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Role of speed vs. grade in relation to muscle pump function at locomotion onset

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Sheriff, Don D., and Amy L. Hakeman. Role of speed vs. grade in relation to muscle pump function at locomotion onset. *J Appl Physiol* 91: 269–276, 2001.—We sought to clarify the roles of contraction frequency (speed) and contraction force (grade) in the rise in muscle blood flow at the onset of locomotion. Shoemaker et al. (*Can J Physiol Pharmacol* 76: 418–427, 1998) explored this relationship in human handgrip exercise and found that the time course of the rise in muscle vascular conductance was similar when a light weight was lifted in a fast cadence and a heavy weight was lifted in a slow cadence (total work constant). This indicates that muscle pumping (contraction frequency) was of limited importance in governing the time course. Rather, vasodilator substances released in proportion to the total work performed appeared to determine the pattern and extent of the rise in conductance. We hypothesized that conductance would rise faster during locomotion at a high speed (frequency) and low grade (force) than at a low speed and high grade, despite similar total increases in conductance, owing to more effective muscle pumping at faster contraction rates. Seven male rats performed nine 1-min bouts of treadmill locomotion across a combination of three speeds (5, 10, and 20 m/min) and three grades (−10, 0, and +15°) in random order. Locomotion at 10 m/min and 0° grade and 20 m/min and −10° grade led to an equal rise in terminal aortic vascular conductance. However, the equal rise was achieved more quickly at the higher running speed, suggestive of more effective muscle pumping. Across the nine combinations of exercise, speed began to exert a statistically significant influence on conductance by the 3rd s of locomotion. Grade did not begin to exert an influence until the 12th s of locomotion (similar to the delays reported for arteriolar dilation to muscle contraction). Additional experiments in dogs provided similar results. Thus the muscle pump appears to initiate the increase in blood flow in proportion to contraction frequency at locomotion onset.

dog; rat; nitric oxide; muscle blood flow; iliac artery; terminal aorta; arterial pressure; vasodilation; vascular conductance

THE ONSET OF LOCOMOTION and most forms of dynamic exercise are accompanied by a rapid increase in the blood flow to the muscles engaged in producing movement. The rise in blood flow is largely attributable to a rise in the calculated vascular conductance across muscle, which in turn is attributable to the muscle pump (10, 15, 18, 23) and inhibition of arteriolar smooth muscle after the production, release, diffusion, and transduction of vasodilator chemicals (4, 16). The relative contribution of each of these two mechanisms is unclear. On the basis of the known delay in the onset of arteriolar vasodilation in response to electrically induced twitch contractions in anesthetized animals (6, 12), Sheriff and co-workers (18) reasoned that the onset of locomotion might provide a short window of opportunity before vasodilation occurs in which to evaluate the isolated influence of the muscle pump on muscle blood flow. This possibility is supported by the observation that the isolated muscle pump can elicit immediate increases in muscle blood flow in a setting where vasodilation alone would be ineffective in raising flow (19). On the basis of the biphasic pattern of rise in muscle vascular conductance at the onset of mild treadmill locomotion in dogs with autonomic blockade, Sheriff and co-workers (18) attributed the immediate rise in calculated vascular conductance in response to mild exercise to the action of the muscle pump. A second, delayed rise in conductance was attributed to the action of vasodilator substances, leading to an increase in vessel diameter. Consistent with this idea, they observed in a limited number of animals that a doubling of treadmill speed led to an approximate doubling of the rise in vascular conductance achieved in the first 2–3 s of locomotion. That is, a doubling of contraction frequency appeared to lead to a proportional increase in the effectiveness of muscle pumping. In response to moderate exercise, conductance rose smoothly (monotonically) to steady-state values, suggesting that vasodilation is initiated after a far shorter delay when work rate is increased above a mild intensity (no window of opportunity for evaluating muscle pump function).

Recently, Shoemaker et al. (21) explored the relationship between muscle pumping and vasodilation at the onset of handgrip exercise performed by human subjects. In separate trials, these investigators had subjects lift and lower a light weight in a fast cadence and a heavy weight in a slow cadence, such that the total work performed was equal in the two conditions. They found that the time course of the rise in vascular conductance was similar between the two conditions, indicating that muscle pumping was of limited impor-

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tance in governing the time course of the rise in conductance. Rather, vasodilator substances released in proportion to the total work performed appeared to be the dominant mechanism responsible for the pattern and extent of the increase in muscle vascular conductance.

In the present study, we sought to pursue the following two goals. First, we sought to clarify the relative role of speed (contraction frequency) vs. grade (contraction force) in governing the rise in muscle blood flow at the onset of locomotion. The rationale was that differences in contraction frequency would lead primarily to changes in the effectiveness of the muscle pump (at least in the first few seconds of locomotion), whereas differences in contraction force would lead primarily to changes in arteriolar diameter [perhaps only after some delay (25)]. On the basis of these premises, alterations in speed and grade should provide insight into the relative roles of the muscle pump vs. vasodilation in raising muscle blood flow at locomotion onset. Because the blood flow-raising action of the muscle pump is proposed to be most effective during locomotory exercise (10), we deemed that it would be useful to apply the approach employed by Shoemaker et al. (21) in their handgrip study to locomotory exercise. We hypothesized that conductance would rise faster during locomotion at a high speed and low grade than at a low speed and high grade, despite similar total increases in conductance, owing to more effective muscle pumping at faster contraction rates associated with higher treadmill speeds. Second, because nitric oxide (NO) synthase (NOS) inhibition has recently been reported to slow the vasodilation in response to locomotion (17), we inhibited NOS in an effort to extend the short window of opportunity for evaluating muscle pump function at the onset of locomotion at moderate intensities of locomotion. Experiments were carried out in chronically prepared, conscious rats and dogs.

METHODS

All procedures met National Institutes of Health guidelines and were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Iowa.

Rats

Seven male Sprague-Dawley rats (250–300 g) were selected for their willingness to run on a motor-driven treadmill (model 1010 Modular Treadmill, Columbus Instruments, Columbus, OH). The rats were familiarized with treadmill running before the following aseptic surgical procedures were performed.

Surgical preparation. Rats were anesthetized with isoflurane. An ultrasonic transit-time blood flow transducer (model 1.5RB, Transonic, Ithaca, NY) was implanted in each animal on the terminal aorta through a midline abdominal incision. The probe cable was tunneled to an exit site on the back. The animal was given nalbuphine hydrochloride (1 mg/kg sc) for control of postoperative pain. The animal was allowed to recover until an acceptable blood flow signal was acquired (usually 2–3 days). On the day before or on the morning of a day when an experiment was to be carried out, a femoral artery was ligated, and a PE-10 catheter was directly inserted centrally into the vessel under isoflurane anesthesia for measurement of systemic arterial pressure. Although this procedure likely reduced the blood flow to the distal tissues, even for the brief duration of low-intensity exercise employed in the present study, it is unlikely that the pattern (time course) of blood flow response measured at the level of the terminal aorta would be substantially altered by this procedure. The catheter was tunneled to an exit site on the back. The animal was allowed to recover for ≥3 h or overnight.

Data collection. The animal was lightly anesthetized (1% isoflurane), the catheter was connected to a pressure transducer (model P10EZ, Ohmeda, Madison, WI), and the flow transducer was connected to a flowmeter (model T106, Transonic). The animal was then placed in the treadmill and, after regaining full consciousness, was allowed ≥30 min to recover. The pressure transducer was connected to a signal conditioner (model 6600, Gould Instrument Systems, Valley View, OH). Signals were displayed on a chart recorder (model MT95K2, Astro-Med, West Warwick, RI), digitized at 1 kHz, and written to a fixed disk of a microcomputer with the use of commercially available software (PONEMAH Physiology Platform, P3, Gould Instrument Systems).

Experimental protocols. The animals performed nine 1-min bouts of treadmill exercise across a combination of three speeds (5, 10, and 20 m/min) and three grades (−10, 0, and +15°) in random order. For the high grade, two rats ran at +10° and five rats ran at +15°; the group mean data are reported as +15° for convenience. The stride frequency for rats at these three speeds is ~1, 2, and 3 strides/s (3). The animals were allowed to recover for ≥3 min between bouts.

Data analysis. Terminal aortic vascular conductance was calculated as terminal aortic flow divided by arterial pressure. Arterial pressure, terminal aortic flow, and terminal aortic vascular conductance were each averaged over 1-s periods beginning 10 s before the onset of locomotion until the end of locomotion.

Dogs

Six mongrel dogs (18–24 kg body wt) of either gender were selected for their willingness to run on a motor-driven treadmill (model J6, Proform). The dogs were familiarized with treadmill running in a series of training sessions before the following aseptic surgical procedures were performed.

Surgical preparation. Dogs were anesthetized with thiopental, intubated, ventilated, and maintained with halothane. Ultrasonic transit-time blood flow transducers (Transonic) and vascular occluder cuffs were placed bilaterally on the iliac arteries through a midline abdominal incision. In one dog, these devices were implanted on the terminal aorta, and the measured values of blood flow in this dog were on average three times greater than in the other dogs. Blood flows measured in this dog were divided by a factor of 3 before they were averaged with the results from the remaining animals. A catheter was inserted into the aorta for measurement of systemic arterial pressure and in a femoral artery and vein for measurement of hindlimb perfusion pressure and infusion of drugs, respectively. All leads were tunneled to exit sites on the back. Skin patches delivering a total of 75 μg/h of fentanyl were placed on the dogs for 72 h after surgery to control postoperative pain, and the dogs were given cephalexin (500 mg po twice a day) continually after surgery throughout the time that data were collected.

Data collection. Catheters were connected to pressure transducers (model P10EZ, Ohmeda) and flow transducers were connected to flowmeters (model T106, Transonic). Signals were displayed on a pen recorder and digitized at 250

Hz. Average values of each signal were written to a fixed disk of a microcomputer twice per second.

Experimental protocols. The animals performed treadmill exercise at three intensities (3.2 km/h and 0% grade, 6.4 km/h and 0% grade, and 6.4 km/h and 10% grade) for 3 min in no regular order. The animals were allowed to recover for ≥3 min before exercise was repeated at a different exercise intensity. The animals were then given intravenous hexamethonium (10 mg/kg), atropine (0.1 mg/kg), and captopril (1 mg/kg) to block autonomic function and the renin-angiotensin system, respectively (17, 20). The efficacy of these drugs was inferred from the exaggerated fall in arterial pressure that accompanied locomotion after autonomic blockade. The three bouts of exercise were then repeated. Some animals ran for only 1 min at some workloads, owing to reduced exercise capacity after autonomic blockade. Animals were then given nitro-L-arginine methyl ester (L-NAME, 10 mg/kg iv) to inhibit NO production, and exercise was repeated as described above. The efficacy of NOS inhibition was inferred from the rise in arterial pressure elicited by this drug. On a separate day, animals performed locomotion as described above with and without L-NAME alone. Drugs were acquired from Sigma Chemical (St. Louis, MO).

Data analysis. Iliac vascular conductance was calculated as iliac blood flow divided by arterial pressure. We evaluated the effect of L-NAME treatment on the time course of the rise in iliac vascular conductance graphically on the basis of calculations of iliac vascular conductance for each exercise bout averaged over 1-s periods from 10 s before until the end of the first 60 s of exercise. Values are means ± SE.

Statistical Analysis

Tests for statistical significance were done by paired *t*-tests, except as follows. For each 1-s time period of the data presented in Figs. 3 and 5, treatment effects were tested statistically by multiple linear regression (22) with a computer spreadsheet program (Microsoft Excel 97, Redmond, WA). Dummy variables were used as independent variables to account for interindividual variability among animals (22). For Fig. 3, treadmill speed (5, 10, or 20 m/min), grade (−10, 0, or +10°), and sequence order (1–9) were used to encode treatment effects. For Fig. 5, treadmill speed (3.2 or 6.4 km/h) and treadmill grade (0 or 10%) were used to encode treatment effects. Values are means ± SE.

RESULTS

Rats

Figure 1 depicts arterial pressure, hindlimb blood flow, and hindlimb vascular conductance in a rat performing treadmill exercise graded across nine combinations stemming from three speeds (5, 10, and 20 m/min) and three grades (−10, 0, and +15°). All speeds and grades led to immediate increases in blood flow and calculated vascular conductance. The steady-state values of these variables reveal considerable overlap among the different combinations of speed and grade.

Data collected in response to locomotion at 10 m/min and 0° grade and 20 m/min and −10° grade were selected to illustrate how different combinations of speed and grade can lead to a similar rise in vascular conductance with a variable pattern of response. Resting vascular conductance (averaged over the 10 s immediately preceding the onset of locomotion) was

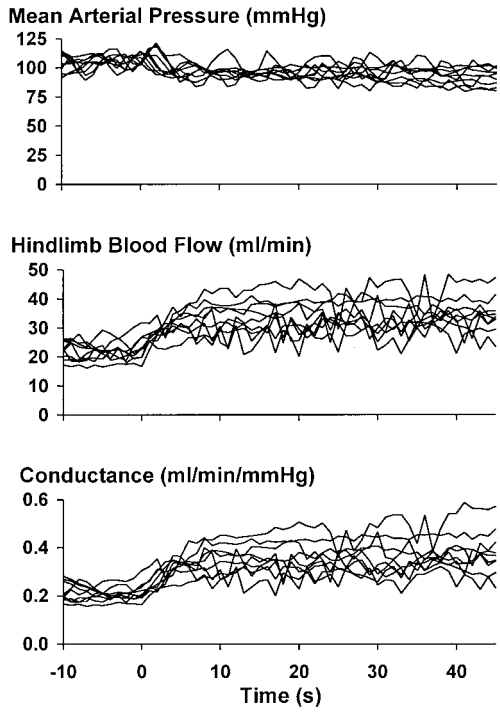

Fig. 1. Hemodynamic responses from 1 rat to 9 bouts of treadmill locomotion graded across 3 speeds and 3 grades. Terminal aortic flow and vascular conductance rose in response to locomotion, whereas femoral arterial pressure tended to fall slightly. There was considerable overlap in the steady-state values of flow and conductance achieved across the 9 conditions.

0.150 ± 0.022 and 0.162 ± 0.023 ml·min^{-1}·mmHg^{-1} for 10 m/min and 0° grade and 20 m/min and −10° grade, respectively (P = 0.19). Exercise vascular conductance (averaged over the final 10 s of locomotion) was 0.271 ± 0.034 and 0.277 ± 0.038 ml·min^{-1}·mmHg^{-1} for 10 m/min and 0° grade and 20 m/min and −10° grade, respectively (P = 0.60). The increase in hindlimb conductance from rest to exercise was 0.121 ± 0.016 and 0.115 ± 0.021 ml·min^{-1}·mmHg^{-1} for 10 m/min and 0° grade and 20 m/min and −10° grade, respectively (P = 0.53). Femoral arterial pressure was similar during the two bouts. Figure 2 depicts the time course of the rise in hindlimb conductance as percent increase, and it can be seen that the similar increases in conductance followed different time courses. That is, conductance at the slower speed and higher grade was significantly less ($P < 0.05$) than conductance at the faster speed and lesser grade early during locomotion.

Figure 3 depicts group mean data showing the changes in hindlimb blood flow, femoral arterial pressure, and hindlimb vascular conductance from seven rats. Figure 3, *left*, shows the isolated influence of speed on the rise in blood flow and conductance; i.e., each trace represents the response to the three individual bouts at the three different grades, averaged together. The statistical analysis (which included the data from the intermediate speed) indicates that speed began to exert a significant influence ($P < 0.05$) on conductance as soon as the 3rd s of locomotion, and this

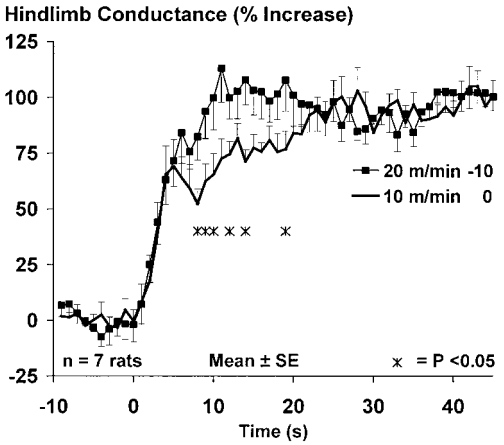

Fig. 2. Time course of rise in hindlimb vascular conductance as percent increase in response to locomotion at 10 m/min and 0° grade and 20 m/min and −10° grade in rats. Resting vascular conductance, exercise vascular conductance, and change in vascular conductance from rest to exercise were not significantly different between the 2 conditions. Rise in vascular conductance follows a slower time course during locomotion at 10 m/min and 0° grade than during locomotion at 20 m/min and −10° grade.

influence persisted throughout the remainder of exercise. Figure 3, *right*, depicts the isolated influence of grade on the rise in blood flow and conductance; i.e., each trace represents the response to the three individual bouts at the three different speeds, averaged together. The statistical analysis (which included the data from the intermediate grade) indicates that grade did not begin to exert a significant effect ($P < 0.05$) on the rise in conductance until the 12th s of locomotion.

Dogs

Figure 4 depicts the time course of the rise in hindlimb vascular conductance averaged from five dogs performing treadmill exercise at 6.4 km/h and 10% grade before and after inhibition of NOS (autonomic function intact). When NOS function was intact, conductance rose progressively over the first 10 s of locomotion with an apparent overshoot. After inhibition of NOS, the time course of the rise in conductance exhibited a biphasic pattern. Conductance initially (1–3 s) rose to a steady level that persisted for ~5 s, after which conductance began a second rise to a steady-state value with no apparent overshoot.

Figure 5 depicts the rise in conductance across the three workloads after inhibition of NOS and autonomic blockade. The immediate rise in conductance in the first few seconds of locomotion at 6.4 km/h was approximately twice as great as the rise that accompanied locomotion at 3.2 km/h. Furthermore, the immediate rise in conductance was identical for locomotion at 6.4 km/h and 0% grade and for locomotion at 6.4 km/h and 10% grade. Thereafter, under all three conditions, conductance underwent further increases.

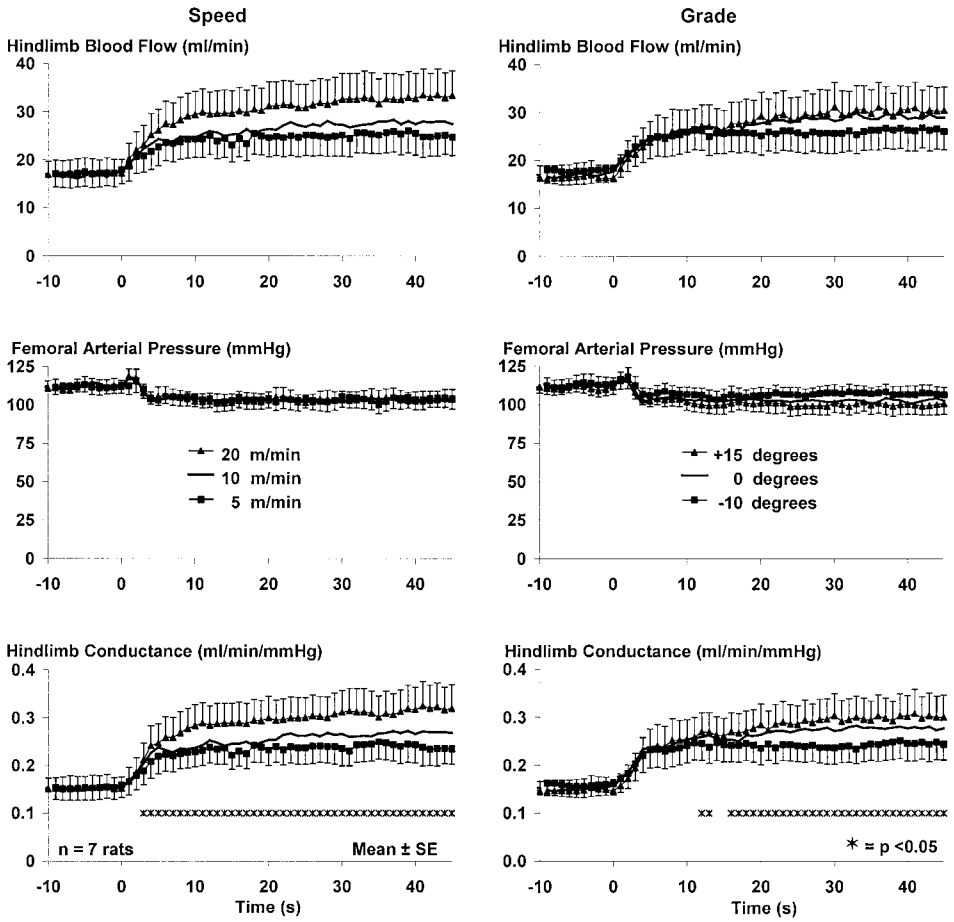

Fig. 3. Isolated influence of speed and grade on the hemodynamic response to locomotion in rats. *Left*: each trace represents responses to individual bouts at the 3 different grades, averaged together. *Right*: each trace represents responses to individual bouts at the 3 different speeds at a single grade, averaged together. Error bars for intermediate speed and grade have been deleted to improve clarity.

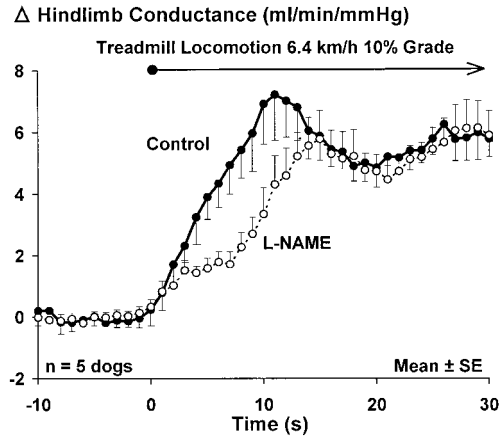

Fig. 4. Influence of nitric oxide synthase (NOS) inhibition on the time course of the rise in hindlimb vascular conductance in response to locomotion of moderate intensity in dogs. NOS inhibition eliminated the rise in conductance normally seen at 3–7 s but did not affect the overall rise in conductance. Nitro-L-arginine methyl ester (L-NAME) exerted a statistically significant effect ($P < 0.01$) at 2–13 s. Resting conductance was 2.60 ± 0.19 and 1.23 ± 1.23 $ml \cdot min^{-1} \cdot mmHg^{-1}$ in control and after L-NAME, respectively.

DISCUSSION

The major new findings of our study are twofold. First, the time course of the rise in muscle vascular conductance is significantly altered by the mechanical factors (speed and grade) by which a given rate of work is achieved during locomotion in rats. Second, augmented NO formation exerts a significant effect on the rise in conductance during the initial stages (3–10 s) in response to a moderate intensity of treadmill locomotion and appears to account for all the dilation that normally occurs over this period in dogs. Moreover, the absence of the normal influence of NO reveals that the muscle pump likely accounts for all the rise in blood flow in the first 8 s of locomotion in these conditions.

Rats

The increases in muscle blood flow and muscle vascular conductance achieved during dynamic exercise are tightly coupled to the amount of work performed by muscle, and metabolic vasodilation is an important regulatory mechanism by which muscle blood flow is coupled to local energy demands. The common view is that vasodilator substances within active muscle accumulate in a manner governed by the balance between the energy expended by the muscle and the blood flow through the muscle and that metabolic vasodilation constitutes the primary determinant of the vascular conductance achieved during locomotion. Because arterial pressure changes far less than does muscle blood flow in the transition from rest to locomotion (20% vs. >200%), the blood flow achieved is also tightly coupled to the rise in vascular conductance. For these reasons, we chose muscle vascular conductance as an index of the work rate imposed by the various combinations of speed and grade employed in our study. Also, the time course of the relaxation of vascular smooth muscle appears to be constrained by relatively fixed dynamic characteristics, which are relatively slow (half-relaxation times of 5–40 s) (11). Therefore, we reasoned that a fixed rise in vascular conductance should follow a similar time course, regardless of the specific mechanics of locomotion if vasodilation were the sole or overwhelming cause of the rise in conductance. In support of this rationale is the observation that vasodilatory responses induced by four different chemical substances follow a relatively similar time course (25). Also, even though a change in arteriolar diameter can be detected immediately after an electrically induced 1-s tetanic contraction of surrounding skeletal muscle fibers, the vasodilation that is expressed lasts for ~50 s (6). These observations imply that the microvasculature possesses relatively sluggish response characteristics and thus functions as a low-pass filter.

The muscle pump exerts important influences on the pressure-volume (capacitive) (20) and the pressure-flow characteristics (18, 19) of the peripheral circulation. A number of investigators employing a broad mixture of different exercise conditions have concluded that the muscle pump can augment blood flow across muscle (5, 15, 18, 19, 23). Laughlin (10) postulated that the blood flow-raising function of the muscle pump is most effective during locomotory-type exercise. A common assumption is that contraction frequency constitutes a major determinant of muscle pump efficacy (5, 8, 18, 21) just as cardiac frequency can constitute a major determinant of cardiac pump efficacy, and several studies have provided evidence in support of this idea (5, 8, 18). For example, Gotschall and co-workers (8) examined the importance of cycling cadence at a fixed workload and found that higher cadences were associated with a higher total vascular conductance, which they attributed to more effective muscle pump-

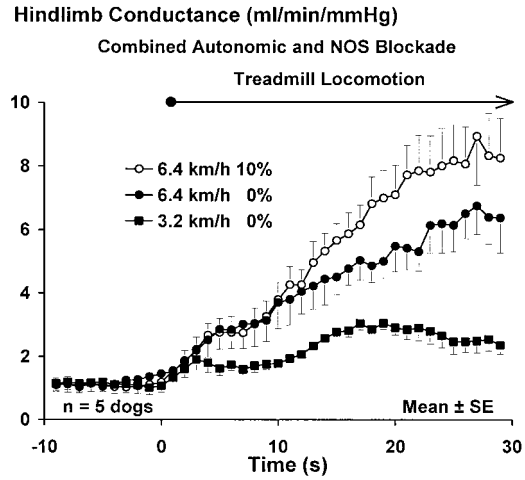

Fig. 5. Rise in hindlimb vascular conductance in response to locomotion graded across 2 speeds and 2 grades after inhibition of NOS and autonomic blockade. Doubling of treadmill speed from 3.2 to 6.4 km/h led to an approximate doubling of the rise in conductance achieved in the first 5 s of locomotion. Increasing treadmill grade from 0 to 10% at a fixed treadmill speed did not affect the rise in vascular conductance over this time period. Speed began exerting a significant effect ($P < 0.05$) on conductance at 4 s, whereas grade did not begin to exert an effect until 18 s.

ing. The importance of contraction force on the blood flow-raising function of the muscle pump is unclear, although this variable is known to be relatively unimportant in determining the efficacy of the muscle pump in altering the pressure-volume characteristics of the circulation [i.e., mild contractions are as effective as forceful contractions in emptying muscle veins (2)].

We found that contraction frequency exerts an influence on muscle vascular conductance as soon as the 3rd s of locomotion. In contrast, the influence of muscle contraction frequency on arteriolar dilation per se is indistinct. For example, the changes in arteriolar diameter in response to contractions at 2 and 4/s (similar to the contraction frequencies of the rats in the present study) are identical for the first 40 s, whereas the rise in diameter is more extensive over this period during contractions at 8/s (6). On the basis of this finding, the observation that conductance is different across various treadmill speeds relatively early during locomotion argues that this difference stems from the influence of contraction frequency on the effectiveness of muscle pumping, and not on arteriolar vasodilation.

We found that treadmill grade (contraction force) did not begin to exert a significant effect on conductance until 13 s after the onset of locomotion. If contraction force constituted an important determinant of the efficacy of muscle pumping, we would expect this variable to exert an effect on conductance with little or no time delay. The delayed nature of the influence of contraction force on conductance is consistent with the idea that this factor exerts its influence by modulating the release of vasodilator substances. The fact that the delay we report here is similar to the delays reported for arterioles to begin dilating in response to twitch contractions at similar frequencies strengthens this argument (6).

Recently, Shoemaker et al. (21) found that contraction frequency did not affect the pattern or extent of the rise in muscle blood flow during forearm exercise performed by human subjects. Rather, the rise in muscle blood flow during work at a fixed rate followed the same time course, regardless of whether the work was performed with a light weight at a fast cadence or with a heavy weight at a slow cadence. This led these investigators to conclude that vasodilator responses are important early during dynamic exercise, and this influence likely obscures any muscle pump effect that is dependent on contraction frequency. In contrast, we found that the time course of the rise in vascular conductance varied significantly with changes in contraction rate and grade, even when the eventual rise in vascular conductance, and thus presumably the total work rate, was the same (Fig. 2). Furthermore, the faster rise in conductance at the faster treadmill speed is consistent with the idea that the muscle pump is more effective at higher contraction rates. To illustrate these points graphically, Fig. 6 replots the vascular conductance data from the nine bouts of exercise as follows. Over the period of time when neither speed nor grade exerted a significant influence (from the beginning until the 3rd s), all the runs were averaged together into a single trace. At the point in time when speed begins to exert a significant influence (3rd s), the single trace splits into three traces, one new trace for each of the three speeds. Finally, at the point in time when grade begins to exert a significant influence (12th s), the traces representing locomotion at the low and high speeds are each split into three, one new trace for each of the three grades. The responses from the three grades at the intermediate speed remain averaged together to improve clarity. As can be seen, a similar rise in conductance can be achieved via many paths, depending on loading conditions.

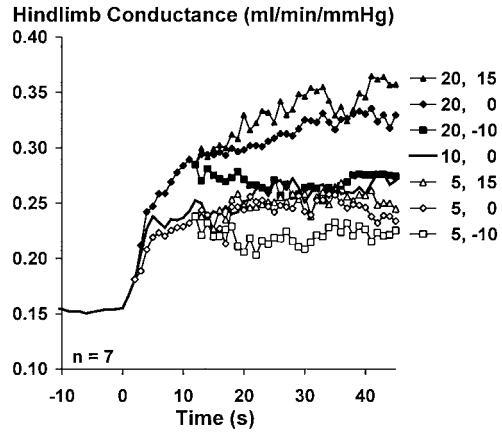

Fig. 6. Influence of speed and grade on the rise in hindlimb vascular conductance in response to locomotion in rats. Results are averaged together over time periods when a factor (speed or grade) does not exert a statistically significant influence, and the results are split over time periods when a factor does exert a significant influence.

The qualitatively different results from the study by Shoemaker et al. (21) and the present study likely stem from the different modes of exercise employed in the two studies. In the study by Shoemaker et al., the subjects raised and then lowered a weight over a 1-s period and then relaxed the muscle for 1 s before another duty cycle was begun. Thus for each duty cycle, the muscle was continuously activated for 1 s, during which it performed a concentric contraction followed by an eccentric contraction, after which it relaxed at fixed length. In contrast, the pattern of activation and the mode of shortening and lengthening are substantially different during locomotion. Generally, muscles engaged in producing locomotion tend to perform brief concentric contractions, relax, and then undergo forced reextension by antagonistic muscles while they are relaxed (or are relaxing). For example, during locomotion at 8.5 km/h (2.2 strides/s) in dogs, the gluteus medius muscle (a hip extensor) expresses electromyogenic activity for 27% (120 ms) of the stride period and the muscle shortens while it is active (7). The muscle is then quiescent for >150 ms before it begins to relengthen as the leg is pulled forward by the hip flexors. For this muscle to accumulate 1 s of electromyographic activity (as presumably occurs in the 1st duty cycle in the study of Shoemaker et al.), it would take over eight strides and thus would not occur until the 4th s of exercise. The forceful reextension of muscle may be a

critical factor in promoting effective muscle pumping during locomotion (10). Histological evaluation of the influence of changes in muscle length on lymphatic volume (13) and microscopy evaluation of the influence of changes in muscle length on the geometry of microvessels (14) lend credence to the suggestion that skeletal muscle and its vasculature function as a "bellows pump" (1).

Dogs

In a previous study using a limited number of animals, Sheriff et al. (18) found that doubling treadmill speed from a low to a moderate level led to a doubling of the immediate rise in hindlimb conductance in dogs walking on the flat. These investigators attributed this initial (2- to 3-s) rise in blood flow and calculated conductance to more effective muscle pumping at the higher contraction frequency. After a delay of ~10 s, during which time conductance was relatively unchanged, they observed that conductance underwent a second rise that they attributed to the action of vasodilator substances. They also found that vascular conductance rose more smoothly to its steady-state level in dogs walking uphill at a moderate speed, suggesting that vasodilation might begin much sooner at this workload (thereby encroaching on the short window of opportunity for gauging the effectiveness of the muscle pump). Importantly, recent evidence indicates that NOS inhibition slows the rate of vasodilation during locomotion (17). It is believed that the muscle pump elicits an immediate increase in blood flow and, as a consequence, there is a rise in shear stress on endothelial cells, which augments the release of NO. In this way, NO acts as an amplifier that reinforces the influence of the muscle pump on blood flow. In the present study, we sought to shut off this amplifier by inhibiting NOS as a means of possibly extending the window of opportunity for evaluating muscle pump effectiveness at the onset of locomotion at this higher work rate.

We found that NO exerts an important influence early during the transition from quiet standing to locomotion as reported previously (17). The focus of our previous study was on evaluating the contribution of NO to the rise in vascular conductance that accompanies locomotion; the focus here is on evaluating what the absence of NO reveals about muscle pump function. As seen in Fig. 4, NOS inhibition does not alter the initial (1- to 2-s) rise in conductance. However, NOS inhibition appears to eliminate the entire rise in conductance normally seen in *seconds 3–7* at this intensity of locomotion. These results are consistent with the idea that the muscle pump initiates an immediate increase in blood flow (virtual conductance) that is subsequently reinforced by augmented NO formation induced by an increase in endothelial wall shear stress that arises as a consequence of the increase in blood flow. Beyond 7 s, conductance begins a second rise during NOS inhibition that is similar in rate and extent to the rise seen in control conditions but delayed in time, an observation that underscores the view that redundant control mechanisms are involved in the regulation of muscle blood flow (9).

In the present study, we confirm and extend the previous findings of Sheriff et al. (18). Figure 5 shows, on the basis of the measurement of iliac artery blood flow and without the competing influence of the autonomic nervous system and the early contribution of NO, that an approximate doubling of stride frequency (treadmill speed) leads to an approximate doubling of the initial (2- to 3-s) rise in conductance. Importantly, when the influence of NO is lacking, increasing treadmill grade at a fixed speed does not alter the initial (1- to 10-s) rise in conductance. Thus all the other numerous potential vasodilator substances (16) fail to elicit any measurable change in conductance until ~8 s into the exercise bout. Beyond 8 s, these other dilators eventually exert a profound effect, eliciting a second rise in conductance that is approximately twice as large for work at 10% grade as on the flat. In our opinion, it is unlikely that vasodilation causes (or even contributes to) the initial (1- to 2-s) rise in conductance under these conditions, and it is even more unlikely that vasodilation ceases for 5 s and then begins again. For example, recent elegant studies by Welsh and Segal (24) provide compelling evidence that there is a 2- to 3-s delay attributable to electromechanical coupling within vascular smooth muscle cells in response to vasodilator substances. Also, Wunsch et al. (25) recently demonstrated a delay of ~4 s before the onset of dilation of isolated arterioles after direct application of chemical vasodilator substances. On the basis of these arguments, our results support the proposal of Sheriff et al. that the muscle pump elicits all the initial (1- to 3-s) increase in calculated conductance (blood flow) during mild intensities of locomotion and extends this to moderate intensities of locomotion when NO function is inhibited. The discrepancy between the delayed vasodilatory responses resulting from direct application of vasodilator substances to isolated arterioles (25) and the more immediate vasodilation observed in arterioles after 1-s electrically evoked contractions of surrounding skeletal muscle (6) remains to be explained.

Finally, our results may help explain why the search for the chemical substances that account for active hyperemia has proven so elusive (9). Our results strongly suggest that the muscle pump can initiate a significant (2-fold) increase in blood flow, which in turn stimulates augmented NO release and a further rise in blood flow. If feedforward control such as this matches the rise in blood flow to the rise in oxygen demand in a close temporal relationship, a chemical error signal may not arise over a wide range of intensities of locomotion. Indeed, the tendency for vascular conductance to exhibit an overshoot early during mild locomotion when sympathetic function is intact (18) indicates that muscle may be relatively overperfused early during this form of exercise.

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REFERENCES

1. **Almen T and Nylander G.** Serial phlebography of the normal lower leg during muscular contraction and relaxation. *Acta Radiol* 57: 264–272, 1962.
2. **Barendsen GJ and van den Berg JW.** Venous capacity, venous refill time and the effectiveness of the calf muscle pump in normal subjects. *Angiology* 35: 163–172, 1984.
3. **Clarke KA and Parker AJ.** A quantitative study of normal locomotion in the rat. *Physiol Behav* 38: 345–351, 1986.
4. **Delp MD.** Control of skeletal muscle perfusion at the onset of dynamic exercise. *Med Sci Sports Exerc* 31: 1011–1018, 1999.
5. **Folkow B, Gaskell P, and Waaler BA.** Blood flow through limb muscles during heavy rhythmic exercise. *Acta Physiol Scand* 80: 61–72, 1970.
6. **Gorczynski RJ, Klitzman B, and Duling BR.** Interrelations between contracting striated muscle and precapillary microvessels. *Am J Physiol Heart Circ Physiol* 235: H494–H504, 1978.
7. **Goslow, GE Jr, Seeherman HJ, Taylor CR, McCutchin MN, and Heglund NC.** Electrical activity and relative length changes of dog limb muscles as a function of speed and gait. *J Exp Biol* 94: 15–42, 1981.
8. **Gotshall RW, Bauer TA, and Fahrner SL.** Cycling cadence alters exercise hemodynamics. *Int J Sports Med* 17: 17–21, 1995.
9. **Joyner MJ and Proctor DN.** Muscle blood flow during exercise: the limits of reductionism. *Med Sci Sports Exerc* 31: 1036–1040, 1999.
10. **Laughlin MH.** Skeletal muscle blood flow capacity: role of muscle pump in exercise hyperemia. *Am J Physiol Heart Circ Physiol* 253: H993–H1004, 1987.
11. **Liu X, Jiang H, and Stephens NL.** Use of a new index to study relaxation in a vascular model of anaphylactic shock. *J Appl Physiol* 74: 2621–2626, 1993.
12. **Marshall JM and Tandon HC.** Direct observations of muscle arterioles and venules following contraction of skeletal muscle fibres in the rat. *J Physiol (Lond)* 350: 447–459, 1984.
13. **Mazzoni MC, Skalak TC, and Schmid-Schönbein GW.** Effects of skeletal muscle fiber deformation on lymphatic volumes. *Am J Physiol Heart Circ Physiol* 259: H1860–H1868, 1990.
14. **Nakao M and Segal SS.** Muscle length alters geometry of arterioles and venules in hamster retractor. *Am J Physiol Heart Circ Physiol* 268: H336–H344, 1995.
15. **Radegran G and Saltin B.** Muscle blood flow at onset of dynamic exercise in humans. *Am J Physiol Heart Circ Physiol* 274: H314–H322, 1998.
16. **Shepherd JT.** Circulation to skeletal muscle. In: *Handbook of Physiology. The Cardiovascular System. Peripheral Circulation and Organ Blood Flow*. Bethesda, MD: Am. Physiol. Soc., 1983, sect. 2, vol. III, pt. 1, chapt. 11, p. 319–370.
17. **Sheriff DD, Nelson CD, and Sundermann RK.** Does autonomic blockade reveal a potent contribution of nitric oxide to locomotion-induced vasodilation? *Am J Physiol Heart Circ Physiol* 279: H726–H732, 2000.
18. **Sheriff DD, Rowell LB, and Scher AM.** Is rapid rise in vascular conductance at onset of dynamic exercise due to muscle pump? *Am J Physiol Heart Circ Physiol* 265: H1227–H1234, 1993.
19. **Sheriff DD and Van Bibber R.** Flow-generating capability of the isolated skeletal muscle pump. *Am J Physiol Heart Circ Physiol* 274: H1502–H1508, 1998.
20. **Sheriff DD, Zhou XP, Scher AM, and Rowell LB.** Dependence of cardiac filling pressure on cardiac output during rest and dynamic exercise in dogs. *Am J Physiol Heart Circ Physiol* 265: H316–H322, 1993.
21. **Shoemaker JK, Tschakovsky ME, and Hughson RL.** Vasodilation contributes to the rapid hyperaemia with rhythmic contractions in humans. *Can J Physiol Pharmacol* 76: 418–427, 1998.
22. **Slinker BK and Glantz S.** Multiple linear regression is a useful alternative to traditional analyses of variance. *Am J Physiol Regulatory Integrative Comp Physiol* 255: R353–R367, 1988.
23. **Tschakovsky ME, Shoemaker JK, and Hughson RL.** Vasodilation and muscle pump contribution to immediate exercise hyperemia. *Am J Physiol Heart Circ Physiol* 271: H1697–H1701, 1996.
24. **Welsh DG and Segal SS.** Endothelial and smooth muscle cell conduction in arterioles controlling blood flow. *Am J Physiol Heart Circ Physiol* 274: H178–H186, 1998.
25. **Wunsch SA, Muller-Delp J, and Delp MD.** Time course of vasodilatory responses in skeletal muscle arterioles: role in hyperemia at the onset of exercise. *Am J Physiol Heart Circ Physiol* 279: H1715–H1723, 2000.