in the capillary bed. The presence and consequences of these oscillations on gas exchange in the capillary network must await further study.

In conclusion, these data demonstrate that muscle tension and time for relaxation can have a profound effect on blood flow into exercising limbs in humans. At the same power output, a contraction frequency of 80 cpm provided less MBF than 40 cpm because of the reduced time available for flow between contractions. Retrograde blood flow due to muscle contraction was a function of muscle tension and appears to be independent of contraction frequency. MBF\textsubscript{NET} did not rise linearly but rather was attenuated at higher power outputs for all three kicking frequencies. We speculate that this attenuation in blood flow was due to the high muscle tension developed during knee extension and/or a reduced perfusion pressure caused by the supine body position.

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