Mechanics of feline soleus: I. Effect of fascicle length and velocity on force output

STEPHEN H. SCOTT‡, IAN E. BROWN and GERALD E. LOEB*

The MRC Group in Sensory-Motor Neuroscience, Department of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6

Summary

The aim of the present study was to quantify how fascicle length and velocity modify force production in cat soleus. A computerized muscle puller controlled the length and velocity of the whole-muscle. We recorded the force output at the tendon and the length of muscle fascicles using sonomicrometry during whole-muscle isometric and isokinetic contractions. Peak muscle stress was estimated as $31.8 \pm 4.1 \, \mathrm{N\,cm^{-2}}$ (mean and SD) and optimal fascicle length, L_0, was estimated as 3.8 ± 0.6 cm which corresponds to an optimal sarcomere length of 2.49 ± 0.08 µm. The isometric force-length data followed closely the expected force-length relationship for cat sarcomeres. The force-velocity relationship was found to be similar in shape between cats, but the per cent increment of force over isometric levels for lengthening contractions was highly variable. Estimates of the kinematics of the fascicles based on whole-muscle length were systematically incorrect; whole-muscle velocity was 21% greater than fascicle velocity. The force-velocity data demonstrated consistent dependencies on fascicle length. At lengths below $0.7 \, L_0$ (1.74 µm), the shape of the force-velocity relationship was altered by the inclusion of a passive, repulsive force in the estimate of active isometric force. The shape of the force-velocity relationship changed at lengths greater than $0.7 \, L_0$, but was restricted to lengthening velocities where the increment of force with respect to isometric levels was found to increase with fascicle length. This change in shape in the force-velocity relationship for lengthening contractions reveals a systematic, but previously unknown interdependence between fascicle length and velocity on muscle force production.

Introduction

In the field of motor control, mathematical models of the musculoskeletal system have been developed to understand its inherent mechanical properties as well as to facilitate understanding of how the CNS might control posture and movement (e.g. Winters & Stark, 1985; Zajac & Gordon, 1989; He *et al.*, 1991). Such models require quantitative knowledge of how factors such as muscle fibre length and velocity affect muscle force output (see Brown *et al.*, 1996). This is especially true for human muscles where direct measurements are not possible and when all of the mechanical properties must be extrapolated from non-human data or inferred from indirect measures.

The force output of mammalian muscle represents a sum of properties of muscle fascicles attached in series with connective tissue and has been recorded under a variety of conditions (Joyce *et al.*, 1969; Rack & Westbury, 1969; Hatcher & Luff, 1986; Gareis *et al.*, 1992; Herzog *et al.*, 1992b). However, the measured mechanical properties vary between muscles (Hatcher & Luff, 1986; Gareis *et al.*, 1992) reflecting the wide range of architectural and anatomical organizations seen in skeletal muscle (Gans & Gaunt, 1991). Such a variable organization results in widely varying relationships between the kinematics of whole-muscle and muscle force generation (e.g. Gareis *et al.*, 1992). Therefore, such studies only provide quantitative knowledge of the mechanics for the particular muscle and conditions of study. Moreover, previous studies have generally been confined to a limited subset of the kinematic conditions under which muscles normally work. For example, the interdependence between muscle length and velocity has been analysed only during concentric and not eccentric contractions (Hatcher & Luff, 1986).

It is possible that the mechanical properties of

*To whom correspondence should be addressed at: Bio-Medical Engineering Unit, Queen's University, Kingston, Ontario, Canada K7L 3N6.
‡Present address: Dépt. de physiologie, Université de Montréal, Montréal, Québec, Canada H3C 3J7.

whole muscles could be extrapolated from the many studies on single muscle fibres or simple muscles that contain no in-series connective tissue (Katz, 1939; Abbott & Wilkie, 1953; Gordon *et al.*, 1966; Edman and Reggiani, 1987; Edman, 1988). However, most of these studies have focused on understanding the molecular and biochemical basis of muscle contraction. These experiments are often performed in non-mammalian muscles at low temperatures and with unphysiological forms of activation; they are not easily extrapolated to whole mammalian muscle under normal physiological conditions. Such studies can be used to identify physiological properties of mammalian muscle at least qualitatively, but even then, a complete characterization of the mechanics of muscle fibres has not been performed. For example, the interdependence between sarcomere length and velocity has only been analysed for shortening and not lengthening contractions (Abbott & Wilkie, 1953; Edman, 1988).

The aim of the present study was to examine the way in which fascicle length and velocity modify force production in the cat soleus muscle. A second aim of the present study was to compare the mechanical properties of fascicles based on direct measures of fascicle length with estimates based on measures of whole-muscle length. The soleus muscle was selected because it has a relatively simple and uniform, unipennate architecture (Murphy & Beardsley, 1974) and because it is composed exclusively of a homogeneous population of slow, fatigue-resistant muscle fibres (Ariano *et al.*, 1973).

Materials and methods

The mechanical properties of the soleus were recorded in cats of either sex (2.75–4.70 kg). They were anaesthetized initially with 35 mg kg^{-1} of sodium pentobarbital IP with supplemental doses IV as needed to suppress withdrawal reflex. The cat was secured to an adjustable frame by clamps that were attached to one mid-thoracic and the seventh lumbar dorsal vertebral spines. A heating blanket maintained core body temperature at 37° C based on feedback control from a rectal probe.

An incision was made along the posterior surface of the right leg from the calcaneum to the popliteal fossa. The popliteal fat-pad was removed and the plantaris and gastrocnemii were resected to expose the soleus. Care was taken to denervate and separate the tibial nerve from the lateral gastrocnemius and to dissect all of the connective tissue surrounding the soleus.

The length of the soleus was related to the range of motion of the ankle joint. The length of the whole-muscle was recorded at 30 and 150° of ankle joint plantarflexion, essentially the full anatomical range from maximal dorsiflexion to maximal plantarflexion, respectively. The lengths of the tendon, aponeurosis and fascicles were measured using calipers when the ankle joint was held at an angle at which the fascicles became slack (~ 110°; Table 1).

The soleus tendon and a small piece of the posterior calcaneus were removed from the foot and clamped to a custom-made force transducer (calibrated and determined to be linear up to 55 N), which was attached to a stepping-motor (Aerotech 1000DC servo-motor) (Fig. 1). The servocontroller for the step-motor was based on proportional error control, which results in a lag between actual and expected motor position that is proportional to velocity. The actual position of the motor was recorded

Table 1. Anatomical and physiological properties of the cat soleus muscle.

| Muscle	S8	S9	S10	S11	S12	Mean	SD
Symbol	◇	○	□	△	▽		
Sex	f	m	m	f	m		
Cat mass (kg)	2.9	4.1	4.7	2.8	3.5	3.6	0.8
Leg length (cm)	11.6	11.3	11.4	13.1	10.4	11.6	1.0
Contralateral muscle mass (g)	2.2	4.2	4.9	2.8	2.8	3.4	1.1
Range of motion (cm)	2.2	2.6	2.2	3.0	2.6	2.5	0.3
Slack lengths							
Fascicle (cm)	3.0	2.7	3.3	3.3	2.6	3.0	0.3
Aponeurosis (cm)	4.3	4.9	4.4	4.7	4.5	4.6	0.2
Tendon (cm)	2.2	2.5	2.4	2.9	2.6	2.5	0.3
F_0 (N)	18.1	31.7	28.4	24.7	26.3	25.8	5.1
L_0 (cm)	3.3	3.5	4.9	3.8	3.7	3.8	0.6
Length of whole-muscle at L_0 (cm)	9.8	10.9	11.7	11.4	10.8	10.9	0.7
L_0 relative to range of motion (% from shortest length)	100	92*	94	75	63	85	15
PCSA (cm^2)	0.62	1.14	0.96	0.69	0.72	0.83	0.22
Peak stress (N cm^{-2})	29.1	27.8	29.7	35.9	36.3	31.8	4.1
Passive force at L_0 (N)	1.5	0.7	0.1	0.0	0.1	0.5	0.6
Sarcomere length at L_0 (cm)	–	2.54	2.52	2.36	2.52	2.49	0.08
Change in slope of force-velocity relationship about isometric (lengthening/shortening)	1.0	3.6	2.5	2.8	3.9	2.7	1.1

*Longest length tested.

digitally and a relationship between motor velocity and positional error was calculated and used to compensate motor position lag during the development of the experimental templates (see below). Preliminary tests demonstrated that the motor could accelerate to 90% of test velocities in under 70 ms for velocities below 35 cm s^{-1}. Loads added to the system of 40 N did not measurably affect the response properties of the motor.

A bone screw was driven into the proximal end of the tibia and secured to the frame of the motor. The total compliance of the entire system (bone screw, motor, and force transducer) was measured to be 38 µm N^{-1}; the majority of this compliance could be attributed to bending of the bone screw which was set at 90° to the line of action of the motor.

A tri- or bi-polar nerve cuff was secured around the tibial nerve. The nerve was stimulated with a bi-phasic constant current pulse with a duration of 0.2 ms and a magnitude four times the threshold to elicit a just-detectable muscle twitch.

The length of the most proximal fascicle was recorded by piezoelectric crystals attached to the surface of the muscle (Sonomicrometer 120; Triton Technology; 5 MHz pulse, repetition rate 1543 Hz). An ultrasonic pulse was transmitted between one crystal attached to the origin of the fascicle and another attached to its insertion onto the aponeurosis: the transit time between crystals was converted into a measure of length. The crystals transmitted ultrasonic pulses through the pool of paraffin oil parallel to rather than through the muscle itself. Variations in signal output were less than 0.0125 mm under steady-state conditions. Direct measures using calipers of the distance between two crystals attached to a muscle were almost identical to values computed from the ultrasonic signals ($r = 0.99$, $p < 0.01$, $n = 18$). The pool was created by tethering the skin flaps to a metal support ring; a heating coil and a thermistor probe maintained the temperature of the pool at 36 ± 1° C. Crystals were attached directly to the muscle with a drop of cyanoacrylate adhesive (Accu-Flo Crazy Glue, Lepage). Crystals attached by the adhesive were found to remain secure for long periods of time, although constant monitoring was required in order to ensure the stability and alignment of the crystals and thus the quality of their output signals.
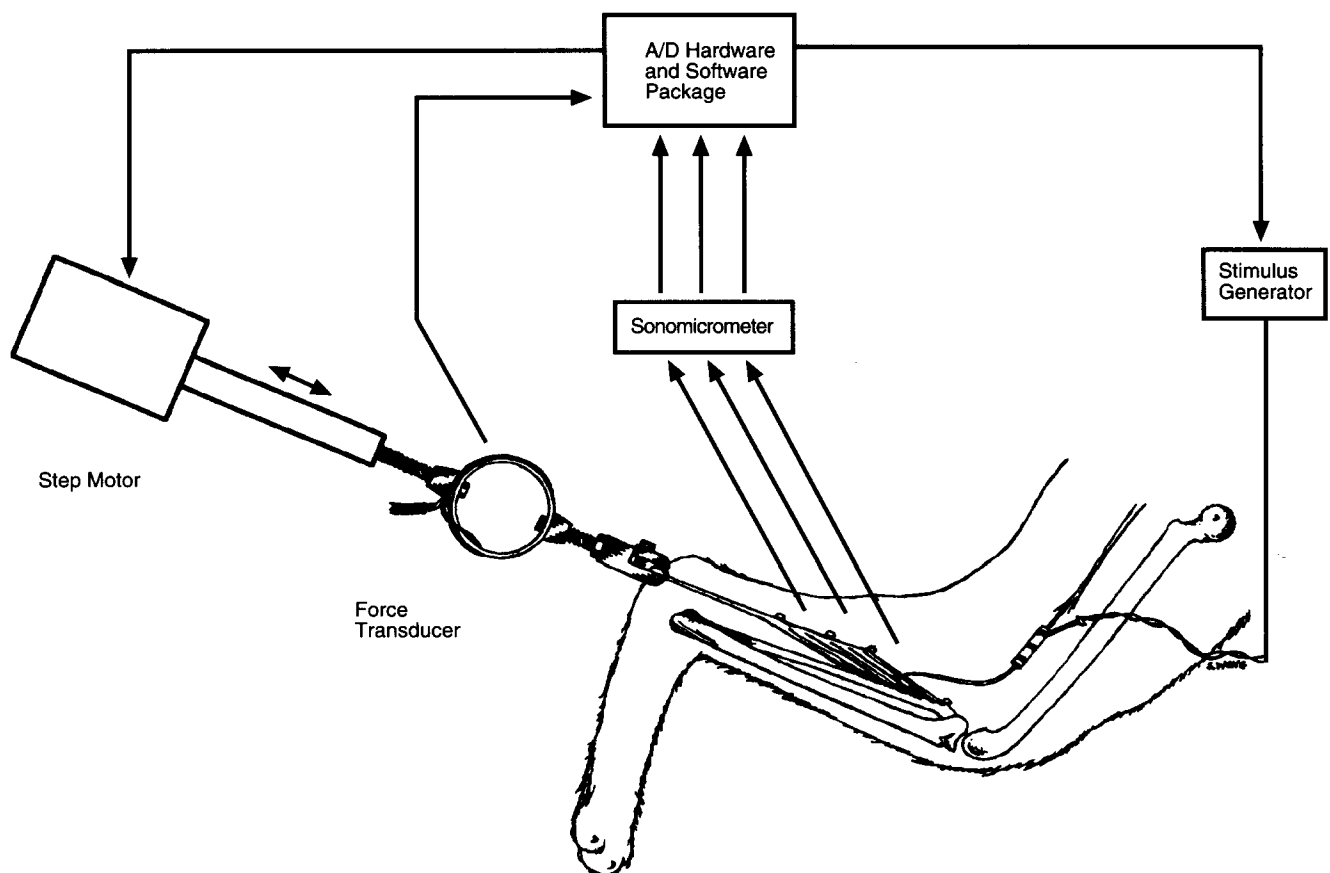
Fig. 1. The experimental setup for recording the mechanical properties of the soleus muscle. Whole-muscle length and velocity were controlled by a muscle puller as the muscle nerve was stimulated. Muscle force was recorded, as well as the length of the individual portions of the muscle based on sonomicrometry using piezoelectric crystals attached to the surface of the muscle. The transit time of ultrasonic pulses between crystals was converted into measures of length. The piezoelectric crystals attached at each end of the proximal fascicle of soleus monitored fascicle length, while crystals attached on the aponeurotic sheet (two crystals at left) monitored aponeurotic length (see Scott & Loeb, 1995).

Data collection

A generalized, data-collection software package was developed to control and record the experiment on a Macintosh II computer. The package used pregenerated experimental templates to define the step-by-step information to control the exact timing of nerve stimulation as well as the position and velocity of the step-motor for multiple sequences of motion and stimulation. This method allows the muscle to be worked over physiological ranges of lengths and stimulation intervals, avoiding stress relaxation (creep) that occurs when a muscle is quiescent or immobilized for long periods of time such as may be required to adjust equipment manually. During data collection, the software recorded motor position and the output signals from the force transducer and the sonomicrometer. The recorded motor position was converted into a measure of whole-muscle length. The interval between steps, or sample periods, in the template was set at 150 samples s^{-1} (6.67 ms interval).

A number of experiments were performed to study the force generated by the muscle under isometric and isokinetic conditions. In the first experiment, isometric contractions were recorded at a total of 16 muscle lengths evenly spanning the estimated 2.2 cm range of motion of the soleus (Rack & Westbury, 1969; Hatcher & Luff, 1986). Each trial started at the shortest length; the muscle was then lengthened to the test length at 2.2 cm s^{-1} and paused for 300 ms. The nerve was then stimulated for 400 ms at 50 pulses s^{-1}, the maximal firing rate observed for feline motor units during normal movement (Hoffer et al., 1987). After a further 300 ms, the muscle was shortened to the shortest length at 2.2 cm s^{-1}. The shortest length was tested first followed by progressively longer lengths. Individual trials were separated by five oscillations of imposed muscle stretch and release through the estimated range of motion of the soleus (2.2 cm) at 0.5 Hz (total time = 10 s). The muscle was left at its shortest length between sessions of data collection with the tendon submerged in the paraffin oil pool.

Subsequent experiments on each muscle recorded the force generated by the muscle under lengthening and shortening conditions at a number of muscle lengths. Test velocities ranged from shortening velocities at 26 cm s^{-1} to lengthening velocities at 13 cm s^{-1}. Each trial contained two full passes through the entire range of motion of the muscle; the muscle was first lengthened while the nerve was stimulated, then shortened passively, then lengthened passively and finally shortened while the nerve was again stimulated. Nerve stimulation (50 pulses s^{-1} for 400 ms) was timed to end just as the muscle passed through a target whole-muscle length. Nerve stimulation was preceded and followed by a 300 ms period of no stimulation while the test velocity was maintained. Before and after this 1000 ms block, the muscle length was changed at 2.2 cm s^{-1} from and to its limits of range of motion, respectively. The first trial was isometric and subsequent trials were conducted at progressively faster velocities.

In all experiments, the first and last trials recorded isometric contractions at identical whole-muscle lengths in order to monitor whether the muscle had fatigued between trials. In most cases, there was no drop in the force recorded between these two trials, but the data collected in the entire block was discarded if the force dropped by more than 5% from the first to the last trial.

Cats were killed at the end of the experiment with an overdose of sodium pentobarbital. The soleus muscle was allowed to go into rigor, the muscle was then excised and the length of its fascicles were measured. Small samples of muscle tissue were taken at several sites throughout the muscle. The length of sarcomeres in each sample were measured by light microscopy at several different sites and averaged. No regional differences were found for the length of the fascicles or sarcomeres, suggesting a relatively uniform anatomical organization (Murphy & Beardsley, 1974). Sarcomere length was normalized to fascicle length in the rigored muscle in order to compensate for any change in sarcomere length or decrease in sarcomere uniformity related to the rigored state. The contralateral muscle was also excised and its muscle mass was recorded and used as an estimate of the mass of the ipsilateral muscle. The mass of the ipsilateral muscle was not used since its magnitude was always slightly larger than that of the contralateral unstimulated muscle, presumably because of edema produced by exposure and work (Murphy & Beardsley, 1974). Although the mass of the stimulated muscle was larger, no changes in the physiological properties of the soleus were found during the course of the experiments.

Data analysis

Preliminary results demonstrated that there were considerable differences between the kinematics imposed on the whole muscle and the resultant kinematics of the fascicle during nerve stimulation (Fig. 2). Although the largest discrepancies in the kinematics occurred at the beginning and end of the contraction, we have focused on the mechanical properties of the muscle under steady-state conditions. The steady-state values during isometric contractions were taken as the sample period in which maximal force was generated by the muscle. For the isokinetic trials, the steady-state properties were taken from the sample period corresponding to the last stimulus of the tetanic train. The derivative of each signal (e.g. fascicle velocity) was estimated from the values recorded in the preceding and following sample periods.

The mechanical properties of the muscle under passive conditions were calculated for both isometric and isokinetic conditions. The relationship between fascicle length and passive muscle force was defined as the force recorded just prior to nerve stimulation for the isometric trials. The passive force generated by the muscle under isokinetic conditions was recorded for each test length during the passes through the range of motion of the muscle when the nerve was not stimulated (see above).

The passive force generated by the muscle had to be subtracted from the isometric contractions in order to estimate the active force-length and force-velocity relationships. The magnitude of the passive force during the contraction was linearly interpolated from the passive force-length data. Each isokinetic contraction was normalized to the isometric force that could be generated by the muscle at that fascicle length. Optimal fascicle length, L_0,

A

stim 400 ms at 50 Hz

B

C
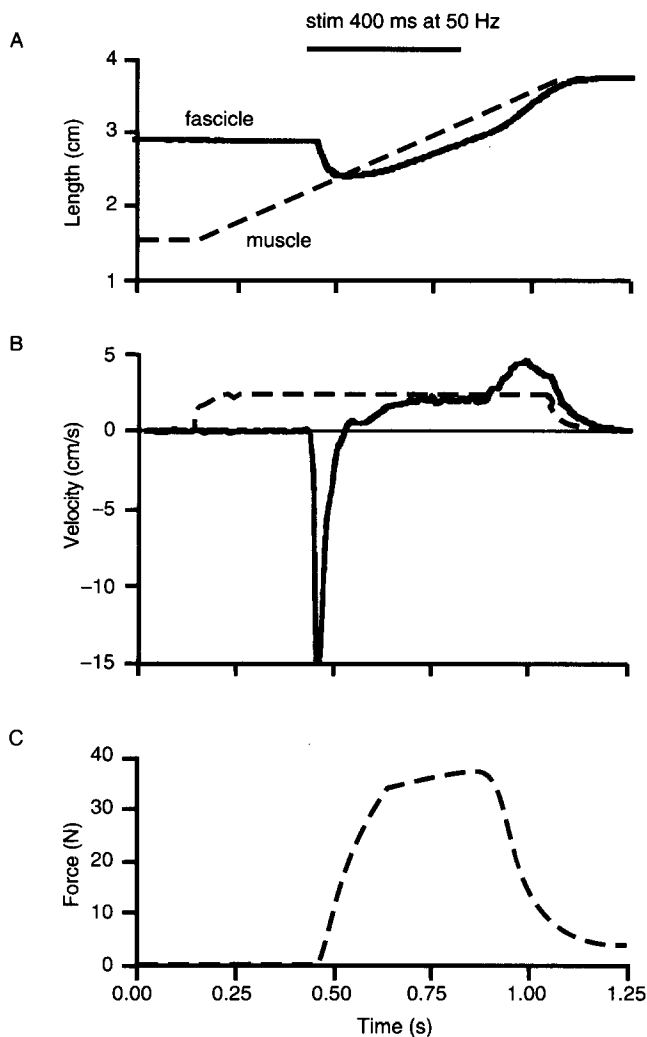
Fig. 2. (A) and (B). A comparison between recorded fascicle length and velocity (solid lines) and their estimated values based on whole-muscle length (dashed lines) when the muscle was lengthened through the anatomical range of motion. There is considerable discrepancy between the imposed kinematics of the whole-muscle and the resultant kinematics of the fascicles. (C) Force generated by the muscle during the contraction.

equalled the length at which maximal isometric force, F_0, was generated. The physiological cross-sectional area (PCSA) of the muscle was estimated by dividing the contralateral muscle mass by the density of muscle (1.06 gm cm^{-3}, from Méndez & Keys, 1960) and by L_0. The pennation angle for soleus is between 5 and 10° and was assumed to have negligible effects on these calculations. Peak stress generated by the muscle equalled F_0 divided by the PCSA. Measures of sarcomere length were adjusted for the difference between L_0 and the fascicle length obtained from the rigored muscle in order to estimate optimal sarcomere length.

The classical approach to estimate the force-length and force-velocity relationship of mammalian whole-muscle is based on the kinematics of the whole-muscle and not the fascicle (Rack & Westbury, 1969; Hatcher & Luff, 1986).

This alternate approach was also used to estimate the force-length and force-velocity relationship of the soleus muscle. Active muscle force generated by the muscle during isometric and isokinetic contractions equalled the peak force measured during stimulation minus the passive force generated by the muscle under similar whole-muscle kinematic conditions. In these calculations, the muscle mechanical properties were adjusted for the compliance of the test equipment, but not that of its own connective tissue.

Results

The passive force generated by each muscle was relatively small within its normal range of motion (Table 1, Fig. 3). However, the passive force generated by each muscle was not consistent between animals; some produced negligible passive force at L_0, whereas others generated 1 N or more. Analysis of the isokinetic data demonstrated that passive force generated by the muscle was not highly dependent on fascicle velocity. On average, muscle force under passive conditions dropped 0.1 ± 0.22 N during shortening and increased 0.14 ± 0.13 N during lengthening.

The average maximal active force generated by the muscles was 25.8 ± 5.1 N (mean $\pm$ SD) when optimal fascicle length equalled 3.8 ± 0.6 cm and optimal sarcomere length was estimated as 2.49 ± 0.08 μm (Table 1). Peak stress produced by the muscle was estimated to be 31.8 ± 4.1 N cm^{-2}. Maximal force was produced near the longest anatomical lengths (Fig. 3A). The force decreased linearly as fascicle length decreased until $0.7 L_0$. At lengths shorter than $0.7 L_0$, the force-length relationship had a steeper slope. The data follow closely the hypothesized force-length relationship for cat muscle based on sliding-filament theory (Herzog et al., 1992a; but see Brown et al., 1996) except at lengths longer than L_0 where force generation was greater than expected theoretically.

The force generated by the muscle during whole-muscle isokinetic contractions was recorded at 24 different velocities (Fig. 4A). Force dropped to half isometric levels at roughly $-0.5 L_0$ s^{-1} (negative for shortening). At a velocity of $-1 L_0$ s^{-1}, the force ranged from 0.2 to 0.4 times isometric force. V_{max}, the maximal velocity at which force could be generated, was extrapolated to about $-4.5 L_0$ s^{-1}. There was some variability between the force generated during lengthening contractions; the force plateaued at values ranging from 1.25 to 1.55 times isometric force for different muscles.

The force-length relationship based on whole-muscle length consistently underestimated peak muscle force (6.2 $\pm$ 4.1%) and overestimated optimal fascicle length (10.5 $\pm$ 4.4%). As expected, there was a systematic error between the recorded fascicle
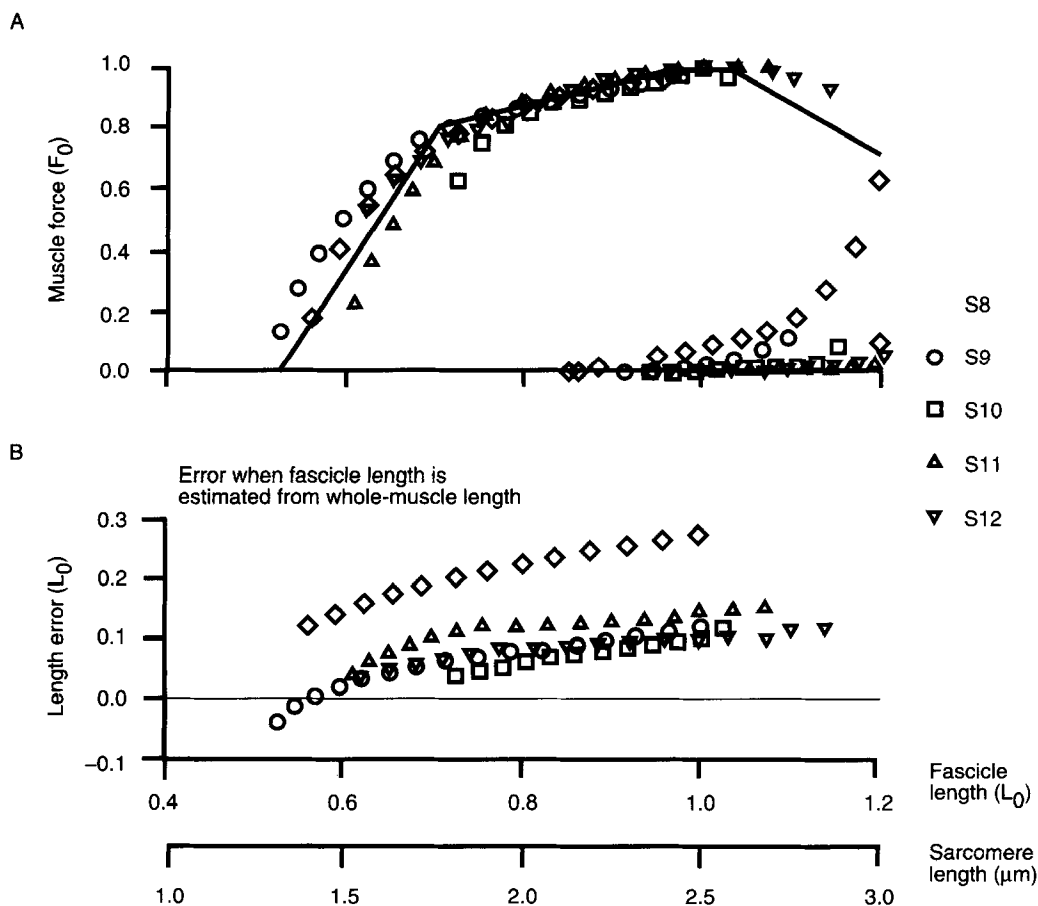
Fig. 3. (A) The relationship between fascicle length and muscle passive and active force within the anatomical range of motion of soleus. Each muscle is denoted by a unique symbol in this and in the following figures in this study. Solid line denotes hypothesized force-length relationship for cat muscle based on sliding-filament theory (Herzog et al., 1992a; but see Brown et al., 1996). (B) The error between the recorded fascicle length and its estimated value based on whole-muscle length.

length and its estimated value based on whole-muscle length (Fig. 3B). The magnitude of the error was consistent for all muscles except S8, which also generated the largest passive force within the range of motion of the muscle.

The velocity of the whole muscle was consistently larger than the velocity of the fascicle (Fig. 4B). Above $\pm 1\ L_0\ s^{-1}$, the whole-muscle velocity was 28 $\pm$ 25% larger than that of the fascicles ($n = 5$; paired t-test, $p < 0.01$). Therefore, force-velocity relationships constructed from whole-muscle measures overestimate the force generating capabilities of the muscle fascicles. However, in a related study on the mechanics of soleus tendon and aponeurosis, it was determined that the crystal at the distal end of the fascicle moved slightly (Scott & Loeb, 1995). It was estimated that fascicle velocity was 5–10% greater than recorded initially. When fascicle values were adjusted for this discrepancy, whole-muscle velocity was still, on average, 21% higher than fascicle velocity.

The slope of the force-velocity relation is different for small lengthening versus shortening velocities (Fig. 5, Table 1). The change in slope was more pronounced when the force was related to fascicle velocity rather than whole-muscle velocity. Using fascicle velocity, the change of force from isometric to slowly lengthening contractions was 2.9 $\pm$ 1.1 $F_0/$ ($L_0\ s^{-1}$), whereas the slope from isometric to slowly shortening contractions was 1.0 $\pm$ 0.2 $F_0/(L_0\ s^{-1})$, only 37% of the slope for lengthening.

The normalized force generated for active shortening was fairly congruent for all muscle lengths (Fig. 6). However, there was an apparent difference in the force that could be generated by the muscle during active lengthening at different fascicle lengths. For this muscle, greater normalized force could be generated at longer fascicle lengths than at shorter lengths.

Force generated by the muscle was estimated for active shortening and lengthening at $1\ L_0\ s^{-1}$. The force generated at these velocities was interpolated
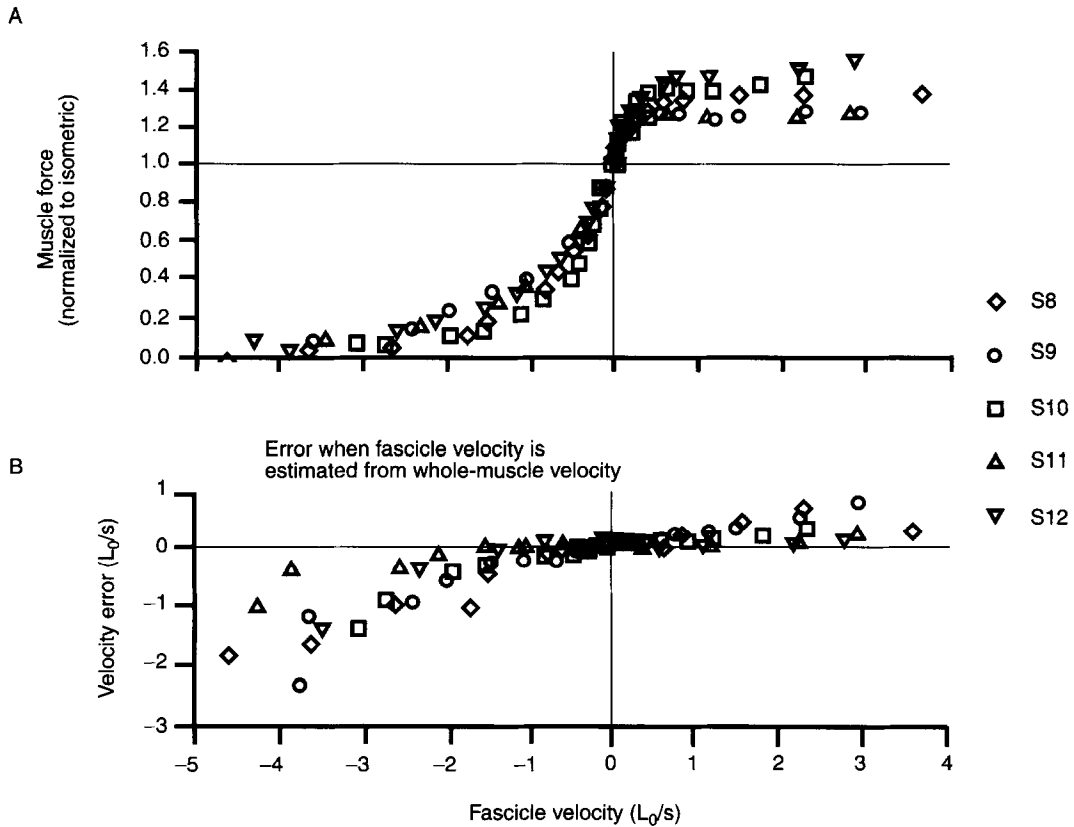
Fig. 4. (A) The relationship between fascicle velocity and muscle force recorded at a whole-muscle length corresponding to an ankle joint angle of about 70°. Muscle force is normalized to the isometric force at that fascicle length. (B) The error between the recorded fascicle velocity and its estimated value based on whole-muscle velocity.
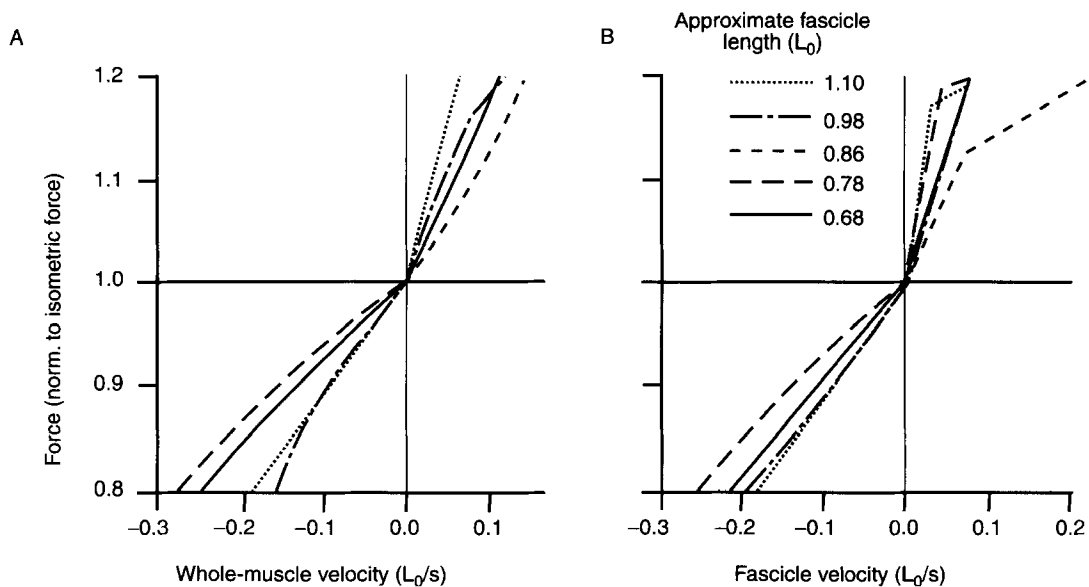
Fig. 5. The relationship between muscle force and the velocity of the whole-muscle (A) and the fascicle (B) around isometric, for a single muscle preparation. Both relationships show a discontinuity in the slope between shortening and lengthening conditions. However, there was a 2.8 times increase in the slope from shortening to lengthening velocities based on fascicle measures compared to only 1.8 times for the slope change based on whole-muscle.
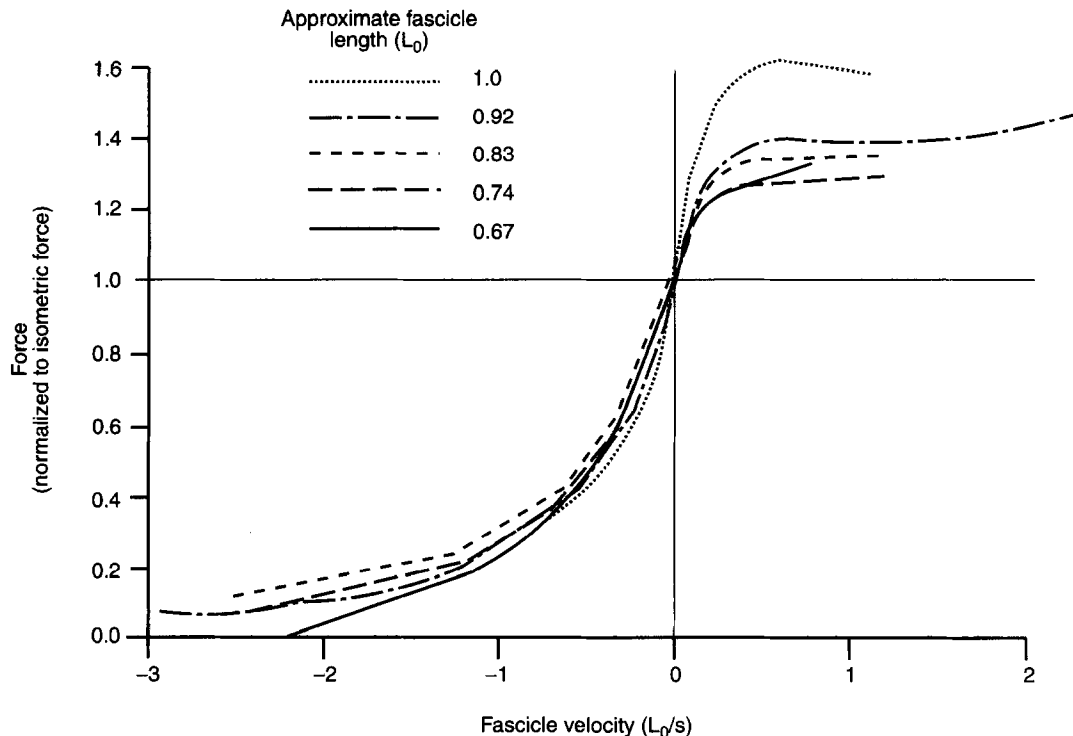
Fig. 6. The relationship between fascicle velocity and muscle force at five different whole-muscle lengths. Force is normalized to the isometric force at each fascicle length. A systematic difference exists between muscle force during lengthening contractions at different fascicle lengths.

from the experimental data and plotted against fascicle length. A complex yet consistent relationship was found between fascicle length and muscle force for non-isometric conditions (Fig. 7A,B). The force generated during active lengthening at $1\,L_0\,s^{-1}$ was minimal at around 0.7–$0.8\,L_0$; greater normalized force was generated at both longer and shorter fascicle lengths. In contrast, the magnitude of force during active shortening at $1\,L_0\,s^{-1}$ were consistent at most fascicle lengths with perhaps a tendency for the force to decrease at shorter lengths.

The increase in the relative force during active lengthening at lengths greater than $0.8\,L_0$ cannot be explained from erroneous estimation of the passive muscle force. This suggests a special interdependence between the effects of fascicle length and velocity on force generation in lengthening muscle. However, this interdependence might arise artefactually as a result of systematic differences in the kinematic history prior to the measurement of muscle force and fascicle velocity at the different fascicle lengths. Therefore, the magnitude and duration of total fascicle stretch were calculated for each lengthening contraction. These kinematic parameters remained constant for contractions at different muscle lengths for the slower lengthening velocities (Fig. 8). However, the magnitude and duration of

fascicle stretch varied systematically with fascicle length at the faster velocities; at a given velocity, both the time and magnitude of fascicle stretch increased with fascicle length. Thus, the observed variations in the shape of the force-velocity relationships in Figs 6 and 7 could have been related to the kinematic history of the contractions. To verify whether the kinematic conditions during the contraction affected the measured force-velocity relationships, muscle force was estimated at lengthening contractions at $0.3\,L_0\,s^{-1}$. At this speed, the kinematic history of the contractions remained constant at different fascicle lengths. Muscle force, and the magnitude and duration of fascicle stretch were interpolated from the series of isokinetic contractions performed at each muscle length. Figure 9 shows that the kinematic conditions during fascicle stretch did, in fact, remain relatively constant. However, the normalized force continued to rise with fascicle length suggesting that fascicle length does modify the shape of the force-velocity relationship.

Additional templates were also developed to test further whether the recorded forces for the isokinetic contractions were modified by the magnitude of the muscle stretch. Isokinetic contractions were performed at a number of muscle velocities for starting lengths ranging from 0.28 to 1.1 cm from the test
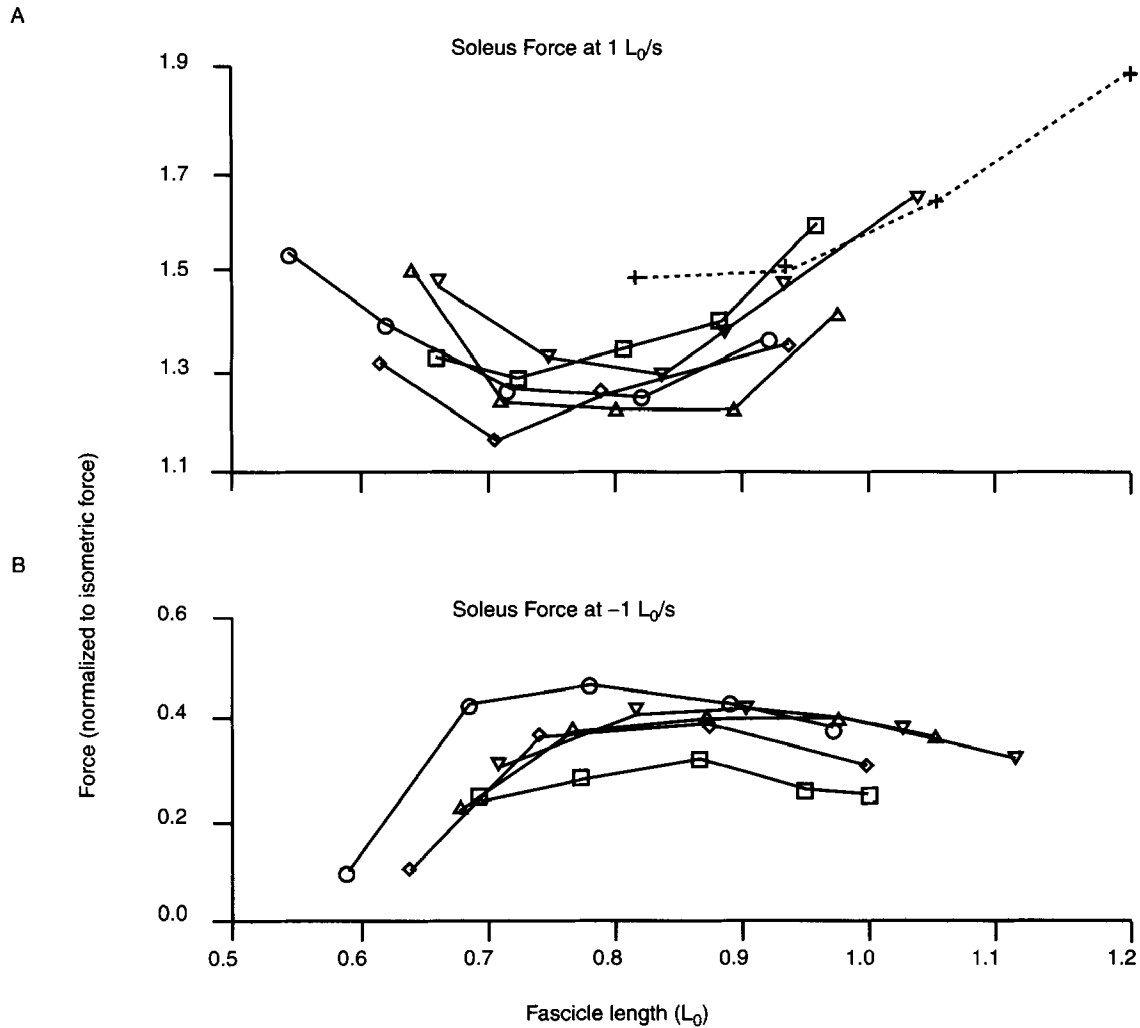
A

B

Fig. 7. The relationship between fascicle length and normalized muscle force at $1 L_0 s^{-1}$ of fascicle lengthening (A) and shortening (B) (solid lines, data from present study; dashed line, data from Edman *et al.*, 1978 for constant, but unspecified velocity). (A) Force normalized to isometric at each fascicle length is minimal between $0.7 L_0 s^{-1}$ and $0.8 L_0 s^{-1}$ and rises at longer and shorter lengths for lengthening contractions. (B) Normalized force for shortening contractions is constant for all fascicle lengths, except below $0.7 L_0$.

length. Analysis of the results demonstrated no systematic alteration in the force generated by the muscle.

Discussion

The purpose of the present study was to compare the kinematics of the sarcomere to the force generated by the whole muscle under systematically controlled, but physiological conditions. This approach assumes implicitly that the recorded length of the fascicle is a scaled version of the length of its sarcomeres, and, in fact, a scaled version of all the sarcomeres within the muscle. The suitability of this assumption for soleus appears to be valid for all but the longest muscle lengths. Alternatively, direct measurements of sarcomere length could have been performed in these

experiments, but such techniques have been successful only in parallel-fibred muscles and mostly in nonmammalian species (e.g. Julian *et al.*, 1986). As well, direct sarcomere measurements in a whole-muscle preparation like the cast soleus must also assume implicitly that such measures represent the length of all sarcomeres within the muscle.

The force-length relationship of cat soleus

The force-length relationship obtained from measurements of fascicle length followed the expected properties based on sliding filament theory. Maximal active force was estimated to occur at a sarcomere length of 2.49 ± 0.04 μm (mean and SEM) based on fascicle length, which is similar to estimates based on measures of sarcomere thick and thin filament length (between 2.35 and 2.51 μm, Herzog *et al.*, 1992a). In
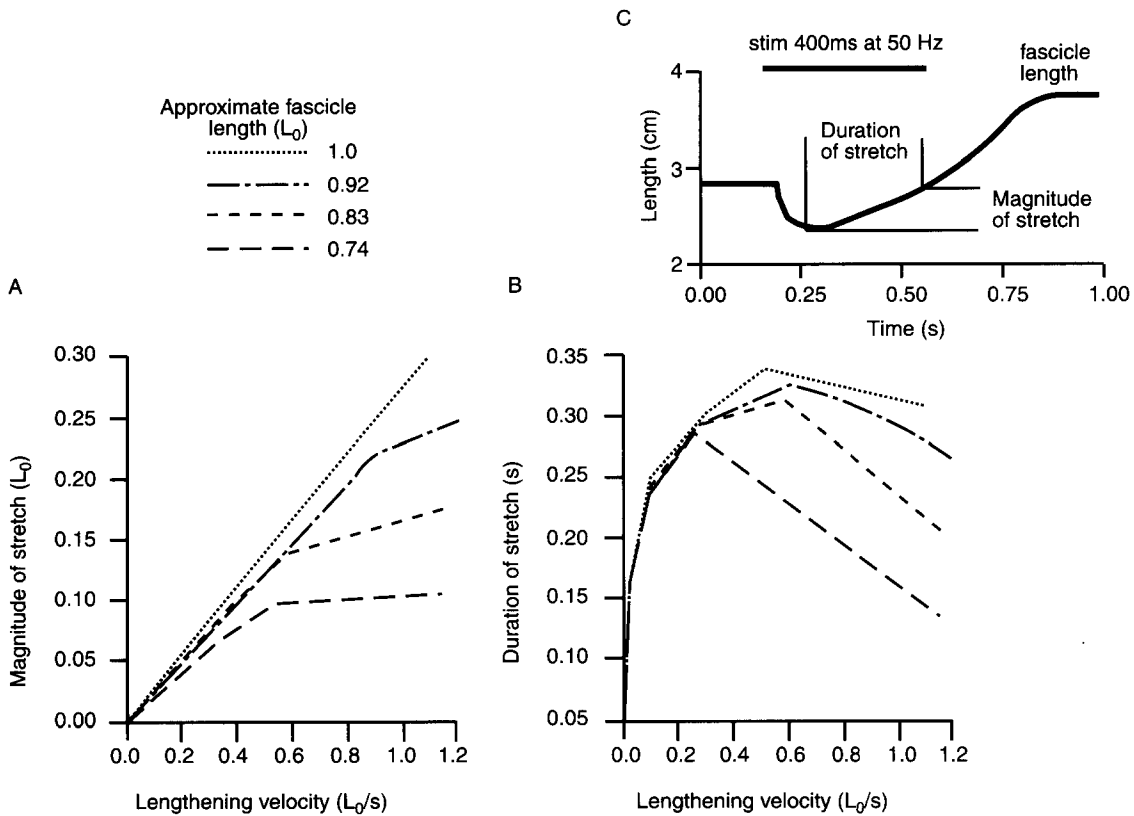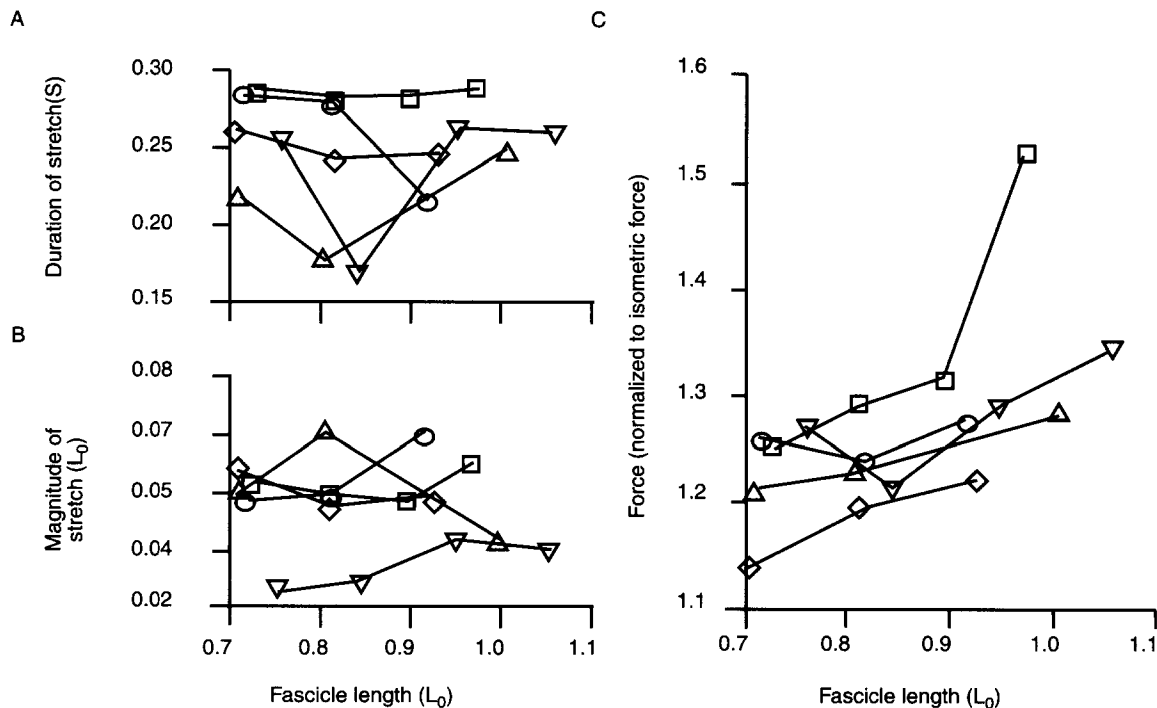
Fig. 8. The relationship between the lengthening velocity of the isokinetic contractions and the magnitude of fascicle stretch (A) and the duration of fascicle stretch (B). (C) The kinematic properties were measured directly from the recorded fascicle lengths during the contractions. At different fascicle lengths, the kinematic conditions were similar for the lower, but not higher velocity contractions. At these higher velocities, there is a systematic change in the time and duration of fascicle stretch prior to recording muscle force and fascicle velocity.

contrast, optimal sarcomere length based on measures of whole-muscle length are 10–20% larger, from 2.7–2.9 μm (present study; Rack & Westbury, 1969; Murphy & Beardsley, 1974). Gordon and colleagues (1966) demonstrated a sharp change in the slope of the ascending limb of the force-length relationship corresponding to the sarcomere length at which the thick filament hits the Z-disc (Herzog et al., 1992a). Our results show a similar sharp change in slope at 1.7 μm, the sarcomere length for cats at which the thick filament is projected to hit the Z-disc. However, there was no discernable plateau region in the present study due to the limited range of lengths analysed. Force generated by the soleus at long fascicle lengths appears to be higher than expected from the overlap of thin and thick filaments as seen in previous studies (Gordon et al., 1966). Such discrepancies have been attributed to non-uniform sarcomere lengths along the muscle fibres (Morgan, 1985). These instabilities in sarcomere lengths along the length of the fibre might also be expected in the cat soleus, although the effect may be smaller than in isolated fibres because of the stabilizing effect of

adjacent fibres and endomysial connective tissue (Trotter, 1993).

The observed error in estimating sarcomere or fascicle length from the length of the whole-muscle was relatively small in soleus, but has the potential to be larger under different physiological conditions (e.g. during rapid changes in activation; see Fig. 2) or in other muscles that are architecturally different (e.g. more highly pennate). In the present study, the error in estimating the maximal stress of the muscle fibres based on whole-muscle measures was quite small because the errors in F_0 (which were underestimated) offset errors in L_0 (which were overestimated). In general, the errors in estimating the mechanical properties of the cat soleus based on whole-muscle kinematics were minimized because of two major factors: (1) passive muscle force at optimal muscle length is small and (2) the in-series connective tissue is only twice as long as the fascicles. The errors may be larger or may even reverse sign in muscles that possess long tendons and aponeuroses and/or produce higher passive force at L_0 (Zajac, 1989; Lieber et al., 1992) or

A

0.30

Duration of stretch(S)

0.25

0.20

0.15

B

0.08

Magnitude of stretch (L₀)

0.07

0.05

0.04

0.02

0.7 0.8 0.9 1.0 1.1

Fascicle length (L₀)

C

1.6

Force (normalized to isometric force)

1.5

1.4

1.3

1.2

1.1

0.7 0.8 0.9 1.0 1.1

Fascicle length (L₀)

Fig. 9. (A) and (B) The duration and magnitude of fascicle stretch relative to fascicle length for lengthening contractions at $0.3\,L_0\,s^{-1}$. (C) Muscle force relative to fascicle length for lengthening contractions at $0.3\,L_0\,s^{-1}$. Note that although the kinematic characteristics of the fascicle stretch remain constant, muscle force increases with fascicle length.

demonstrate large changes in their architectural organization (Zuurbier & Huijing, 1992).

The force-velocity relationship of cat soleus

Measures of fascicle length allow the force-velocity relationship of the sarcomeres in whole-mammalian muscles to be estimated from data recorded under physiological conditions. Although not explicitly calculated, V_{max} was around -4 to $-5\,L_0\,s^{-1}$ in the present study, which is somewhat lower than previous estimates that were derived from whole-muscle velocity (Murphy & Beardsley, 1974; Hatcher & Luff, 1986). Our results show that this difference is due to a systematic difference between fascicle and whole-muscle velocity. On average, whole-muscle velocity was 21% greater than fascicle velocity. A large discrepancy between whole-muscle and fascicle velocity has been observed previously in the extensor digitorum longus muscle of the rat by Zuurbier and Huijing (1992) and may be due to several factors. First, the angle of pennation increases as fascicle length decreases (Huijing *et al.*, 1989). This relationship means that whole-muscle velocity is greater than fascicle velocity (Scott & Winter, 1991), but this effect is probably insignificant for the soleus muscle due to its small pennation angle (roughly 5–10°). More importantly, the change of force during the isokinetic contractions would change the length of the in-series connective tissue. The in-series connective tissue is

approximately twice as long as the fascicles. As force decreases during shortening contractions, the connective tissue would also shorten and reduce the shortening velocity imposed on the fascicles. The discrepancies between whole-muscle and fascicle velocity would be expected to be much greater in muscles such as the gastrocnemii of the cat which possess a large angle of pennation and a relatively long length of connective tissue in-series with the fascicles.

In comparison to the large number of studies on shortening contractions in cat soleus, few studies have looked at the force-velocity relationship for lengthening velocities (Joyce *et al.*, 1969). In that work, the maximal force attained during controlled lengthening contractions was around 1.1 times the isometric force (which is generally lower than recorded in the present study). However, our results obtained near optimal fascicle length are close to the values in previous studies in other preparations (Katz, 1939; Edman, 1988). The smaller difference between maximal isometric and isokinetically lengthening forces reported by Joyce and colleagues (1969) may be related to at least two factors. First, Joyce and colleagues used a stimulation frequency of 35 Hz; 50 Hz stimulation was used in the present study. The rate of stimulation appears to have a significant effect on the force generated by a muscle under lengthening conditions (Joyce *et al.*, 1969).

Second, the force recorded during lengthening contractions stretches the tendon and shortens the fascicles relative to their isometric lengths. Muscle length corresponded to an angle of 70° at the ankle joint which would correspond to a fascicle length at $0.9 L_0$. Because the muscle was on the ascending limb of the force-length relationship, the absolute force recorded during lengthening contractions in their study would have been lower than expected.

Interdependence between fascicle length and velocity on force production

Perhaps the most interesting finding in this study was that the shape of the force-velocity relationship for lengthening contractions was found to change with fascicle length. Surprisingly, no previous study has looked systematically at the effects of fascicle or sarcomere length on force production during lengthening contractions. A study on single fibres of the semitendinosus muscle of *Rana temporaria* has shown that force during stretch at a constant (but unspecified) velocity was not proportional to the isometric tension over the range of sarcomere lengths (Edman et al., 1978; their Fig. 4A). Their results, re-plotted in Fig. 7A, also show that muscle force increases with fascicle length and is qualitatively similar to our results. At least two details of sarcomere structure and function that have been noted previously might account for this phenomenon:

(1) Width changes in the myofilament lattice. The variation in the shape of the force-velocity relationship with fascicle length may be based on changes in the width of the myofilament lattice. The width of the myofilament lattice has been shown to increase with fibre shortening (Elliott et al., 1963). Edman and Reggiani (1987) suggested that changes in the width of the lattice might decrease the force output of the contractile systems at lengths below optimum. They discounted this idea because the isometric length-tension relation was not altered by artificially shrinking or swelling the fibres (Edman & Andersson, 1968). However, expansion of the myofilament lattice may reduce a directionally sensitive rate constant for cross-bridge attachment when the thick and thin filaments slide away from each other, and thus change force output during active lengthening at different fascicle lengths.

(2) Inappropriate cross-bridge attachment to overlapping actin filaments. Diminished force production during lengthening contractions at fascicle lengths shorter than L_0 may be related to increased overlap of opposing thin filaments. At shorter sarcomere lengths, the thin filaments attached to opposing Z-discs overlap and the opposing actin filaments will tend to bunch together. It is possible that cross-bridges at one end of a thick filament interact with the thin filaments arising from the Z-disc at the opposite end of the sarcomere. Such inappropriate cross-bridges have not been found to occur under shortening conditions (Fig. 7; Edman, 1979), but these inappropriate bridges may only occur when the filaments slide away from each other. It is doubtful that the thin filaments could transmit a 'pushing' force, but the simple interaction of a myosin cross-bridge with the inappropriate thin filament would temporarily engage the cross-bridge and annul its contribution to force production. The total number of inappropriate cross-bridges would tend to increase as thin filament overlap increased. This is consistent with our observed decrease in normalized force for lengthening contractions as filament overlap increases. This hypothesis suggests that the interdependence between fascicle length and velocity on muscle force output would not occur at lengths at or above optimal where opposing thin filaments do not overlap; unfortunately more data at lengths greater than L_0 are needed to determine whether the interdependence between fascicle length and velocity is localized only to the ascending portion of the force-length relationship.

References

ABBOTT, B. C. & WILKIE, D. R. (1953) The relation between velocity of shortening and the tension-length curve of skeletal muscle. *J. Physiol.* **120**, 214–23.

ARIANO, M. A., ARMSTRONG, R. B. & EDGERTON, V. R. (1973) Hindlimb muscle fiber populations of five mammals. *J. Histochem. Cytochem.* **21**, 51–5.

BROWN, I. E., SCOTT, S. H. & LOEB, G. E. (1996) Mechanisms of feline soleus: II. Design and validation of a mathematical model of muscle mechanics. *J. Muscle Res. Cell Motil.* **17**, 221–33.

EDMAN, K. A. P. (1979) The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibres. *J. Physiol.* **291**, 143–59.

EDMAN, K. A. P. (1988) Double-hyperbolic force-velocity relation in frog muscle fibres. *J. Physiol.* **404**, 301–21.

EDMAN, K. A. P. & ANDERSSON, K. E. (1968) The variation in active tension with sarcomere length in vertebrate skeletal muscle and its relation to fibre width. *Experentia* **24**, 134–5.

EDMAN, K. A. P. & REGGIANI, C. (1987) The sarcomere

length-tension relation determined in short segments of intact muscle fibres of the frog. *J. Physiol.* **385**, 709–32.

EDMAN, K. A. P., ELZINGA, G. & NOBLE, M. I. M. (1978) Enhancement of mechanical performance by stretch during tetanic contractions of vertebrate skeletal muscle fibres. *J. Physiol.* **281**, 139–55.

ELLIOTT, G. F., LOWRY, J. & WORTHINGTON, C. R. (1963) An X-ray and light diffraction study of the filament lattice of striated muscle in the living state and in rigor. *J. Mol. Biol.* **6**, 295–305.

GANS, C. & GAUNT, A. S. (1991) Muscle architecture in relation to function. *J. Biomechanics* **24**, 53–66.

GAREIS, H., SOLOMONOW, M., BARATTA, R., BEST, R. & D'AMBROSIA, R. (1992) The isometric length-force models of nine different skeletal muscles. *J. Biomechanics* **25**, 903–16.

GORDON, A. M., HUXLEY, A. F. & JULIAN, F. J. (1966) The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *J. Physiol.* **184**, 170–92.

HATCHER, D. D. & LUFF, A. R. (1986) The effect of initial length on the shortening velocity of cat hind limb muscles. *Pflugers Arch.* **407**, 396–403.

HE, J., LEVINE, W. S. & LOEB, G. E. (1991) Feedback gains for correcting small perturbations to standing posture. *IEEE Trans. Automatic Control* **36**, 322–32.

HERZOG, W., KAMAL, S. & CLARKE, H. D. (1992a) Myofilament lengths of cat skeletal muscle: theoretical considerations and functional implications. *J. Biomechanics* **25**, 945–8.

HERZOG, W., LEONARD, T. R., RENAUD, J. M., WALLACE, J., CHAKI, G. & BORNEMISZA, S. (1992b) Force-length properties and functional demands of cat gastrocnemius, soleus and plantaris muscles. *J. Biomechanics* **25**, 1329–35.

HOFFER, J. A., SUGANO, N., LOEB, G. E., MARKS, W. B., O'DONOVAN, M. J. & PRATT, C. A. (1987) Cat hindlimb motoneurons during locomotion. II. Normal activity patterns. *J. Neurophysiol.* **57**, 530–53.

HUIJING, P. A., VAN LOOKERAN CAMPAGNE, A. A. H. & KOPER, J. F. (1989) Muscle architecture and fibre characteristics of rat gastrocnemius and semimembranosus muscles during isometric contractions. *Acta Anat.* **135**, 46–52.

JOYCE, G. C., RACK, P. M. H. & WESTBURY, D. R. (1969) The mechanical properties of cat soleus muscle during controlled lengthening and shortening movements. *J. Physiol.* **204**, 461–74.

JULIAN, F. J., ROME, L. C., STEPHENSON, D. G. & STRIZ, S. (1986) The maximum speed of shortening in living and skinned frog muscle fibres. *J. Physiol.* **370**, 181–99.

KATZ, B. (1939) The relation between force and speed in muscular contraction. *J. Physiol.* **96**, 45–64.

LIEBER, R. L., BROWN, C. G. & TRESTIK, C. L. (1992) Model of muscle-tendon interaction during frog semitendinosis fixed-end contractions. *J. Biomechanics* **25**, 421–8.

MÉNDEZ, J. & KEYS, A. (1960) Density and composition of mammalian muscle. *Metabolism* **9**, 184–8.

MORGAN, D. L. (1985) From sarcomeres to whole muscles. *J. Exp. Biol.* **115**, 69–78.

MURPHY, R. A. & BEARDSLEY, A. C. (1974) Mechanical properties of the cat soleus muscle *in situ. Amer. J. Physiol.* **227**, 1008–13.

RACK, P. M. H. & WESTBURY, D. R. (1969) The effects of length and stimulus rate on tension in the isometric cat soleus muscle. *J. Physiol.* **204**, 443–60.

SCOTT, S. H. & LOEB, G. E. (1995) The mechanical properties of the aponeurosis and tendon of the cat soleus muscle during whole-muscle isometric contractions. *J. Morph.* **224**, 73–86.

SCOTT, S. H. & WINTER, D. A. (1991) Technical note: a comparison of three muscle pennation assumptions and their effect on isometric and isokinetic force. *J. Biomechanics* **24**, 163–7.

TROTTER, J. A. (1993) Functional morphology of force transmission in skeletal muscle: a brief review. *Acta Anat.* **146**, 205–22.

WINTERS, J. M. & STARK, L. (1985) Analysis of fundamental human movement patterns through the use of in-depth antagonistic muscle models. *IEEE Trans. Biomed. Engng* **BME-32**, 826–39.

ZAJAC, F. E. (1989) Muscle and tendon: properties, models, scaling and applications to biomechanics and motor control. *Crit. Rev. Biomed. Engng* **17**, 359–411.

ZAJAC, F. E. & GORDON, M. E. (1989) Determining muscle's force and action in multi-articular movement. *Exer. Sport Sci. Rev.* **17**, 187–230.

ZUURBIER, C. J. & HUIJING, P. A. (1992) Influence of muscle geometry on shortening speed of fibre, aponeurosis and muscle. *J. Biomechanics* **25**, 1017–26.