Relationship between short latency stretch reflex and fascicle behavior in the soleus muscle \textit{in vivo}

Keitaro Kubo

Department of Life Science, The University of Tokyo, Meguro, Tokyo, Japan

Abstract

\textbf{Objectives:} Stretch reflex responses were considered to be affected by the velocity of muscle fiber lengthening and angular velocity. However, the results of previous studies \textit{in vivo} and \textit{in vitro} are inconsistent in this regard. The purpose of the present study was to investigate the effects of the velocity of fascicle lengthening on the amplitude of the stretch reflex for each trial with a high angular velocity and wide range of motion. \textbf{Methods:} Thirteen healthy men volunteered for this study. While the ankle was passively moved from 100 to 80 deg at five different angular velocities (100, 200, 300, 500, and 600 deg$\cdot$s$^{-1}$), the velocity of fascicle lengthening in the soleus muscle was measured using ultrasonography. In addition, the amplitude of the short latency stretch reflex in the soleus muscle was also measured. \textbf{Results:} As angular velocity increased, the amplitude of the stretch reflex and velocity of fascicle lengthening significantly increased (both $p<0.001$). For each trial in all subjects, the amplitude of the stretch reflex was not correlated with the velocity of fascicle lengthening at any of the angular velocities. \textbf{Conclusion:} In conclusion, the stretch reflex size is not related to the fascicle behavior in each trial.

\textbf{Keywords:} Angular Velocity, Electromyography, Plantar Flexor Muscles, Ultrasonography, Human

Introduction

Previous studies \textit{in vivo} and \textit{in vitro} demonstrated that stretch reflex responses were affected by the velocity of muscle fiber lengthening and angular velocity$^{1-3}$. Eng and Hoffer$^1$ reported that the electromyographic amplitude of the stretch reflex in the cat medial gastrocnemius muscle was correlated with the corresponding velocity of muscle fiber lengthening but not with that of whole muscle. On the other hand, Cronin et al.$^4$ showed that the short latency stretch reflex size in the human triceps surae muscles was poorly correlated with the amplitude and velocity of fascicle lengthening measured by ultrasonography. However, they determined this at a relatively low angular velocity (120 deg$\cdot$s$^{-1}$), although the amplitude of the stretch reflex rose as the angular velocity increased$^{5,6}$. In addition, it cannot be ruled out that the change in fascicle length may be obscured within the measurement error, since the stretch (dorsiflexion) range was very narrow (1.5–6.0 deg).

In most previous studies concerning the stretch reflex$^{4,5}$, the averaged data on the stretch reflex from several trials were used. However, the amplitude of the stretch reflex varied markedly among several trials. Therefore, we need to determine the relationship between the stretch reflex size and fascicle behavior for each trial, not the average. In the present study, we aimed to investigate the effects of the velocity of fascicle lengthening on the amplitude of the stretch reflex for each trial with a high angular velocity and wide range of motion. We hypothesized that the velocity of fascicle lengthening at higher angular velocities would be correlated with the size of the stretch reflex.

Materials and Methods

Participants

The sample size was estimated using data from my previous study$^7$ in which the differences in the stretch reflex size and amplitude of fascicle lengthening among the five
angular velocities (see below) were determined. On the basis of an α level of 0.05 and a power (1–ß) of 0.8, it was shown that at least 11 participants were necessary for this study. Thirteen healthy men participated in this study. Their mean age, height, and body mass were 36.1±7.3 yr, 172.0±5.1 cm, and 73.5±15.0 kg, respectively. They did not have experience with regular exercise training for at least 1 year before testing. None of them reported any current or recent lower limb injuries in the 3 years before testing. They were fully informed about the procedures, purpose, and risks associated with the study. Written informed consent was obtained from all participants. This study was approved by the Ethics Committee for Human Experiments, Department of Life Science (Sports Sciences), The University of Tokyo. Data (stretch reflex at all angular velocities and amplitudes of fascicle lengthening at 100 and 200 deg·s⁻¹) were presented previously⁷.

Velocity of fascicle lengthening

The knee joint of the participant was flexed at 120 deg (0 deg=full extension) in order to remove the contribution of the gastrocnemius muscle to plantar flexion torque⁸. Their trunk and waist were fixed to a wooden base on a test bench by adjustable belts. The ankle joint was set at 100 deg (90 deg was the neutral anatomic position, with angles more than 90 deg on plantar flexion), and the foot tightly secured by two straps to the footplate of a specially designed dynamometer (T.K.K.S-18035, Takei Scientific Instruments Co., Ltd., Niigata, Japan). The joint angle signals were digitized at a sampling rate of 1 kHz and stored for later analysis. After a standardized warm-up, the participants performed two or three isometric maximal voluntary contractions (MVC). Each MVC was maintained for around 2 s, and the mean amplitude of the electromyographic activity during MVC was used to normalize the amplitude of the stretch reflex (see below).

After at least a 15-min rest following the MVC measurement in order to eliminate the effect of fatigue, the ankle was passively moved from 100 to 80 deg at five different angular velocities (100, 200, 300, 500, and 600 deg·s⁻¹). The acceleration of the used dynamometer was constant, and the changes in angular velocities at five conditions were already presented in Figure 1 of my previous study⁹. The “peak angular velocities” reached the specified velocity at the end of the analysis range of 500 and 600 deg·s⁻¹, although the specified angular velocities were reached in the range of half or more of the analysis range at 100, 200, and 300 deg·s⁻¹. This was related to the limitation of the torque motor of the dynamometer used in the present study. The order of tasks (100, 200, 300, 500, and 600 deg·s⁻¹) was randomized to avoid any systematic effects in the present study. The rest periods between trials and different angular velocities were 1 min and 2 min, respectively. A real-time ultrasonic apparatus (SSD-6500, Aloka, Tokyo, Japan) was used to obtain longitudinal ultrasonic images of the soleus muscle (SOL) at the level of 40% of the lower leg length, i.e., from the popliteal crease to the center of the lateral malleolus (Figure 1). During the measurement, ultrasonic images were stored at 100 Hz at 100, 200, and 300 deg·s⁻¹ and 125 Hz at 500 and 600 deg·s⁻¹ in the computer memory of the apparatus⁸. The fascicle length was defined as the distance between the insertion of the fascicle into the superficial and deep aponeuroses. In the present study, the amplitude and mean velocity of fascicle lengthening were calculated from 100 to 87 deg. The measurement was performed three times per each angular velocity. The measured values were the means of three trials, and the measured values for each trial were also used to analyze the relationship between the stretch reflex size and velocity of fascicle lengthening. In the present study, I excluded one trial at 100, 200, and 600 deg·s⁻¹ from the analysis due to unclear ultrasound images.

Stretch reflex

Electromyographic activity (EMG) during the measurements of fascicle behavior (see above) was recorded using a wireless EMG telemetric system (BioLog DL-5500, S&ME, Japan) at a sampling rate of 1 kHz. Surface electrodes (DL-510, S&ME, Japan) were placed over the muscle bellies of SOL. The raw data were band-pass filtered between 10 to 500 Hz. According to the procedure of Cronin et al.⁴, I manually identified the onset of the short latency stretch reflex from the EMG data by visual inspection. The peak-to-peak EMG amplitude was normalized by the amplitude of the reflex response to that during MVC⁵. In the present study, I excluded one trial at 100 and 600 deg·s⁻¹ and two trials at 200, 300, and 500 deg·s⁻¹ from the analysis due to noise in EMG data.
Statistical Analysis

All descriptive data are reported as the means ± SD. A one-way analysis of variance (ANOVA) with repeated measures was used for comparison among the angular velocities of measured variables. If the F statistic of the analysis of variance was significant, differences among means were assessed using multiple comparisons with Bonferroni’s correction. To assess the relationship among the measured variables for each trial, Pearson product-moment correlations were computed. The level of significance was set at p<0.05.

Results

At all angular velocities, the stretch reflex responses could be observed for all participants. The latency of the stretch reflex was 42.6±7.7 ms at 100 deg⋅s\(^{-1}\), 40.4±7.1 ms at 200 deg⋅s\(^{-1}\), 39.8±6.6 ms at 300 deg⋅s\(^{-1}\), 40.2±6.5 ms at 500 deg⋅s\(^{-1}\), and 40.6±8.2 ms at 600 deg⋅s\(^{-1}\), respectively. There was no difference in the latency of the stretch reflex among the five angular velocities (p=0.216). The amplitude of the stretch reflex significantly increased as the angular velocity increased (p<0.001, Figure 2A). The amplitude of fascicle lengthening significantly decreased (Figure 2B) and the velocity of fascicle lengthening significantly increased (Figure 2C) as the angular velocity increased (both p<0.001). For each trial in all subjects, the amplitude of the stretch reflex was not correlated with the velocity of fascicle lengthening at any angular velocities (Figure 3).

Discussion

The main result of the present study was that the amplitude of the stretch reflex was not related to the velocity of fascicle lengthening in any trial with a high angular velocity and wide range of motion. To date, human studies have demonstrated that the amplitude of the stretch reflex is associated with the angular velocity\(^2,3\). On the other hand, Cronin et al.\(^4\) reported that there was a weak association between the amplitude of the stretch reflex and velocity of fascicle lengthening measured by ultrasonography. However, they determined the relation between the measured variables at a relatively low angular velocity (120 deg⋅s\(^{-1}\)) and narrow range of stretch (1.5–6.0 deg). In the present study, we obtained similar results to those of Cronin et al.\(^4\), even with a high angular velocity (up to 600 deg⋅s\(^{-1}\)) and wide range of motion (13 deg). In addition, we found the same results for each trial (Figure 2), whereas mean measured variables were presented in the previous study\(^4\). There are two reasons for this, as follows.

Firstly, the length of the fascicle, not the length of the muscle fiber, was measured using ultrasonography in the present study. In the study of Eng and Hoffer\(^1\), the stretch velocity of muscle fibers was directly measured based on the ultrasound transit-time using piezoelectric crystals surgically implanted in a cat’s hindlimb. In addition, they reported that the velocity of muscle fibers was correlated poorly with that of the whole muscle. Therefore, it is likely that the fascicle length measured by ultrasonography does not always correspond with the length of muscle fibers and spindles.
Secondly, the present result may be related to the slack within the fascicle. Cronin et al.\textsuperscript{10} demonstrated that the amplitude of the stretch reflex enhanced from 0 to 50% of maximum strength due to the removal of slack from extrafusal muscle fibers and tendinous tissues, although the velocity of fascicle lengthening decreased. In my recent study\textsuperscript{7}, I examined the relationships between the stretch reflex size and muscle stiffness under passive (0% of MVC) and active (30% of MVC) conditions. As shown in Figure 3 of my recent study\textsuperscript{7}, stretch reflexes during muscle contraction may not be clearly detected, unlike at rest. This result agreed with the finding (Figure 3) of Ogawa et al.\textsuperscript{6}. In my recent study\textsuperscript{7}, the relationships between the stretch reflex size and muscle stiffness were examined “on an average of 3 trials”. In the present study, however, I hoped to target clear data concerning the stretch reflex (i.e., stretch reflex during rest), since the purpose of the present study was to examine the relationship between stretch reflex size and fascicle lengthening velocity for “each trial”. In the present study, the velocity of fascicle lengthening increased only 2 times from 59 to 130 mm⋅s\textsuperscript{-1} on average, whereas the angular velocity increased 6-fold from 100 to 600 deg⋅s\textsuperscript{-1}. Furthermore, this result would be related to the tendon elasticity. Herbert et al.\textsuperscript{12} also reported that only 27% of the total change in muscle-tendon length (corresponding to the change in joint angle) in the medial gastrocnemius muscle was transmitted to the

\textbf{Figure 3.} The relationships between the maximal amplitude of the stretch reflex and velocity of fascicle lengthening at 100 (A), 200 (B), 300 (C), 500 (D), and 600 (E) deg⋅s\textsuperscript{-1}.
fascicles during passive dorsiflexion. This result indicated that much of the change in muscle-tendon length occurred in the tendon during passive lengthening. From a functional point of view, it can be said that tendon elasticity prevents excessive muscle lengthening during suddenly stretching.

In this study, we need to discuss the limitations of the methodology used. We obtained ultrasonic images at sampling rates of 100 and 125 Hz. Certainly, ultrafast ultrasound\textsuperscript{10-13} must be used to obtain the instantaneous velocity of fascicle lengthening. In the present study, however, we analyzed the mean velocity of fascicle lengthening at a given range of motion. In future studies, we must investigate the relationship between the instantaneous velocity of fascicle lengthening and the stretch reflex.

In conclusion, the size of the stretch reflex was not associated with the velocity of fascicle lengthening. This result suggests that the amplitude of the short latency stretch reflex was controlled by factors other than the behavior of the fascicle.

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References

1. Eng JJ, Hoffer JA. Regional variability of stretch reflex amplitude in the cat medial gastrocnemius muscle during a postural task. J Neurophysiol 1997;78:1150-1154.
2. Gottlieb G, Agarwal GC. Response to sudden torques about ankle in man: myotatic reflex. J Neurophysiol 1979;42:91-106.
3. Lee RG, Tatton WG. Long latency reflexed to imposed displacements of the human wrist: dependence on duration of movement. Exp Brain Res 1982;45:207-216.
4. Cronin NJ, Rantalainen T, Avela J. Triceps surae fascicle stretch is poorly correlated with short latency stretch reflex size. Muscle Nerve 2015;52:245-251.
5. Obata H, Kawashima N, Ohtsuki T, Nakazawa K. Aging effects on posture-related modulation of stretch reflex excitability in the ankle muscles in humans. J Electro Kinesiol 2012;12:31-36.
6. Ogawa T, Kawashima N, Suzuki S, Nakazawa K. Different modulation pattern of spinal stretch reflex excitability in highly trained endurance runners. Eur J Appl Physiol 2012;112:3641-3648.
7. Kubo K. Effect of short latency stretch reflex on passive and active muscle stiffness in the soleus muscle \textit{in vivo}. Eur J Appl Physiol 2020;122:1303-1312.
8. Maganaris CN. Force-length characteristics of \textit{in vivo} human skeletal muscle. Acta Physiol Scand 2001;172:279-285.
9. Kubo K, Ikebukuro T, Yata H. Effect of angular velocity on active muscle stiffness \textit{in vivo}. J Biomech 2020;111:110007.
10. Cronin NJ, Peltonen J, Ishikawa M, et al. (2008) Effects of contraction intensity on muscle fascicle and stretch reflex behavior in the human triceps surae. J Appl Physiol 2008;105:226-232.
11. Kawashima N, Nakazawa K, Yamamoto S, Nozaki D, Akai M, Yano H. Stretch reflex excitability of the anti-gravity ankle extensor muscle in elderly humans. Acta Physiol Scand 2004;180:99-105.
12. Herbert RD, Moseley AM, Butler JE, Gandevia SC. Change in length of relaxed muscle fascicles and tendons with knee and ankle movement in humans. J Physiol 2002;539:637-645.
13. Haurait H, Nordez A, Guilhem G, Rabita G, Dorel S. \textit{In vivo} maximal fascicle-shortening velocity during plantar flexion in humans. J Appl Physiol 2015;119:1262-1271.