THERMAL DEPENDENCE OF CONTRACTILE PROPERTIES OF SKELETAL MUSCLE FROM THE LIZARD *SCELOPORUS OCCIDENTALIS* WITH COMMENTS ON METHODS FOR FITTING AND COMPARING FORCE–VELOCITY CURVES

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SUMMARY

The isometric and isotonic contractile properties of fast-twitch glycolytic fibres of the iliofibularis muscle (FG–IF) in the lizard *Sceloporus occidentalis* were measured *in vitro* at 5°C intervals from 10 to 40°C. The mean isometric parameters at 35°C, the preferred body temperature of this species, were as follows: maximum isometric force (P_0), 187 ± 8 (S.E.M.) $kN\,m^{-2}$; ratio of twitch force to tetanic force (P_{TW}/P_0), 0.46 ± 0.02; time to peak twitch tension (tP_{TW}), 7.0 ± 0.3 ms; and time from peak twitch force to 50% relaxation (t50%R), 8.2 ± 0.3 ms. From 20 to 35°C P_0 was almost constant (within 8% of the value at 35°C). At 10 and 15°C P_0 dropped to approximately 80% of the value at 35°C. P_0 was very unstable at 40°C. P_{TW}/P_0 was almost constant at all temperatures. The time-related isometric parameters were positively modified by temperature at all temperatures measured ($Q_{10} > 1.9$).

The force–velocity curves of the FG–IF deviated from the simple hyperbolic relation of A. V. Hill's characteristic equation. We present two alternative equations for fitting these data. These equations resulted in residual sums of squares from nonlinear least-squares analysis that were at least seven-fold lower than those from Hill's equation. The equation that best describes our data is a hyperbola modified by the addition of a linear component:

$$V = B(1 - P/P_0)/(A + P/P_0) + C(1 - P/P_0).$$

To describe the curvature of this or any other force–velocity relationship, we propose the power ratio, $\dot{W}_{max}/V_{max}P_0$ (where $\dot{W}_{max}$ is the maximum power calculated from the force–velocity relationship and V_{max} is the predicted maximum velocity of shortening at zero force).

V_{max} of the FG–IF was $21.9\,L_0\,s^{-1}$ at 35°C (where L_0 is muscle length). This parameter was directly related to temperature between 10 and 35°C with $Q_{10} > 1.8$. The shape of the force–velocity curve is not influenced by temperature ($\dot{W}_{max}/V_{max}P_0 = 0.11$).

INTRODUCTION

Temperature has profound effects on the time-dependent contractile properties of skeletal muscle (Bennett, 1984, 1985). Thus, the organismal performance of ectothermic animals is also expected to be greatly influenced by body temperature, and it has been assumed that this influence has been one of the evolutionary forces resulting in accurate behavioural thermoregulation by these animals (Dawson, 1975). Contrary to simple predictions, recent studies have found that the burst running performance of lizards may show a rather low thermal dependence over a broad range of body temperatures (Bennett, 1980; Christian & Tracy, 1981; Hertz, Huey & Nevo, 1982, 1983; Marsh & Bennett, 1985, 1986). Studies of the isolated muscles from these animals have not revealed any exceptional properties that would explain the low temperature dependence of the behavioural measures (Putnam & Bennett, 1982; Marsh & Bennett, 1985). However, Marsh & Bennett (1985), working with the iguanid lizard *Dipsosaurus dorsalis*, did suggest that the lower limit of the zone of low thermal dependence may be determined by temperature effects on the twitch properties of the muscles. The present study was undertaken to test this hypothesis by examining the thermal effects on contractile properties of fast-glycolytic fibres from another species of small iguanid, *Sceloporus occidentalis*. These measurements were performed in conjunction with a study on the effects of temperature on running performance of the same species (Marsh & Bennett, 1986).

As in our previous study on the fast-glycolytic fibres of *Dipsosaurus*, we found that the simple hyperbolic equation of Hill (the 'characteristic equation'; Hill, 1938) did not adequately describe our data on isotonic contractile properties of these fibres from *Sceloporus*. Numerous other researchers have also found deviations from the hyperbolic relationship of Hill (Ritchie & Wilkie, 1958; Allen & Stainsby, 1973; Close & Luff, 1974; Cecchi, Colomo & Lombardi, 1978; Edman, Mulieri & Scubon-Mulieri, 1976; Lännergren, 1978; Rome, 1983). These investigations suggest that most vertebrate fast-twitch fibres display nonhyperbolic force–velocity curves, particularly at very high and very low relative forces. Because comparative studies depend on accurate descriptions of the data, we present here more fully the rationale and procedures for arriving at equations to describe our data and for comparing the shapes of the fitted curves.

MATERIALS AND METHODS

Animals

Adult *Sceloporus occidentalis* of both sexes were collected in Orange County, CA, under a California scientific collector's permit to AFB and maintained in the laboratory for less than 1 week before use. Prior to the experiments they were maintained in cages allowing behavioural thermoregulation and fed crickets and mealworms *ad libitum*. Animals were collected from July to October and had a mean body mass of $13 \cdot 7 \pm 1 \cdot 0$ g and snout–vent length of $70 \cdot 0 \pm 1 \cdot 3$ mm (mean $\pm$ s.e.m., $N = 14$).

Contractile properties

Contractile properties of the fast-glycolytic (white) region of the iliofibularis (FG–IF) muscle were measured with methods very similar to those described in detail in Marsh & Bennett (1985). In *S. occidentalis* the IF muscle of most animals does not have such visually distinct red and white regions as are found in *Dipsosaurus dorsalis* (Gleeson, Putnam & Bennett, 1980). Nevertheless, in preliminary experiments with intact muscles, we identified significant numbers of tonic fibres by a slow decline in force after isometric contractions and the presence of a contracture response to acetylcholine. For all preparations reported here, we split the muscle and used the superficial portion, as was done previously for *Dipsosaurus* (Marsh & Bennett, 1985). The resulting preparations were composed almost exclusively of FG fibres and were free of any detectable tonic fibres, as judged by the application of acetylcholine. The fibre bundles were arranged in a thermostatted bath of Ringer solution (in $mmol\,l^{-1}$: NaCl, 145; KCl, 4; imidazole, 20; $CaCl_2$, 2·5; glucose, 11) saturated with 100 % oxygen. The pelvis was tied to a rigid stainless-steel rod and the distal end of the muscle attached *via* a light silver chain to the transducer arm of a Cambridge Technology Model 300-H servo-controlled ergometer. Force, position and velocity outputs from the ergometer were monitored with a Tektronix Model 5111 storage oscilloscope.

Muscles were stimulated *via* parallel platinum plate electrodes with 0·2-ms pulses produced by a Grass S-44 stimulator and amplified with a d.c.-coupled power amplifier. Stimulus frequency during tetanic contractions varied from 50 Hz at 10 °C to 400 Hz at 40 °C. In all cases, muscles were first placed in the chamber at 35 °C and stimulus voltage and length were adjusted to give maximal force during isometric tetanic contractions. The maximum isometric force during tetanic contractions (P_0) tended to increase following the placement of the muscles in the bath, presumably due to recovery from the dissection procedure. Muscles were initially held at 35 °C and contractions were recorded approximately every 15 min until no increase in P_0 was noted between subsequent contractions. After twitch and tetanic contractions had been measured at 35 °C, the temperature was switched to the experimental temperature and a series of both isometric and after-loaded isotonic tetanic contractions were recorded. Following this series of measurements, most preparations were switched to a second experimental temperature and another series of contractions recorded. P_0 was recorded before, in the middle of, and after each series of 8–12 isotonic contractions. The force during the isotonic contractions is reported as a fraction of P_0, assuming that the change in force was linear between isometric contractions. The twitch parameters reported are for twitches potentiated by a previous tetanus.

Following the contractile measurements, the muscle length (L_0) was measured in place. The muscle was then removed, damaged fibres were dissected away from the intact fibre bundle, the pelvis and distal tendon were removed, and the remaining bundle of fibres was blotted and weighed. The bundles had a mean mass of

16.8 ± 1.4 mg and a length of 14.0 ± 0.3 mm ($N = 14$). The cross-sectional area of each bundle was calculated by dividing the mass of the muscle by the muscle length.

Curve-fitting and statistics

One common strategy in dealing with deviations from Hill's characteristic equation has been to continue to use the equation but to ignore, or not report, a portion of the data at high levels of force (e.g. Lännergren, 1978; Fitts *et al.* 1980; Spector *et al.* 1980; Rome, 1983). The reason for retaining Hill's equation in these cases (although this reason is not often explicitly stated) appears to be a desire to use the fitted constants to compare different muscles. However, this potential benefit is obviated if significant parts of the data are not described well by the fitted curve. Modifying the curve by including P_0 as a fitted value (Edman *et al.* 1976) does not correct this situation because the constants from this modified equation cannot be compared meaningfully to those from Hill's equation, in which P_0 is the measured value. Recent theoretical discussions of the contractile process have become increasingly complex and no clear consensus has emerged in favour of any particular force–velocity equation (e.g. Hill, Eisenberg, Chen & Podolsky, 1975; Morel, 1978; Wood & Mann, 1981; Eisenberg & Hill, 1985). As a result, investigators are left in the uncomfortable situation of choosing from among an almost infinite number of multi-parameter equations, all of which could describe their data (Riggs, 1970). However, the law of parsimony dictates that the number of constants be kept to the minimum necessary to describe the data. We tried a number of alternative equations to describe our data, including the equations of Hill (1938), Polissar (1952), Close & Luff (1974) and Morel (1978). All three constants in Polissar's equation were found to be highly correlated when nonlinear least-squares procedures were applied. Consequently, the equation was difficult to fit accurately and will not be considered further. The six-parameter equation of Close & Luff was found to contain a number of redundant constants, as judged by the correlation matrix resulting from the nonlinear procedures. By reducing the number of constants we arrived at equation 1 below. We would like to note in particular that we rejected the use of a hyperbola, in which P_0 is a fitted value, such as has been suggested by Edman *et al.* (1976). The use of this modification of Hill's equation necessitates a rather arbitrary truncation of the data and results in an equation which extrapolates to zero velocity at very high levels of force.

Two three-parameter equations accurately describe our data on lizard muscle and will be compared below with each other and with the equations of Hill (1938) and Morel (1978). The first of these is the exponential–linear equation (EXP–LIN) which we used in a previous study (Marsh & Bennett, 1985):

$$V = \alpha e^{-\beta P/P_0} + \gamma(1 - P/P_0), \tag{1}$$

where V is velocity of shortening in $L_0 s^{-1}$, P/P_0 is force as a fraction of maximum isometric force, α and γ are constants with dimensions of velocity and β is

a dimensionless constant. The second equation is a hyperbolic–linear equation (HYP–LIN):

$$V = \frac{B(1 - P/P_0)}{A + P/P_0} + C(1 - P/P_0),\qquad(2)$$

where V and P/P_0 are as above, B and C have dimensions of velocity, and A is dimensionless.

To compare the fit of alternative equations, we employed iterative, nonlinear curve-fitting procedures. These techniques are necessary because all the equations used are intrinsically nonlinear (Draper & Smith, 1981). It is worthwhile emphasizing that the correct application of Hill's equation also requires an iterative procedure to arrive at a statistically valid fit to the data. Commonly, force–velocity data have been linearized by plotting $(P_0-P)/V$ *versus* P and the constants of Hill's equation derived from this plot (e.g. Lännergren, 1978; Fitts *et al.* 1980; Rome, 1983). Statistical procedures cannot be validly applied to these transformed data because the y-variable contains elements of the x-variable. Iterative procedures avoid these problems by calculating a least-squares regression on the untransformed data. Other authors have applied iterative methods to fit their data to Hill's equation (Edman *et al.* 1976; Binkhorst, Hoofd & Vissers, 1977; Cecchi *et al.* 1978; van Mastrigt, 1980).

We employed the iterative, nonlinear curve-fitting program NLLSQ, which is an adaptation of the Marquardt method developed for use on the Apple II series microcomputers and available from CET Research Group, Ltd, Norman, OK. For a general discussion of nonlinear methods see Kennedy & Gentle (1980) and Draper & Smith (1981). A disadvantage of nonlinear methods is the lack of a well-developed body of statistical theory such as that found for linear models. However, the programs available do generate estimates of the standard errors of the parameters. These estimates and the parameter correlation matrix can be used along with plots of the residuals as guides to the adequacy of the models used (Draper & Smith, 1981).

In describing the force–velocity data in the present study, we have applied the curve-fitting procedure to the data on individual muscles and then arrived at average parameter values by taking the mean of values determined from the individual muscles. This procedure is appropriate because it correctly reflects the degree of variability among muscles from different animals. For an individual muscle, all of the data points can be described with very little error, given an appropriate equation (see below).

Simple F-tests are not valid for testing the significance of differences between alternative models fitted by nonlinear least squares to a single data set. Consequently, we have compared the equations discussed above by testing whether a significant difference exists between the residual mean squares resulting from fitting a series of curves. The residual mean squares were calculated as the residual sums of squares divided by the degrees of freedom, where the degrees of freedom in each case are determined by the number of data points minus the number of constants in the

model. After calculating the residual mean squares, we applied both paired t-tests and sign tests to determine significant differences.

All means are presented ± 1 S.E.M.

RESULTS

Isometric contractile properties

The thermal dependence of isometric contractile properties is reported in Table 1. All preparations of the FG–IF were first equilibrated at 35 °C. The mean maximum isometric tetanic tension (P_0) at this temperature was $187\cdot6 \pm 8\cdot4\,\mathrm{kN\,m^{-2}}$. When the temperature was increased to 40 °C, the isometric tension was not stable in most cases. The values in Table 1 for this temperature are for two preparations that remained viable long enough for measurements to be made. After approximately 15 min at 40 °C P_0 had dropped to 72 % of the value at 35 °C (Table 1), and subsequently continued to decline rapidly. At all other temperatures P_0 was stable (within 10 %) after a short period of equilibration at the new temperature. At temperatures of 30 °C and below the measured P_0 values showed significant variation (analysis of variance, ANOVA, $F = 4\cdot25$, $P < 0\cdot025$). Application of the T-method for multiple comparisons (Sokal & Rohlf, 1981) indicated that for pairwise comparisons only the values at 15 and 30 °C were significantly different (MSD $= 0\cdot24$, $P = 0\cdot05$). The twitch to tetanic tension ratios ranged from $0\cdot42$ to $0\cdot52$ and were not significantly different at the various temperatures (ANOVA, $F = 0\cdot34$, $P > 0\cdot75$).

The time-related parameters of twitch contractions were affected by temperature throughout the range of temperatures studied (Table 1). Both the time from onset to peak tension ($\mathrm{tP_{TW}}$) and the 50 % relaxation time (t50%R) showed a similar thermal dependence (Fig. 1). The Q_{10} values for the temperature range from 10 to 25 °C were

Table 1. *Isometric contractile properties of the fast-twitch glycolytic region of the iliofibularis muscle of* Sceloporus occidentalis

T_M (°C)	$P_0/P_{0,35C}$	P_{TW}/P_0	$\mathrm{tP_{TW}}$ (ms)	t50%R (ms)	N
40	0·72	0·52	5·53	5·58	2
35	1·0	0·46 ± 0·02	6·99 ± 0·30	8·17 ± 0·33	13
30	1·02 ± 0·04	0·42 ± 0·07	10·00 ± 0·54	11·50 ± 0·79	4
25	0·98 ± 0·02	0·46 ± 0·04	13·25 ± 1·20	15·38 ± 1·14	4
20	0·92 ± 0·43	0·47 ± 0·03	21·50 ± 3·97	29·13 ± 4·60	4
15	0·76 ± 0·10	0·52 ± 0·05	35·25 ± 1·89	41·50 ± 2·87	4
10	0·79 ± 0·04	0·49 ± 0·12	52·50 ± 5·74	72·00 ± 6·93	4

T_M, muscle temperature.

$P_0/P_{0,35C}$, maximal isometric tension in a tetanic contraction expressed as a fraction of the maximal isometric tension in the same muscle preparations at 35 °C. Absolute tension at 35 °C was $187\cdot6 \pm 8\cdot4\,\mathrm{kN\,m^{-2}}$.

P_{TW}/P_0, ratio of twitch to tetanic tension.

$\mathrm{tP_{TW}}$, time from onset to peak tension in a twitch.

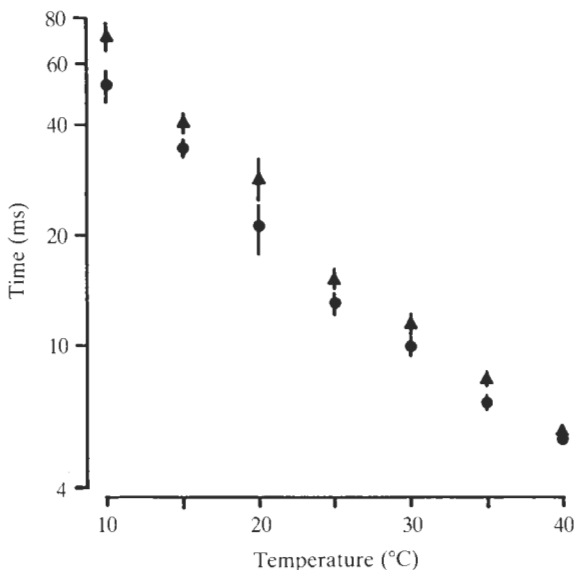t50%R, time from the peak tension in a twitch to 50 % relaxation.

N, sample size.

Fig. 1. Relationship between the time-related parameters of twitch contractions [tP$_{TW}$, time from onset to peak tension in a twitch (●); t50%R, time from peak tension in a twitch to 50% relaxation (▲)] and temperature of the fast-twitch glycolytic region of the iliofibularis muscle of the lizard *Sceloporus occidentalis*. Vertical scale is logarithmic.

2·5 and 2·8 for $1/tP_{TW}$ and $1/t50\%R$, respectively; from 25 to 35°C these values were 1·89 and 1·88, respectively.

Isotonic contractile properties

As described in Materials and Methods, we used nonlinear curve-fitting procedures to describe the force–velocity data and compare the fit of several equations. The use of either equation 1 or 2 resulted in a dramatic reduction of the residual sums of squares (RSS) in comparison with Hill's equation (Table 2). Adding the RSS from fitting all 24 curves at six temperatures gave values of 25·6, 5·57, 3·59 and 3·11 for Hill's equation, Morel's equation, equation 1 and equation 2, respectively. The superiority of the fit of the alternative equations when compared to Hill's equation can be seen in Fig. 2, which shows data from one of the muscles measured at 35°C. Using paired *t*-tests or sign tests (see Materials and Methods), the residual mean squares for equations 1 and 2 were found to be significantly less than those for the equations of Hill or Morel ($P < 0.01$). Equations 1 and 2 were not found to be significantly different on this basis.

Two considerations led us to favour equation 2 (HYP–LIN) over equation 1 (EXP–LIN) for describing the data in the present study. First, its use resulted in an average 15% reduction in the residual sums of squares when compared to equation 1. Second, equation 2 predicts a more consistent V_{max} in the absence of data at very low forces. We compared the prediction of V_{max} using all the data collected with the prediction of this parameter when the data at forces below 0·04P$_0$ were excluded from the statistical analysis. In every case, excluding the data at low forces resulted in

equation 1 predicting a lower V_{max} than the same equation applied to all of the data. The average difference in this case was $5 \cdot 4 \%$ and was statistically significant (paired t-test, $N = 22$, $t = 7 \cdot 30$, $P < 0 \cdot 001$). In contrast, equation 2 predicted nearly the same V_{max} with or without the data collected at forces below $0 \cdot 04 P_0$; the average difference was $1 \cdot 3 \%$ and was not statistically significant (paired t-test, $N = 22$, $t = 0 \cdot 88$, $P > 0 \cdot 5$). Therefore, it appears that equation 2 more accurately predicts the shape of the present data set at low forces, a point of particular significance in estimating the maximal velocity of shortening (V_{max}).

The force–velocity curves of the FG–IF were influenced by temperature throughout the range of temperatures studied. Fig. 3 shows representative data obtained at three temperatures. The mean constants from equation 2 for each temperature are given in Table 3 along with the derived parameters. Except for minor variations, which are probably due to sampling errors, the predicted maximal V_{max} has a

Table 2. *Residual sums of squares for force–velocity data described with four different equations using nonlinear least-squares procedures*

T_M (°C)	Muscle no.	N	Residual sums of squares			
			EXP–LIN*	HYP–LIN†	Hill‡	Morel§
35	13	9	0·294	0·211	2·270	0·591
	2	8	0·195	0·212	2·617	0·292
	8	8	0·211	0·201	2·585	0·424
	4	8	0·507	0·436	3·873	0·664
30	6	8	0·106	0·066	1·204	0·234
	7	8	0·900	1·021	1·690	0·801
	9	8	0·064	0·026	2·585	0·238
	8	8	0·289	0·175	1·616	0·379
25	4	8	0·113	0·063	1·155	0·196
	3	8	0·174	0·284	0·984	0·260
	1	8	0·080	0·041	0·538	0·203
	2	8	0·054	0·057	1·133	0·167
20	12	8	0·073	0·053	0·609	0·110
	9	9	0·239	0·093	0·686	0·363
	10	8	0·038	0·009	0·653	0·126
	6	8	0·037	0·017	0·229	0·104
15	1	6	0·008	0·005	0·019	0·047
	11	8	0·050	0·034	0·205	0·044
	5	8	0·017	0·005	0·268	0·057
	10	8	0·055	0·008	0·366	0·106
10	5	8	0·005	0·011	0·092	0·007
	12	8	0·020	0·029	0·132	0·047
	11	8	0·042	0·043	0·048	0·079
	3	8	0·018	0·010	0·078	0·028

* $V = \alpha e^{-\beta P/P_0} + \gamma(1 - P/P_0)$.
† $V = B(1 - P/P_0)/(A + P/P_0) + C(1 - P/P_0)$.
‡ $V = b(1 - P/P_0)/(a + P/P_0)$.
§ $V = K/\ln[(1 + a)/(1 - P/P_0)]$.
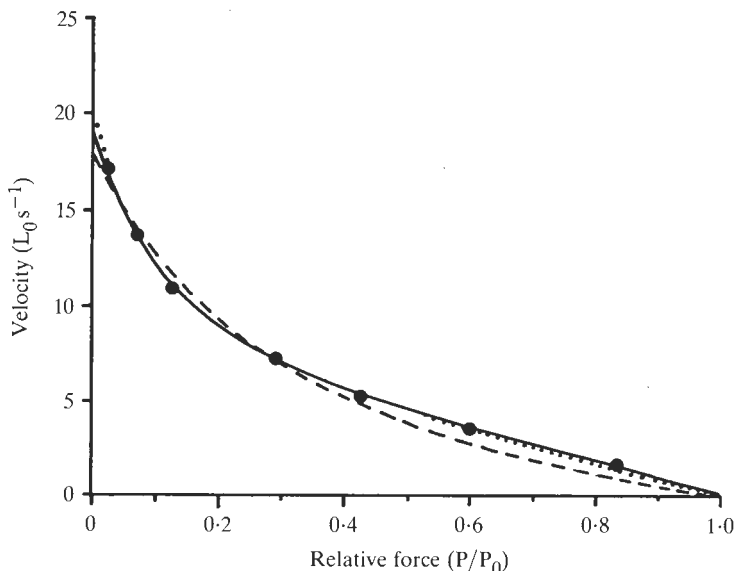T_M, muscle temperature.

Fig. 2. Representative force–velocity relationship from the fast-twitch region of the ilio-fibularis muscle of the lizard *Sceloporus occidentalis*. Data were obtained from a single muscle at 35 °C and fitted by nonlinear least squares to three equations: —— = equation 2 (HYP–LIN); · · · · = equation 1 (EXP–LIN); − − − = Hill's characteristic equation. The residual sums of squares for these three equations fitted to the data shown were 0·201, 0·211 and 2·585, respectively. See Materials and Methods for a description of the alternative equations and the fitting procedure.
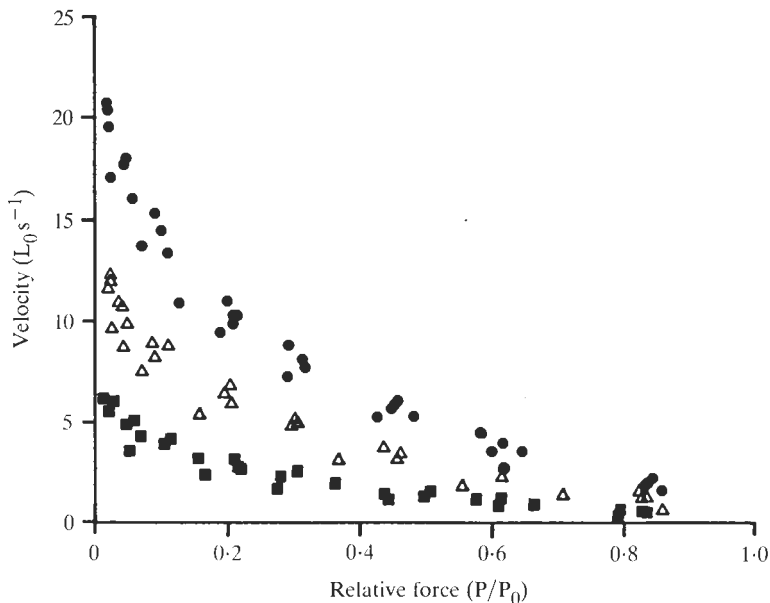

Fig. 3. Force–velocity data from the fast-twitch glycolytic region of the iliofibularis muscle of the lizard *Sceloporus occidentalis* obtained from four muscles at each of three temperatures (● = 35 °C, △ = 25 °C, ■ = 15 °C).

constant thermal dependence ($Q_{10} = 1.80$) from 15 to 35 °C (Fig. 4). The shape of the force–velocity curve, as indicated by the power ratio $\dot{W}_{max}/V_{max}P_0$ (see Discussion), is not influenced by temperature (Table 3).

DISCUSSION

Fitting force–velocity curves

The alternative equations used in this study and in our previous paper on *Dipsosaurus* (Marsh & Bennett, 1985) offer a clear improvement over Hill's characteristic equation in the accuracy with which the fitted curves describe the force–velocity data (Fig. 2). Nevertheless, in the absence of a theoretical justification for these alternative equations, a natural conservatism may lead investigators to continue to use Hill's equation. However, we feel that the use of an accurate, empirically based description of force–velocity data has important consequences for our understanding of the function of skeletal muscle, particularly in the context of attempts to relate *in vitro* contractile properties to *in vivo* function.

A number of studies of vertebrate fast-twitch muscle have noted that at high relative forces the measured velocities are higher than those predicted from Hill's equation (Ritchie & Wilkie, 1958; Cecchi *et al.* 1978; Edman *et al.* 1976; Lännergren, 1978; Rome, 1983; Marsh & Bennett, 1985, and this study). The practice of truncating the high-force sections of the curve (see Fitts *et al.* 1980; Spector *et al.* 1980) makes it difficult to judge the frequency of this deviation among the various vertebrate muscles studied. However, sufficient data exist to indicate that it is a common, if not general, phenomenon. Greater velocity at high forces results in higher predicted power output. For example, the power output of *Sceloporus* FG–IF at $0.8P_0$ is 39% higher than the value predicted by Hill's equation. Additionally, the relative force at which maximum power is produced is

Table 3. *Force–velocity relationships of the fast-twitch glycolytic region of the iliofibularis muscle of* Sceloporus occidentalis

T_M (°C)	A	B ($L_0 s^{-1}$)	C ($L_0 s^{-1}$)	V_{max} ($L_0 s^{-1}$)	$\dot{W}_{max}/V_{max}P_0$
35	0.113 ± 0.010	1.64 ± 0.20	7.52 ± 0.36	21.9 ± 0.6	0.118 ± 0.002
30	0.116 ± 0.016	1.22 ± 0.27	5.26 ± 0.29	15.5 ± 0.6	0.119 ± 0.003
25	0.116 ± 0.012	1.02 ± 0.12	4.21 ± 0.48	12.9 ± 0.5	0.114 ± 0.007
20	0.104 ± 0.022	0.642 ± 0.120	3.15 ± 0.19	9.41 ± 0.29	0.114 ± 0.005
15	0.098 ± 0.007	0.458 ± 0.027	2.12 ± 0.18	6.79 ± 0.26	0.107 ± 0.003
10	0.131 ± 0.034	0.381 ± 0.123	1.19 ± 0.28	3.95 ± 0.18	0.114 ± 0.005

Values presented are means ± 1 S.E.M. determined after data for four muscles at each temperature had been fitted to the equation:

$$V = B(1 - P/P_0)/(A + P/P_0) + C(1 - P/P_0),$$

where V is velocity in $L_0 s^{-1}$ and P/P_0 is force expressed as a fraction of maximum isometric force.

The power ratio ($\dot{W}_{max}/V_{max}P_0$) is a measure of the degree of curvature of the force–velocity relationship (see Discussion).

T_M, muscle temperature; L_0, muscle length; V_{max}, maximum shortening velocity at zero force; $\dot{W}_{max}$, maximum power.
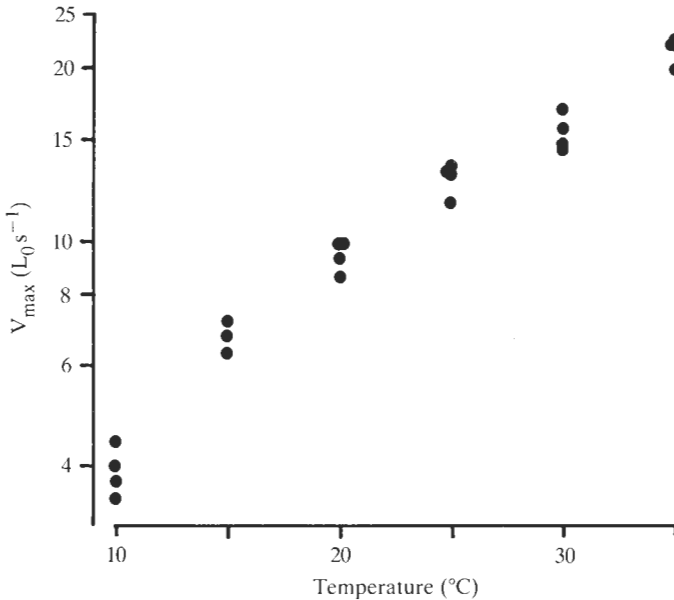
Fig. 4. Relationship between the maximum predicted velocity of shortening at zero force (V_{max}) and muscle temperature of the fast-twitch glycolytic region of the iliofibularis muscle of the lizard *Sceloporus occidentalis*. Vertical scale is logarithmic. L_0 is muscle length.

$0\cdot44P_0$ for this muscle compared to $0\cdot35P_0$ predicted by Hill's equation. Greater power output at high forces may be important to *in vivo* performance due to the high relative forces developed during terrestrial locomotion (Goslow *et al.* 1981).

In addition to the significance of accurate descriptions of force–velocity curves for analysing the functional adaptations of skeletal muscle, accurate empirically based curve-fitting may have an indirect effect on theoretical models of the contractile mechanism. One test of the adequacy of these models has been comparison with steady-state force–velocity curves (e.g. Eisenberg & Greene, 1980). Because of the common use of Hill's equation, it has been the standard of comparison in most cases. If, as appears to be the case, the force–velocity curves of many muscles do not follow Hill's equation precisely, it will be important to examine the extent of congruence between the actual contractile data and the curves derived from the models.

We do not intend to propose any particular equation as the most appropriate model for describing the force–velocity properties of all muscles. Instead, the conclusion we draw from the above analysis is that investigators should apply statistically justifiable procedures to fit an equation that accurately describes all of their data. We see no reason that force–velocity data should be an exception to this pragmatic principle applicable to all empirical data.

Describing the shape of force–velocity curves

The shape of the force–velocity relationship has been of considerable interest to muscle physiologists. This interest is justifiable because the degree of curvature of

this relationship has important consequences for the maximum mechanical power available from the muscle and has been found to be correlated with the efficiency of work output (Woledge, 1968). If Hill's characteristic equation is used to describe the data, the ratio a/P_0, provides a convenient way to compare the shapes of various curves independently of the absolute values of V_{max} and P_0. This ratio can be used because it uniquely describes the course of Hill's equation. The ratio loses its usefulness if additional constants are added to the equation, e.g. making P_0 a fitted value (Edman et al. 1976).

Because force–velocity curves often deviate significantly from Hill's equation and because of the variability in the estimates of a/P_0 (see below), an alternative to this ratio is needed for describing the curvature. Despite variations in the precise shape of the curve, all force–velocity curves measured to date are similar in that velocity decreases regularly with increasing force following a curve that is downwardly convex. To describe these curves it is desirable to have a dimensionless ratio which reflects the degree of curvature. The power ratio:

$$\frac{\dot{W}_{max}}{V_{max}P_0},$$

where $\dot{W}_{max}$ is the maximum power output defined by the curve of $V \times P$ *versus* P, provides an alternative to a/P_0 for this purpose. There are several advantages of this ratio compared to a/P_0. First, it can be calculated regardless of the equation used to fit the data, or even if the data are fitted by eye. Second, it accurately reflects the position of the curve at intermediate forces at which power is maximal. For example, the power ratios for the fast-glycolytic portions of the iliofibularis muscles of lizards (Marsh & Bennett, 1985, and this study) are ≈ 0.11. Data on frog muscle (Hill, 1970) yield a power ratio of 0.096 and tortoise muscle, which is known for its great degree of curvature, has a value of 0.042 (Woledge, 1968). Third, it is subject to less variability in estimation than is a/P_0. At least some of the large variability reported in the values of a/P_0 from similar muscles (Hill, 1938; Julian & Sollins, 1973; Edman et al. 1976; Rome, 1983) is due to differences in the fitting procedures and does not represent true variation in the isotonic data among the various studies. For example, when the force–velocity data on single fibres obtained by Edman et al. (1976) are compared directly with similar information for whole muscles (Hill, 1970), the two sets of data are remarkably similar. However, the values of a/P_0 for the single fibre data range from 0.177 to 0.290 depending on how the data are fitted to Hill's equation and whether measured or estimated values of P_0 are used in the ratio. The more conservative nature of estimates of the power ratio can be seen by re-examining the data on single fibres. A power ratio of 0.100 is calculated from the equation presented in Edman et al. (1976) that best fits the data. This value is very close to the ratio of 0.096 based on the whole muscle data of Hill (1970), and accurately reflects the similarity in the two sets of data. Power ratios calculated from data on single fibres (see table I in Edman et al. 1976) differ by less than 6% compared to the 60% variation in the values of a/P_0. The power ratio has an additional advantage in that it is readily and intuitively interpretable because it directly reflects one of the more

important results of varying the curvature of the force–velocity relationship, i.e. the relative power output. The ratio has been calculated previously at least once in the context of comparing relative power outputs in frog and tortoise muscles (Woledge, 1968). To our knowledge, we are the first to suggest its general use as a description of the curvature of the force–velocity relationship.

Thermal effects on contractile properties

The effects of temperature on the isometric contractile properties of the FG–IF of *Sceloporus* are similar to those described for the intact iliofibularis, which contains some tonic and fast-oxidative fibres (Putnam & Bennett, 1982). The Q_{10} values of the time to peak tension and the 50 % relaxation time in the twitch were greater than 1·9 over the entire range of temperatures studied and were higher at temperatures below 20°C (Table 1; Fig. 1). As might be expected, the use of only the fast-glycolytic region resulted in somewhat faster twitches than in the earlier study by Putnam & Bennett. Marsh & Bennett (1985, 1986) have concluded that the high thermal dependence of the time course of twitch contractions in lizards may help determine the range of temperatures over which these animals have low thermal dependence of running performance.

The thermal dependence of shortening velocity of the FG–IF fibres of *Sceloporus* is also quite high, $Q_{10} > 1·8$. The Q_{10} is similar to that found for V_{max} of living or skinned fibres from the FG–IF of the lizard *Dipsosaurus* (Marsh & Bennett, 1981, 1985; Johnston & Gleeson, 1984). Similar thermal dependencies of isotonic shortening have also been found for mammalian muscles measured over the same temperature range (reviewed by Bennett, 1984). In the two lizard species we have studied, the major difference in thermal effects on their skeletal muscles is that *Sceloporus* FG fibres are not stable *in vitro* at 40°C; whereas this temperature is optimal for *Dipsosaurus* FG fibres. This difference was noted previously for isometric properties (Putnam & Bennett, 1982) and correlates with differences in the preferred body temperatures and upper critical temperatures of the two species. Interestingly, despite the instability of its muscles *in vitro* at 40°C, *Sceloporus* tolerates this body temperature for several hours (Bennett & Gleeson, 1976) and performs well in burst runs (Bennett, 1980; Marsh & Bennett, 1986). This difference between *in vitro* and *in vivo* tolerance may relate to deleterious effects of maintaining fibre bundles at elevated temperatures *in vitro* without an intact blood supply (Essig, Segal & White, 1985).

Sceloporus muscle has a higher shortening velocity and faster twitch than does *Dipsosaurus* muscle measured at similar temperatures. This difference may be partially associated with differences in body mass between the two species. Lizards have been shown to have an inverse relationship between body mass and contractile speed (R. L. Marsh, H. B. John-Alder & A. F. Bennett, unpublished data); a similar relationship has been noted for several species of mammals (Close, 1972). However, an alternative explanation is an adaptive shift in the thermal dependence of muscle function, tending to equalize performance interspecifically at naturally experienced

body temperatures. Partial adaptations of this type have been found in closely related genera of scincid lizards (H. B. John-Alder & A. F. Bennett, unpublished data).

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