In this study, the force-length characteristics of the in vivo medial (GM) and lateral (GL) heads of the human gastrocnemius muscle were estimated from measurements in eight healthy male subjects. This involved: 1) dynamometry-based measurements of the moment generated during maximal isometric plantar flexion; 2) ultrasound-based measurements of fascicular length and pennation angle; and 3) ultrasound-based calculations of moment arm lengths. All measurements were taken over the ankle angle range from 20° of dorsiflexion to 30° of plantar flexion. Tendon forces were calculated by dividing the moments recorded by the muscle moment arm lengths, and fascicular forces were calculated by dividing the tendon forces estimated by the cosine of pennation angle. In the transition from 30° of plantar flexion to 20° of dorsiflexion, the GM muscle fascicular length and force increased linearly from 24 to 39 mm and from 222 to 931 N, respectively. Over the same ankle angle range, the GL muscle fascicular length and force increased linearly from 30 to 47 mm and from 139 to 393 N, respectively. Estimates of the sarcomeric lengths corresponding to the fascicular lengths measured indicated that the two muscles operated in the range 1.4–2.2 μm, below the optimal length region for force generation according to the cross-bridge mechanism of contraction. These results indicate that the force–length relation of the in vivo human gastrocnemius muscle is limited to the ascending limb of the bell-shaped force–length curve obtained from experiments on isolated material. Clin. Anat. 16:215–223, 2003. © 2003 Wiley-Liss, Inc.

Key words: ultrasound; length-tension; muscle contraction; moment arm; sarcomere

INTRODUCTION

The classic experiments by Gordon et al. (1966) showed that the force–length relation of isolated muscle fibers approximated a bell-shaped pattern. The level of contractile force elicited was related to the extent of myofilament overlap in the sarcomere, a relationship that gave support to the cross-bridge theory of contraction introduced by Huxley (1957).

As opposed to isolated fiber experiments, the operating length range of muscle fibers under in vivo conditions is confined by the anatomical constraints of the skeleton and the architectural features of the muscle. Due to lack of technology for measuring muscular architecture in vivo, determining the force–length characteristics of an intact muscle has necessitated either presuming that cadaver-based measurements of fascicular length and pennation angle are representative of those during muscle contraction (Cutts, 1988) or neglecting pennation effects and approximating the mechanical behavior of a pennate structure to that of a parallel-fiber structure (Herzog and ter Keurs, 1988a,b; Herzog et al., 1991). The assumptions in both approaches, however, are clearly unrealistic. The architecture of a muscle changes during contraction due to a stretch in the serial tendons and aponeuroses (Narici et al., 1996; Kawakami et al., 1998; Maganaris et al., 1998; Martin et al., 2001) and the fascicles in a pennate muscle shorten upon contraction less than those in a parallel fiber muscle (for review see Gans, 1982). Thus, the force–length relations reported
should be viewed with caution when referring to in vivo muscle function.

Ultrasoundography, however, has recently made it possible to measure in vivo muscular architecture with high accuracy and reproducibility (Narici et al., 1996; Kawakami et al., 1998; Maganaris et al., 1998). We used ultrasonography and dynamometry to estimate the force–length characteristics of the intact human gastrocnemius muscle.

MATERIALS AND METHODS

Experimental Design

The force–length relation of the gastrocnemius muscle of the right limb was examined in eight healthy male volunteers (age, 22 ± 2 years; height, 173 ± 3 cm; and body mass, 75 ± 3 kg; mean ± SD) with approval from the institutional ethics committee. Measurements were taken at ankle angles of −20° (dorsiflexion direction), −10°, 0° (the sole of the foot at right angles to the longitudinal axis of the tibia), +10° (plantar flexion direction), +20°, and +30°. All subjects could passively rotate the foot from +30 to −10°, but a slight force was applied externally to bring the foot to −20°. At each ankle angle studied, the following parameters were either measured or calculated: 1) the moment elicited upon isometric plantar flexion maximal voluntary contraction (MVC); 2) the gastrocnemius muscle fascicular length and pennation angle; and 3) the gastrocnemius muscle moment arm length.

Measurement of MVC Moment

MVC contractions were elicited in the prone position on the bench of an isokinetic dynamometer (Lido Active, Loredan Biomedical, Davis, CA). The pivot point of the dynamometer lever was aligned with the lateral malleoli and the foot was firmly secured at the dynamometer footplate with Velcro straps. Measurements were taken after compensating for gravitational and passive moments about the ankle. After a warming up protocol consisting of three submaximal contractions, the subjects generated four MVCs with the knee fully extended (0°) and two MVCs with the knee flexed at 120°. MVCs were elicited randomly for knee and ankle angles, and were separated by a 2-min rest. Measurements were repeated until obtaining the MVC moments established in a familiarization trial (±5%) 2 to 3 days earlier.

Conventional dynamometers record moments in only one plane in each test. In the present experiment, moment measurements were taken in the sagittal plane. In vitro and in vivo experiments have shown that the lateral side of the tibiotalar joint axis points downwards and posteriorly by 10° relative to the medial side (Isman and Inman, 1969; van den Bogert et al., 1994). This geometric arrangement results in a triplanar orientation in the moment produced about the tibiotalar joint; however, ankle moment measurements in the sagittal plane include only the cosine component of this moment. Trigonometry-based calculations using the above axis angle values indicate that the measurable component of moment accounts for 97% of the entire moment vector. Thus, the present MVC moment measurements were considered to be valid.

Measurements of Fascicular Length and Pennation Angle

Fascicular lengths and pennation angles were measured in vivo from sonographs taken during MVCs elicited at the knee extension angle (Narici et al., 1996; Kawakami et al., 1998; Maganaris et al., 1998; Martin et al., 2001). A linear-array, 7.5 MHz, B-mode ultrasound probe (Esaote Biomedica, Florence, Italy), with width and depth resolutions of 1 mm and 0.62 mm, respectively, was coated with transmission gel and placed over the midlongitudinal axes of the medial (GM) and lateral (GL) heads of the gastrocnemius muscle, 5 cm above (first and third MVCs) and 5 cm below (second and fourth MVCs) the midlength of the muscle belly during MVC (Fig. 1). The architecture of the two muscle heads in the above regions has been shown to be representative of that along and across the muscle belly (Narici et al., 1996; Maganaris et al., 1998). In the muscle scans recorded, fascicular, interfascicular, and aponeurotic echoes were identified. The criterion adopted for ensuring that the scanning plane coincided with the plane in which the fascicles lay was that the echo of the entire fascicular path could be seen in the scans (Narici et al., 1996; Kawakami et al., 1998; Maganaris et al., 1998). This criterion was met in most of the scans taken with the scanning head placed in the midsagittal plane of the muscle. If, however, this probe position generated echoes in only a part of the fascicular path, the probe was oriented manually to a plane that included the entire length of the fascicles. In each scan, the length of one to three fascicles was digitized using computerized image analysis (NIH Image, National Institute of Health, Bethesda, MD), taking into account any visible curvature along the fascicular path. Pennation angle measurements were taken at the fascicular insertions in the deep aponeurosis (Fig. 2).
Calculation of the Moment Arm Length

Moment arm lengths were obtained using the excursion method (An et al., 1984), adapted under in vivo conditions as described by Ito et al. (2000) and Maganaris (2000). This method necessitates identifying a reference landmark along the action line of the muscle and measuring displacement of the landmark over a given rotation in the joint that the muscle spans. Because the action lines of the GM and GL muscles do not coincide and the axis of rotation in the tibiotalar joint is not perpendicular to the sagittal plane, separate measurements were taken to quantify the displacements of the reference landmarks in the two muscles. The reference landmark in each muscle was the end point of the echo generated by the tendon in the distal myotendinous junction of the muscle. To locate the myotendinous junction, five axial-plane sonographs were recorded (with the scanning probe described above) along the central portion of the GM and GL muscle bellies at 2-cm intervals. The medial and lateral borders of each muscle in each scan were marked on the skin to identify the midlongitudinal axis of the muscle, which was also drawn on the skin. The probe was positioned in the sagittal plane along the midlongitudinal axis of each muscle to locate the exact position of the myotendinous junction. The probe was oriented in a plane reflecting clear echoes and secured with adhesive tape on the skin. Scans were first recorded having the ankle plantar flexed by 5° more than the angle at which the moment arm length was to be obtained (Figs. 2, 3A). The same procedure was repeated with the ankle plantar flexed by 5° less, compared to the angle at which the moment arm length was to be obtained. The displacement $\Delta x$ of the tendon endpoint in each myotendinous junction over the $10^\circ$-rotation ($\Delta \theta$) was then digitized in the scans taken. The absence of mediolateral shifts in the gastrocnemius muscle during plantar flexion-dorsiflexion rotation (Fukunaga et al., 2001) indicates that all $\Delta x$ measurements in each muscle were taken in a single muscular section. The moment arm lengths of the GM and GL muscles were calculated from the ratios $\Delta x/\Delta \theta$ ($\Delta \theta$ in rad).

Data Analysis

The moment-producing potential of the gastrocnemius muscle at each ankle angle was estimated by subtracting the MVC moment at the knee flexion angle from that at the knee extension angle. For each knee angle, the MVC that generated the highest moment was considered. The above calculation assumes that at any given ankle angle: 1) the gastrocnemius muscle is fully active during an MVC with the knee extended; 2) the biarticular gastrocnemius musculotendon unit is slack at a knee flexion angle of $120^\circ$ and thus it transmits upon MVC negligible contractile forces to the calcaneus; and 3) the moment produced by other muscles crossing the ankle joint is the same between MVCs generated at knee angles of 0 and $120^\circ$. The validity of the first assumption could be tested by superimposing electrical current upon the MVCs carried out, referred to as the “twitch superimposition technique” (Belanger and McComas, 1981; Allen et al., 1995). Unfortunately, we found the application of this method in the experimental trial impractical: placement of stimulating electrodes over the muscle prohibited muscle scanning, and placement of stimulating electrodes over the tibial nerve in the popliteal fossa proved to be ineffective at the knee flexion angle due to loose contact between the electrodes and the skin. However, to assess whether our subjects were capable of activating fully the gastrocnemius muscle upon volition, the twitch superimposition technique was applied in the familiarization...
trial. For these measurements, twitch doublets of 150–175 V were applied to the GM and GL muscles through two self-adhesive rubber electrodes of dimensions 7.5 × 12.5 cm. Our pilot measurements showed no evidence of incomplete motor unit activation during MVC. The validity of the second assumption is supported by previous EMG-based studies (Hof and van den Berg, 1977; Gravel et al., 1987) and by recent results showing that knee flexion above 115° yields no measurable decrease in the moment generated during plantar flexion contraction (Maganaris, 2001). The third assumption has been discussed in detail by Herzog and ter Keurs (1988a) and Herzog et al. (1991); because all the synergists and antagonists of the gastrocnemius muscle span the ankle joint only, their moment contribution to the net MVC moment at any given ankle angle would not differ between knee angles. This view has been challenged by moment calculations from in vivo measurements of Achilles tendon force in a recent study (Arndt et al., 1998); however, these measurements were taken in only one subject. More importantly, the authors assumed that the length of the Achilles tendon moment arm does not change as a function of muscle-tendon length and contraction-state, which later proved to be incorrect (Maganaris et al., 1998).

The moment contribution of the GM and GL muscles to the entire gastrocnemius muscle moment was obtained from the relative physiological cross-sectional areas of the GM and GL muscles to that of the entire gastrocnemius muscle: 70 and 30%, respectively (Huijing, 1985; Fukunaga et al., 1992). Tendon forces in each muscle were calculated by dividing the estimated moment by the moment arm length of the muscle (Fig. 3B). Fascicular forces were estimated by representing the muscle as a parallelogram with aponeuroses and tendons operating over the same line (Gans, 1982; Cutts, 1988; Narici et al., 1996;
The fascicular force elicited upon contraction was therefore obtained by dividing the tendon force by the cosine of pennation angle (Fig. 3B). The selection of the above model as opposed to that developed by Huijing and Woittiez (1985) was based on visual inspection of the aponeuroses and tendons in the scans taken over the myotendinous junction region (Fig. 2). Nevertheless, it should be recognized that although simple unipennate muscle models (with or without an angle between the tendons and the aponeuroses) are computationally efficient for estimating changes in forces when altering muscle length, they are mechanically unstable. Modelling mechanically stable architectures requires detailed information about the arcs over which the fascicles and the tendinous sheets operate (Van Leeuwen and Spoor, 1992), which cannot be obtained in vivo at present.

Direct measurements on cadaveric human muscles have previously shown that ultrasonography provides valid measurements of fascicular length and pennation angle (Kawakami et al., 1993; Narici et al., 1996). Inter- and intraobserver variations of the scanning procedures and image analyses involved have been confirmed to be less than 8% (Maganaris et al., 1998; Ito et al., 2000; Maganaris, 2000, 2001). In the present study, all scan morphometrics were carried out three times and mean values were used for further analysis. In each muscle, average values of pennation angle across all fascicles traced and both muscular regions scanned were considered in the analysis.

RESULTS

Tables 1 and 2 show the measured and calculated values of the parameters examined. The following observations can be made from the data shown:

1. The MVC moment at the knee extension angle was higher by 57–65% than that at the knee flexion angle. This difference is in line with previous results (Sale et al., 1982; Kawakami et al., 2000a) and reflects the ineffectiveness of the gastrocnemius musculotendon unit to transmit contractile forces and generate calcaneal displacements at knee angles above 90° (Hof and van den Berg, 1977; Gravel et al., 1987; Maganaris, 2001).

2. Both muscles exhibited similar architectural features between the two regions scanned, in agreement with previous studies reporting a uniform geometry along the muscle belly (Narici et al., 1996; Maganaris et al., 1998).

3. The two muscles exhibited similar patterns of
TABLE 1. Parameters Measured as a Function of Ankle Angle*

<table>
<thead>
<tr>
<th>Ankle angle (degrees)</th>
<th>Dorsiflexion ↔ Plantar flexion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−20</td>
</tr>
<tr>
<td>MVC moment at knee extension (Nm)</td>
<td>201 ± 17</td>
</tr>
<tr>
<td>MVC moment at knee flexion (Nm)</td>
<td>124 ± 11</td>
</tr>
<tr>
<td>GM muscle fascicular length A (mm)</td>
<td>39 ± 6</td>
</tr>
<tr>
<td>GM muscle fascicular length B (mm)</td>
<td>39 ± 6</td>
</tr>
<tr>
<td>GL muscle fascicular length A (mm)</td>
<td>46 ± 7</td>
</tr>
<tr>
<td>GL muscle fascicular length B (mm)</td>
<td>47 ± 8</td>
</tr>
<tr>
<td>GM muscle pennation angle A (deg)</td>
<td>30 ± 4</td>
</tr>
<tr>
<td>GM muscle pennation angle B (deg)</td>
<td>32 ± 5</td>
</tr>
<tr>
<td>GL muscle pennation angle A (deg)</td>
<td>20 ± 3</td>
</tr>
<tr>
<td>GL muscle pennation angle B (deg)</td>
<td>22 ± 3</td>
</tr>
</tbody>
</table>

*GM, maximum voluntary contraction; GM and GL, medial and lateral heads of the gastrocnemius muscle, respectively; A and B, 5 cm above and 5 cm below the midlength of the muscle belly, respectively. Fascicular lengths and pennation angles are average values from one to three fascicles. Values are mean ± SD (n = 8).

changes in any given parameter examined as a function of ankle angle, not surprising given that both muscles are anatomic subunits of the same actuator (the gastrocnemius muscle).

4. Neither muscle exerted its maximal force at the ankle angle of 0°, in contrast with the general notion that skeletal muscles generate maximal forces at neutral anatomical positions (see also Lieber et al., 1994).

The observed changes in all parameters examined as a function of ankle angle agree with several previous studies (Sale et al., 1982; Rugg et al., 1990; Herzog et al., 1991; Narici et al., 1996; Kawakami et al., 1998; Maganaris et al., 1998, 2000).

As shown in Figure 4A, the contractile force of each muscle increased as a function of fascicular length. In either muscle, fitting the force–length data with linear models produced $R^2 > 0.98$. No further improvement in $R^2$ ($P > 0.05$; Student’s t-test) was obtained by fitting the data using second and third order polynomials. The starting point and the slope of the force–length relation were different between the two muscles, but the normalized force–length curves were very similar (Fig. 4B), thus indicating that the two muscles operated over similar relative length regions.

**DISCUSSION**

Determination of force-length characteristics in intact human muscles has often been attempted; however, the following unrealistic assumptions have been made: 1) the architecture of cadaveric muscles represents muscular architecture during contraction (Cutts, 1988); 2) pennate muscles behave mechanically like parallel fiber muscles (Herzog and ter Keurs, 1988b; Herzog et al., 1991); 3) antagonist muscles are inactive during contraction (Ichinose et al., 1997); and 4) the operating range of a muscle during submaximal and maximal contractions is the same (Leedham and Dowling, 1995). In the present experiment, these limitations have been eliminated. An additional advantage of the present methodology is that it allows estimates to be derived of the theoretical operating range of the average sarcomere. Dividing the GM and GL fascicular lengths in the present study by 17,600 and

TABLE 2. Parameters Calculated as a Function of Ankle Angle*

<table>
<thead>
<tr>
<th>Ankle angle (degrees)</th>
<th>Dorsiflexion ↔ Plantar flexion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−20</td>
</tr>
<tr>
<td>GM muscle muscle moment (Nm)</td>
<td>54 ± 4</td>
</tr>
<tr>
<td>GL muscle moment (Nm)</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>GM muscle moment arm length (mm)</td>
<td>51 ± 4</td>
</tr>
<tr>
<td>GL muscle moment arm length (mm)</td>
<td>55 ± 4</td>
</tr>
<tr>
<td>GM tendon force (N)</td>
<td>1055 ± 82</td>
</tr>
<tr>
<td>GL tendon force (N)</td>
<td>416 ± 35</td>
</tr>
<tr>
<td>GM fascicular force (N)</td>
<td>931 ± 95</td>
</tr>
<tr>
<td>GL fascicular force (N)</td>
<td>393 ± 42</td>
</tr>
</tbody>
</table>

*GM and GL, medial and lateral heads of the gastrocnemius muscle, respectively. Values are mean ± SD (n = 8).
21,300 in-series sarcomeres, respectively (Huijing, 1985), indicated that the average sarcomere operated at between 1.4 and 2.2 \text{\mu m}. The optimal sarcomeric length region of 2.6–2.8 \text{\mu m} (Walker and Schrodt, 1974; Lieber et al., 1994) would be obtained at fascicular length regions between 46 and 49 mm in the GM muscle and between 55 and 60 mm in the GL muscle. Extrapolating the fascicular length–ankle angle relations in the present experiment (GM muscle: \( y = -3.4x + 110.4, R^2 = 0.99 \); GL muscle: \( y = -2.859x + 114.61, R^2 = 0.99 \)) indicated that the above fascicular lengths would be reached over the ankle angle range between \(-43^\circ\) and \(-57^\circ\). These angles far exceed the physiological end-range dorsiflexion ankle angle in man, \(-25^\circ\) (Sammarco et al., 1973; Nigg et al., 1992). It must be emphasized, however, that the above calculations assume that the lengths of a fascicle and the fibers contained in it coincide. Indeed, there is evidence from staining-based studies on the number and position of motor end-plate in cadaveric specimens that the gastrocnemius muscle fibers span the entire fascicular length (Kawakami et al., 2000b).

The fascicular force–length relation and the theoretical sarcomeric operating range obtained indicate that the gastrocnemius muscle has more in-series sarcomeres than those required to generate maximal activation forces at optimal lengths. This finding, however, should not be taken to imply that physiological function is associated with operation of the gastrocnemius muscle outside its plateau region. Physiological functioning requires generating smaller muscular forces than those produced during maximal isometric contraction, thus resulting in less myofilament overlap and longer sarcomeric lengths. During locomotion, for example, the human gastrocnemius muscle contracts near-isometrically, close to its theoretical optimal length (sarcomeric operating range: 2.75–2.92 \text{\mu m}; Fukunaga et al., 2001). This propels the body ahead at minimal metabolic cost. Similar results have been reported from sarcomeric length measurements during physiological movement in several other species (Burkholder and Lieber, 2001). Numerous experiments have shown that the number of serial sarcomeres is highly adaptable to changes in muscle length (Tabary et al., 1972; Williams and Goldspink, 1973; Herring et al., 1984; Williams, 1990). For many muscles, the muscle length that dictates sarcomere number seems to be the length corresponding to maximal isometric force (Herring et al., 1984; Williams, 1990). The above experiments on sarcomeric length, however, indicate that in some muscles adjustments in sarcomeric number may be made to optimize habitual function rather than maximize contractile force generation (see also Herring et al., 1984).

The present results are of clinical relevance. Information about force–length properties of in vivo muscles is important in surgical procedures involving tendon transfer/lengthening (Delp et al., 1995; Loren et al., 1996). Laser diffraction has allowed direct length measurements to be taken in sarcomeres of the affected muscle (Lieber and Friden, 1998); however, this intraoperative method is highly invasive, thus impractical for measuring sarcomeric length in a healthy muscle. Therefore, providing reference values according to which corrective operation would be planned may not be always feasible. Moreover, laser diffraction necessitates making appropriate corrections in the length measurements taken to account for...
REFERENCES


