Oxygen consumption and blood flow in resting mammalian skeletal muscle

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DURÁN, WALTER N., AND EUGENE M. RENKIN. Oxygen consumption and blood flow in resting mammalian skeletal muscle. Am. J. Physiol. 226(1): 173-177. 1974. Of a total of 35 isolated blood-perfused, resting dog gracilis muscles, 25 maintained constant oxygen uptake (\(V_02\)) at all blood flows (\(Q\)) above 2 ml/min \(\times 100\) g. In nine muscles, \(V_02\) varied with \(Q\) up to at least 10 ml/min \(\times 100\) g, reaching values up to 60\% above \(V_02\) at \(Q = 3\) ml/min \(\times 100\) g, which was the same as that of constant-\(V_02\) muscles. One muscle converted spontaneously from \(V_02\)-dependent to \(V_02\)-independent relation to flow. Constancy of \(V_02\) was associated with the presence of resistance vessel autoregulation, particularly if transient response criteria for the latter were used. However, abolition of autoregulation by vasodilator drugs did not change the relation of \(V_02\) to \(O_2\) in any preparation. It is concluded that the \(V_02\)-Q relation at flows higher than 3 ml/min \(\times 100\) g does not depend on vascular autoregulation, but that it is a characteristic of muscle cell metabolism. Blood flow autoregulation may depend on a metabolic pattern which itself limits \(O_2\) uptake.

Different workers have reported different relations between oxygen uptake (\(V_02\)) and blood flow (\(Q\)) in mammalian skeletal muscles. Verzar (21) maintained that \(V_02\) of the cat's gastrocnemius muscle in situ varied directly with blood flow. His conclusion was derived indirectly from observations of the effect of decreasing oxygen supply by reducing arterial oxygen saturation, and was supported, not by direct experimental variation of \(Q\) in individual muscles at a constant level of metabolism, but by a close correlation of \(V_02\) with spontaneous flow in a series of different preparations (20). In such a series it is impossible to tell whether high levels of \(Q\) were a consequence of high \(V_02\)'s or vice versa. Nakamura (13) found that \(V_02\) in the cat leg was independent of blood flow except at very low flows, but Rein and Schneider (16) reported that \(V_02\) in muscle and deep tissue of the dog's hindleg was reduced when blood flow was decreased by half. Pappenheimer (14) measured \(V_02\) in isolated perfused legs of dogs as \(Q\) was varied at constant arterial \(O_2\) saturation. \(V_02\) increased with increasing \(Q\) through what is now considered the normal range for resting mammalian skeletal muscle (4-10 ml/min \(\times 100\) g (3, 8, 9)). At flows above 20 ml/min \(\times 100\) g, \(V_02\) tended to level off independent of \(Q\). Stainsby and Otis (18), however, reported for the dog's gastrocnemius-plantaris muscle preparation in situ that \(V_02\) remained constant as \(Q\) was lowered by reducing perfusion pressure until \(Q\) fell to values below 1.2 ml/min \(\times 100\) g. They suggested that maintenance of \(V_02\) at low values of \(Q\) and low oxygen tension (\(P_02\)) depends on the phenomenon of blood flow autoregulation which may have been absent in some earlier preparations. Evaluation of their results relative to those cited previously should not be confused by their observation (like Verzar (20, 21)) that in their series of preparations \(V_02\) was closely correlated with the spontaneous level of blood flow observed at the animal's normal arterial pressure. Recently, Whalen, Buerk, and Thuning (22) reported that for both red (soleus) and white (gracilis) muscles of cats, \(V_02\) increased with perfusion rate over the physiological flow range for each muscle type. Their preparations showed little or no sign of autoregulation. A further complication is the possibility of species differences in the \(V_02\)-Q relation. Honig, Frierson, and Nelson (3) reported that doubling of blood flow by acute decravialization in gracilis muscles of dogs did not increase \(V_02\), whereas the same procedure in rats increased \(V_02\) substantially.

In a series of experiments, we measured \(V_02\) in resting isolated, blood-perfused gracilis muscles of dogs at several different perfusion rates, with constant arterial \(O_2\) saturation. In some of our preparations, \(V_02\) was independent of blood flow over the normal range for resting skeletal muscles; in others it increased with increasing flow. Each pattern was present in a substantial fraction of our preparations, and both were persistent for the several-hour duration of our experiments. In this report, we examine the circumstances associated with each type of relation in our experiments and describe the results of our attempts to influence this relation experimentally.

Materials and Methods

Experiments were performed on 35 dogs, anesthetized with sodium pentobarbital (30 mg/kg, supplemented as required). The gracilis muscle of one leg was completely isolated vascularity, but left in situ, and perfused with blood at constant flow from the contralateral femoral artery with a peristaltic pump (Sigmamotor) adjusted for output independent of pressure. The gracilis is a mixed muscle in dogs, but is composed predominantly of white fibers. Arterial perfusion pressure and venous outflow pressure were measured with strain-gauge transducers (Statham) and recorded on a Grass polygraph. Venous outflow was measured and recorded via a drop counter calibrated with stopwatch and graduated cylinder. The temperature of flowing blood was kept at 37°C by a warm-water jacket.
around the arterial perfusion line. A thermostat-controlled heat lamp kept the surface of the perfused muscle at the same temperature. For calculation of oxygen uptake, simultaneous samples of arterial and venous blood were drawn and analyzed spectrophotometrically for hemoglobin concentration and oxygen saturation according to the method of Gordy and Drabkin (4). Then, using the Fick principle

$$V_O_2 = Q(C_a - C_v)$$

where $C_a$ represents the concentration of oxygen (ml/ml blood) in arterial blood and $C_v$ represents that in blood taken from the gracilis vein. In order to be certain that $V_O_2$ was steady when samples were taken, venous blood $O_2$ saturation was monitored continuously by a cuvette oximeter (Waters-Grass). At the end of each experiment the muscles were weighed and both $V_O_2$ and $Q$ were expressed in ml/min X 100 g. In 4 experiments, the gracilis nerve remained intact throughout; in 4 it was cut between two pressure-flow runs; in 27, it was cut before the first run.

RESULTS

In 25 out of 35 muscles perfused, oxygen consumption remained nearly constant over a wide range of blood flows, falling only at flows below 2 ml/min X 100 g. Nine muscles exhibited a dependence of oxygen consumption on blood flow over the entire range tested. The group of muscles maintaining constant $V_O_2$ is termed hereafter group I. The group of muscles showing increasing $V_O_2$ with flow is termed group II. One muscle initially showing the group II relation shifted to group I during the course of perfusion. It is not included in tabulation of either group and will be discussed separately.

Figure 1 shows graphs of mean $V_O_2$ as a function of $Q$ for the muscles of the two groups. To permit statistical comparison, individual $V_O_2$'s representing four ranges of $Q$ were pooled. Examples of individual $V_O_2$-$Q$ curves are shown in Fig. 2. For group I muscles, $V_O_2 = 0.245 \pm 0.006$ (SEM) at flows of 3.3 ml/min X 100 g and higher. At lower flows $V_O_2$ falls. The critical $Q$ at which $V_O_2$ begins to fall is lower than 3.3 ml/min X 100 g; the process of pooling flows blurs the sharpness of the decline (see Fig. 2). Comparison of $V_O_2$ relative to its constant rate for individual group I muscles indicates that $V_O_2$ remains constant down to flows of 1.0-2.0 ml/min X 100 g. Venous blood $O_2$ saturation at these flows was close to 20% corresponding (according to the nomogram for dog blood of Rahn and Fenn (15)) to an oxygen tension of about 18 mm Hg. The relations of $V_O_2$ to $Q$ and to venous $P_O_2$ are essentially the same as described for individual muscles by Stainsby and Otis (16).

Oxygen uptake of group II muscles rises over the range of $Q$ from 1 to 10 ml/min X 100 g. At mean flows of 1.4 and 3.3 ml/min X 100 g, $V_O_2$ was not significantly different from that of group I, but at higher flows, $V_O_2$ continued to increase, reaching 0.400 \pm 0.012 (SEM) ml/min X 100 g at a mean flow of 10.2 ml/min X 100 g. Examination of individual curves shows a tendency for $V_O_2$ to level off at flows above this range. The behavior of these muscles closely resembles that described by Pappenheimer (14) in the dog hindlimb and by Whalen et al. (22) in cat muscles. $V_O_2$ started to fall at venous $P_O_2$'s of 40 mm Hg or greater. For both groups of muscles, at all rates of perfusion, $V_O_2$...
remained constant for periods of 10–20 min, and we did not attempt to follow it further.

The distinction between the two groups was not related to arterial O2 content or saturation. In group I muscles, mean arterial O2 content was 15.9 ml O2/100 ml blood (range 13.0–20.7), and in group II it was 15.2 ml O2/100 ml blood (12.7–18.0). Nor does it depend on innervation. Both groups I and II contain innervated and acutely denervated muscles. Denervation during the experiment usually altered oxygen consumption slightly but not in a consistent direction, and it did not change the relation between oxygen consumption and blood flow initially observed. An example of the effect of acute denervation on the relation of VO2 and Q, as well as on vascular resistance is included in Fig. 2. (muscle d, innervated and subsequently denervated, D). The experiments were distributed over the course of more than 1 year and included dogs of different breed, sex, size, and age. There was no obvious correlation of results with these factors. Figure 2 shows VO2-Q and pressure-flow curves for some muscles of groups I and II. Muscle L represents one of the three cases in group I in which VO2 was constant at a value slightly lower than 0.200 ml/min X 100 g. As to the VO2-Q pattern, the curves show the same characteristics of groups I and II described in Fig. 1. There is a clear difference in their ability to control VO2 independently of Q. Muscles of group II tended to be more vasodilated (pressure-flow curves shifted to the left). However, much overlapping of the two groups was found. Many muscles of group I showed vascular autonomy of blood flow by the criterion of a rise in resistance to flow with increased perfusion pressure (8, 9), but there was also a substantial number of muscles which did not meet this criterion (e.g., A). Some muscles of group II showed high levels of spontaneous tone (e.g., G), and their pressure-flow curves looked very much like those of autoregulating muscles. A better correlation between constant VO2 and autoregulation is found if the transient response is used as the criterion (19). All group I muscles showed transient responses, whereas group II muscles did not (Fig. 3).

The single muscle which exhibited characteristics of both groups I and II during the course of an experiment started out with a typical group II preparation with strong dependence of VO2 on Q/VO2 = 0.21 ml/min X 100 g at Q = 5.2 ml/min X 100 g; 0.30 at 10.6). It met neither steady nor transient criteria of flow autoregulation. After 1.5 h of perfusion, the pressure-flow curve started to shift to the right, and pressure transients indicative of autoregulation were observed when flow was changed. The autoregulation improved with time, and by 3.5 h, both criteria were met. From the first appearance of autoregulatory transients, VO2 at flows above 5.2 ml/min X 100 g remained constant at 0.21 ml/min X 100 g.

To test the hypothesis that the distinction between constant VO2 muscles (group I) and variable VO2 muscles (group II) depends on autoregulation of blood flow, we infused vasodilating agents intra-arterially to abolish autoregulation after completing the control VO2-Q run in 18 preparations. In 4 experiments we used chloral hydrate (10 mg/ml blood); in 11 we used papaverine (0.25 mg/ml blood); in 3 we used bradykinin (2.5 mg/ml blood). By all criteria blood flow autoregulation was lost during infusion of vasodilator. All but one of these preparations fell initially into group I, and in none of these was the relation between oxygen consumption and Q changed to that of group II by near maximal vasodilatation (Fig. 4A). In the single experiment in which the initial pattern was group II, there was also no change with vasodilatation. Infusions of short duration (2–3 min) did not change the rate of VO2. However, prolonged vasodilatation (5 min–1 h) decreased VO2 at all flows but did not alter the relation of VO2 to Q initially observed (Fig. 4B).

DISCUSSION

It has been suggested that the ability of a muscle to maintain constant VO2 independent of Q might be associated with the way in which the experimental preparation is handled, i.e., how well its normal physiological properties are preserved (13, 18). In our series, 8 of the 9 muscles showing VO2 dependence on Q belonged to the first 16 experiments, falling more or less randomly within this group. Of the subsequent 19 experiments, only 2 showed VO2 dependence after 2–3 h of perfusion. Though it is possible that our technique improved with experience, we have not been able to identify any specific aspects of the technique which might be responsible for the distinction between the two groups of muscles with respect to VO2 and blood flow. We do not wish to claim that one or the other relation is normal for the dog gracilis, but to point out that both are possible within the usual conditions of laboratory experimentation.
Muscles of group I were able to maintain \( \dot{V}O_2 \) constant at all values of \( Q \) above 2 ml/min × 100 g. These muscles also showed autoregulation of blood flow (Fig. 3) as manifested in transient vascular responses to step changes in perfusion rate (19). In group II muscles, \( \dot{V}O_2 \) was the same as for group I at \( Q = 3 \) ml/min × 100 g and increased with blood flow up to flows of at least 10 ml/min × 100 g. At flows between 2 and 3 ml/min × 100 g, according to the shapes of the curves in individual experiments, \( \dot{V}O_2 \) must have been higher in group I muscles than in group II. At \( Q = 1 \) ml/min × 100 g, however, \( \dot{V}O_2 \)'s for the two groups did not differ significantly. Group II muscles did not show transient resistance changes when blood flow was suddenly increased or decreased. If autoregulatory properties of the muscles were evaluated by the criteria of steady-state pressure-flow curves (8, 9), better autoregulation was shown by most group I muscles, but there was considerable overlap of the two groups (Fig. 2). It seems possible that our experimental condition of constant-flow perfusion impairs the steady-state response of the arterioles to disten-
sions, since their constriction must further increase the load which they sustain.

Both steady and transient criteria for autoregulation are based on the activity of arterioles (resistance vessels). Oxygen supply to the tissue at constant blood flow depends on the action of terminal arterioles and precapillary sphincters, which contribute little to resistance, but determine the number of open capillaries and the distance between them (10, 11). Muscles with effective autoregulatory responses of the terminal vascular bed will be able to open more capillaries as blood flow goes down and to distribute available blood flow uniformly through the capillary network. Consequently, they will be able to extract more oxygen from the blood at low flows or low oxygen tensions (17, 18).

Constant oxygen uptake by group I muscles may be due to a) limitation of oxygen supply by closure of capillaries or terminal arterioles as blood flow increases or b) limitation of the supply of ADP or other substrate to the respiratory chain. If a is the controlling mechanism, \( \dot{V}O_2 \) in a substantial part of the muscle must be less than the saturation tension of cytochrome \( a_3 \). Direct measurements of muscle cell \( \dot{V}O_2 \) (23) and of tissue \( \dot{V}O_2 \) (24) gave values in the range of 0–45 mm Hg with the highest frequency in the range of 0–5 mm Hg, which makes this a distinct possibility. Similar results were presented for myoglobin oxygen tension (2). Under these conditions, the only change required to produce group II characteristics is loss of the precapillary vasoconstrictor activity which maintains low local \( \dot{V}O_2 \). However, blood flow and \( \dot{V}O_2 \) were not measured in these experiments, and the relation between these parameters is not known. More recently, Whalen et al. (22) have reported higher \( \dot{V}O_2 \)'s in autoperfused cat gracilis muscles which clearly showed group II behavior. However, even at the lowest flows, most measurements of tissue \( \dot{V}O_2 \) were above 10 mm Hg. Furthermore, in our experiments, vasodilator responses to minimal or near minimal resistance levels did not alter the \( \dot{V}O_2 \)-Q relation in group I muscles. It is possible that the precapillary vessels, which determine the number of open capillaries, are less sensitive to the vasodilator agents we used than are those controlling vascular resistance. However, it seems more likely that something other than tissue \( \dot{V}O_2 \) limits oxygen uptake (6, 7, 22).

If \( \dot{V}O_2 \) uptake in group I muscles is limited by the supply of ADP to the respiratory chain (State 4 respiration (1)), increased \( \dot{V}O_2 \) of group II muscles at blood flows higher than 3 ml/min × 100 g could be attributed to 1) increased utilization of ATP, 2) uncoupling of oxidative phosphorylation (thereby removing ADP limitation), and 3) activation of other \( O_2 \) consuming reactions (not requiring ATP or producing ADP). Whatever the mechanism, it must interact with blood flow in such a way as to produce group II characteristics.

It is hard to imagine how mechanisms 1 and 2 could meet this requirement, at least under the conditions of our experiments. Flow-dependent oxygen uptake by mechanism 3 is possible if the affinity of the reactions for molecular oxygen is sufficiently low. Possible reactions include \( O_2 \) uptake by flavoproteins (auto-oxidation) and lipid peroxidation (6, 12).
According to this view, the distinction between group I and group II muscles is primarily metabolic. It is possible that vascular autoregulation is a consequence of a particular pattern of metabolism rather than a factor controlling metabolism. What it is that alters the metabolic pattern in group II muscles remains unknown.

REFERENCES


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