Simulation of motor unit recruitment and microvascular unit perfusion: spatial considerations

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Fuglevand, Andrew J., and Steven S. Segal. Simulation of motor unit recruitment and microvascular unit perfusion: spatial considerations. J. Appl. Physiol. 83(4): 1223–1234, 1997.—Muscle fiber activity is the principal stimulus for increasing capillary perfusion during exercise. The control elements of perfusion, i.e., microvascular units (MVUs), supply clusters of muscle fibers, whereas the control elements of contraction, i.e., motor units, are composed of fibers widely scattered throughout muscle. The purpose of this study was to examine how the discordant spatial domains of MVUs and motor units could influence the proportion of open capillaries (designated as perfusion) throughout a muscle cross section. A computer model simulated the locations of perfused MVUs in response to the activation of up to 100 motor units in a muscle with 40,000 fibers and a cross-sectional area of 100 mm². The simulation increased contraction intensity by progressive recruitment of motor units. For each step of motor unit recruitment, the percentage of active fibers and the number of perfused MVUs were determined for several conditions: 1) motor unit fibers widely dispersed and motor unit territories randomly located (which approximates healthy human muscle), 2) regionalized motor unit territories, 3) reversed recruitment order of motor units, 4) densely clustered motor unit fibers, and 5) increased size but decreased number of motor units. The simulations indicated that the widespread dispersion of motor unit fibers facilitates complete capillary perfusion of muscle at low levels of activity. The efficacy by which muscle fiber activity induced perfusion was reduced 7- to 14-fold under conditions that decreased the dispersion of active fibers, increased the size of motor units, or reversed the sequence of motor unit recruitment. Such conditions are similar to those that arise in neuromuscular disorders, with aging, or during electrical stimulation of muscle, respectively.

The microvasculature of skeletal muscle is also organized into anatomically defined units of control (7, 14, 56). The entry of red blood cells into a capillary (defined here as capillary perfusion) is controlled at the level of the terminal arteriole, the dilation of which results in simultaneous perfusion of all capillaries supplied by the arteriole (14, 40). This control element of oxygen transport to muscle fibers is referred to as a microvascular unit (MVU) (14, 17) or capillary module (4). Each MVU encompasses 1–2 mm along a muscle fiber bundle composed of fibers that derive from many motor units.

Whereas the overall delivery of blood to skeletal muscle closely parallels the intensity of muscular activity (1), neither the pattern nor mechanisms by which the control elements of capillary perfusion are coordinated to meet the metabolic requirements of active fibers are completely understood. For example, little is known of how blood flow may be directed to the fibers of a motor unit, all of which have similar metabolic demands but are dispersed throughout the muscle. Typically, the control of muscle blood flow has been studied by recording hemodynamic and vascular responses to electrical stimulation of whole muscle (26, 42) or to microstimulation of a few muscle fibers (4, 21, 43). With whole muscle stimulation, all fibers may be activated simultaneously, thereby eliminating differences in activity levels that normally exist across a population of motor units. Furthermore, with graded electrical stimulation of the muscle or its motor nerve, motor units tend to be recruited in an order that is reversed from that which occurs naturally (46). Microstimulation, on the other hand, engages a small bundle of contiguous fibers, none of which may belong to the same motor unit. Whereas these approaches have yielded valuable insights into the coupling between oxygen delivery and metabolic demand, these methods fail to emulate the complex spatial and temporal features of natural muscle activation (35, 37). Such limitations have constrained our understanding of how blood flow is actually regulated in response to exercise (35).

As a first step toward addressing the complexity of interaction between motor unit recruitment and oxygen delivery to muscle fibers, we developed a computer model to simulate the perfusion of MVUs in response to different levels of motor unit recruitment. This was done to evaluate how differences in the spatial organization of muscle fibers into motor units could influence the pattern and magnitude of capillary perfusion throughout a muscle cross section. Simulations included voluntary activation and electrical stimulation of healthy muscle. Additional simulations were performed to predict the pattern of MVU perfusion in muscles that have undergone motor unit reorganiza-
tion due to peripheral nerve damage and aging. Our results indicate that the widespread dispersion of motor unit fibers promotes complete perfusion of healthy muscle at low levels of activity. This phase advance of perfusion over contraction implies a form of “safety factor” in blood delivery: the capillaries of many inactive fibers are perfused; therefore, upon additional fiber recruitment, oxygen may be available without delay. This putative safety factor was found to be compromised in simulations of muscle pathology, aging, and electrical stimulation.

METHODS

Definitions. Perfusion and blood flow are distinguished as follows: the term perfusion is used to denote the existence of blood flow through a capillary. Perfusion, therefore, was considered an all-or-nothing phenomenon; i.e., either a capillary was perfused or not. On the other hand, blood flow indicates the quantity of blood moving through a vessel or group of vessels per unit time and is represented by a continuous variable. This nomenclature for describing vascular networks is analogous to that used to describe motor unit pools; a motor unit may be recruited or not, but it is inappropriate to indicate “how much” a unit is recruited. Rather, a separate, continuous variable is reserved to represent the intensity of motor unit activity, namely, motor unit firing rate. By distinguishing perfusion from flow in this manner, it is possible to describe a vascular control system composed of two coupled processes that reflect the Fick principle (rate of oxygen consumption = extraction × flow). One process involves the perfusion of MVUs, which functions to alter the surface area for exchange and is a primary determinate of oxygen extraction. The second process is controlled by the resistance network proximal to the terminal arteride and modulates the quantity of blood moving through vessels and thereby regulates the total delivery of oxygen. These two processes interact to ensure that oxygen demands are met during physical activity. The present work focuses on the perfusion of capillaries in accord with the recruitment properties of skeletal muscle fibers.

Model constraints. A model was developed to simulate the location and number of perfused capillaries in response to recruitment of up to 100 motor units innervating 40,000 muscle fibers in a muscle with cross-sectional dimension of 10 × 10 mm. The general constraints of the model were as follows: 1) the simulations were performed entirely in the spatial domain; therefore, the time courses of muscle activation and perfusion were not features of the model; 2) only increases in perfusion (i.e., fraction of open capillaries) above that existing at rest were simulated; 3) the magnitude of blood flow through muscle or MVUs was not determined; and 4) contraction intensity was graded in terms of muscle fiber recruitment only; thus firing-rate modulation was not considered. Specific assumptions underlying parameter assignments for simulations of human muscle are detailed below.

Motor unit size. The number of fibers assigned to each motor unit was determined on the basis of the following experimental observations and assumptions (19). The twitch or tetanic forces of motor units within a muscle are known to vary over a wide range, typically ≥100-fold (45, 48). Differences in force exerted by motor units could be due to variations in 1) the cross-sectional areas of muscle fibers, 2) intrinsic force capacity of the fibers, or 3) the number of fibers innervated (10, 18, 33). In human muscle there appear to be no systematic differences in fiber diameter associated with fiber type (39, 50). Although some differences have been reported in intrinsic force capacity for different motor unit types (11), the dominant factor underlying variation in motor unit force is fiber number (8, 33, 59).

In the model, therefore, all fibers were allotted the same cross-sectional dimensions (50 × 50 µm). Furthermore, it was assumed that the number of fibers innervated by different motor units was distributed across the motor unit population in a manner similar to the way in which force is distributed. The frequency distribution of motor units according to force is skewed such that many units produce small forces and relatively few units exert large forces (20, 41, 45, 48). Therefore, fiber number was varied across the motor unit population as an exponential function that assigned the largest unit to have 100-fold more fibers than the smallest unit, and one-half of the population had fiber numbers that were <10% of the fiber number assigned to the largest unit (19).

Motor unit territory. The fibers of a motor unit are scattered over a broad region of the muscle cross section (2, 8, 11, 16, 59) and interdigitate with fibers belonging to many other motor units. The distribution of fibers within a motor unit territory (i.e., subarea of muscle cross section containing fibers belonging to a single unit) is relatively uniform (2, 11, 59). However, the fibers of small motor units tend to be more scattered relative to one another than the fibers of large motor units (2, 33; cf. Ref. 49). The density of motor unit fibers ranges from 10 to 40 unit fibers/mm² for large units (2, 33). Therefore, unit fiber density was defined in the model as a linear function of the number of fibers assigned to each unit and ranged from 10 to 40 unit fibers/mm². The area encompassed by motor unit territories was then computed for each unit as the ratio of unit fiber number to unit fiber density.

Regionalization of muscle fiber types is seen in some muscles of small mammals, where slow-twitch fibers tend to predominate in the deep interior of the muscle (3, 34, 46). The degree of regionalization in human muscle, however, is small (30, 38, 44). It was assumed, therefore, that a motor unit territory could be located anywhere within the muscle cross section.

Motor unit territories were modeled to be square, with motor unit fibers randomly distributed throughout the territory. The x-y coordinates (representing 1 corner of a motor unit territory) were randomly selected from a square simulation space (representing the muscle cross section) and were used to locate the motor unit territory. If the selected territory location was such that it overlapped the muscle boundary, the position of the territory was shifted until it lay just within the muscle boundary. This approach facilitated “filling” of fibers near the muscle boundary. Nonetheless, in some cases, there appeared to be a slight bias toward recruiting fibers in the interior of the muscle at low levels of activity. This effect, however, did not appear to affect the overall pattern of MVU perfusion.

Motor unit and microvascular unit recruitment. Normally, motor units are recruited in an orderly sequence, progressing from small to large force units (25, 45, 61). This property was also a feature of the model; recruitment occurred in a fixed order from the unit innervating the fewest to the unit innervating the most fibers. As a motor unit was recruited, the locations of its fibers were registered on a computer display of the muscle cross section.

Embedded in the display was a network of nonoverlapping MVUs. Each MVU was assumed to encompass 1-mm² area of muscle or 400 muscle fibers. This dimension derived from a
compilation of morphometric data and theoretical arguments presented by Bloch and Iberall (7).

The collective action of several mechanisms makes the microvasculature exquisitely sensitive to muscle fiber contraction (37, 53). Indeed, activity in one to several muscle fibers appears sufficient to induce vasodilation and perfusion of MVUs (4, 21, 43). In the model, therefore, when any fibers within the domain of an MVU were active, the MVU was considered to be “recruited” with all its capillaries perfused (14, 40, 52). Perfusion was indicated on the computer display by highlighting the boundaries of the MVU.

Simulation procedures. Each simulation trial involved a stepwise increment in the number of motor units recruited. At each step of the simulation, the percentage of active fibers and the number of perfused MVUs were determined. This was done for six conditions: 1) with parameters assigned as above to represent normal, healthy human muscle; 2) with decreased area and increased number of MVUs to assess the sensitivity of the simulation to MVU dimensions; 3) with motor unit territories regionalized to correspond to the organization of some rat hindlimb muscles: small-force, low-threshold motor units located deep within the muscle and large-force, high-threshold units located nearer the surface; 4) with motor unit recruitment order reversed to emulate electrical stimulation of muscle; 5) with decreased dispersion of motor unit fibers to simulate the dense clustering of motor unit fibers consequent to peripheral nerve damage and reinnervation; and 6) with decreased number but increased size of motor units and reduced dispersion of unit fibers to represent age-related alterations in motor unit organization. The specific changes in parameter values from the normal condition for each of the conditions are described in detail below.

Data analysis. Because motor unit territories were located randomly and changed from trial to trial, there was variability in the pattern of perfusion for repeated runs of the same condition. Therefore, five trials were simulated for each condition, from which the mean and standard deviation of the number of perfused MVUs were calculated for each step of motor unit recruitment.

To compare quantitatively the change in perfusion as a function of muscle fiber activity across conditions, the percentage of active fibers associated with perfusion of 90% of the MVU population was measured for each condition. Ninety rather than 100% was chosen for this parameter because of the quasi-asymptotic advance toward complete perfusion exhibited in preliminary simulations. Conceptually, this parameter reflects the effectiveness by which muscle fiber activity induced perfusion: the fewer fibers active to cause perfusion of a given proportion (90%) of MVUs, the greater the perfusion efficacy. The statistical significance of the difference in this parameter between the condition representing normal healthy muscle and the other five conditions was assessed using a Mann-Whitney U-test for unpaired measures (P < 0.05).

RESULTS

Figure 1 shows the frequency distribution based on fiber number for the 100 motor units in the model of normal muscle. This distribution was skewed, with many motor units having relatively small numbers of muscle fibers. The smallest motor unit was composed of 19 muscle fibers, and the largest unit included 1,818 fibers. The median motor unit (unit 50) had 182 fibers, which corresponded to 10% of the fiber number of the largest motor unit. Because of the skewed distribution, the arithmetic mean (400 fibers/motor unit) was representative of a motor unit in the upper one-third of this population.

An example output of the model is presented in Fig. 2A, which shows active muscle fibers at steps of simulation when 20, 40, 60, and 80 motor units had been recruited. For clarity, MVUs are not shown in this example. Figure 2B depicts, in isolation, the territories for motor units 20, 40, 60, and 80.

Normal muscle. The results of simulations representing voluntary activation of motor units and consequent perfusion of the microvasculature in normal muscle are depicted in Fig. 3A. An example display of the motor unit recruitment step at which 90% of the MVU population was perfused is shown in Fig. 3A. Figure 3B shows the mean number of perfused MVUs plotted as a function of the number of active muscle fibers for each step of motor unit recruitment. Perfusion increased dramatically with the onset of muscle activity, such that 90% of the MVUs were perfused when 2.7 ± 0.6% (SD) of the fibers were active (Fig. 3B, arrow). This potent effect of muscle fiber activity on MVU perfusion in simulations of normal muscle results from the widespread dispersion of motor unit fibers and the random placement of motor unit territories throughout the muscle cross section.

Size of microvascular units. Unlike the case for motor units, limited information is available on the dimensions of MVUs. Recent findings (17, 40) indicate that the cross-sectional areas of MVUs in some muscles may be considerably smaller than 1 mm², which was estimated by Bloch and Iberall (7). Therefore, the effect of MVU dimension on the pattern of perfusion was assessed by repeating the simulations for normal muscle but with MVU area reduced by fourfold (i.e., set to 0.25 mm²). Each MVU in these simulations enclosed 100 muscle fibers, and the entire muscle cross section (100 mm²) was supplied by a network of 400 MVUs.
Figure 4 shows the result of these simulations. Figure 4A depicts an output of the model at the recruitment step at which $90\%$ of the MVUs were perfused. With smaller MVUs, slightly more muscle fibers were recruited to elicit perfusion in $90\%$ of the MVUs than in the control condition (Fig. 3). Nevertheless, the perfusion of MVUs once again greatly outpaced muscle fiber activity (Fig. 4B), with $90\%$ of the MVUs recruited when only $4.8 \pm 0.9\%$ of the muscle fibers were active. Therefore, the particular dimensions attributed to MVUs in these simulations did not markedly affect the overall response of MVU perfusion to motor unit recruitment.

Regionalization of motor unit territories. In contrast to human muscle, slow-twitch oxidative and fast-twitch glycolytic fibers predominate in different regions of some muscles in small mammals. Usually, the deep interior has a higher proportion of fatigue-resistant oxidative fibers, whereas the outer surface region is composed primarily of fatigable glycolytic fibers (3, 34, 46). In general, motor units with low recruitment thresholds innervate slow-twitch, fatigue-resistant fibers, whereas high-threshold units innervate fast-twitch, fatigable fibers (61). Therefore, a spatial gradient of fiber activity normally exists in these muscles. To ascertain the effect of such a gradient on muscle perfusion, simulations were performed in which low-threshold units tended to be located in one part of the muscle and high-threshold units in another. This was done by applying a weighting function in which the probability that a motor unit was located near the deep border of the muscle was inversely related to the number of fibers comprising the motor unit.

Because low-threshold motor units were concentrated in the deep (lower) part of the muscle (Fig. 5A), perfusion of MVUs in this region was complete at relatively low levels of activity. Consequently, further increases in motor unit recruitment within this region were not accompanied by changes in the number of perfused MVUs. This is seen as an intermediate plateau at $\sim 50\%$ perfusion in Fig. 5B. As muscle activity intensified, motor units with territories located in the upper part of the muscle eventually were recruited, leading to a gradual perfusion of MVUs supplying this region of the muscle cross section.

When the muscle is considered as a whole, regionalization of motor unit territories had a pronounced effect on perfusion, in that a much larger proportion ($P < 0.05$) of the muscle fiber population needed to be active ($26.6 \pm 9.9\%$; Fig. 5B, arrow) to realize the perfusion of $90\%$ of the MVU population. Alternatively, if the muscle is considered to be composed of two functionally distinct compartments, then perfusion of the lower compartment roughly followed the pattern arising in nonregionalized muscle: $90\%$ of the MVUs in the lower compartment were perfused when $3.2 \pm 0.5\%$ of the fibers in that compartment were active, which is similar to that obtained in the simulations of normal muscle.

Perfusion of MVUs in the upper compartment followed a more variable pattern, as evident from the large standard deviation bars in Fig. 5B. This can be explained as follows. At some point in a trial, a high-threshold motor unit with a relatively large territory was recruited within the previously quiescent upper
part of the muscle. This usually would cause several MVUs to be engaged and lead to an abrupt increment in perfusion. Because the placement of motor unit territories was determined as a random process, the recruitment step at which this occurred differed from trial to trial. In addition, subsequent motor units often had territories located within previously perfused areas of the muscle. This meant that perfusion leveled off temporarily for subsequent recruitment steps, the number of which varied from trial to trial. Together, these factors contributed to the relatively high variability of perfusion in the upper (i.e., superficial) region of the muscle.

Reversal of recruitment order. Motor units composed of small motoneurons, small-diameter axons, and small numbers of muscle fibers generally have lower voluntary recruitment thresholds than do motor units made up of larger components (25). Large-diameter axons, however, are more susceptible to activation by extracellular electrical stimulation than are small-diameter axons (46). Thus activation of muscle by electrical stimulation will tend to reverse the normal order of motor unit recruitment. Because electrical stimulation is commonly used to induce contraction in many types of experiments, including those designed to study blood flow, simulations were carried out to assess how reversal of motor unit recruitment might affect MVU perfusion.

When motor units were recruited in a sequence progressing from largest to smallest, only a few units needed to be active for most of the MVUs to be perfused (Fig. 6A). However, because these units innervated large numbers of fibers and because of the greater density of unit fibers in large than in small motor units (40 vs. 10 unit fibers/mm²), there was a sevenfold increase (P < 0.05 vs. control) in the number of active fibers (18.9 ± 5.9% of total fibers; Fig. 6B) needed to cause 90% of the MVUs to be perfused compared with when motor units were recruited in a small-to-large
sequence. These findings suggest that, for submaximal contractions, muscle activated by electrical stimulation may have fewer perfused capillaries than muscle activated naturally for a given level of developed force.

Decreased size of motor unit territory. Muscle function can recover almost completely after many types of peripheral nerve injuries (22, 57). For example, the number of motor units, the number of fibers innervated by a motoneuron, and the force capacity of motor units return to near normal levels within 1 yr after surgical repair of a sectioned nerve (59). However, the spatial pattern of fiber innervation changes with nerve regeneration: motor unit fibers tend to be clustered together, with a reduction in size of motor unit territories (59). It was of interest, therefore, to explore how this spatial reorganization after injury might impact on the distribution of blood flow during muscle activity. For these simulations, it was assumed that the spatial density of unit fibers increased to 100 fibers/mm² for all motor units. Motor unit size (in terms of fiber number), the number of motor units, and recruitment sequence, however, were unchanged from those used in the simulations of normal muscle. Although capillary number may decrease in muscle denervated as a consequence of neuropathy or motoneuron disease (13), for the present simulations, capillarity was assumed to be the same as in normal muscle.

Motor unit territories were much compressed compared with normal muscle (Fig. 7A), similar to the clustering of motor unit fibers seen after nerve damage. For example, the area encompassed by the smallest motor unit (19 fibers) in the simulation of damaged muscle was 0.19 mm² compared with 1.90 mm² in normal muscle. A direct consequence of smaller motor unit territories was a reduction in the number of perfused MVUs for submaximal contractions. Whereas MVU perfusion would be complete when 5% of the muscle fibers were active under normal conditions (Fig. 3), simulations of denervated-reinnervated muscle indicated that the same level of muscle fiber activity would
be maintained with only about one-half of the MVUs perfused (Fig. 7B). To attain perfusion of 90% of MVUs in damaged muscle, 18.1 ± 3.4% of the muscle fibers were required to be active, which is a sevenfold increase over that occurring in normal muscle (P < 0.05).

Aging. Beyond the age of 60 yr in humans, there is a significant decline in the number of motor units (9, 12), which is attributed to degeneration and death of a motoneuron in the spinal cord (58). Muscle fibers that lose their parent motoneurons, however, appear to induce sprouting and reinnervation from axons of surviving motoneurons (32, 55). The net effect of this reorganization is a reduction in the number of motor units but an increase in the number of fibers per motor unit (32). Orderly recruitment of motor units according to size, however, remains intact with aging (20). In addition, age-related reinnervation leads to clustering of motor unit fibers (32, 55). To determine how these adaptations might influence muscle perfusion, simulations were performed in which the number of motor units was reduced to 25, the number of fibers innervated by motor units was increased on average by fourfold, and unit fiber density was elevated to 100 fibers/mm². Because capillary density does not appear to change with age in human skeletal muscle (24), microvascular parameters were the same as those used for the normal condition.

The results of simulations to emulate motor unit recruitment and muscle perfusion in aged muscle are shown in Fig. 8. In normal muscle (Fig. 3) the steep rise in perfusion results from widespread scattering of fibers within single motor units and by the broad distribution of motor unit territories, each composed of relatively few fibers, across the muscle cross section. These two forms of fiber dispersion were attenuated in the simulations of aged muscle. Motor unit territories were more densely packed, and because each motor unit supplied more fibers (Fig. 8A), there were fewer recruitment steps involving territory distribution. Acti-

![Fig. 7. Pattern of MVU perfusion in response to recruitment of motor units for simulation in which fiber dispersion was decreased (unit fiber density = 100 fibers/mm² for all units); see Fig. 3 legend for details. A: locations of active fibers and perfused MVUs (squares) at recruitment step that caused ≈90% of MVUs to be perfused. B: plot of number of perfused MVUs as a function of number of active fibers (mean ± SD). Perfusion of 90% of MVUs occurred when ≈18% of total fibers were active (arrow, 64 of 100 units recruited).](http://jap.physiology.org/)

![Fig. 8. Pattern of MVU perfusion in response to recruitment of motor units for simulation of aged muscle in which size of motor unit population was decreased to 25, number of fibers innervated by individual motor units was increased (e.g., units 1, 12, and 25 innervated 229, 943, and 5,030 fibers, respectively), and fiber density was increased to 100 fibers/mm² for all units; see Fig. 3 legend for details. A: locations of active fibers and perfused MVUs (squares) at recruitment step that caused ≈90% of MVUs to be perfused. B: plot of number of perfused MVUs as a function of number of active fibers (mean ± SD). Perfusion of 90% of MVUs occurred when 38% of total fibers were active (arrow). This corresponded with 18–19 of 25 motor units recruited.](http://jap.physiology.org/)
vation of 38.2 ± 13.7% of the aged muscle fiber population was needed to elicit perfusion of 90% of the MVUs (Fig. 8B), representing a 14-fold reduction in perfusion efficacy compared with control (P < 0.05). Furthermore, whereas 100% of the MVUs were perfused when 5% of the muscle fibers were active in normal muscle, only ~25% of the MVUs were perfused at this level of muscle activity in the simulation of aged muscle (Fig. 8B). In other words, these results suggest that the average number of active fibers within a perfused MVU and, hence, the metabolic demand on an MVU could be four times higher in aged than in normal muscle for the same submaximal level of muscle contraction.

Summary. Some of main concepts raised by the simulation results are shown in Fig. 9, which depicts a magnified cross section of a small muscle region. Figure 9A represents “normal” muscle, in which active fibers are widely scattered; Fig. 9B depicts a clustered, abnormal arrangement of active fibers. In both cases, 4 of 48 total fibers in the region are active. Because of the dispersion of active fibers in the normal muscle case, all four MVUs are recruited, and thereby all capillaries are perfused. In Fig. 9B the active fibers are clustered within a single MVU, and consequently only capillaries within that MVU are perfused.

The following points are highlighted by the illustration in Fig. 9. First, in normal muscle during submaximal contraction, many inactive fibers are likely to be surrounded by perfused capillaries. Second, the average number of active fibers within an MVU and, therefore, the metabolic load on an MVU may be markedly higher when motor unit fibers are clustered together. Third, the average number of perfused capillaries that directly supply (i.e., are contiguous with) active fibers is diminished in a clustered arrangement. This point can be derived by counting the total number of perfused capillaries in direct contact with active fibers. For the normal case (Fig. 9A), there are 12 such capillaries; in the clustered arrangement (Fig. 9B), only 8 perfused capillaries contact active fibers. On average then, in this simplified representation, 50% more perfused capillaries would directly supply active fibers in the normal, dispersed case than in the clustered case. Fourth, the number of perfused capillaries not directly in contact but likely close enough to supply some oxygen and remove metabolites would also be substantially greater in the dispersed than in the clustered condition. Taken together, these three latter points suggest that the normal, dispersed arrangement of motor unit fibers may serve to expand the exchange capacity and thereby augment the capability of the muscle to do work.

**DISCUSSION**

In this study, perfusion was defined as distinct from blood flow to distinguish two features of circulatory control. The term perfused was used to indicate whether blood moves through a vessel; blood flow, on the other hand, represents how much blood traverses a vessel (or group of vessels) per unit time. The present investigation focused on how the number of perfused vessels changed as a function of muscle fiber recruitment.

The microvascular unit of skeletal muscle represents the functional element by which capillary perfusion is regulated (7, 14, 56). Activity in only a few of the many muscle fibers enmeshed within an MVU appears sufficient to elicit perfusion of the MVU (4, 21, 43). The smallest increment by which muscle fibers are activated (the motor unit), however, normally involves many fibers scattered over a broad region of the muscle cross section. Consequently, recruitment of a single...
motor unit may well lead to perfusion of several MVUs, distributed both radially (i.e., in muscle cross section, investigated in the present study) and longitudinally [i.e., along muscle fibers (17)] throughout the muscle. Control of perfusion in skeletal muscle, therefore, would seem to occur on a coarser scale than that realized at the level of single MVUs. Furthermore, with perfusion of MVUs in response to motor unit recruitment, it follows that blood will be distributed to many inactive as well as active fibers. In addition, because of the intermingling of fiber types (particularly in human muscle) and because MVUs enclose contiguous sets of fibers, it does not seem feasible that blood could be selectively directed to a particular fiber type. This apparent imprecision of perfusion was highlighted in the present study, and the functional significance of such an arrangement is considered below.

The most striking result of the present work was the effectiveness with which motor unit recruitment induced MVU perfusion. Simulations of normal muscle indicated that all MVUs would be perfused when only ~5% of the muscle fibers were active. This result is similar to that of Honig et al. (27), in which capillary perfusion was shown to be near maximal in dog gracilis at the lowest work rate examined. Blood flow, however, was little changed from the resting level. With further increments in exercise intensity, blood flow increased as a linear function of work rate with only minor variation in capillary perfusion (27). Although some of the large increase in perfusion at the lowest work rate may have been related to the use of maximal electrical stimulation in the study of Honig et al., similar responses have been shown for humans during graded voluntary exercise (1). The primary role of the steplike increment in capillary (i.e., MVU) perfusion to near-maximal levels with light exercise, therefore, would not seem to be directly related to increasing blood flow. Rather, it may 1) act to immediately expand the diffusion capacity in muscle at the onset of exercise, 2) ensure that virtually all fibers receive flow without the need for a complicated feedback system, and 3) minimize the lag in supplying oxygen for motor units recruited subsequently. Regulation of blood flow, on the other hand, is mediated by resistance vessels proximal to the terminal arteriole (23, 27, 54), which serve to govern the volume of blood delivered in accord with metabolic demand.

Several factors were shown to contribute to the steep rise in MVU perfusion relative to muscle fiber activity, including 1) widespread dispersion of motor unit fibers, 2) distribution of motor unit territories throughout muscle, 3) recruitment of motor units in a small-to-large sequence, and 4) partition of muscle into many, smaller motor units rather than fewer, larger units. Some idea of the magnitudes by which these factors may influence perfusion were revealed in simulations that mimicked aspects of certain pathologies, experimental conditions, and interspecies differences. These effects are summarized in Fig. 10, where the mean level of fiber recruitment required to induce perfusion in 90% of MVUs is illustrated for each condition.

Fiber dispersion. A curious though invariable anatomic feature of healthy mammalian muscle is the large volume over which fibers belonging to individual motor units are scattered (2, 8, 11, 59). Whether this dispersion has functional significance or simply represents the outcome of a competitive process by which polyinnervated muscle fibers are trimmed to single connection during development (5) is an issue that has received little attention. In the present study, when fiber dispersion was decreased to simulate the pattern of reinnervation after nerve injury, the intensity of muscle contraction needed to attain perfusion of 90% of the MVUs increased by sevenfold over the control condition (Fig. 10). This finding suggests that one role of fiber clustering may be to promote the thorough perfusion of muscle early during exercise. Conversely, the clustering of fibers characteristic of certain neuromuscular disorders could give rise to relatively impaired perfusion (Fig. 9). This prediction, however, seems contrary to experimental observations of increased blood flow in resting denervated muscle (27, 28, 47). The increased flow (3- to 4-fold) associated with denervation, however, may be explained by loss of vasomotor tone (and thereby physiological control) present in healthy muscle. Therefore, the heterogeneous perfusion of MVUs across the muscle cross section associated with clustering of motor unit fibers might still contribute to perfusion deficiencies in daring
vated muscle, even though the overall resting blood flow is increased.

Distribution of motor unit territories. The pattern of MVU perfusion was markedly altered in simulations where motor unit territories were regionalized rather than distributed uniformly over the muscle cross section. In regionalized muscle, low-threshold motor units were confined to one part of the muscle and high-threshold units to another. Consequently, a spatial gradient of activity occurred within the muscle (Fig. 5) that led to a considerable increase in the overall fiber activity needed to perfuse a given proportion of MVUs in the entire cross section (Fig. 10). Therefore, unrestricted distribution of motor unit territories throughout muscle, combined with wide-ranging dispersion of motor unit fibers, may serve to spread the perfusion stimulus (i.e., active fibers) to many MVUs and thereby facilitate red blood cell flow in capillaries throughout the muscle. On the other hand, the allocation of those motor units most often used to the deep interior of muscle may be an important adaptation for small mammals in which heat conservation is crucial for maintenance of body temperature (10).

Related to the issue of regionalization is the question of whether the metabolic profile per se of muscle fibers can influence capillary recruitment and flow. A number of studies have shown preferential distribution of blood flow during exercise to muscles and muscle compartments composed of a high proportion of oxidative fibers (3, 36, 37, 42). One interpretation of these findings is that blood flow can be conveyed selectively to oxidative fibers. As pointed out above, much mammalian skeletal muscle (particularly in humans) can be characterized as a mosaic of fibers of different types. MVUs envelop bundles of fibers; therefore, a given MVU normally will supply fibers of different types and belonging to many motor units. Because dilation of a terminal arteriole appears to cause perfusion of all capillaries supplied by the arteriole (14, 40), it does not seem possible to selectively direct blood flow to any particular type of fiber in heterogeneous muscle. If fiber types are segregated into different compartments in a muscle, clearly then, flow could be augmented to one type of fiber over another (Fig. 5). However, this would not necessarily indicate a fiber-type-specific influence on perfusion and flow—it may arise simply because one compartment of the muscle is more active than the other.

Recruitment order. Much has been written describing the importance of orderly recruitment of motor units in a small-to-large sequence for the control of muscle force (6, 18, 25). Orderly recruitment may also influence the way in which MVUs are perfused throughout a muscle. When recruitment order was reversed in the model, MVU perfusion was shown to lag significantly behind that observed when recruitment followed the normal small-to-large sequence (Fig. 10). This lag was also related to the two factors described above: 1) the relative fiber dispersion was less in large motor units than in smaller units, and 2) because the earliest activated motor units contained large numbers of fibers, far fewer recruitment steps were required to attain a particular level of contraction. Both factors would reduce the distribution of active fibers throughout the muscle and in this way could limit the number of perfused MVUs.

The putative effect of motor unit recruitment order on MVU perfusion is not only of theoretical interest, but it may also be a consideration for clinical and experimental studies in which electrical stimulation is used to activate muscle. The threshold for activating an axon with extracellular electrodes is inversely related to the diameter of the axon (46). Therefore, when graded stimulation is applied to a peripheral nerve, large-diameter axons supplying large numbers of fatigueable fibers will tend to be recruited before motor axons of small motor units innervating fatigue-resistant fibers (25). Reversal of recruitment order will probably also occur when electrodes are placed directly over the muscle, inasmuch as it has been shown that this type of stimulation activates muscle indirectly via nerve branches rather than by direct activation of muscle fibers (29). The simulations presented here indicate that, compared with physiological recruitment, capillary perfusion throughout a muscle would be depressed when motor units were recruited in a large-to-small sequence. Therefore, muscle activated by electrical stimulation may have somewhat fewer MVUs perfused than muscle activated naturally. Such an effect, when combined with preferential recruitment of fatigueable motor units, may contribute to the rapid fatigue observed in electrically stimulated muscle (31, 46).

Aging. Three adaptations in motor unit organization accompany senescence: 1) the number of motor units declines (9, 12); 2) surviving motor units increase in size (20, 32); and 3) the density of motor unit fibers increases (32, 55). The first two alterations give rise to a fiber population in aged muscle that is divided into fewer, but larger, contractile units. This change has been shown previously to have a deleterious effect on the ability of older individuals to control muscle force (20).

Results from the present study also suggest that these age-related adaptations in motor unit organization could compromise oxygen delivery to muscle fibers. Our simulations indicated that an ~14-fold increase in muscle fiber activity would be required to attain the same level of MVU perfusion as in normal muscle (Fig. 10). This was the largest change of any of the conditions tested and can be attributed to the additive effect of two factors: 1) decreased dispersion of motor unit fibers (analogous to the denervation condition, which caused a 7-fold change in perfusion efficacy), and 2) the increased size (number of fibers) of motor units, leading to fewer recruitment steps involving territory distribution (analogous to the reversed recruitment condition, which also caused a 7-fold change in perfusion). This decrease in MVU perfusion predicted by the model, combined with thickening of capillary basement membrane (51), could impair oxygen extraction and contribute to the diminished work capacity known to affect older individuals (60).
Hypothesis. Two general circulatory adjustments can be made to help satisfy the marked increase in metabolic demand in skeletal muscle during exercise: 1) increase in the overall delivery of blood to muscle and 2) improvement in the efficacy by which oxygen is extracted from the circulation. Within muscle, these two processes seem to be controlled at different sites (23, 26, 27, 52). Proximal resistance vessels appear important for controlling the magnitude and distribution of blood flow during a hyperemic response. In contrast, terminal arterioles regulate the number of capillaries through which blood moves (i.e., the number perfused) and thereby alter the functional surface area for exchange, a key factor underlying oxygen extraction.

The present findings indicate that perfusion cannot be exclusively directed to active muscle fibers. Instead, because of the widespread dispersion of motor unit fibers and the distribution of motor units throughout muscle, we suggest that MVU perfusion will increase throughout large regions of a muscle, even at light workloads requiring only a portion of the total motor unit pool. We hypothesize that the supply of blood to an active fiber requires perfusion of many surrounding (and inactive) fibers. Indeed, such an arrangement may constitute a "feedforward" mechanism, whereby perfusion of MVUs and the availability of oxygen may precede the activation of corresponding muscle fibers. Hence, upon the recruitment of additional motor units, much of the microvasculature would already be perfused, and this could minimize lags in elevating oxidative metabolism. Given the low oxygen extraction of resting skeletal muscle (1), such a rapid increase in capillary surface area would facilitate oxygen consumption (via increased extraction) before substantial increases in total muscle blood flow occur (1, 26, 27).

This work was supported by National Institutes of Health Grants AR-42893 (A. J. Fuglevand) and HL-56786 (S. S. Segal) and was performed during the tenure of an Established Investigator Award from the American Heart Association and Genentech (S. S. Segal). Address for reprint requests: A. J. Fuglevand, Dept. of Physiology, University of Arizona, PO Box 210093, Tucson, AZ 85721-0093 (E-mail: fuglevan@U.Arizona.edu).

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Received 15 May 1996; accepted in final form 9 June 1997.

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