The biphasic force-velocity relationship in whole rat skeletal muscle in situ

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Devrome AN, MacIntosh BR. The biphasic force-velocity relationship in whole rat skeletal muscle in situ. J Appl Physiol 102: 2294–2300, 2007.—Edman has reported that the force-velocity relationship (FVR) departs from Hill’s classic hyperbola near 0.80 of measured isometric force (J Physiol 404: 301–321, 1988). The purpose of this study was to investigate the biphasic nature of the FVR in the rested state and after some recovery from fatigue in the rat medial gastrocnemius muscle in situ. Force-velocity characteristics were determined before and during recovery from fatigue induced by intermittent stimulation at 170 Hz for 100 ms each second for 6 min. Force-velocity data were obtained for isotonic contractions with 100 ms of 200-Hz stimulation, including several measurements with loads above 0.80 of measured isometric force. The force-velocity data obtained in this study were fit well by a double-hyperbolic equation. A departure from Hill’s classic hyperbola was found at 0.88 ± 0.01 of measured isometric force, which is higher than the ~0.80 reported by Edman et al. for isolated frog fibers. After 45 min of recovery, maximum shortening velocity was 86 ± 2% of prefatigue, but neither curvature nor predicted isometric force was significantly different from prefatigue. The location of the departure from Hill’s classic hyperbola was not different after this recovery from the fatiguing contractions. Including an isometric point in the data set will not yield the same values for maximal velocity and the degree of curvature as would be obtained using the double hyperbola approach. Data up to 0.88 of measured isometric force can be used to fit data to the Hill equation.

fatigue; contractile properties; double hyperbola; maximal velocity

THE RELATIONSHIP BETWEEN MUSCLE shortening velocity and the load that is imposed on muscle is known as the force-velocity relationship (FVR), where velocity of shortening decreases for progressively higher loads. In 1938, A. V. Hill reported the classic equation that is most often used by researchers to characterize the FVR in skeletal muscle (17): (P + a)(V + b) = (P0* + a)b; where P is force at any velocity of shortening, V; P0* is predicted isometric force, and a and b are constants. The maximal velocity of shortening (Vmax) can be estimated by solving the equation for P = 0, once the constants are known. The ratio a/P0*, describes the magnitude of curvature of the relationship, with a higher value indicating a lesser curvature.

Although Hill’s equation is used most frequently when force-velocity data are fit to an equation, several studies have shown that the force-velocity properties of muscle do not fit the simple rectangular hyperbola over the full range of values for force and velocity (1, 12–15). Rather, there is a departure of measured values from the predicted hyperbola at high forces where velocity of shortening is low. According to Edman et al. (14), this departure from the traditional rectangular hyperbola occurs at ~0.80 of measured isometric force for isolated frog muscle fibers, and data below this force fit the Hill equation to a high degree. The force at which this departure occurs will be referred to herein as the breakpoint.

Edman et al. (12–15) have further characterized this departure from the classic single hyperbolic relationship. In several studies, Edman has described two distinct curvatures in the FVR, one at low to moderate forces, and the other in the high-force region, indicating that the relationship is biphasic, rather than a single rectangular hyperbola (12–15). Edman (15) developed an extended version of Hill’s (17) hyperbolic equation, which proved to be a better fit for this biphasic data:

\[ V = \frac{(P_0^* - P)b}{P + a} \left(1 - \frac{1}{1 + e^{-k_1(P - k_2P_0)}}\right) \]  

where abbreviations are as defined above, and k1 and k2 are constants. In this version of the equation, P0 is the measured isometric force.

Other than one abstract report (1), the biphasic FVR has only been reported in amphibian single muscle fibers at cool temperatures and in mouse single fibers at slightly higher than room temperature (12, 13). Those studying the FVR generally assume that the breakpoint in the FVR occurs at ~0.80 of measured isometric force, and they use values up to this force to fit to the Hill equation (6, 21, 28). Whether or not this is appropriate for whole mammalian muscles at 37°C has not been determined. This would be important to know for accurate curve fitting of the FVR.

Another area of great interest to muscle researchers is how repetitive fatiguing contractions and subsequent partial recovery alter the force-velocity properties of muscle. It is known that fatigue measured with low frequencies of stimulation persists (19, 23) while the muscle is capable of generating maximal force with high-frequency stimulation, but the impact of this long-lasting fatigue on the FVR is unknown. Although there is some controversial research regarding the precise effects of fatigue on the FVR, many researchers suggest a decrease in Po and Vmax, and an increase in a/P0 (2, 3, 7, 16). Edman et al. (14) have shown that in single fibers, fatigue does not alter the breakpoint but this has not been assessed in whole muscle, and it has not been assessed at a time when low-frequency fatigue is present.

The purpose of this study was to systematically investigate the biphasic nature of the FVR in rested whole muscle and muscle that had been permitted some recovery after a series of repetitive isometric contractions at physiological temperature. There were four specific aims: 1) determine if the biphasic curve is present under these conditions; 2) if it is present, find the relative force at which the breakpoint occurs; 3) determine if the position of the breakpoint changes in fatigued mammalian muscle; and 4) evaluate curve-fitting procedures for force-velocity data obtained with whole mammalian muscle at 37°C.

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METHODS

Female Sprague-Dawley rats weighing 196–240 g were used in this study. A University of Calgary Animal Care Committee approved all procedures. Rats were given standard rat chow and water ad libitum. They were kept in an environmentally controlled room (20–22°C). The rats were anesthetized with an intramuscular ketamine-xylazine injection (100 mg/ml each, mixed 85:15, and given at a dose of 1 ml/kg). Subsequent injections were given, if needed, to maintain the rats under a surgical level of anesthesia. On completion of the experiment, animals were euthanized by an overdose of anesthetic.

Muscle preparation. The surgical procedures and experimental apparatus used in this study have been described previously (22). Briefly, the left medial gastrocnemius muscle was surgically isolated from all other muscles and connective tissue, leaving only the innervation, blood supply, and muscle origin intact. The sciatic nerve was cut proximally, and distal branches were cut except the branch for the medial gastrocnemius muscle. The remaining section of nerve was draped over two stainless steel stimulating electrodes. A Grass model S88 stimulator (Grass Instruments, Quincy, MA) was used to stimulate the nerve with square pulses (50 μs). Maximal voltage (the minimal voltage that activates all motor units) was determined (0.5–1.0 V) and all subsequent stimulation was supramaximal at 3 V. To immobilize the hindlimb, a steel dissecting probe was placed longitudinally into the medulla of the severed tibia, and a steel drill bit was put perpendicularly into the middle of the shaft of the femur. The dissecting probe and drill bit were then fixed to the base of the myograph. A large pocket, made from the skin surrounding the muscle, was used to keep the muscle immersed in paraffin oil for the duration of the experiment. Rectal and muscle (paraffin) temperatures were maintained within 1° of 37°C.

Once the rat was immobilized in the apparatus, the tendon was connected to the titanium lever with silk suture (no. 1). The lever had a strain gauge (model ESU-060-350, Entran) bonded to the side to permit measurement of the force of contraction. The opposite end of the lever was connected to a piston that inserted into a cylinder that was connected by reinforced tubing (1.25 cm ID) to a tank in which a pneumatic resistance. Optimal muscle length, defined as the length at which the muscle gave the largest amplitude contraction, was set using double-pulse stimulation (27). A series of double-pulse isometric contractions were obtained at ~20-s intervals, changing muscle length between contractions. This length adjustment was permitted by a screw that limited travel of the lever, on the side opposite from the connection of the muscle. Following the initial setting of optimal length, a conditioning isometric tetanic contraction (200 Hz, 500 ms) was elicited to ensure that the connections between the muscle and lever were tight and secure. The muscle was then left to recover for 5 min, to allow dissipation of any potentiating effects (24). After the 5 min of recovery, optimal length was reset, using double-pulse stimulation.

Experimental protocol. To investigate the presence of a breakpoint in the FVR, contractions were obtained by stimulating the sciatic nerve at 200 Hz for 100 ms. A total of 17–20 contractions were obtained at 1-min intervals, with loads ranging from nearly unloaded to maximum isometric force. This interval was used to minimize the possibility of fatigue. An effort was made to obtain several contractions above 0.80 of maximum isometric force to permit evaluation of a departure from the single hyperbolic FVR. The load on the muscle was set by adding or releasing air from the pressure tank, which provided a constant resistance to shortening (22). A length sensor (LVDT model GSA 750-200, Macro Sensors, Pennsauken, NJ) detected the change in muscle length during isometric contractions. Sample contractions are presented in Fig. 1 to illustrate the constant force during shortening and the duration of relatively constant shortening. Velocity of shortening for any contraction was determined by finite difference after determination of the greatest absolute length change in 7.5 ms (30 consecutive data points).

To cause fatigue, muscles were made to contract isometrically using a stimulation frequency of 170 Hz for 100 ms at 1/s for 6 min. Twitch and tetanic (170 Hz) contractions were obtained at 10 s, 20 s, 30 s, 1, 2, 3, 4, 5, 15, 25, 35, and 45 min during the period after the fatigue protocol to monitor the rate and extent of recovery. Force-frequency and force-velocity measurements were obtained after this 45 min of recovery. This period of time allowed the rapid recovery to be complete so a number of contractions could be obtained without noticeable change in contractile capability.

Determining the force at departure from Hill’s hyperbolic equation. It was clear that there was a departure from the hyperbolic shape of Hill’s equation at high forces. To determine the precise location of the breakpoint, we plotted the log of V as a function of measured force as done by Edman (15). It was clear that there were two distinct linear relationships: one in the high-force region, and one in the low- and moderate-force region. Regression equations were determined.
Nonlinear regression (least squares) was used to obtain estimates of \( k \). Superimposed on the Edman double hyperbola from one experiment fit to Edman’s double hyperbolic equation are (12, 15). There are three additional constants in Edman’s equation:

\[ \frac{a}{b} = \frac{k_1}{k_2} \]

approach, including only values up to \( 0.80 \) of maximum force were fit to the Hill equation. Then the percent difference between measured and predicted force values was calculated for each contraction. The breakpoint for any FVR was defined as the lowest force in the high-force range at which there was a >3% difference between measured and predicted values. The location of the breakpoint was similar using both methods, but the second one showed greater variability. This is probably because estimates using this technique can only identify a breakpoint at a force represented by a contraction, whereas the semilog approach can find a value at an appropriate force by interpolation. For this reason, and because Edman (15) used the semilog approach described above, we will present values only from the semilog method.

Curve fitting. To investigate curve fitting of force-velocity data, three different approaches were initially used: 1) data were fit to the Hill equation (nonlinear least squares method) using the traditional approach, including only values up to ~0.80 of observed isometric force; 2) data were fit to the Hill equation using values that included measured isometric force; and 3) data were fit to Edman’s double-hyperbolic equation (15). In retrospect, considering that a breakpoint was found at 0.88, we included a fit to 0.88 of observed isometric force. The constants \( a, b \), and \( P_{o*} \) were the same for the Edman equation and the Hill equation fit to 0.80 of measured isometric force (12, 15). There are three additional constants in Edman’s equation: \( k_1 \), \( k_2 \), and \( P_o \). Measured isometric force was substituted for \( P_o \), and nonlinear regression (least squares) was used to obtain estimates of \( k_1 \) and \( k_2 \).

Statistics. Comparison of two means before and after the fatiguing contractions was by paired t-test (Excel, Microsoft, Redmond, WA). Mean values are presented with standard errors. Linear regression was done in Excel, and nonlinear regression by least squares was done with Sigma Plot (Jandel Scientific, San Rafael, CA).

RESULTS

The biphasic FVR and the breakpoint. Force-velocity data from one experiment fit to Edman’s double hyperbola are shown in Fig. 3. Superimposed on the Edman double hyperbola is the result for the classical means of fitting data through only 0.80 of measured isometric force. It is clear from this diagram that the FVR is biphasic in whole skeletal muscles under physiological conditions. This general pattern was always observed. Values for \( k_1 \) and \( k_2 \) obtained by fitting the data to Edman’s double-hyperbolic equation are presented in Table 1. There was no significant difference in these constants between prefatigue and postfatigue (\( P > 0.1 \)).

The intersection of the regression lines from the high- and low-moderate force regions of the semilog plots was identified by the method described above, and this was considered to be the location of the breakpoint (see Fig. 2). When this procedure was followed for all experiments, the breakpoint in the force-velocity relationship was found at 0.88 ± 0.01 of measured isometric force. This can be seen in the example shown in Fig. 3, where the breakpoint is clearly close to 0.88 of measured isometric force. This value is higher than the ~0.80 of measured isometric force described by Edman and colleagues (13–15) for frog muscle and mouse fibers (12).

Fatigue. After 6 min of repetitive isometric contractions (see Fig. 4A), active force was reduced to 32 ± 2% of original isometric force. During the recovery period, there was a rapid increase in isometric force over the first 5 min to 85 ± 1%, followed by a slower recovery (see Fig. 4B). After 45 min of recovery the twitch amplitude was 71 ± 3% of the original, and fatigue was still evident at all test frequencies except 200 Hz (see Fig. 5). After 45 min of recovery, \( V_{max} \) was 86 ± 2% of the prefatigue values. Table 1 presents a summary of the Hill force-velocity parameters comparing the prefatigue values with those obtained after 45 min of recovery. The mean breakpoint after repetitive fatiguing contractions and subsequent recovery was located at 0.88 ± 0.01 of measured isometric force. This was not significantly different (\( P = \))

<table>
<thead>
<tr>
<th>Force (N)</th>
<th>Measured force-velocity data</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.2</td>
<td>50</td>
</tr>
<tr>
<td>0.4</td>
<td>100</td>
</tr>
<tr>
<td>0.6</td>
<td>150</td>
</tr>
<tr>
<td>0.8</td>
<td>200</td>
</tr>
<tr>
<td>1.0</td>
<td>250</td>
</tr>
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</table>

Edman’s double hyperbola

Classic Hill hyperbola

Fig. 3. Demonstration of the biphasic curve. Data are from one experiment, fit to the Hill equation (17), as well as Edman’s double-hyperbolic equation (15), superimposed with actual measured values. This experiment clearly demonstrates how measured values at low velocities depart from those predicted by the Hill equation when values up to 0.8 are used to fit to the Hill equation.
0.924) from the breakpoint before the fatiguing contractions. There was no difference between the absolute velocities ($P = 0.87$) at which the breakpoint occurred before the repetitive contractions, compared with after 45 min of recovery from fatiguing contractions (22.1 ± 1.0 and 20.9 ± 1.5 mm/s, respectively). These values represent 7.3 ± 0.6 and 8.3 ± 0.6% of $V_{\text{max}}$. These relative values are significantly different ($P = 0.05$).

**Curve fitting.** The final goal of this study was to evaluate the impact of different approaches for curve-fitting force-velocity data. When the force-velocity data obtained before the fatiguing contractions included data up to 0.80 of measured isometric force as well as measured isometric force, mean Hill parameters were significantly different from those obtained when isometric force was not included (see Table 1). The $a/P_o*$ ratio was significantly higher, whereas $P_o*$ and $V_{\text{max}}$ were lower, when measured isometric force was included in the data set. It is clear that the parameters are dramatically different with these two approaches. Table 1 also contains the constants for fitting the data up to 0.88 of maximal isometric force to the Hill equation. These values are significantly different from both the values fit to 0.8 and the values fit with isometric force included, but are clearly closer to the values fit to 0.8 than to the values that were obtained with isometric force included.

**DISCUSSION**

This study clearly shows a biphasic shape to the FVR in whole mammalian skeletal muscle in situ. This is similar to the findings of Edman and colleagues (13–15) for frog muscle at

Table 1. Hill parameters for force-velocity relationship

<table>
<thead>
<tr>
<th>Condition</th>
<th>$P_o*$, N</th>
<th>$V_{\text{max}}$, mm/s</th>
<th>$a/P_o*$</th>
<th>$k_1$</th>
<th>$k_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefatigue 0.8</td>
<td>15.5±0.7</td>
<td>283±9</td>
<td>0.31±0.05</td>
<td>1.27±0.1</td>
<td>0.92±0.01</td>
</tr>
<tr>
<td>Prefatigue 0.88</td>
<td>14.7±0.6</td>
<td>277±9</td>
<td>0.34±0.03</td>
<td>1.70±0.4</td>
<td>0.94±0.01</td>
</tr>
<tr>
<td>Postfatigue 0.8</td>
<td>14.5±0.5</td>
<td>244±6*</td>
<td>0.34±0.03</td>
<td>1.67±0.4</td>
<td>0.94±0.01</td>
</tr>
<tr>
<td>Postfatigue 0.88</td>
<td>13.9±0.4</td>
<td>239±6*</td>
<td>0.40±0.04</td>
<td>1.70±0.4</td>
<td>0.94±0.01</td>
</tr>
<tr>
<td>Prefatigue</td>
<td>12.7±0.4*</td>
<td>258±6*</td>
<td>0.64±0.05*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 7$. Force-velocity data up to 0.80 or 0.88 of measured isometric force were fit to the Hill equation, with the exception of the final row, which gives the constants obtained when isometric force was included in the curve-fitting procedure. Prefatigue values were found for the Hill parameters when isometric force was included in the data to be fit to the equation. $P_o*$, maximum isometric force predicted from curve fitting; $V_{\text{max}}$, maximum velocity of shortening; $a/P_o*$, curvature of the force-velocity relation, where $a$ is the constant; $k_1$ and $k_2$, constants. *Mean Hill values significantly different from prefatigue conditions, $P < 0.001$. 

Fig. 4. Time course of fatigue (A) and recovery of force (B). Sample contractions were obtained during the 1 per s repetitive contractions and at specific times after the 6 min of intermittent tetanic contractions. Active force for 170-Hz stimulation decreased to 32% at the end of the 6 min, and it recovered nearly to the prefatigue level (12.3 N) during the 45 min of recovery.

Fig. 5. Force-frequency relationship. Control measurements (●) obtained at specific frequencies before the repetitive stimulation and after 45 min of recovery are shown. All values obtained after 45 min of recovery (□) were significantly less than prefatigue values ($P < 0.05$) with the exception of the contraction at 200 Hz. The relative depression of force at frequencies <100 Hz was greater than the relative depression at 100 or 200 Hz, indicating low-frequency fatigue.
1.5–3°C and for mouse single fibers at 21°C (12). In one aspect, the results of the present study were different from previous work. The location of the breakpoint was at a different force relative to maximum isometric force. The position of the breakpoint relative to maximal isometric force was not different when measured after a recovery period following the repetitive fatiguing contractions. The position of the breakpoint did change when expressed relative to the maximal velocity.

It is of interest to compare the present results with previously published data. The most logical comparison is with the rat medial gastrocnemius muscle in situ, as presented by de Haan and colleagues (9). These authors have published force-velocity data for rats of similar size (slightly larger males with a mean mass = 270 g), and they report similar values for V_max (270–300 mm/s), maximal tetanic force (11.1 N), and a/P_o* (0.5, presumably fit with isometric force included). Of particular interest, these authors present the impact of muscle length on the force-velocity properties. They present curves for measurements at various lengths, and their data obtained at optimal length and at 2 mm less than optimal length are virtually superimposed. In our experiments, measurements were obtained within 2 mm of optimal length. These values for the parameters of the FVR are also consistent with those published by Rijkelijkhuizen et al. (28) and by Ranatunga (26), who found values for V_max that persists in the fatigued muscles may be due to fatigue being predominantly present in the fastest fibers.

It is worth considering whether or not the fiber-type differences might contribute to the double hyperbola. Edman (15) has demonstrated that the double hyperbola is present in mouse fast-twitch fibers, without identifying whether these were type IIa, b, or x, so there is no basis for direct resolution of this question. Considering that the force per cross section is similar for these fast-twitch fibers, there is no justification for thinking that fiber type would influence this.

There are many graphs of the force-velocity relationship for whole muscle that are presented in the literature, but most do not show values plotted in the range of force where the departure from the hyperbola would be seen. However, in some cases, there is a clear indication of the break (2, 21, 28). A departure from the hyperbolic relationship has also been reported for isolated myosin (25) as well as for cardiac trabeculae (10).

Fatigue. There was no alteration in the location of the breakpoint after partial recovery from repetitive isometric fatiguing contractions. By this time, metabolic recovery should be complete (30), but the force for a given stimulation is still attenuated, particularly with low frequencies of stimulation. There is considerable evidence suggesting that fatigue, particularly low-frequency fatigue, is due to reduced Ca^{2+} release (11, 31, 32). Our findings are therefore consistent with Edman...
et al.’s (13) findings in experiments using dantrolene, a drug that reduces \( \mathrm{Ca}^{2+} \) release. These observations in fatigued muscle could be related to the fatigue-related decrease in \( \mathrm{Ca}^{2+} \) release. The reduced activation from the dantrolene mimics fatigue, which results in fewer bound cross bridges. In fatigue, as with dantrolene, the location of the breakpoint did not change. However, it should be pointed out that the magnitude of reduction in \( P_o \) in our experiments was not significant. Considering that the maximal velocity was still decreased 45 min after the fatiguing contractions, it could be that cross-bridge kinetics were altered. However, it has been shown that a higher level of activation is needed to achieve maximal velocity of shortening than is needed for maximal isometric force in this muscle preparation (8).

Curve fitting. Because of the biphasic nature of the FVR, the method of curve fitting will influence the values obtained for the parameters of the equations used to fit the force-velocity data. In recent studies examining the force-velocity properties of muscle, force-velocity data were fit to the Hill equation using data up to 0.80 of measured isometric force (6, 21, 28). This choice is a consequence of Edman’s work (15), which showed a breakpoint from Hill’s classical equation at ~0.80 of measured isometric force. Our results show that the breakpoint is located at 0.88 of measured isometric force in whole mammalian skeletal muscle at 37°C. This observation would indicate that values up to 0.88 could be used when selecting data to fit to Hill’s equation. The advantage of including values up to 0.88 of maximal isometric force is that with very few values in total, this might permit a more complete curve with otherwise limited data.

The present study examined four different approaches to fit force-velocity data: 1) we fit force-velocity data to the Hill equation using up to 0.80 of measured isometric force; 2) we fit force-velocity data to the Hill equation using values in method 1) but included measured isometric force; 3) we fit force-velocity data to Edman’s double-hyperbolic equation (15), including several points in the high force range and isometric force; and finally, 4) we fit data up to 0.88 of measured isometric force to the Hill equation.

The present study showed that if data are fit according to method 1, then both \( P_o^* \) and \( V_{\text{max}} \) are much higher than when isometric force is included (method 2). Extrapolation of the line obtained by method 1 overestimates forces at low velocities. This results in a value for \( P_o^* \) that is much greater than measured isometric force. For a given force above the breakpoint, velocities predicted by Edman’s equation (15) are matched better to measured values than the data predicted by the Hill equation obtained with data fit through 0.80 of measured isometric force (see Fig. 3). The \( a/P_o^* \) ratio is also affected by the curve-fitting procedure. If data are only fit to 0.80 or 0.88 of measured isometric force using the Hill equation, then \( a/P_o^* \) is substantially lower than when isometric force is included. When the data including isometric force were fit to the Hill equation, \( V_{\text{max}} \) was frequently close to, and occasionally less than, the fastest measured shortening velocity. In this circumstance, it is likely that true maximal velocity was underestimated. Clearly, the data used to fit the Hill equation affect the values of the force-velocity parameters.

Physiological considerations. Realizing that the FVR is biphasic in nature is necessary in understanding muscle function. Edman (15) has suggested that this is an important property of skeletal muscle that imparts stability for length control at high forces. Considering that this property has now been demonstrated in situ, it is reasonable to assume that it occurs in vivo. Studies performed in humans have not shown evidence for this phenomenon, but this is likely because multiple measurements at forces >0.88 of measured isometric force are rarely taken. It is also possible that some central mechanisms such as motivation, fatigue, and pain sensations are involved, with high-force voluntary contractions in human experiments. Ultimately, it is apparent that skeletal muscle in vivo, in situ, and in vitro may share fundamental biphasic force-velocity properties that reflect similarities at the molecular level.

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GRANTS

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