

Hill's equation of muscle performance and its hidden insight on molecular mechanisms

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Muscles shorten faster against light loads than they do against heavy loads. The hyperbolic equation first used by A.V. Hill over seven decades ago to illustrate the relationship between shortening velocity and load is still the predominant method used to characterize muscle performance, even though it has been regarded as purely empirical and lacking precision in predicting velocities at high and low loads. Popularity of the Hill equation has been sustained perhaps because of historical reasons, but its simplicity is certainly attractive. The descriptive nature of the equation does not diminish its role as a useful tool in our quest to understand animal locomotion and optimal design of muscle-powered devices like bicycles. In this Review, an analysis is presented to illustrate the connection between the historic Hill equation and the kinetics of myosin cross-bridge cycle based on the latest findings on myosin motor interaction with actin filaments within the structural confines of a sarcomere. In light of the new data and perspective, some previous studies of force–velocity relations of muscle are revisited to further our understanding of muscle mechanics and the underlying biochemical events, specifically how extracellular and intracellular environment, protein isoform expression, and posttranslational modification of contractile and regulatory proteins change the interaction between myosin and actin that in turn alter muscle force, shortening velocity, and the relationship between them.

Muscle cells convert chemical energy into mechanical work. Mechanical performance of a muscle is often assessed by how fast the muscle shortens against a range of external loads. Such an assessment is best summarized by a plot of muscle force versus shortening velocity that can be described mathematically by a hyperbolic equation of the form:

$$(F + a)(V + b) = c, \quad (1)$$

where F and V are force and velocity, and a , b , and c are constants. The equation was introduced by A.V. Hill (1938), who also suggested that the mechanics of muscle contraction is closely linked to the muscle's energy metabolism, because in his experiments the same hyperbolic force–velocity relationship could be derived from heat measurements, and the constant a was found to match closely to an empirically derived thermal constant of shortening heat, α (Hill, 1938). However, α was later found not to be a constant but dependent on shortening velocity and load (Hill, 1964). It appears, therefore, that the force–velocity behavior of a muscle is not an unfiltered manifestation of energetic events occurring inside the muscle, as Hill originally thought.

Today, the canonical explanation for the characteristic force–velocity behavior is based on the kinetics of cyclic interaction between myosin cross-bridges and actin filaments within the contractile units of a muscle,

first proposed by A.F. Huxley (1957), followed by improved models capable of explaining complex behavior of the muscle in transient and steady states (Huxley and Simmons, 1971; Eisenberg et al., 1980; Pate and Cooke, 1989; Slawnych et al., 1994; Piazzesi and Lombardi, 1995; Edman et al., 1997; Smith et al., 2008; Månsson, 2010; Barclay et al., 2010). Enormous amounts of data from muscle experiments accumulated over the past decades have served to refine the models. In particular, the data provided by Piazzesi et al. (2007) has led to a quantum increase in our understanding of the molecular basis of force–velocity relations in muscle contraction. Interestingly, the data also provide justification for the use of a hyperbolic equation not as a mere empirical description but as a meaningful explanation for force–velocity behavior based on cross-bridge kinetics, and may serve to revive the Hill equation after decades of under-appreciation as a result of incomplete comprehension.

Force–velocity relationship

The relationship between muscle force and shortening velocity can be visualized by plotting the velocity of a shortening muscle as a function of the load (or force) pulling on the muscle. A force–velocity curve is usually obtained from curve-fitting of force–velocity data. The data in turn are usually obtained with a protocol involving

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Abbreviation used in this paper: EDL, extensor digitorum longus.

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isotonic quick releases. (A complete description of force–velocity relations in muscle should include negative loads and positive loads greater than the maximum isometric force [F_{\max}]. In this Review, only the force–velocity relations within the force range of 0 to F_{\max} , where the Hill hyperbola is most relevant, are considered.) Before an isotonic quick release, a muscle is activated at a fixed length. The contraction is therefore isometric. During an isotonic quick release, the muscle is suddenly released from its isometric force to a lower and constant force (i.e., isotonic load). In response to the sudden change in load, the muscle shortens in a characteristic fashion (Civan and Podolsky, 1966) as illustrated in Fig. 1. After an initial period of transient changes in velocity, muscle shortening settles to a steady velocity. The slope of the steady phase (phase 4) of length change (Fig. 1) is taken as the shortening velocity of the muscle corresponding to the isotonic load. Repeated velocity measurements from quick releases with different isotonic loads yield force–velocity data points; the hyperbolic Hill equation can then be used to fit the data and, often by extrapolation, to obtain the maximum velocity of shortening (V_{\max}) and maximum force (F_{\max}). An example is shown in Fig. 2 for skeletal muscle; similar hyperbolic relations are also seen in cardiac and smooth muscles. Although the velocity transient (Fig. 1) is thought to originate from myosin cross-bridges, it should be pointed out that filament compliance could alter the transient, especially if it is coupled with a viscous component. It is therefore essential to identify the steady shortening phase (phase 4), preferably with direct measurement of sarcomere length (Goldman and Simmons, 1984; Seow and Ford, 1992), to accurately determine the velocity of shortening under an isotonic load following the transient.

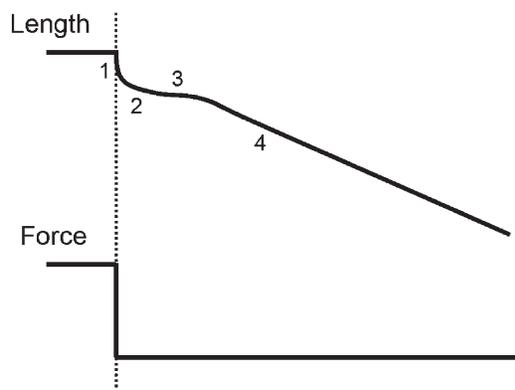


Figure 1. Time course of length response to a force step during an isotonic quick release. The length response can be divided into four phases. Phases 1–3 are responses mainly caused by the recoil of the elastic muscle elements and cross-bridge transients. Typically, within a few milliseconds, the length response settles to steady-state shortening (phase 4) characterized by a linear slope.

Despite its relatively accurate description of the force–velocity relations, the Hill equation historically has been regarded as purely empirical and devoid of any insight into the molecular mechanism of contraction (Abbott and Wilkie, 1953). Although this has not prevented widespread use of the equation, it may have discouraged exploration of physiological significance associated with the equation.

Deviation from Hill's hyperbola at low and high loads

Careful measurements of force–velocity properties of single skeletal muscle fibers have revealed that at low loads (less than $\sim 5\%$ F_{\max}), the measured velocities exceed those predicted by the Hill hyperbola. At the extrapolated zero load, Hill's equation underestimates the value of V_{\max} by $\sim 6\text{--}7\%$ (Edman et al., 1976; Julian et al., 1986). For whole muscle preparations with mixed fiber types, the underestimation of V_{\max} by the Hill hyperbola is found to be much greater (Claflin and Faulkner, 1989), but the bulk of the deviation is likely caused by heterogeneity in the intrinsic shortening velocities of the individual fibers within the whole muscle, because when the shortening velocity of the whole muscle exceeds the maximum shortening velocity of the slower fibers within the bundle, the shared load in the faster fibers increases because the slower fibers can no longer keep up with the overall pace of shortening. For a muscle preparation with mixed fibers, the force–velocity relations are better described by a composite of different hyperbolic force–velocity curves (Claflin and Faulkner, 1989).

The Hill hyperbola also failed to accurately describe the force–velocity relationship at loads greater than $\sim 80\%$ of isometric force (Edman et al., 1976; Lännergren, 1978). Studies specifically designed to examine the deviation of force–velocity data at high loads from Hill's hyperbola have revealed double hyperbolic features (Edman, 1988; Edman et al., 1997) and reversal of curvature (Wang et al., 1994; Devrome and MacIntosh, 2007). Theoretical models of cross-bridge cycling (Edman et al., 1997; Negroni and Lascano, 2008; Månsson, 2010) were able to simulate the nonhyperbolic features of the force–velocity relationship, suggesting that the deviation from Hill's hyperbola has its origin in the kinetics of interaction between myosin cross-bridges and the actin filaments.

Practical applications of the Hill equation

Despite the discrepancies at low and high loads, velocity measurements within the force range of $0.05\text{--}0.8 F_{\max}$ are remarkably close to the values predicted by the Hill equation. Because the maximum power output of a muscle occurs at $\sim 0.3 F_{\max}$, Hill's equation is often used in data interpolation to obtain the value of maximum power; an example is shown in Fig. 2 (solid circles and dashed line). It is not uncommon to use values of V_{\max}

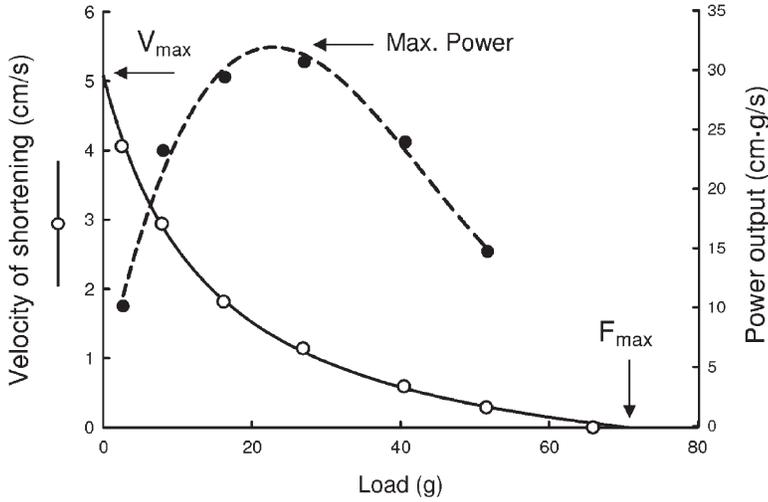


Figure 2. Velocity (solid line and open circles) and power (dashed line and closed circles) as functions of load in an isotonic contraction. Modified from Hill (1938) with permission from *Proceedings of the Royal Society B: Biological Sciences*. The curve is calculated from the same equation $(F + 14.35)(V + 1.03) = 87.6$ used in the original fit to data (circles). Power output = $F \cdot V$.

and F_{\max} extrapolated from the Hill hyperbola as approximations for the muscle's maximum shortening velocity and maximum isometric force. Changes in the curvature of the Hill hyperbola, represented mathematically by the Hill constant a over the maximum isometric force (i.e., a/F_{\max}), are often used to signify changes in the intrinsic properties of the muscle, although the interpretations are often vague and superficial because of the lack of understanding of the force–velocity behaviors in terms of the kinetics of actomyosin interaction.

Connection between Hill's equation and cross-bridge kinetics

Studies on the molecular basis of cross-bridge kinetics and force–velocity relationship (Bárány, 1967; Cooke and Bialek, 1979; Cooke and Pate, 1985; Nosek et al., 1987; Cooke et al., 1988; Dantzig et al., 1992; Rayment et al., 1993; Potma et al., 1995; Seow and Ford, 1997; Piazzesi et al., 2007; Elangovan et al., 2012; Caremani et al., 2013) have provided crucial data that allow us to make connections between changes in the force–velocity properties and the changes in the kinetics of actomyosin interaction. To illustrate this, we need to rewrite the Hill equation in a normalized form and compare it to an equation derived from actomyosin kinetics. Because maximal velocity (V_{\max}) occurs when externally applied force (F) is zero, and because at maximum isometric force (F_{\max}) the shortening velocity (V) is zero, it follows from Eq. 1 that $c = (F_{\max} + a)b = (V_{\max} + b)a$, or $a/F_{\max} = b/V_{\max}$. Therefore, in the normalized form, a single constant (K) can be used to represent a/F_{\max} and b/V_{\max} :

$$K = a / F_{\max} = b / V_{\max}. \quad (2)$$

Now it can be shown that in the normalized form, ($F = F/F_{\max}$, $V = V/V_{\max}$), the Hill equation (Eq. 1) becomes:

$$F = \frac{K(1-V)}{K+V}. \quad (3)$$

Force–velocity properties of a muscle are steady-state properties. This is because in determining their relationship, force and the corresponding velocity are measured after the transient response of the muscle has settled (Fig. 1). This point becomes important later in the analysis of the kinetic model.

The simplest cross-bridge kinetic model is a two-state model, as shown in Fig. 3 A. The entire cross-bridge population is assumed to reside in either the detached state or the attached state, and the corresponding fractional cross-bridge populations (D and A) add up to unity; i.e., $D + A = 1$. Force–velocity relations can be obtained from such models because of the intimate relationship between

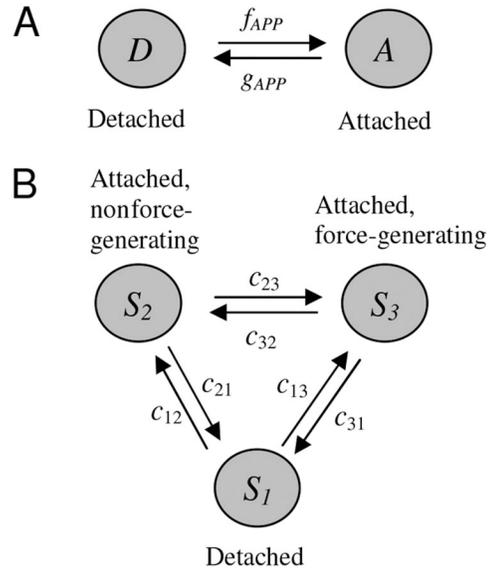


Figure 3. A two-state kinetic model of actomyosin interaction. (A) The steady-state distributions of cross-bridge populations in detached (D) and attached (A) states are determined by the apparent forward (f_{APP}) and reverse (g_{APP}) rate constants. (B) A three-state model with a detached state (S_1), an attached nonforce-generating state (S_2), and an attached force-generating state (S_3). c_{ij} denotes transition rates from state i to j .

force and the fraction of attached cross-bridges, and between velocity and the transition rates between the states (Huxley, 1957).

A linear first-order differential equation is used to describe the rate of change of the fraction of cross-bridges in each state. The rate of change in a particular state is taken as the difference between the rates at which the cross-bridges move into and out of the state. That is,

$$dD/dt = g_{APP} \cdot A - f_{APP} \cdot D \quad (4)$$

$$dA/dt = f_{APP} \cdot D - g_{APP} \cdot A, \quad (5)$$

where f_{APP} and g_{APP} are apparent forward and reverse transition rates, respectively. In a steady state, dD/dt and dA/dt are zero. However, setting Eqs. 4 and 5 to zero would result in an ill-posed condition with no unique solution for the simultaneous equations. This problem is avoided by replacing either Eq. 4 or 5 with the following:

$$D + A = 1. \quad (6)$$

From Eq. 4 (with $dD/dt = 0$), we have:

$$A = D \left(\frac{f_{APP}}{g_{APP}} \right). \quad (7)$$

Substituting A in Eq. 6 with Eq. 7:

$$D = \frac{g_{APP}}{g_{APP} + f_{APP}}. \quad (8)$$

Similarly,

$$A = \frac{f_{APP}}{g_{APP} + f_{APP}}. \quad (9)$$

By designating p as force per attached cross-bridge (or motor), the total force (F) produced by the muscle is:

$$F = pA = p \left(\frac{f_{APP}}{g_{APP} + f_{APP}} \right). \quad (10)$$

To convert Eq. 10 to a hyperbolic function of velocity, three assumptions need to be made: (1) the detachment rate is linearly proportional to the shortening velocity, i.e., $g_{APP} = kV$, where k is a constant of proportionality; (2) the attachment rate is independent of shortening velocity; and (3) force per bridge declines linearly with shortening velocity, i.e., $p = 1 - V$. With the above assumptions, Eq. 10 becomes

$$F = (1 - V) \left(\frac{f_{APP}}{kV + f_{APP}} \right). \quad (11)$$

By defining

$$K = f_{APP}/k, \quad (12)$$

and substituting f_{APP}/k with K in Eq. 11, we obtain $F = (1 - V)K/(V + K)$, which is exactly the same as Eq. 3 (the Hill equation).

The hyperbolic relationship can be demonstrated in two-state and multistate models (Baker and Thomas, 2000; Landesberg and Sideman, 2000; Chin et al., 2006). A more general approach in establishing a connection between the Hill equation and cyclic actomyosin interaction is illustrated below using a three-state model (Fig. 3 B). Assuming that S_1 is the detached state, S_2 is the attached state before power stroke (non-force-generating), and S_3 is the attached state after power stroke (force-generating), simultaneous equations can be set up to describe the cross-bridge transitions through the states:

$$1 = S_1 + S_2 + S_3$$

$$dS_1/dt = -(c_{12} + c_{13}) \cdot S_1 + c_{21} \cdot S_2 + c_{31} \cdot S_3$$

$$dS_2/dt = +c_{12} \cdot S_1 - (c_{21} + c_{23}) \cdot S_2 + c_{32} \cdot S_3,$$

where c_{ij} is the rate constant from state i to j , and $dS_i/dt = 0$ in a steady state. In matrix form,

$$\mathbf{D} \cdot \mathbf{S} = \mathbf{Y},$$

where

$$\mathbf{D} = \begin{bmatrix} 1 & 1 & 1 \\ -(c_{12} + c_{13}) & c_{21} & c_{31} \\ c_{12} & -(c_{21} + c_{23}) & c_{32} \end{bmatrix},$$

and $\mathbf{S} = [S_1, S_2, S_3]^T$, and $\mathbf{Y} = [1, 0, 0]^T$. Solving for S_3 (fraction of cross-bridges in the force-generating state),

$$S_3 = \frac{c_{12}c_{23} + c_{13}c_{21} + c_{13}c_{23}}{|D|}, \quad (13)$$

where the determinant $|D| = c_{21}c_{32} + c_{21}c_{31} + c_{23}c_{31} + c_{12}c_{32} + c_{13}c_{32} + c_{12}c_{31} + c_{13}c_{21} + c_{13}c_{23}$. If we assume that the transition from S_3 to S_1 (detachment of cross-bridge by ATP after the power stroke) is irreversible, then $c_{13} = 0$, and Eq. 13 becomes

$$S_3 = \frac{f_{APP}}{[c_{12}c_{31} + c_{12}c_{32} + c_{21}c_{31} + c_{21}c_{32} + c_{23}c_{31}] + f_{APP}}, \quad (14)$$

where $f_{APP} = c_{12}c_{23}$. Set $g_{APP} = [c_{12}c_{31} + c_{12}c_{32} + c_{21}c_{31} + c_{21}c_{32} + c_{23}c_{31}]$, we obtain

$$S_3 = \frac{f_{APP}}{g_{APP} + f_{APP}}. \quad (15)$$

Eq. 15 therefore is identical to Eq. 9, and as illustrated above for Eq. 9, Eq. 15 can be converted to a hyperbolic equation when the same assumptions regarding velocity

dependence of g_{APP} and force per cross-bridge (p) are applied. Note that the terms in g_{APP} all contain one transition rate associated with movement of cross-bridges away from the force-generating state S_3 . To obtain a hyperbolic force–velocity relationship in a seven-state model with multiple force-generating states, only the initial attachment rate (from detached state to the first attached state) is set to be velocity independent; all other transition rates are linear functions of shortening velocity (Chin et al., 2006) and as a whole can be represented by g_{APP} as a function of shortening velocity.

Now let us examine the validity of the three assumptions needed to convert Eq. 10 to the Hill equation in light of the findings from Piazzesi et al. (2007). The first assumption is supported by their findings showing that the detachment rate increases linearly from $\sim 15/s$ at isometric condition to $\sim 275/s$ at a sliding velocity of 2,000 nm/s, $\sim 2/3 V_{max}$ (their results are reproduced in Fig. 4 A). If we choose not to ignore the small detachment rate under isometric condition (i.e., $g_i = 15/s$), Eq. 11 becomes:

$$F = (1 - V) \left(\frac{f_{APP}}{kV + f_{APP} + g_i} \right). \quad (16)$$

From Eq. 16 (and Fig. 4 A) it is apparent that at high velocities with g_i relatively small compared with kV , the equation is essentially the same as the Hill equation. But when V approaches zero, overestimation of force by the Hill equation becomes obvious. This may account for, at least partially, the deviation of force–velocity data from the Hill hyperbola in the low velocity region (Edman, 1988; Wang et al., 1994; Devrome and MacIntosh, 2007).

The second assumption partly agrees with the findings of Piazzesi et al. (2007). They found that for sliding velocities greater than $\sim 20\% V_{max}$ (use 3,000 nm/s for the value of V_{max} as assumed by Piazzesi et al., 2007), the attachment rate (f_{APP}) is virtually constant (Fig. 4 B). At low velocities, f_{APP} falls below the constant level. For the third assumption, the force per attached bridge (p) found by Piazzesi et al. (2007) showed linear decline in the high velocity region (Fig. 4 C), but again there is discrepancy in the low velocity region: the actual value for p under isometric conditions falls below the expected value by linear extrapolation from the rest of the data (Fig. 4 C).

All three assumptions involved in the transformation of the kinetic equation (Eq. 11) to the Hill equation are therefore not entirely correct. However, the discrepancies are all concentrated in the low velocity region where the Hill hyperbola deviates most from the force–velocity data.

Explanations for the deviations from the Hill hyperbola

As discussed above, if the assumptions regarding the apparent rates of cross-bridge attachment and detachment,

and the force per bridge as functions of shortening velocity are true, then the Hill equation should describe perfectly the relationship between muscle force and velocity. The fact that the description is not perfect simply means that the assumptions are not perfect, but ironically, from the deviations some unique behavior of the muscle can be understood in terms of molecular mechanisms. The assumption regarding the apparent rate of detachment (g_{APP}) being directly proportional to the shortening velocity is true only when the small detachment rate at isometric condition (g_i) is set to zero (see Eq. 16 and Fig. 4 A). The implicit assumption embedded in the Hill equation that g_{APP} is zero under isometric

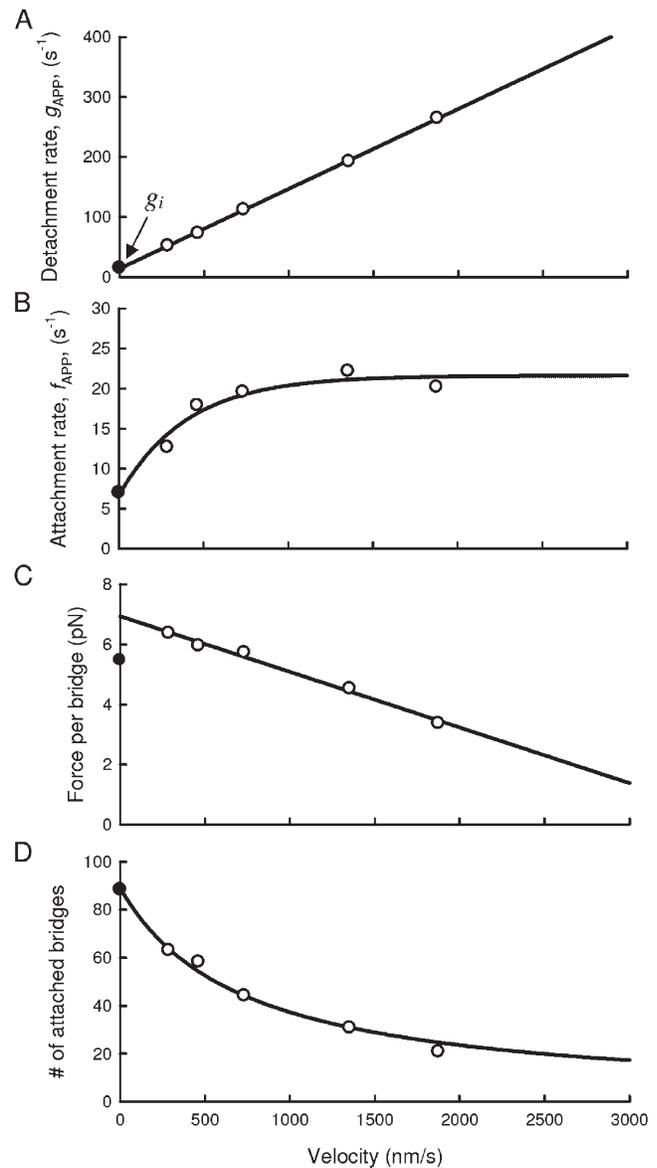


Figure 4. The apparent rates of detachment (A), attachment (B), force per attached bridge (C), and the number of attached bridges (D) as functions of shortening velocity. Redrawn from Piazzesi et al. (2007) with permission from *Cell*.

conditions (i.e., $g_i = 0$) is therefore one of the sources that contribute to the equation's overestimation of force at low velocities. A corollary of this analysis is that a muscle with higher energetic efficiency in maintaining isometric force (i.e., small g_i) will have less force deviation from the Hill hyperbola in the high force range, provided of course all other factors remain the same.

The apparent rate of attachment (f_{APP}) is constant only in the intermediate to high velocity range; at low velocities, the rate is slower and not constant (Piazzesi et al., 2007) (Fig. 4 B). This means at low velocities the fraction of attached bridges is lower than expected from extrapolation of the Hill hyperbola (which assumes f_{APP} remains constant throughout the entire force range). This is another source of discrepancy between the measured force at the low velocity region and that predicted by the Hill hyperbola. To account for the curvature reversal at low velocities seen in force-velocity plots (Wang et al., 1994; Devrome and MacIntosh, 2007), Chin et al. (2006) used a $f_{APP}(v)$ function (Fig. 5 A) very similar to what Piazzesi et al. (2007) found (Fig. 4 B) and were able to reproduce the feature (Fig. 5 B). Chin et al. (2006) were also successful in reproducing the double hyperbolic feature (Edman, 1988) with a different $f_{APP}(v)$ function (Fig. 5, C and D) that is also characterized by slower attachment rates at low velocities. The more elaborate feature of this function was not observed by Piazzesi et al. (2007), but this could be the result of insufficient data points in the low velocity region.

The deviation of p (force per bridge) from the linear function in the low velocity region (Fig. 4 C) is yet another source of discrepancy between the measured

force in the low velocity region and that expected from the Hill hyperbola (Edman, 1988; Wang et al., 1994; Devrome and MacIntosh, 2007). Therefore, it appears that there are at least three causes that could explain why the Hill equation overestimated both the force and velocity values in the low velocity (or high force) region in the force-velocity relationship. It is likely that a combination of and interaction among these three causative factors result in the deviation of actual measurements from the Hill hyperbola.

It is not surprising that the mid portion of the Hill hyperbola fits the force-velocity data quite well, because all of the conditions regarding the velocity dependence of p , g_{APP} , and f_{APP} inherent to the Hill equation agree with experimental evidence (Piazzesi et al., 2007).

The reason for the underestimation of shortening velocity at the other end of the force-velocity curve (i.e., at low loads) (Edman et al., 1976; Julian et al., 1986) by the Hill equation is less clear. No data on p , f_{APP} , and g_{APP} in the very high velocity region clearly show deviations that could be used to explain the higher than expected velocity at low loads. However, that does not mean the deviation of the kinetic functions ($p(v)$, $f_{APP}(v)$, and $g_{APP}(v)$) from linearity does not exist in the high velocity region.

Force-velocity performance over the range of 0.05–0.8 F_{max}

Most force-velocity studies of muscle performance have been performed in a force range that excludes very low and high loads. Force-velocity data from these studies are generally well described by the Hill hyperbola and

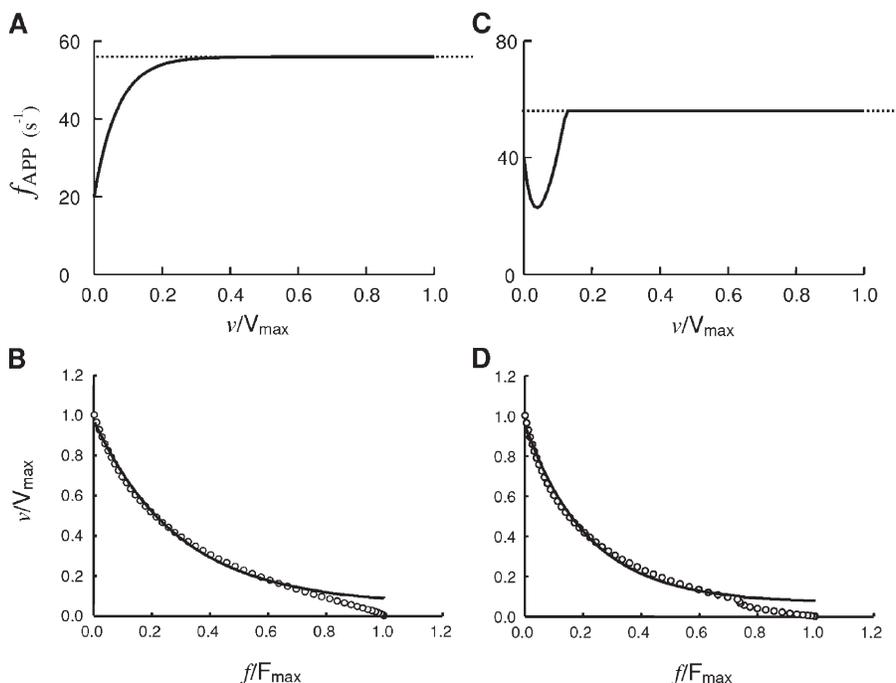


Figure 5. Deviation of the apparent attachment rate (f_{APP}) from constancy (dotted line) at low velocities (A and C) leads to the deviation of the simulated force-velocity data (circles) from Hill's hyperbola (solid line) at high loads (B and D). Modified from Chin et al. (2006) with permission from *Biophysical Journal*. The g_{APP} and force per bridge (p) used in the simulation by Chin et al. (2006) were assumed to be linear functions of velocity. See text for more details.

from the discussions above; a hyperbolic force–velocity relationship is expected and consistent with the results from kinetic studies of actomyosin interaction in muscle cells within this force range. Because the maximum power output of muscles occurs within the mid force range, variation of this parameter can now be understood more clearly in terms of the kinetics of actomyosin interaction.

Maximum power and the curvature of the force–velocity curve. The shape of the force–velocity curve is often described by its curvature, which in a hyperbolic curve can be specified by the Hill constant a normalized to F_{\max} (i.e., a/F_{\max} , or K in Eqs. 2 and 3). As shown in Fig. 6 A, an increase in K (or a/F_{\max}) is associated with a decrease in curvature, and as shown in Fig. 6 B, a decrease in curvature is associated with an increase in the relative power. (Relative power is obtained in a force–velocity plot in which force and velocity are normalized to their respective maximum values.) Because $K = f_{\text{APP}}/k$ (Eq. 12), it is clear that the curvature is determined by the ratio of the apparent attachment rate (f_{APP}) and the slope (k) of the $g_{\text{APP}}(v)$ function. It follows that any intervention that increases f_{APP} and decreases the dependence of g_{APP} on shortening velocity will make the force–velocity curve less curved, and will increase the relative power output of the muscle. The increase in K value therefore could be caused by an increase in f_{APP} , a decrease in k , or a combination of both. Intuitively, an increase in the fraction of attached bridges (Eq. 9) could lead to an increase in the power output, because the power stroke occurs in between attached states. A decrease in k also favors accumulation of bridges in attached states because of the slower rate of detachment of cross-bridges as a muscle shortens. A reduced force–velocity curvature therefore is related to an increase in the fraction of cross-bridges in the attached force-generating states at all velocities. Although the explanation is derived from a two-state kinetic model, it applies to multistate models as well. Similar insight can be derived from a three-state model (Fig. 3 B). In a seven-state model with five attached and two detached states (Chin et al., 2006), the attachment rate is also found to be crucial in determining the force–velocity curvature and hence relative power.

The curvature of the force–velocity relation can also be derived from Huxley’s two-state model (Huxley, 1957) in terms of kinetic rate constants. It can be shown that a/F_{\max} is equivalent to the ratio of $(f_1 + g_1)/g_2$ in Huxley’s model, where f_1 is related to the attachment rate, g_1 is related to the detachment rate of cross-bridges generating positive force, and g_2 is the rate constant for detachment of compressively strained cross-bridges. Although there is resemblance to Eq. 12, $(f_1 + g_1)/g_2$ is not equivalent to f_{APP}/k . This is because in this particular case, Huxley’s definition of force–velocity curvature

is model specific. In a different model, Huxley and colleagues (Slawnych et al., 1994) used very different rate functions for cross-bridge attachment and detachment; the definition of the force–velocity curvature for this model would therefore be very different from that of the Huxley 1957 model. On the other hand, f_{APP}/k can be used to describe the force–velocity curvature as long as the data can be satisfactorily described by the Hill equation.

Fraction of attached bridges as a function of velocity. One important finding of Piazzesi et al. (2007) is that the decrease in force as shortening velocity increases is partially caused by a decrease in the number of attached bridges. Using their f_{APP} and g_{APP} as functions of velocity in Eq. 9 and ignoring the low velocity region, one can see that the fraction of attached bridges (A) decreases in a fashion that can be described by a rectangular hyperbola, because f_{APP} is constant (except for the low velocity region) and g_{APP} increases approximately linearly with velocity. Fig. 4 D shows a rectangular hyperbola (solid line) fitted to the data from Piazzesi et al. (2007). Because of the relatively good fit and because Eq. 9 is part of the link between the Hill equation and the cross-bridge kinetics analysis, this implies that an implicit assumption embedded in the Hill equation is that the

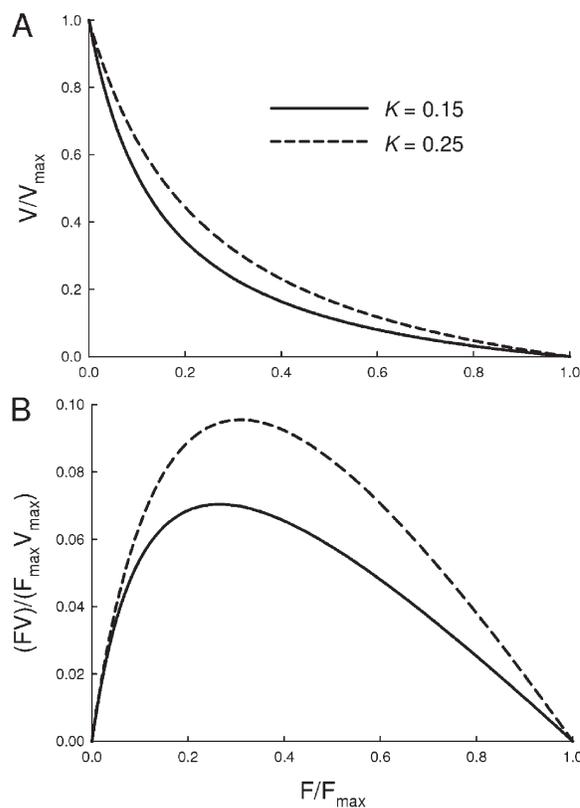


Figure 6. Force–velocity (A) and force–power (B) curves with different degrees of curvature (a/F_{\max} or K values). Note that Eq. 3 can be rewritten in the form: $V = K(1 - F)/(K + F)$.

fraction of attached bridges decreases with velocity, as Piazzesi et al. (2007) have found experimentally.

Force–velocity relations in fast and slow muscle fibers. It is well known that V_{\max} is related to the intrinsic actin- and calcium-activated myosin ATPase activity of the muscle (Bárány, 1967), which in turn is largely determined by the enzymatic properties of the isoforms of the myosin family (Moss et al., 1995; Schiaffino and Reggiani, 1996). Myosin isoforms in different animal species have evolved so that their intrinsic ATPase activities match the power requirements imposed by animal body size (Seow and Ford, 1991). Within a single species, muscle fibers containing predominantly fast or slow myosin isoforms possess, respectively, higher and lower V_{\max} values (Seow and Ford, 1991; Pellegrino et al., 2003). It is not entirely clear how nature modifies ATPase activity, but it is likely that the rate of ADP release from its myosin-binding site is the target for modification (Bárány, 1967; Siemankowski et al., 1985; Rayment et al., 1993; Elangovan et al., 2012). This implies that at maximal shortening velocity, the rate-limiting step in a cross-bridge cycle is the hydrolysis of ATP and the release of P_i and ADP from the myosin head. The fast and slow myosin isoforms, besides determining V_{\max} , also shape the force–velocity curve. Woledge et al. (1985), after compiling force–velocity data from many independent studies (Katz, 1939; Close, 1964; Woledge, 1968; Cecchi et al., 1978; Lännergren, 1978; Luff, 1981; Lännergren et al., 1982; Ranatunga, 1982), found a positive correlation between a/F_{\max} and V_{\max} . Later studies examining shortening velocities from fast and slow muscle fibers also showed a similar correlation (Brooks and Faulkner, 1988; Stienen et al., 1988; Barclay et al., 1993; Wahr and Metzger, 1998; Gilliver et al., 2009). This indicates that a fast muscle like extensor digitorum longus (EDL), compared with a slow one like soleus, not only shortens faster but also has greater power output because of less curvature in its force–velocity curve. According to Eq. 12, in order for a fast muscle to have less curvature in its force–velocity relation, either the apparent attachment rate (f_{APP}) of the myosin to actin needs to be increased or the rate of increase in g_{APP} as a function of increasing velocity needs to be decreased, or a combination of both.

In cardiac myocytes containing α -MHC and β -MHC isoforms, it has been found that the faster isoform (α -MHC) is associated with higher shortening velocity and power output, as well as a greater value for a/F_{\max} , when compared with the slower β -MHC isoform (Herron et al., 2001).

Variation in force–velocity properties exists within a single fiber type of the same species and also across species. Examination of contractile properties of type I fibers from different species has revealed the same positive correlation between a/F_{\max} and V_{\max} (Seow and

Ford, 1991; Widrick et al., 1997). Interestingly, the relationship between a/F_{\max} and V_{\max} obtained from a single fiber type of the same species is opposite to that from different fiber types or the same type that belongs to different species (Gilliver et al., 2009, 2011); that is, the ratio a/F_{\max} decreases with increasing V_{\max} . This indicates that modification of actomyosin kinetics resulting in variation of force–velocity properties within a fiber type of a single species is different from the modification that occurs in the fast and slow types in the same species, or within the same type from different species.

Effects of temperature. In summarizing the data from Lännergren et al. (1982) and Ranatunga (1982), Woledge et al. (1985) noticed that V_{\max} is more sensitive to temperature than F_{\max} is. Because shortening velocity is mostly determined by the apparent detachment rate (g_{APP}), which in turn is closely related to the rate of ADP release after the power stroke under low loads, the high temperature sensitivity of V_{\max} is likely a reflection of the same temperature sensitivity of actomyosin interaction, especially in the conformational change step within the myosin molecule leading to ADP release (Rayment et al., 1993). Ranatunga (1984) specifically examined the temperature effect on V_{\max} and a/F_{\max} in fast (EDL) and slow (soleus) muscles from rats. At each of the six temperatures examined, a/F_{\max} was found to correlate positively with V_{\max} , and across a temperature range of 10–35°C, the value of a/F_{\max} increases with increasing temperature in both the fast and slow muscles (Ranatunga, 1984). The increase in a/F_{\max} with temperature could be a secondary effect of increasing V_{\max} with temperature, because as discussed earlier, a/F_{\max} and V_{\max} are positively correlated. However, a closer examination of the data from Ranatunga (1984) suggests that this is not the case. Fig. 7 shows that although the values of a/F_{\max} in both slow and fast muscles increase with temperature, they do not have the same velocity dependence. The a/F_{\max} value for a fast muscle increases faster with velocity compared with the relationship for a slow muscle (Fig. 7). The increase in a/F_{\max} with temperature is therefore muscle-type specific. The a/F_{\max} value of the fast muscle has an overall greater sensitivity to temperature compared with that of slow muscle. Increasing temperature does not merely increase the shortening velocity. If the velocity values in a force–velocity curve are scaled up by a constant factor, the a/F_{\max} value does not change. The fact that a/F_{\max} is altered by temperature suggests that f_{APP} , k , or both are temperature sensitive, at least in mammals. Zhao and Kawai (1994) have found that the fraction of detached cross-bridges declines as temperature increases, indicating an increase in f_{APP} (and thus a/F_{\max}) with increasing temperature and providing an answer to the question of why force–velocity curvature decreases with

increasing temperature. In some cold-water fish species it has been found that a/F_{\max} is not temperature sensitive, even though V_{\max} increases with temperature (Johnston and Salamonski, 1984; Johnston and Sidell, 1984). Fish myosin isoforms may have adapted to temperature ranges to minimize loss of power at low temperatures.

Effects of [ADP], [ATP], and [P_i]. An increase in ADP concentration surrounding the site of actomyosin interaction will lead to a decrease in g_{APP} because it interferes with the release of ADP from the cross-bridges (Bárány, 1967; Cooke and Pate, 1985; Siemankowski et al., 1985; Seow and Ford, 1997; Elangovan et al., 2012). The expected effects are an increase in the fraction of attached bridges (therefore isometric force) and a decrease in shortening velocity. These effects have been observed in experiments (Cooke and Pate, 1985; Seow and Ford, 1997) and simulated with a multistate model of cross-bridge kinetics (Chin et al., 2006). There is an increase in the a/F_{\max} value as a result of the increase in [ADP] (Seow and Ford, 1997). Because reducing the rate of ADP release directly affects g_{APP} , and because of the close relationship between g_{APP} and shortening velocity (Piazzesi et al., 2007), the increase in a/F_{\max} (or reduction of curvature of the force–velocity curve) is likely caused by a reduced dependency of g_{APP} on shortening velocity (i.e., a smaller k value). It should be pointed out that the ATP-regenerating system (phosphocreatine and creatine kinase) is omitted in experiments examining the effects of ADP to control ADP concentration.

A decrease in [ATP] has very similar effects as an increase in [ADP] without the ATP-regenerating system. It causes an increase in isometric force and a decrease in shortening velocity (Ferenczi et al., 1984; Cooke and Pate, 1985; Stienen et al., 1988; Seow and Ford, 1997), and an increase in a/F_{\max} (Ferenczi et al., 1984; Stienen et al., 1988; Seow and Ford, 1997; Wakayama and

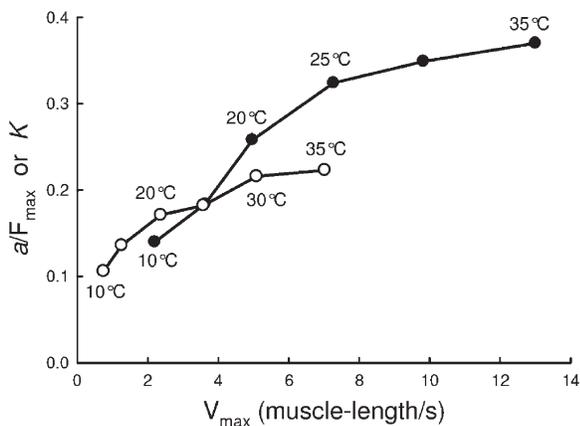


Figure 7. The relationship between a/F_{\max} and V_{\max} of fast (EDL) muscle (closed circles) and slow (soleus) muscle (open circles) at different temperatures. Data source from Ranatunga (1984) with permission from *The Journal of Physiology*.

Yamada, 2000). An increase in [ADP] associated with the experimental conditions creating low [ATP] could be an explanation for the observed effect of ATP (Cooke and Bialek, 1979). But even without an increase in [ADP] as a secondary effect, lowering [ATP] will reduce g_{APP} and hence give rise to an effect similar to that caused by increased [ADP].

Amrinone (a bipyridine compound) has been shown to increase ADP affinity to actomyosin (Albet-Torres et al., 2009). Not surprisingly, the effects of amrinone on the force–velocity relationship are very similar to the effects of high [ADP] described above: an increase in F_{\max} , a decrease in V_{\max} , and a characteristic increase in a/F_{\max} .

An increase in [P_i] reduces F_{\max} (Dawson et al., 1978; Nosek et al., 1987; Wilson et al., 1988; Potma et al., 1995) without changing V_{\max} (Cooke et al., 1988), especially at near in vivo temperature (Debold et al., 2004; Karatzaferi et al., 2008). The action of inorganic phosphate on the cross-bridge cycle is more complicated than a two-state kinetic model can explain. It has been suggested that a partial power stroke can occur before the release of P_i from the cross-bridge (Dantzig et al., 1992; Linari et al., 2010; Caremani et al., 2013). This could at least partially account for the depressive effect of P_i on F_{\max} . It has also been suggested that a myosin cross-bridge could detach from actin filament, even when the ATP hydrolysis products (P_i and ADP) are still attached to the bridge (Linari et al., 2010). This is a crucial feature used by Chin et al. (2006) to simulate and explain the unique effects of P_i that uncouples F_{\max} and V_{\max} . A simplified explanation is that P_i reduces f_{APP} without affecting g_{APP} . This would imply a decrease in a/F_{\max} with increasing [P_i], which is supported by Debold et al. (2004) but not Karatzaferi et al. (2008).

In experiments where intracellular concentrations of ATP, ADP, and P_i must be controlled, it is necessary to permeabilize the cell membrane. To preserve the structural integrity of the “skinned” cells, the experiments are usually conducted at temperatures much lower than the body temperature. Readers must be aware that the results discussed above may be different at higher temperatures.

Effects of muscle fatigue. Muscle fatigue is associated with reduced muscle performance, especially in power output, which in turn is determined by muscle force and velocity and the curvature of the force–velocity relationship. At the level of actomyosin interaction, muscle fatigue is likely a result of altered chemical environment caused by rapid use of ATP and a reduction in intracellular [Ca²⁺] (Fitts, 2008; Jones, 2010). The products of ATP hydrolysis include ADP, P_i, and H⁺. Changes in intracellular [ATP], [ADP], [P_i], [H⁺], and [Ca²⁺] associated with fatigue are likely direct modifiers of cross-bridge kinetics. To complicate the matter, the effects of some of these modifiers are temperature

dependent, for example, the effect of $[H^+]$ (Knuth et al., 2006). To study mammalian muscle fatigue, experiments should be conducted at near body temperature (Jones, 2010). The decrease in F_{\max} seen in fatigue is likely a consequence of high intracellular $[P_i]$ and $[H^+]$, and low $[Ca^{2+}]$. P_i , besides its direct effect on the cross-bridge cycle mentioned above, has the additional effect of reducing myofibrillar Ca^{2+} sensitivity (Debold et al., 2006). High intracellular $[H^+]$ is known to cause cross-bridges to accumulate in low force states (Seow and Ford, 1993; Debold et al., 2008), which could reduce F_{\max} . Low intracellular pH is also a likely player in reducing V_{\max} during muscle fatigue. The myosin ATPase activity is lowered at low pH (Cooke et al., 1988), perhaps because of lower rates of release of ADP from the myosin head (Debold et al., 2008), which has been found to reduce V_{\max} (Bárány, 1967; Siemankowski et al., 1985; Elangovan et al., 2012). It has been noticed that in the initial phase of muscle fatigue, small decreases in F_{\max} could occur in the absence of appreciable change in V_{\max} (Edman and Mattiazzi, 1981; Jones et al., 2006). Because phosphocreatine is the energy reserve (~ 20 mM) in muscle cells used first to replenish ATP in a process that produces inorganic phosphate, short-term fatigue is likely mainly caused by an increase in intracellular $[P_i]$ (Wilson et al., 1988). As mentioned above, high $[P_i]$ is able to reduce F_{\max} without affecting V_{\max} , making it a likely candidate for causing the initial decrease in F_{\max} seen in muscle fatigue. The cause for the reduction in a/F_{\max} (increase in force–velocity curvature) seen in muscle fatigue is less clear and more controversial (Fitts, 2008). However, the individual or combined effect of high $[P_i]$ and $[H^+]$ is likely the culprit. Low $[Ca^{2+}]$ appears to have no effect on a/F_{\max} (Langeron et al., 1999).

Reduction in a/F_{\max} seen in muscle fatigue is a major factor contributing to the loss of muscle power (Jones et al., 2006). Because both elevated $[P_i]$ and $[H^+]$ reduce the cross-bridge transition rate from low to high force states (Cooke et al., 1988; Seow and Ford, 1993; Debold et al., 2004, 2008; Karatzaferi et al., 2008), the decrease in a/F_{\max} is likely a result of a reduction in f_{APP} (according to Eq. 12; and according to Fig. 3 B and Eq. 14, a reduction in c_{23} leads to reduction in f_{APP}). Jones (2010) has reached a similar conclusion that there must be a substantial decrease in the rate constant for attachment in the Huxley (1957) model to account for the observed decrease in power and increase in force–velocity curvature in fatigued muscle.

Effects of myosin light chain phosphorylation in striated muscle. In submaximally activated striated muscle fibers, isometric twitch and tetanic force are substantially augmented by the phosphorylation of the regulatory myosin light chain (Persechini et al., 1985; Sweeney and Stull, 1986; Grange et al., 1995). One of the effects of

light chain phosphorylation is that it pushes myosin heads toward the actin filaments, and this has been speculated to facilitate the actomyosin interaction (Metzger et al., 1989; Levine et al., 1996; Yang et al., 1998). Analysis of data on isometric tension potentiation and redevelopment (Metzger et al., 1989; Sweeney and Stull, 1990) suggests that the apparent rate of attachment (f_{APP}) is enhanced by light chain phosphorylation. Examination of the changes in force–velocity properties and power output caused by phosphorylation of the regulatory light chain reveals an increase in the a/F_{\max} ratio (Grange et al., 1995), consistent with the hypothesis that f_{APP} is enhanced by phosphorylation of the myosin light chain that increases the muscle power in part by reducing force–velocity curvature.

Hill's hyperbola, no longer just descriptive

Despite being regarded as purely empirical and lacking physiological insight soon after its inception three quarters of a century ago, the Hill hyperbola has continued to be the predominant descriptor of the mechanical performance of muscle. Although the connection between the hyperbolic equation and cross-bridge cycling kinetics could have been made long ago, soon after A.F. Huxley's publication of his 1957 model, a solid connection was only possible after the state-of-the-art study on the mechanics of myosin motors by Piazzesi et al. (2007) at nanometer and piconewton resolution. For physiologists and kinesiologists who use Hill's equation to approximate force–velocity relationships, this connection provides molecular mechanistic insights to inform their interpretations of data. The connection also provides a new perspective for appreciating a seminal contribution to muscle physiology that is of both historical and contemporary interest.

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REFERENCES

- Abbott, B.C., and D.R. Wilkie. 1953. The relation between velocity of shortening and the tension-length curve of skeletal muscle. *J. Physiol.* 120:214–223.
- Albet-Torres, N., M.J. Bloemink, T. Barman, R. Candau, K. Frölander, M.A. Geeves, K. Golker, C. Herrmann, C. Lionne, C. Piperio, et al. 2009. Drug effect unveils inter-head cooperativity and strain-dependent ADP release in fast skeletal actomyosin. *J. Biol. Chem.* 284:22926–22937. <http://dx.doi.org/10.1074/jbc.M109.019232>
- Baker, J.E., and D.D. Thomas. 2000. A thermodynamic muscle model and a chemical basis for A.V. Hill's muscle equation. *J. Muscle Res. Cell Motil.* 21:335–344. <http://dx.doi.org/10.1023/A:1005615925390>
- Bárány, M. 1967. ATPase activity of myosin correlated with speed of muscle shortening. *J. Gen. Physiol.* 50:197–218. <http://dx.doi.org/10.1085/jgp.50.6.197>

- Barclay, C.J., J.K. Constable, and C.L. Gibbs. 1993. Energetics of fast- and slow-twitch muscles of the mouse. *J. Physiol.* 472:61–80.
- Barclay, C.J., R.C. Woledge, and N.A. Curtin. 2010. Inferring cross-bridge properties from skeletal muscle energetics. *Prog. Biophys. Mol. Biol.* 102:53–71. <http://dx.doi.org/10.1016/j.pbiomolbio.2009.10.003>
- Brooks, S.V., and J.A. Faulkner. 1988. Contractile properties of skeletal muscles from young, adult and aged mice. *J. Physiol.* 404: 71–82.
- Caremani, M., L. Melli, M. Dolfi, V. Lombardi, and M. Linari. 2013. The working stroke of the myosin II motor in muscle is not tightly coupled to release of orthophosphate from its active site. *J. Physiol.* 591:5187–5205. <http://dx.doi.org/10.1113/jphysiol.2013.257410>
- Cecchi, G., F. Colomo, and V. Lombardi. 1978. Force-velocity relation in normal and nitrate-treated frog single muscle fibres during rise of tension in an isometric tetanus. *J. Physiol.* 285:257–273.
- Chin, L., P. Yue, J.J. Feng, and C.Y. Seow. 2006. Mathematical simulation of muscle cross-bridge cycle and force-velocity relationship. *Biophys. J.* 91:3653–3663. <http://dx.doi.org/10.1529/biophysj.106.092510>
- Civan, M.M., and R.J. Podolsky. 1966. Contraction kinetics of striated muscle fibres following quick changes in load. *J. Physiol.* 184:511–534.
- Claffin, D.R., and J.A. Faulkner. 1989. The force-velocity relationship at high shortening velocities in the soleus muscle of the rat. *J. Physiol.* 411:627–637.
- Close, R. 1964. Dynamic properties of fast and slow skeletal muscles of the rat during development. *J. Physiol.* 173:74–95.
- Cooke, R., and W. Bialek. 1979. Contraction of glycerinated muscle fibers as a function of the ATP concentration. *Biophys. J.* 28:241–258. [http://dx.doi.org/10.1016/S0006-3495\(79\)85174-7](http://dx.doi.org/10.1016/S0006-3495(79)85174-7)
- Cooke, R., and E. Pate. 1985. The effects of ADP and phosphate on the contraction of muscle fibers. *Biophys. J.* 48:789–798. [http://dx.doi.org/10.1016/S0006-3495\(85\)83837-6](http://dx.doi.org/10.1016/S0006-3495(85)83837-6)
- Cooke, R., K. Franks, G.B. Luciani, and E. Pate. 1988. The inhibition of rabbit skeletal muscle contraction by hydrogen ions and phosphate. *J. Physiol.* 395:77–97.
- Dantzig, J.A., Y.E. Goldman, N.C. Millar, J. Lacktis, and E. Homsher. 1992. Reversal of the cross-bridge force-generating transition by photogeneration of phosphate in rabbit psoas muscle fibres. *J. Physiol.* 451:247–278.
- Dawson, M.J., D.G. Gadian, and D.R. Wilkie. 1978. Muscular fatigue investigated by phosphorus nuclear magnetic resonance. *Nature.* 274:861–866. <http://dx.doi.org/10.1038/274861a0>
- Debold, E.P., H. Dave, and R.H. Fitts. 2004. Fiber type and temperature dependence of inorganic phosphate: implications for fatigue. *Am. J. Physiol. Cell Physiol.* 287:C673–C681. <http://dx.doi.org/10.1152/ajpcell.00044.2004>
- Debold, E.P., J. Romatowski, and R.H. Fitts. 2006. The depressive effect of Pi on the force-pCa relationship in skinned single muscle fibers is temperature dependent. *Am. J. Physiol. Cell Physiol.* 290:C1041–C1050. <http://dx.doi.org/10.1152/ajpcell.00342.2005>
- Debold, E.P., S.E. Beck, and D.M. Warshaw. 2008. Effect of low pH on single skeletal muscle myosin mechanics and kinetics. *Am. J. Physiol. Cell Physiol.* 295:C173–C179. <http://dx.doi.org/10.1152/ajpcell.00172.2008>
- Devrome, A.N., and B.R. MacIntosh. 2007. The biphasic force-velocity relationship in whole rat skeletal muscle in situ. *J. Appl. Physiol.* 102:2294–2300. <http://dx.doi.org/10.1152/jappphysiol.00276.2006>
- Edman, K.A. 1988. Double-hyperbolic force-velocity relation in frog muscle fibres. *J. Physiol.* 404:301–321.
- Edman, K.A., and A.R. Mattiazzi. 1981. Effects of fatigue and altered pH on isometric force and velocity of shortening at zero load in frog muscle fibres. *J. Muscle Res. Cell Motil.* 2:321–334. <http://dx.doi.org/10.1007/BF00713270>
- Edman, K.A., L.A. Mulieri, and B. Scubon-Mulieri. 1976. Non-hyperbolic force-velocity relationship in single muscle fibres. *Acta Physiol. Scand.* 98:143–156. <http://dx.doi.org/10.1111/j.1748-1716.1976.tb00234.x>
- Edman, K.A., A. Månsson, and C. Caputo. 1997. The biphasic force-velocity relationship in frog muscle fibres and its evaluation in terms of cross-bridge function. *J. Physiol.* 503:141–156. <http://dx.doi.org/10.1111/j.1469-7793.1997.141bi.x>
- Eisenberg, E., T.L. Hill, and Y. Chen. 1980. Cross-bridge model of muscle contraction. Quantitative analysis. *Biophys. J.* 29:195–227. [http://dx.doi.org/10.1016/S0006-3495\(80\)85126-5](http://dx.doi.org/10.1016/S0006-3495(80)85126-5)
- Elangovan, R., M. Capitanio, L. Melli, F.S. Pavone, V. Lombardi, and G. Piazzesi. 2012. An integrated in vitro and in situ study of kinetics of myosin II from frog skeletal muscle. *J. Physiol.* 590:1227–1242.
- Ferenczi, M.A., Y.E. Goldman, and R.M. Simmons. 1984. The dependence of force and shortening velocity on substrate concentration in skinned muscle fibres from *Rana temporaria*. *J. Physiol.* 350:519–543.
- Fitts, R.H. 2008. The cross-bridge cycle and skeletal muscle fatigue. *J. Appl. Physiol.* 104:551–558. <http://dx.doi.org/10.1152/jappphysiol.01200.2007>
- Gilliver, S.F., H. Degens, J. Rittweger, A.J. Sargeant, and D.A. Jones. 2009. Variation in the determinants of power of chemically skinned human muscle fibres. *Exp. Physiol.* 94:1070–1078. <http://dx.doi.org/10.1113/expphysiol.2009.048314>
- Gilliver, S.F., D.A. Jones, J. Rittweger, and H. Degens. 2011. Variation in the determinants of power of chemically skinned type I rat soleus muscle fibres. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* 197:311–319. <http://dx.doi.org/10.1007/s00359-010-0613-6>
- Goldman, Y.E., and R.M. Simmons. 1984. Control of sarcomere length in skinned muscle fibres of *Rana temporaria* during mechanical transients. *J. Physiol.* 350:497–518.
- Grange, R.W., C.R. Cory, R. Vandenboom, and M.E. Houston. 1995. Myosin phosphorylation augments force-displacement and force-velocity relationships of mouse fast muscle. *Am. J. Physiol.* 269:C713–C724.
- Herron, T.J., F.S. Korte, and K.S. McDonald. 2001. Loaded shortening and power output in cardiac myocytes are dependent on myosin heavy chain isoform expression. *Am. J. Physiol. Heart Circ. Physiol.* 281:H1217–H1222.
- Hill, A.V. 1938. The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc. Lond. B Biol. Sci.* 126:136–195. <http://dx.doi.org/10.1098/rspb.1938.0050>
- Hill, A.V. 1964. The effect of load on the heat of shortening of muscle. *Proc. R. Soc. Lond. B Biol. Sci.* 159:297–318. <http://dx.doi.org/10.1098/rspb.1964.0004>
- Huxley, A.F. 1957. Muscle structure and theories of contraction. *Prog. Biophys. Biophys. Chem.* 7:255–318.
- Huxley, A.F., and R.M. Simmons. 1971. Proposed mechanism of force generation in striated muscle. *Nature.* 233:533–538. <http://dx.doi.org/10.1038/233533a0>
- Johnston, I.A., and J. Salamonski. 1984. Power output and force-velocity relationship of red and white muscle fibres from the Pacific blue marlin (*Makaira nigricans*). *J. Exp. Biol.* 111:171–177.
- Johnston, I.A., and B.D. Sidell. 1984. Differences in temperature dependence of muscle contractile properties and myofibrillar ATPase activity in a cold-temperature fish. *J. Exp. Biol.* 111:179–189.
- Jones, D.A. 2010. Changes in the force-velocity relationship of fatigued muscle: implications for power production and possible

- causes. *J. Physiol.* 588:2977–2986. <http://dx.doi.org/10.1113/jphysiol.2010.190934>
- Jones, D.A., C.J. de Ruiter, and A. de Haan. 2006. Change in contractile properties of human muscle in relationship to the loss of power and slowing of relaxation seen with fatigue. *J. Physiol.* 576:913–922. <http://dx.doi.org/10.1113/jphysiol.2006.116343>
- Julian, F.J., L.C. Rome, D.G. Stephenson, and S. Striz. 1986. The maximum speed of shortening in living and skinned frog muscle fibres. *J. Physiol.* 370:181–199.
- Karatzafieri, C., K. Franks-Skiba, and R. Cooke. 2008. Inhibition of shortening velocity of skinned skeletal muscle fibers in conditions that mimic fatigue. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 294:R948–R955. <http://dx.doi.org/10.1152/ajpregu.00541.2007>
- Katz, B. 1939. The relation between force and speed in muscular contraction. *J. Physiol.* 96:45–64.
- Knuth, S.T., H. Dave, J.R. Peters, and R.H. Fitts. 2006. Low cell pH depresses peak power in rat skeletal muscle fibres at both 30 degrees C and 15 degrees C: implications for muscle fatigue. *J. Physiol.* 575:887–899. <http://dx.doi.org/10.1113/jphysiol.2006.106732>
- Landesberg, A., and S. Sideman. 2000. Force-velocity relationship and biochemical-to-mechanical energy conversion by the sarcomere. *Am. J. Physiol. Heart Circ. Physiol.* 278:H1274–H1284.
- Langeron, O., C. Coirault, S. Frate, G. Orliaguet, P. Coriat, and B. Riou. 1999. The effects of dantrolene on the contraction, relaxation, and energetics of the diaphragm muscle. *Anesth. Analg.* 89:466–471.
- Lännergren, J. 1978. The force-velocity relation of isolated twitch and slow muscle fibres of *Xenopus laevis*. *J. Physiol.* 283:501–521.
- Lännergren, J., P. Lindblom, and B. Johansson. 1982. Contractile properties of two varieties of twitch muscle fibres in *Xenopus laevis*. *Acta Physiol. Scand.* 114:523–535. <http://dx.doi.org/10.1111/j.1748-1716.1982.tb07020.x>
- Levine, R.J., R.W. Kensler, Z. Yang, J.T. Stull, and H.L. Sweeney. 1996. Myosin light chain phosphorylation affects the structure of rabbit skeletal muscle thick filaments. *Biophys. J.* 71:898–907. [http://dx.doi.org/10.1016/S0006-3495\(96\)79293-7](http://dx.doi.org/10.1016/S0006-3495(96)79293-7)
- Linari, M., M. Caremani, and V. Lombardi. 2010. A kinetic model that explains the effect of inorganic phosphate on the mechanics and energetics of isometric contraction of fast skeletal muscle. *Proc. Biol. Sci.* 277:19–27. <http://dx.doi.org/10.1098/rspb.2009.1498>
- Luff, A.R. 1981. Dynamic properties of the inferior rectus, extensor digitorum longus, diaphragm and soleus muscles of the mouse. *J. Physiol.* 313:161–171.
- Månsson, A. 2010. Actomyosin-ADP states, interhead cooperativity, and the force-velocity relation of skeletal muscle. *Biophys. J.* 98:1237–1246. <http://dx.doi.org/10.1016/j.bpj.2009.12.4285>
- Metzger, J.M., M.L. Greaser, and R.L. Moss. 1989. Variations in cross-bridge attachment rate and tension with phosphorylation of myosin in mammalian skinned skeletal muscle fibers. Implications for twitch potentiation in intact muscle. *J. Gen. Physiol.* 93:855–883. <http://dx.doi.org/10.1085/jgp.93.5.855>
- Moss, R.L., G.M. Diffie, and M.L. Greaser. 1995. Contractile properties of skeletal muscle fibers in relation to myofibrillar protein isoforms. *Rev. Physiol. Biochem. Pharmacol.* 126:1–63. <http://dx.doi.org/10.1007/BFb0049775>
- Negróni, J.A., and E.C. Lascano. 2008. Simulation of steady state and transient cardiac muscle response experiments with a Huxley-based contraction model. *J. Mol. Cell. Cardiol.* 45:300–312. <http://dx.doi.org/10.1016/j.yjmcc.2008.04.012>
- Nosek, T.M., K.Y. Fender, and R.E. Godt. 1987. It is diprotonated inorganic phosphate that depresses force in skinned skeletal muscle fibers. *Science.* 236:191–193. <http://dx.doi.org/10.1126/science.3563496>
- Pate, E., and R. Cooke. 1989. A model of crossbridge action: the effects of ATP, ADP and Pi. *J. Muscle Res. Cell Motil.* 10:181–196. <http://dx.doi.org/10.1007/BF01739809>
- Pellegrino, M.A., M. Canepari, R. Rossi, G. D'Antona, C. Reggiani, and R. Bottinelli. 2003. Orthologous myosin isoforms and scaling of shortening velocity with body size in mouse, rat, rabbit and human muscles. *J. Physiol.* 546:677–689. <http://dx.doi.org/10.1113/jphysiol.2002.027375>
- Persechini, A., J.T. Stull, and R. Cooke. 1985. The effect of myosin phosphorylation on the contractile properties of skinned rabbit skeletal muscle fibers. *J. Biol. Chem.* 260:7951–7954.
- Piazzesi, G., and V. Lombardi. 1995. A cross-bridge model that is able to explain mechanical and energetic properties of shortening muscle. *Biophys. J.* 68:1966–1979. [http://dx.doi.org/10.1016/S0006-3495\(95\)80374-7](http://dx.doi.org/10.1016/S0006-3495(95)80374-7)
- Piazzesi, G., M. Reconditi, M. Linari, L. Lucii, P. Bianco, E. Brunello, V. Decostre, A. Stewart, D.B. Gore, T.C. Irving, et al. 2007. Skeletal muscle performance determined by modulation of number of myosin motors rather than motor force or stroke size. *Cell.* 131:784–795. <http://dx.doi.org/10.1016/j.cell.2007.09.045>
- Potma, E.J., I.A. van Graas, and G.J. Stienen. 1995. Influence of inorganic phosphate and pH on ATP utilization in fast and slow skeletal muscle fibers. *Biophys. J.* 69:2580–2589. [http://dx.doi.org/10.1016/S0006-3495\(95\)80129-3](http://dx.doi.org/10.1016/S0006-3495(95)80129-3)
- Ranatunga, K.W. 1982. Temperature-dependence of shortening velocity and rate of isometric tension development in rat skeletal muscle. *J. Physiol.* 329:465–483.
- Ranatunga, K.W. 1984. The force-velocity relation of rat fast- and slow-twitch muscles examined at different temperatures. *J. Physiol.* 351:517–529.
- Rayment, I., H.M. Holden, M. Whittaker, C.B. Yohn, M. Lorenz, K.C. Holmes, and R.A. Milligan. 1993. Structure of the actin-myosin complex and its implications for muscle contraction. *Science.* 261:58–65. <http://dx.doi.org/10.1126/science.8316858>
- Schiaffino, S., and C. Reggiani. 1996. Molecular diversity of myofibrillar proteins: gene regulation and functional significance. *Physiol. Rev.* 76:371–423.
- Seow, C.Y., and L.E. Ford. 1991. Shortening velocity and power output of skinned muscle fibers from mammals having a 25,000-fold range of body mass. *J. Gen. Physiol.* 97:541–560. <http://dx.doi.org/10.1085/jgp.97.3.541>
- Seow, C.Y., and L.E. Ford. 1992. Contribution of damped passive recoil to the measured shortening velocity of skinned rabbit and sheep muscle fibres. *J. Muscle Res. Cell Motil.* 13:295–307. <http://dx.doi.org/10.1007/BF01766457>
- Seow, C.Y., and L.E. Ford. 1993. High ionic strength and low pH detain activated skinned rabbit skeletal muscle crossbridges in a low force state. *J. Gen. Physiol.* 101:487–511. <http://dx.doi.org/10.1085/jgp.101.4.487>
- Seow, C.Y., and L.E. Ford. 1997. Exchange of ATP for ADP on high-force cross-bridges of skinned rabbit muscle fibers. *Biophys. J.* 72:2719–2735. [http://dx.doi.org/10.1016/S0006-3495\(97\)78915-X](http://dx.doi.org/10.1016/S0006-3495(97)78915-X)
- Siemankowski, R.F., M.O. Wiseman, and H.D. White. 1985. ADP dissociation from actomyosin subfragment 1 is sufficiently slow to limit the unloaded shortening velocity in vertebrate muscle. *Proc. Natl. Acad. Sci. USA.* 82:658–662. <http://dx.doi.org/10.1073/pnas.82.3.658>
- Slawnych, M.P., C.Y. Seow, A.F. Huxley, and L.E. Ford. 1994. A program for developing a comprehensive mathematical description of the crossbridge cycle of muscle. *Biophys. J.* 67:1669–1677. [http://dx.doi.org/10.1016/S0006-3495\(94\)80639-3](http://dx.doi.org/10.1016/S0006-3495(94)80639-3)
- Smith, D.A., M.A. Geeves, J. Sleep, and S.M. Mijailovich. 2008. Towards a unified theory of muscle contraction. I: Foundations. *Ann. Biomed. Eng.* 36:1624–1640. <http://dx.doi.org/10.1007/s10439-008-9536-6>
- Stienen, G.J.M., W.J. van der Laarse, and G. Elzinga. 1988. Dependency of the force-velocity relationships on Mg ATP in different types of muscle fibers from *Xenopus laevis*. *Biophys. J.* 53:849–855. [http://dx.doi.org/10.1016/S0006-3495\(88\)83165-5](http://dx.doi.org/10.1016/S0006-3495(88)83165-5)

- Sweeney, H.L., and J.T. Stull. 1986. Phosphorylation of myosin in permeabilized mammalian cardiac and skeletal muscle cells. *Am. J. Physiol.* 250:C657–C660.
- Sweeney, H.L., and J.T. Stull. 1990. Alteration of cross-bridge kinetics by myosin light chain phosphorylation in rabbit skeletal muscle: implications for regulation of actin-myosin interaction. *Proc. Natl. Acad. Sci. USA.* 87:414–418. <http://dx.doi.org/10.1073/pnas.87.1.414>
- Wahr, P.A., and J.M. Metzger. 1998. Peak power output is maintained in rabbit psoas and rat soleus single muscle fibers when CTP replaces ATP. *J. Appl. Physiol.* 85:76–83.
- Wakayama, J., and T. Yamada. 2000. Contractility of single myofibrils of rabbit skeletal muscle studied at various MgATP concentrations. *Jpn. J. Physiol.* 50:533–542. <http://dx.doi.org/10.2170/jjphysiol.50.533>
- Wang, J., H. Jiang, and N.L. Stephens. 1994. A modified force-velocity equation for smooth muscle contraction. *J. Appl. Physiol.* 76:253–258.
- Widrick, J.J., J.G. Romatowski, M. Karhanek, and R.H. Fitts. 1997. Contractile properties of rat, rhesus monkey, and human type I muscle fibers. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 272:R34–R42.
- Wilson, J.R., K.K. McCully, D.M. Mancini, B. Boden, and B. Chance. 1988. Relationship of muscular fatigue to pH and di-protonated Pi in humans: a ³¹P-NMR study. *J. Appl. Physiol.* 64:2333–2339.
- Woledge, R.C. 1968. The energetics of tortoise muscle. *J. Physiol.* 197:685–707.
- Woledge, R.C., N.A. Curtin, and E. Homsher. 1985. Mechanics of contraction. In *Energetic Aspects of Muscle Contraction*. Academic Press, London. 47–71.
- Yang, Z., J.T. Stull, R.J. Levine, and H.L. Sweeney. 1998. Changes in interfilament spacing mimic the effects of myosin regulatory light chain phosphorylation in rabbit psoas fibers. *J. Struct. Biol.* 122:139–148. <http://dx.doi.org/10.1006/jsbi.1998.3979>
- Zhao, Y., and M. Kawai. 1994. Kinetic and thermodynamic studies of the cross-bridge cycle in rabbit psoas muscle fibers. *Biophys. J.* 67:1655–1668. [http://dx.doi.org/10.1016/S0006-3495\(94\)80638-1](http://dx.doi.org/10.1016/S0006-3495(94)80638-1)