博士（人間科学）学位論文

Architectural and functional properties of the semitendinosus muscle in the hamstring muscles

ハムストリングスにおける半腱様筋の構造的および機能的意義に関する研究

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GENERAL ABSTRACT

This dissertation was designed to investigate the morphological and functional characteristics of the semitendinosus (ST) muscle. Three studies were conducted to achieve this purpose. The first study evaluated the regional differences of magnetic resonance measurements changes of the ST muscle following eccentric exercise. It was demonstrated that the proximal and middle regions of the ST muscle show CSA increase and higher T2 changes compared as the distal region. The next study examined the electromyography (EMG) properties of the ST muscle depending on force level and joint positions. It was demonstrated that the EMG increasing behaviors were different between the proximal and distal compartments at the lengthened and shortened muscle positions in this study. The last study examined the non-uniform changes in the semitendinosus muscle architecture during isometric knee flexion. It was demonstrated that the shortening of muscle fibers and the increasing muscle thickness was non-uniform in the superficial, middle and deep layers in the ST muscle. The results of these experiments indicated that these regional differences would be contributed the presence of a tendinous intersection within the ST muscle belly, which would compensate for the mechanical and functional disadvantage. The presence of the

“V-shaped” tendinous intersection would be affecting to the ST muscle architectural and functional uniformity and contribute to the effective ST muscle contraction.

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LIST OF ABBREVIATIONS

1RM:	1 repetition maximum
ACL:	anterior cruciate ligament
ANOVA:	analysis of variance
ARV:	average rectified value
BFl:	biceps femoris long head
BFl:	biceps femoris long head
BFs:	biceps femoris short head
CK:	plasma creatine kinase
CSA:	cross-sectional area
CT:	computed topography
DICOM:	the Digital Imaging and Communications in Medicine
EMG:	electromyography
F-MARC:	the medical assessment research center in the Fédération Internationale de Football Association
MR:	magnetic resonance
MTJ:	muscle-tendon junction
MVC:	maximal voluntary contraction
PCSA:	physiological cross-sectional area
SM:	semimembranosus
SM:	semimembranosus
ST:	semitendinosus
T2 time:	transverse relaxation time
TI:	tendinous intersection
TR:	repetition time
US:	ultrasonography
VAS:	visual analog scale

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CHAPTER 1:

INTRODUCTION

The fundamental researches about muscle the morphology or function have long been studied, which contribute to the advance of sports orthopaedic researches. Recent years have seen an increased interest in research into sports injury prevention for children, adults, elderly people, and professional or recreational athletes. Injury prevention is now a priority for sports activities all over the world. For example, the medical assessment research center in the Fédération Internationale de Football Association (F-MARC) has recommended the injury prevention program "The 11", which would not only prevent injury but also improve performance. The training programs to prevent injury and enhance performance have been now developing (Bahr and Krosshaug 2005).

Injury prevention research has been described by van Mechelen et al. (1992) as a four step: (1) establishing the extent of the injury problem, (2) establishing the aetiology and mechanisms of sports injuries, (3) introducing preventive measures, and (4) assessing their effectiveness by repeating step 1. This sequence includes obtaining information on why an athlete may be at risk in a given situation (risk factor) or how injuries happen (injury mechanisms). Complete understanding of injury causation needs to address the multifactor of sports injuries such as internal or external factors. The acquiring information of skeletal-muscle morphological and functional characteristics is important for assessing both the intrinsic risk factors or pathomechanics.

The hamstring muscles are one of the significant contributors to exert athletic high performance. Because the hamstring muscles' primary role is that of locomotion than postural control, it is utilized for intense bursts of speed. Therefore, the hamstring muscles must contract forcefully and repeatedly, a factor heavily dependent on the fitness of the individual (Koulouris and Connell 2006). And acute muscle strains in the hamstring muscles are a common injury in sports involving sprinting, jumping or kicking (Kujala et al. 1997; Gabbe et al. 2002). Muscle strain injuries are characterized by observable disruption of the muscle tendon junction (Koulouris and Connell 2003). The high recurrence rate is more problematic. Approximately 60% of reinjured-athlete recurred within one month of returning to sport activity (Brooks et al. 2006). This injury can cause an athlete to miss a few days to a few weeks of activity, and in this period the injury can frustrate the injured athlete own and the surrounding staffs. These observations highlight the challenge in preventing the initial injury and subsequent reinjury. Because it has long been recognized as a priority for efforts at prevention, many studies have been published since it has discussed the hamstring injury mechanisms, potential risk factors, and rehabilitations. However, we have known little fundamental knowledge of hamstring muscles, and it is insufficient for the hamstring injury prevention.

In the hamstring muscles, the semitendinosus (ST) muscle is a notable muscle. The hamstring muscles, excluding the biceps femoris short head muscle, are bi-articular muscle. However, the biceps femoris long head (BFl) and semimembranosus (SM) muscles have relatively short and pennate fibers, which appear to be specialized for high force production. Whereas the ST has parallel- fibered long muscle fascicle arrangement and smaller cross-sectional area, which appears to be better suited for high excursions and low force (Lieber and Bodine-Fowler 1993). Moreover, the ST muscle has a tendinous intersection (TI) within the muscle belly. It separates the ST into proximal and distal regions. The fibers of the two regions are connected in series by TI (Wickiewicz et al. 1983; Woodley and Mercer 2005). Moreover, the two regions are innervated via two branches from the tibial part of the sciatic nerve: one proximal and one distal to the TI (Woodley and Mercer 2005). Thereby, certain motor units provide a distributed pull on all muscle fibers inserted on the opposite side during contraction

(Richmond et al. 1985). However, the functional importance of TI, its contribution to movement, remains unclear in humans.

The anterior cruciate ligament (ACL) injury or rupture is a common non-contact sports injury. The ST distal tendon is commonly used to replace the ruptured ACL because of its relatively low donor site morbidity. After being harvested for use as an ACL graft, the ST tendon can regenerate with a morphological similarity to the native tendon (Cross et al. 1992). The new formed tendon had the histological features of a normal tendon (Eriksson et al. 2001) and indistinguishable from the normal (Leis et al. 2003). Therefore, to elucidate the morphology or contraction behavior of ST muscle-tendon complex would be important to evaluate the recovery of the ST muscle function after the ST tendon is regenerated.

During the last two decades, the availability and sophistication of the diagnostic apparatuses, such as magnetic resonance (MR) imaging or ultrasonography, have increased enormously. In these days, these advances techniques have been used for not only the morphological evaluations but also the functional evaluations of the skeletal-muscles. It have been showed that the non-uniform shortening of the biceps brachii muscle during low-load elbow flexion (Pappas et al. 2002) or the intramuscular variations of activity within a medial gastrocnemius muscle in the calf-raise exercise

(Kinugasa et al. 2006). These techniques have been able to provide the detailed information of the skeletal muscles and to apply the clinical estimations.

Not only the visualizing techniques, the technique of the neuromuscular activation measurement have also evolved as a tool of analyzing the muscle activity. The direct (needle electrode, fine-wire electrode) or indirect (surface electrode) methods have been used for investigating the muscle coordination (Carson et al. 2002), muscle contractile properties (Macefield et al. 1996), or gait pattern (Bejek et al. 2006). The ST muscle has different electromyographic (EMG) activities as well as morphology. In isometric and concentric contraction, the ST EMG increased as the knee flexion angle increased, whereas the maximum BFl EMG was occurred at slight knee-flexed position and decreased as knee angle increased (Onishi et al. 2002). It is uncertain what factor is mainly influential to the difference in EMG activities of hamstring muscles, but the knowledge of the inherent EMG activity is important to apply for sports orthopaedic researches.

In light of these considerations, a quantitative assessment of the ST muscle’s morphology and function could provide not only the physiological basis of force production or movement but also the practical rationale for injury prevention methods, treatment strategies or rehabilitation programs, and analyze potential hamstring muscles' injury risk factors or pathomechanisms. The purpose of this dissertation was to investigate the ST muscle morphology and function by using magnetic resonance imaging, ultrasonography and EMG. And moreover, it was investigated that the functional properties of the ST muscle in the hamstring muscles.

Overview

Following Chapter 1 as an introduction, Chapter 2 provides comprehensive account of numerous details in the semitendinosus muscle research.

In Chapter 3, it was investigated that the differences of MR measurements (cross-sectional areas (CSAs), T2 values) among hamstring muscles following intensive knee eccentric flexion exercise.

In Chapter 4, it was investigated the effect of joint position on the torque and intramuscular EMG activities of the ST muscle. For this experiment, bipolar urethane-coated stainless steel fine-wire electrodes were used to record intramuscular EMG.

In Chapter 5, it was investigated the changes of the TI architecture of the ST in the direction of the short and long axes during isometric knee flexion by using ultrasonography.

Finally, Chapter 6 presents a general discussion about the present findings from Chapters 3, 4 and 5.

CHAPTER 2:

REVIEW OF LITERATURE

The researches focused on the semitendinosus muscle were published from the 1980s. Anatomical characteristics of the semitendinosus muscle were investigated in the cat (Bodine et al. 1982; English and Weeks 1987), rat (Woolf and Swett 1984), rodent (Roy et al. 1984), dog (Rosenblatt et al. 1988), and goats (Gans et al. 1989).

In the 1990s, improved diagnostic techniques, such as magnetic resonance (MR) imaging and ultrasonography (US), have proven to be valuable in the diagnosis of especially muscle, tendon, or connective tissue injuries in human (De Smet et al. 1990; Niitsu et al. 1991; Aspelin et al. 1992; Takebayashi et al. 1995; El-Khoury et al. 1996). Moreover, surprising studies have shown that the semitendinosus tendon actually regenerate after harvesting for use as anterior cruciate ligament (ACL) autografts. In 1992, Cross et al. first reported the apparent semitendinosus tendon regeneration occurred from the distal cut end of the muscle belly to the fascial planes of the popliteal fossa after the reconstruction of the ACL. After that, some reports have published the evaluations of the semitendinosus muscle and other knee flexor muscles (Simonian et al.

1997; Muneta et al. 1998). In 1997, Simonian et al. found a more shortening of muscle length and a more proximal tendon insertion in the harvested semitendinosus muscle, but the cross-sectional areas (CSAs) of the biceps femoris, semimembranosus, and sartorius muscles were not increased compared as the nonoperated side.

In 2000s, because of the increasing of availability and sophistication of the technological advances, investigations of the tendon regeneration and evaluations of the functional recovery of semitendinosus muscle after ACL reconstruction have became by using diagnostic imaging apparatuses, such as MRI (Eriksson et al. 2001; Hioki et al. 2003; Burks et al. 2005;Takeda et al .2006), computed tomography (CT) (Nakamura et al. 2004; Yasumoto et al. 2006) and US (Papandrea et al. 2000) or knee-flexion torque and electromyographic (EMG) analysis (Makihara et al. 2006; Nishino et al. 2006). In 2003, Hioki et al. investigated the intramuscular movement of hamstring muscles after the ACL reconstruction with semitendinosus and gracilis tendons using a novel MRI technique called the “tagging snapshot” technique. The others of this study concluded that the effect of semitendinosus and gracilis tendons on knee function is not uniform as far as the regeneration of the tendons and the knee muscle strength is concerned.

In these days, other reports investigated kinematics of semitendinosus and other hamstring muscles during splinting by using motion analysis system (Thelen et al.

2005; Heiderscheit et al. 2005). They found that peak hamstring stretch occurred during the late swing phase of sprinting before foot contact and intermuscle differences in hamstring muscle geometry could be a contributing factor to the greater propensity for muscle injuries.

Anatomical characteristics of the semitendinosus muscle

The semitendinosus (ST) muscle is one of the hamstring muscles including the ST, semimembranosus (SM), biceps femoris long head (BFl) and biceps femoris short head (BFs), which located in the posterior of the thigh (Fig. 2-1). The architectures and innervations' patterns of the respective muscles differ (Wickiewicz et al. 1983; Friederich and Brand 1990; Woodley and Mercer 2005). The results of previous studies were surmised in Table 2-1.

The ST, SM and BFl are bi-articular muscle, which involves hip and knee joints. The individual bi-articular hamstring muscles has different moment arm at the hip and knee. The ST and BFl have a slightly larger hip extension moment arm than the SM (Arnold et al. 2000). At the knee, the ST and SM have a larger knee flexion moment arm than the BFl (Buford et al. 1997). Hip flexion causes relatively greater lengthening of the ST and BFl, whereas knee flexion causes a reduction in the overall length of the hamstring bi-articular muscles. The net result of these combined effects generates the difference of stretch degrees for sprinting among the individual hamstring muscle-tendon complex, which could contribute to the difference of injury occurrence in the hamstring muscles (Thelen et al. 2005).

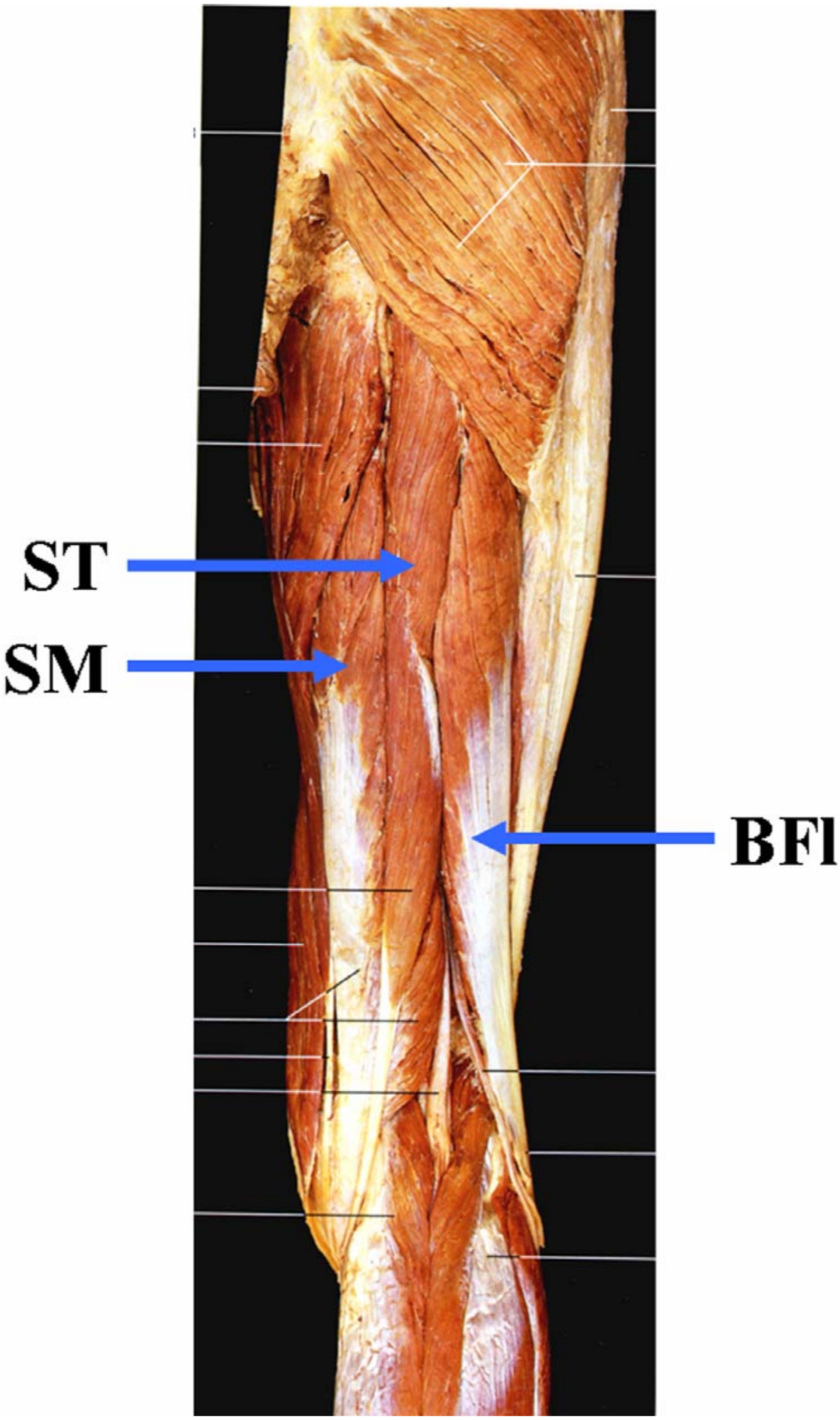

Fig. 2-1 Dorsal muscles of right thigh. *BFl* biceps femoris muscle long head, *ST* semitendinosus muscle, *SM* semimembranosus muscle (Rohen and Yokochi 1988).

Table 2-1 Morphology of the hamstring muscles

			ST	SM	BFl	BFs
Bi-/mono-architecture			Bi-	Bi-	Bi-	Mono-
Knee rotator			Internal	Internal	External	External
Nerve supply			Tibial N.	Tibial N.	Tibial N.	Peroneal N.
Muscle fiber arrangement			Parallel-fiberd	Unipennate	Unipennate	Parallel-fiberd
Muscle weight[1)]	[g]	Wickiewicz et al. (1983)	**76.9**	**119.4**	**128.3**	**-**
Muscle volume	[ml]	Friederich and Brand (1990)	**128.5**	**211.0**	**138.5**	**76.0**
Muscle length	[cm]	Wickiewicz et al. (1983)	31.7	26.2	34.2	27.1
		Friederich and Brand (1990)	28.3	20.8	27.4	22.3
		Woodley and Mercer (2005)	31.6	26.4	28.1	25.8
		Makihara et a. (2006)	26.8	28.5	31.2	-
			29.6	**25.5**	**30.2**	**25.0**
Fiber length	[cm]	Wickiewicz et al. (1983)	15.8	6.3	8.5	13.9
		White (1989)[2)]	6.6	6.6	9.1	11.8
		Friederich and Brand (1990)[2)]	9.0	6.4	7.3	11.7
		Delp et al. (1990)	20.1	8.0	10.9	-
		Woodley and Mercer (2005)[2)]	9.0	5.0	7.0	12.4
		Makihara et a. (2006)	23.8	6.0	7.3	-
			18.1[3)]	**6.4**	**8.3**	**12.4**
Fiber length/muscle length	[%]	Wickiewicz et al. (1983)	50	24	25	52
		Friederich and Brand (1990)	46	27	26	52
			48	**26**	**26**	**52**
Muscle PCSA	[cm^2]	Alexander and Vernon (1975)	8.5	30.0	21.0	5.2
		Wickiewicz et al. (1983)	5.4	16.9	12.8	-
		Freivalds (1985)	4.3	13.0	11.8	-
		Friederich and Brand (1990)	13.2	30.2	9.2	6.4
		Woodley and Mercer (2005)	8.1	15.8	10.1	3.0
			7.9	**21.2**	**13.0**	**4.9**
Pennation angle	[deg.]	Alexander and Vernon (1975)	0.0	16.0	17.0	0.0
		Pierrynowski and Morrison (1985)	0.0	15.0	15.0	0.0
		Spoor et al. (1989)	10.0	15.0	15.0	-
		White (1989)	15.0	0.0	0.0	17.0
		Wickiewicz et al. (1983)	5.0	15.0	0.0	23.3
		Friederich and Brand (1990)	6.0	16.0	7.0	15.0
		Delp et al. (1990)	5.0	15.0	0.0	23.0
		Makihara et a. (2006)	0.0	31.0	28.0	-
			5.1	**15.4**	**10.3**	**13.1**
% typeI fibers	[%]	Pierrynowski and Morrison (1985)	50.0	50.0	65.0	66.9
		White (1989)	50.0	50.0	66.9	50.0
			50.0	**50.0**	**66.0**	**58.5**
Sarcomere length	[μm]	Ward et al. (2007)	**2.9**	**2.6**	**2.4**	**3.3**

ST semitendinosus muscle, *SM* semimembranosus muscle, *BFl* biceps femoris muscle long head, *BFs* biceps femoris muscle short head

1) formalin-fixed muscle

2) average data mesured from the proximal and distal regions

3) from the proximal to distal muscle-tendon junctions

The ST has unique architectural characteristics among the hamstring muscles. The proximal fascicles of ST arose from three locations: the posteromedial aspect of the ischial tubelosity, the medial border of the proximal tendon of BFl, and a proximal aponeurosis appeared to be continuous with the BFl proximal tendon (Fig. 2-2). The proximal tendon of ST was relatively short in general. The ST muscle belly was long, thin and parallel-fibered. Conversely, the physiological cross-sectional area (PCSA) of the ST is smaller than that of either the BFl or SM muscles (Fig. 2-3). The long and thin distal ST tendon passed along the medial aspect of the knee joint, which was the longest tendon of the hamstring muscles (Woodley and Mercer 2005).

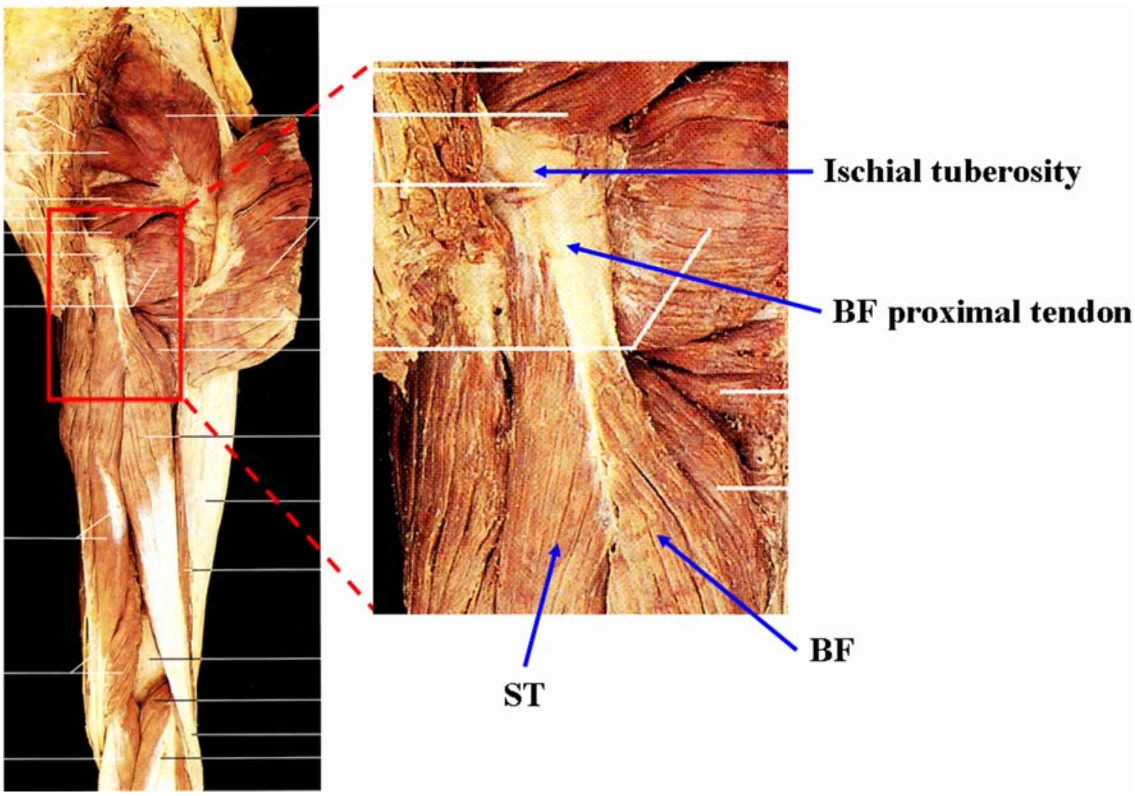

Fig. 2-2 Proximal insertion of the hamstring muscles. *ST* semitendinosus muscle, *BF* biceps femoris muscle (Rohen and Yokochi 1988).

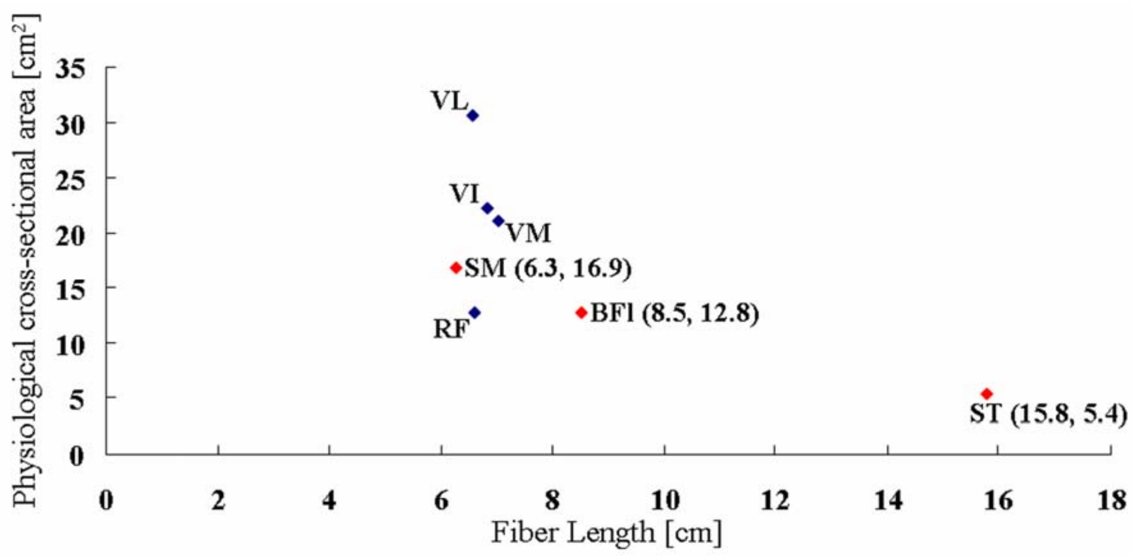

Fig. 2-3 Scatter graph of the fiber length and cross-sectional areas of muscles in the human hamstring (◆) and quadriceps femoris (◆) muscles. Fiber length is proportional to muscle excursion, and cross-sectional area is proportional to maximal muscle force. *RF* rectus femoris muscle, *VL* vastus lateralis muscle, *VM* vastus medialis muscle, *VI* vastus intermedius muscle, *BFl* biceps femoris long head muscle, *ST* semitendinosus muscle, *SM* semimembranosus muscle. Data were from Wickiewicz et al (1983).

Moreover, a tendinous intersection (TI) is present within the muscle belly (Fig. 2-4). It divides the muscle into two (proximal, distal) distinct regions (Wickiewicz et al. 1983; Lee et al. 1988; Woodley and Mercer 2005). Since the fascicular lengths of the proximal and distal regions are almost equal and the fascicles in the two regions are in series, the ST was generally treated as a single muscle. However, almost all of the fascicles in the proximal region inserted to the TI, and those in the distal region arose from the TI (Woodley and Mercer 2005). Moreover, the two regions are innervated via two branches from the tibial part of the sciatic nerve: one proximal and one distal of the TI (Fig. 2-4). In the hamstring muscles, only the ST muscle was partitioned on the basis of both architecture and innervations.

Fig. 2-4 The semitendinosus muscle derived from a cadaver. Two vascular and nerve branches were inserted into the proximal and distal regions of the tendinous intersection (*TI*).

Measurements of human muscle morphology and function *in vivo*

The advance of modern imaging techniques offers a variety of approaches for monitoring not only structure but also function of skeletal-muscles. In these days, magnetic resonance (MR) imaging (Fig. 2-5(A)) and ultrasonographic (US) techniques (Fig. 2-6(A)) have been popular to use morphological and functional investigations in physiological and sports science field.

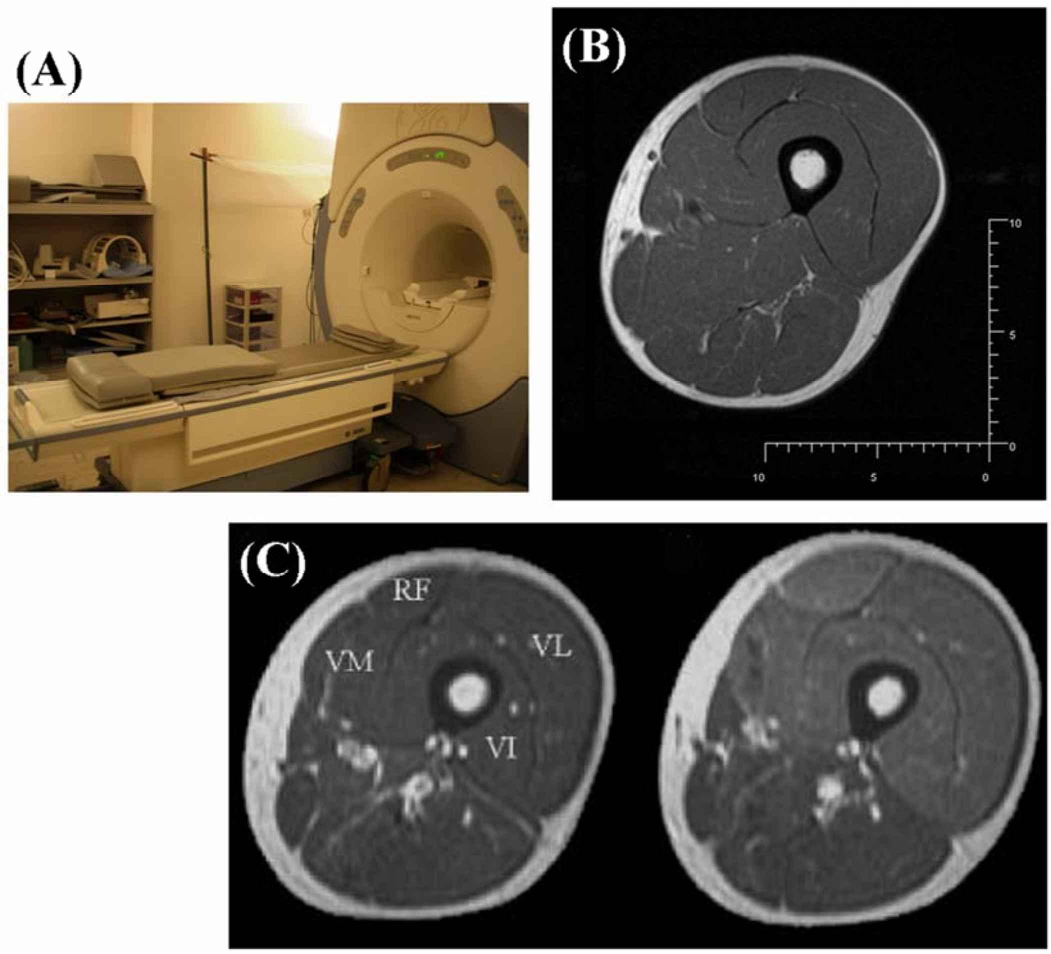

Fig. 2-5 Magnetic resonance (MR) scanner and images. (A) A 1.5-Tesle MR scanner. (B) A T1-weightened spin echo image of the left thigh. (C) T2-weightened spin echo image of the left thigh before (left) and after (right) eccentric exercise of the knee extensor muscles (Prior et al. 2001). *RF* rectus femoris muscle, *VL* vastus lateralis muscle, *VM* vastus medialis muscle,*VI* vastus intermedius muscle.

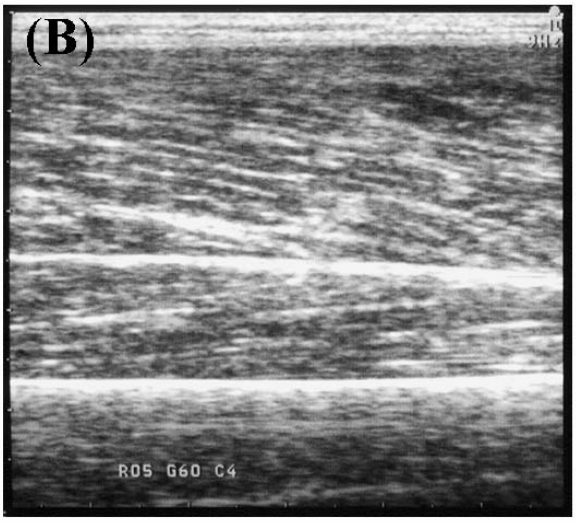

Fig. 2-6 Ultrasonographic (US) apparatus (SSD-1000, Aloka) and image. (A) A US apparatus. (B) A US image of the vastus lateralis muscle.

Imaging technique of human muscle

MR imaging technique

MR imaging is possible to acquire the muscle properties *in vivo*. The MR imaging technique can be applied to measure children, elder people or athletes because this technique is noninvasive and not-painful. Moreover, the images are very clearly and suitable for assessing soft tissues such as brain or skeletal-muscles. These days, this technique has been used not only morphological evaluations but also functional evaluations of the skeletal-muscles. MR imaging enables real-time or near real-rime "functional imaging" of muscles such as brain. Functional MR imaging refers to imaging not only the morphological characteristics but also the extent to which the tissue is involved in performing a task. Furthermore, the computational models made from MR images have emerged as powerful tools for investigating muscle morphology (Blemker and Delp 2005; Blemker and Delp 2006; Tate et al. 2006) and function (Pappas et al. 2002; Hioki et al. 2003; Blemker et al. 2005). Skeletal-muscle models, combined with dynamic simulation, have been used to understand normal (Pappas et al. 2003; Blemker et al. 2005) and pathological human movement (McLean et al. 2003; Manal and Buchanan 2005).

Morphological evaluations

Traditionally, muscle geometry or morphology is typically derived from cadaveric studies (Lieber et al. 1984; Zajac 1989; Murray et al. 2000). However, results of the traditional techniques have limited. For example, it is not clear how musculoskeletal deformities or variations in body size or age. With the integration of MR imaging techniques, more individualized, detailed, and accurate models have begun to emerge. In general, standard pulse sequences, such as T1-weightened spin-echo imaging (Fig. 2-5 (B)), have been used for determining the cross-sectional area or volume of skeletal-muscle or fat tissue noninvasively and *in vivo* (Tate et al. 2006).

Recently, investigators have indicated the feasibility of using diffusion tensor imaging (DTI) to understand the geometric muscle fascicle arrangement, such as pennation angle or fascicle length, and the relationships between structure and function of human skeletal-muscles (Bammer et al. 2003; Sinha et al. 2006; Zaraiskaya et al. 2006; Lansdown et al. 2007) (Fig. 2-7).

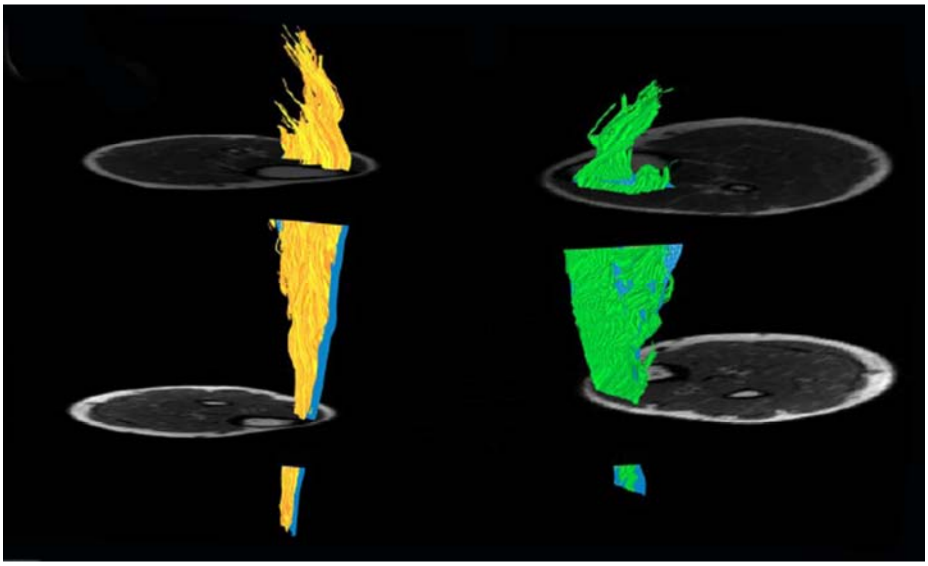

Fig. 2-7 Sample fiber tracking results of the diffusion tensor imaging technique. (Left) Fiber tracts of the deep compartment, indicated as gold (and similarly shaded) lines. (Right) Fiber tracts of the superficial compartment, indicated as green (and similarly shaded) lines (Lansdown et al. 2007).

Functional evaluations

Several reports for examining muscle activity are related to transverse relaxation (T2) times (Adams et al. 1992; Fleckenstein et al. 1993; Foley et al. 1999; Prior et al. 2001; Akima et al. 2003; Kinugasa et al. 2006; Larsen et al. 2007). Muscle MR imaging with analysis of T2 times appears to be capable of examining the relative amount of activity or response of exercised-muscles (Fig. 2-5 (C)). However, it is clear that changes in T2 times with exercise are inherent in a muscle and multifactorial, including fiber type distribution, differences in regional perfusion and aerobic capacity (Prior et al. 2001; Damon and Gore 2005).

These days, dynamic imaging of muscle motion has challenged and provided a powerful source of evaluating muscle contraction behavior (Pappas et al. 2002; Hioki et al. 2003; Blemker et al. 2005). Pappas et al. (2002) acquired cine phase contrast MR images in the biceps brachii muscle during low-load elbow flexion and showed non-uniform shortening along the centerline. Hioki et al. (2003) investigated the intramuscular movement of hamstring muscles after ACL reconstruction of the ACL with ST and gracilis tendons using a novel MRI technique called the “tagging snapshot” during lightly knee flexion. They showed that the effect of hamstring tendons’ harvest on knee function is non-uniform among tendon regeneration patterns.

Ultrasonographic (US) technique

Over the last 20 years, Ultrasonographic (US) techniques have established as a means of imaging soft tissues and joints extensively in Europe and Japan (Kawakami et al. 1993; Fukunaga et al. 1996; Narici et al. 1996; Ito et al. 1998; Sousa et al. 2007). The US technique is used to characterize *in vivo* muscle fascicle orientations and lengths in real-time (Fig. 2-6 (B)). In recent years, the US techniques have demonstrated not only the resting muscle information, such as cross-sectional area (Kanehisa et al. 1994; Ichinose et al. 1998) or whether injury was presence or not (Takebayashi et al. 1995; Schepsis et al. 2002), but also the muscle contraction behaviors (Fukunaga et al. 1996; Ito et al. 1998; Sousa et al. 2007) or follow-up of injured-muscle repairing process (Connell et al. 2004; Genovese et al. 2007). Recently, there has been an upsurge of interest in US examination of the injured athlete due to technological advances in US equipment and small machines that can be used not only in the laboratory but also in the stadium or playing field. The US apparatus is inexpensive or portable compared as other imaging techniques such as MR imaging or computed tomography (CT) techniques. One shortcoming is the limitation to planar and superficial muscles' measurements. In addition, the US technique is only as good as the operator who is performing the examination and the quality of the apparatus that is used.

Neuromuscular activation measurement technique

Muscle force is developed by linkages between the actin and myosin filaments of the sarcomere. This reaction requires ATP and calcium ions, and is brought about by a rapid depolarization of muscle membrane. This depolarizing potential is recorded from electromyography (EMG) in the extra cellular space. Over the last 50 years, the EMG method has evolved as a tool of analyzing electrical activity of muscles. The EMG has been widely used for the studies of muscle coordination (Dietz et al. 1986; Prilutsky et al. 1998; Carson et al. 2002), muscle contractile properties (Milner-Brown et al. 1973; Duchateau and Hainaut Hainaut 1987; Macefield et al. 1996), motor unit recruitment and firing rate (Solomonow et al. 1990; Van Cutsem et al. 1998), and applied to assessing therapeutic procedures such as the rehabilitation process (Maitland et al. 1999; Chmielewski et al. 2005), providing biofeedback to patients (Levitt et al. 1995; Dursun et al. 2001), evaluating gait (Wren et al. 2006; Bejek et al. 2006).

EMG technique

The neuromuscular activity is a direct representation of the outflow of motor neurons to the muscle as a result of voluntary or reflex activation. In the physical training, sports medicine or rehabilitation fields, the EMG technique is applied for practical applications (Duchateau and Hainaut 1991; Hakkinen et al. 2000; Aagaard et al. 2002; Gondin et al. 2004). For example, increase in the EMG signal amplitude appear well before increase in muscle size (Hakkinen et al. 2000; Aagaard et al. 2002), or disuse results in a decrease in muscle electrical activity (Duchateau and Hainaut 1991; Gondin et al. 2004). There are direct and indirect methods in the EMG technique. The former used indwelling electrodes such as needle or fine-wore electrodes, whereas the letter used surface electrodes.

Intramuscular EMG technique

Fine-wire based or needle intramuscular electrodes are used in order to directly detect activities with a high spatial resolution from muscles, which are small or located deep within the body. Wire electrodes are popular for kinesiological issues in dynamic examinations (Hoffer 1993; Rowlands et al. 1995) or neurophysiological studies (Onishi et al. 2000; Onishi et al. 2002; Mohamed et al. 2002) because the electrodes are easily implanted and removed.

The surface EMG technique is susceptible to electrical noise, mechanical artifacts, or crosstalk between muscles. However, the fine-wire technique has the advantage of not-creating major problems shown in surface EMG technique because of recording the same group of motor units.

The electrodes have potential complications such as subject's discomfort and wire fracture. The incidence of these problems is extremely low and is not considered a threat to subjects by experiences investigators. Muscle damage with implanted electrodes was assumed, but we know of no current work on this topic.

Surface EMG technique

The surface electromyography (EMG) comprises the sum of electrical contributions made by the active motor units as detected by electrodes placed on the skin overlying the muscle. The characteristics of surface EMG, such as its amplitude and power spectrum, depend on the membrane properties of the muscle fibers as well as on the timing of motor units' action potentials.

The EMG signal depend on non-physiological factors, such as thickness of the subcutaneous tissue layers or shift of the muscle relative to the EMG electrodes during muscle contractions, and physiological factors, such as the distribution of motor unit conduction velocity or number of recruited motor units. Of the non-physiological factors, the crosstalk from nearby muscles has been the most controversial topic in many investigators (De Luca and Merletti 1988; Aagaard et al. 2000; Dimitrova et al. 2002; Ferina et al. 2002; Lowery et al. 2003; Mogk and Keir 2003). Moreover, the passive surface electrodes have little electrical input resistance. Therefore, the skin surface must be cleaned with using alcohol and rubbed with an abrasive gel preparation to reduce the electrical resistance of the skin.

In the 2000s, the surface EMG technique have been applied to the dynamic contractions, such as concentric or eccentric contractions (Nakazawa et al. 1993; Pasquet et al. 2000; McHugh et al. 2002), or walking (Bird et al. 2003; Warren et al. 2004). Interpretation of the surface EMG in dynamic contraction tasks is complicated by three main additional factors: the signal nonstationarity, the shift of the electrodes relative to muscle fibers, and the changes in the conductive properties of the tissues lying between electrodes and muscle fibers. The analysis techniques of surface EMG in dynamic contractions have developed, and have been a powerful means for assessing muscle function in both research and clinical environments.

CHAPTER 3:

NON-UNIFORM CHANGES IN MAGNETIC RESONANCE MEASUREMENTS OF THE HAMSTRING MUSCLES FOLLOWING INTENSIVE ECCENTRIC EXERCISE

3-1. Introduction

The hamstring muscles include the four muscles [biceps femoris muscle long head (BFl) and short head (BFs), semimembranosus muscle (SM) and semitendinosus muscle (ST)] located in the posterior of the thigh. The architectures and innervation patterns of the respective muscles differ (Friederich and Brand 1990; Wickiewicz et al. 1983; Woodley and Mercer 2005). The BFl has an intermediate fascicle length and a physiological cross-sectional area (CSA) compared with the other hamstring muscles, whereas the BFs have a long fascicular and a small physiological CSA. The SM has a short fascicular length and a large physiological CSA. The ST has unique architectural characteristics among the hamstring muscles. A tendinous intersection (TI) is present within the muscle belly; it divides the muscle into two (proximal and distal) distinct regions (Wickiewicz et al. 1983; Lee et al. 1988; Woodley and Mercer 2005). Since the fascicular lengths of the proximal and distal regions are almost equal and the fascicles in the two regions are in series, the ST was generally treated as a single muscle. However, almost all of the fascicles in the proximal region inserted to the TI, and those in the distal region arose from the TI (Woodley and Mercer 2005). Moreover, the two regions are innervated via two branches from the tibial part of the sciatic nerve: one proximal and one distal of the TI. In the hamstring muscles, only the ST muscle was partitioned on the basis of both architecture and innervations. Consequently, it is assumed that the degree of involvement and/or response of an exercise are different between the proximal and distal regions of the ST.

The results of numerous studies have shown that the anatomical characteristics of a muscle are primary determinants of the functional properties (Lieber and Bodine-Fowler 1993; Lieber and Friden 2000). The hamstring muscles generally activate during knee-flexion and/or hip-extension, and deal with one skeletal muscle group. However, taking into consideration of the different architectural characteristics of each hamstring muscle, it is conceivable that respective muscles have inherent functions and compensate for each other. Many cases of hamstring strain, for example, involve the biceps femoris, although the semitendinosus and semimembranosus muscle are less injured, which might reflect the architectural and functional differences of the muscles (Heiderscheit et al. 2005; Thelen et al. 2005).

Recently, magnetic resonance (MR) imaging has been used to assess skeletal muscle functions, with the transverse relaxation time (T2) indicating a quantitative index of muscle activation. Intensive exercise is known to produce changes in the amount and distribution of water in skeletal muscle. This method can non-invasively monitor the physiological changes of the recruited muscle during exercise. In fact, MR imaging can be used to assess damaged muscles following intensive exercise (Leblanc et al. 1993; Clarkson and Hubal 2002). Many authors have reported that the T2 value increases following eccentric exercise (Jayaraman et al. 2004; Larsen et al. 2007; Prior et al. 2001; Segal and Song 2005; Sesto et al. 2005) and that T2 value is positively correlated with plasma creatine kinase (CK) activity, reflecting exercise-induced muscle damage (Larsen et al. 2007; LeBlanc et al. 1993; Schwane et al. 2000).

Moreover, earlier studies have investigated the inter-muscle differences and intra-muscle regional differences of T2 value changes between proximal and distal regions (Akima et al. 2004; Segal and song 2005). However, to our knowledge, no regionally specific differences of the morphology and T2 value have been reported comprehensively for the hamstring muscles. These muscles are long and multiarticular, representing a complex of fusiform and pennate muscles. Therefore, the hamstring muscles would show each muscle's specific characteristics of architectural change following intensive eccentric exercise. A detailed examination of the changes in MR measurements of the hamstring muscles is applicable to the understanding of the functional difference between the muscles, and of the pathomechanics of the muscles.

In light of these considerations, we hypothesized that the degree of the response following the intensive exercise would be different represented as different changes in MR measurements, such as the CSAs and T2 values, among hamstring muscles and between proximal and distal regions of each muscle. This study was designed to investigate the regional specific differences of MR measurements in the hamstring muscles following eccentric knee-flexion exercise.

3-2. Methods

Subjects

This study examined 12 healthy young male volunteers with no history of neuromuscular or orthopedic disease (age, 23.7±1.8 years; height, 171.8±4.8 cm; weight, 66.9±8.6 kg). None were participating in any regular training regime. Subjects were instructed to avoid activities and not to use icing or anti-inflammatory medication for the week preceding and the week of the experiment. This study was approved by the Human Research Ethics Committee of the School of Sport Sciences of Waseda University and is consistent with their requirements for human experimentation. This study conforms to the Declaration of Helsinki. Written informed consent statements were obtained after participants had read the volunteer information sheet and questions related to the study had been answered to their satisfaction.

Exercise protocol

After a few minutes of warming up, subjects performed eccentric exercise of the hamstring muscles with the right leg using a plate-loaded knee-flexion machine (Prone Leg Curl; Nautilus, USA), which was adjusted to 120% of the 1 repetition maximum (1RM).

Subjects were instructed to lower the weight from a knee-flexed position (100°, Fig. 3-1(A)) to a knee-extended position (0°, Fig. 3-1(B)) in 3 s, maintaining the lowering velocity as constant as possible by following the examiner’s counting of “0” for the beginning and “1, 2, and 3” for the movement with planter flexed of the ankle to reduce the contribution of the gastrocnemius muscle. Subjects were verbally encouraged to generate maximal force at the starting position and to resist maximally against the knee-extending action throughout the range of motion. The weight was raised after each eccentric repetition by an examiner; therefore, the overall exercise task was eccentric only for the subject. This was repeated for five sets of ten repetitions each, with at least a 3-min rest between sets.

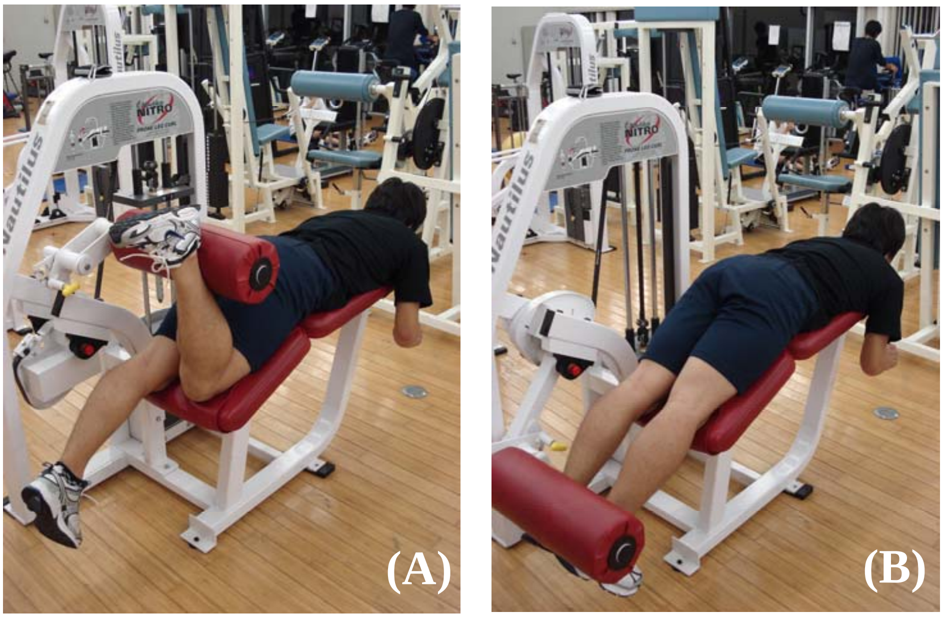

Fig. 3-1 Eccentric exercise of the right hamstring muscles from a knee-flexed position (100, (A)) to a knee-extended position (0°, (B)).

Criterion measures

Before and immediately after exercise, and on the first, second, third and seventh days following the exercise, maximal isometric knee-flexion torques were calculated, and plasma creatine kinase (CK) activity and muscle soreness were assessed. In addition, MR imaging of the thigh was performed.

MVC

Maximal voluntary contractions (MVCs) of the knee-flexor muscles were measured using a modified force-measuring machine that included a force gauge (LTZ-200KA; Kyowa Electronic Instruments, Tokyo, Japan) connected to an analog-to-digital converter (LEG-1000; Nihon Kohden, Tokyo, Japan). The metal cord with the force gauge was mounted to ankle joint. Subjects were prone on a bed with the hip joint at 0° of flexion and abduction, with the knee joint at 15° of flexion. Subjects maintained each MVC for 3 s. They repeated the task two times with at least 3 min rest between tests. Maximal isometric knee-flexion torque was calculated from the MVC value. The highest torque was used for further analyses.

Blood analysis

A 10-ml sample of blood was drawn from a branch of the antecubital vein. The blood was allowed to clot for 30 min at room temperature; it was then centrifuged for 10 min to obtain serum. After separation, all serum samples were stored at -20°C until analysis for CK activity. The CK enzyme activity was measured in the laboratory. Because of the high degree of variability associated with plasma CK, the values were log-transformed to satisfy the analysis of variance (ANOVA) procedure.

Muscle soreness

Muscle soreness was evaluated using a visual analog scale (VAS) consisting of a 100-mm continuous line representing “no pain” at one end (0 mm) and “unbearable pain” at the other (100 mm). Other examiners (Chen et al. 2007; Nosaka and Sakamoto 2001) have used and described this scale previously. Subjects lay prone on a bed and were asked to indicate the muscle soreness level on the line when an investigator pressed the BFl, ST, and SM muscle belly with 4 kg/cm force using a pressure meter (Igarashi Ika Kogyo, Tokyo, Japan). The press points of each muscle were marked to give pressures at a same position over days.

MRI

All MR images of the thigh were performed using a 1.5-T whole body imager (Magnetom Symphony; Siemens-Asahi Medical Technologies Ltd. Tokyo, Japan). For the MR imaging scans, subjects were positioned supine with their knee-flexed. To maximize repeatability of limb placement in the imager, subjects were secured in a leg-holding device that was fitted to the inside of the coil. Then, T2-weightened transverse spin-echo MR axial images [repetition time (TR) = 2,000 ms, echo time (TE) = 30, 45, 60, and 75 ms] were collected beginning at the lower end of ischial tuberosity with a single scan using a 256 × 256 image matrix, with a 270 mm field of view, 10-mm slice thickness, and 12-mm interslice gap using a body coil.

The MRI data were evaluated for anatomical CSA and T2 relaxation time (T2 value) of the hamstring muscles. In the evaluations, the images containing of the areas at 30% (proximal), 50% (middle) and 70% (distal) of thigh length from the upper border of the ischial tuberosity (0%) to the lower border of the tibial plateau (100%) were used. The MR images were transferred to a personal computer in the Digital Imaging and Communications in Medicine (DICOM) file format; image manipulation and analysis software (OSIRIS, University Hospital of Geneva, Switzerland) was used to measure CSA and the signal intensity of each hamstring muscle (BFs, BFl, ST and SM). The region of interest was defined by tracing the outline of the muscles, avoiding visible aponeurosis, vessels, fat, membranes, and the femur. The signal intensity was measured from the same region for all four TEs. A T2 measurement sequence with four TEs was applied to measure the absolute T2 value. Images taken at different TEs were fit to a monoexponential time curve to extract the T2 values based on the formula: $SI = M_0 \times \exp(-TE/T2)$, where SI represents the signal intensity at a given TE and M_0 is the original MRI signal intensity. The same person performed the MR imaging scan and the T2 calculation.

Statistical analysis

Changes in the maximal isometric knee-flexion torque, plasma CK activity, muscle soreness measured over day were compared using one-way ANOVA with repeated measures. Significant differences of the CSAs and T2 values of hamstring muscles following the eccentric exercise were determined using two-way ANOVA with repeated measures (muscle region × day). Bonferroni's post hoc analysis was conducted if the ANOVA showed statistical significant main effects or interaction effects. The statistical significance was set at $P < 0.05$ for the ANOVA and $P < 0.003$ for the post hoc test. All statistical analyses were conducted using a statistical analysis software program (SPSS ver. 14.0; SPSS Japan Inc., Tokyo, Japan). Descriptive data are expressed as mean ± SD.

3-3. Results

MVC

Maximal isometric knee-flexion strength, as measured before, immediately after, and on the first, second, third, and seventh days following the exercise, is shown in Fig. 3-1. The maximal torques showed a significant day effect ($F_{5,55} = 10.9$; $P < 0.001$). The maximal torque was decreased significantly to 25.8% immediately after the exercise ($P < 0.003$). By the seventh day, the torque had not recovered to its initial values; it also showed a trend toward pre-values.

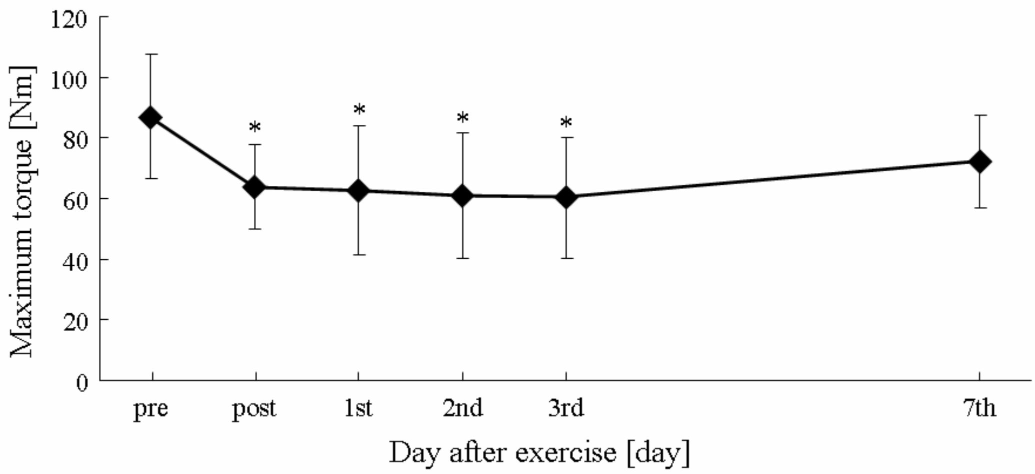

Fig. 3-2 Change in maximal isometric knee flexion torque before (pre), immediately after (post), and on the 1st, 2nd, 3rd, and 7th days following eccentric exercise. Asterisks indicate significant differences from the pre value (*$P < 0.003$).

Plasma CK activity

Plasma CK values were transformed to a natural log scale (Fig. 3-2) and showed a significant day effect ($F_{5,55} = 19.5$; $P < 0.001$). The CK activity was significantly higher on the second, third, and seventh days than the pre-value ($P < 0.003$). The value reached its peak on the third day after exercise.

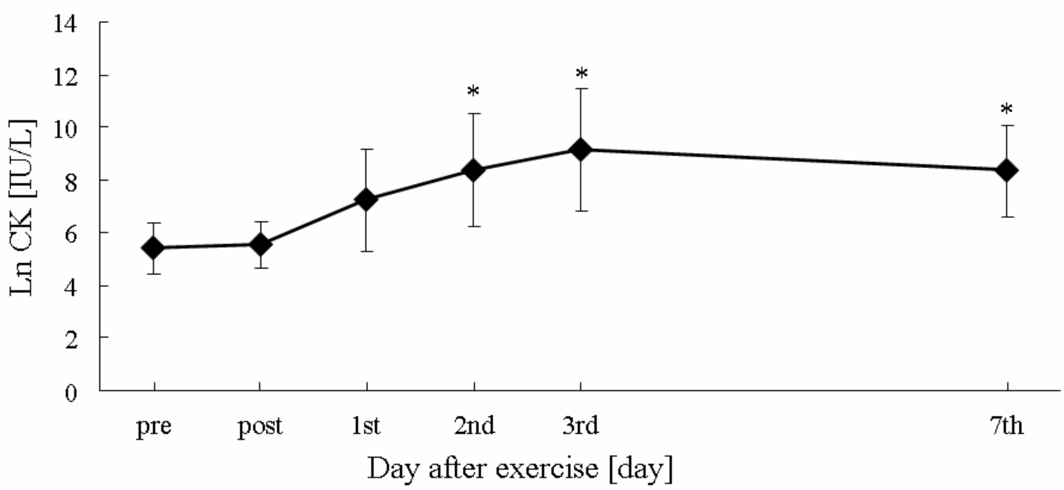

Fig. 3-3 Change in plasma creatine kinase (CK) activity (natural log) before (pre), immediately after (post), and on the 1st, 2nd, 3rd, and 7th days following eccentric exercise. Asterisks indicate significant differences from the pre value (*$P < 0.003$).

Muscle soreness

The changes in muscle soreness of the BFl, ST, and SM are presented in Table 3-1 and showed a significant day effect for the BFl ($F_{5,55}$ = 7.7; P < 0.001), ST ($F_{5,55}$ = 8.3; P < 0.001), and SM ($F_{5,55}$ = 4.1; P < 0.01). The significant increases in muscle soreness were observed in the BFl and ST; it peaked on the second day after exercise (P < 0.003). The soreness in the SM showed no significant change following the exercise.

Table 3-1 Results for muscle soreness following the eccentric exercise

		pre	post	1day	2day	3day	7day
BFl	[mm]	10.0±13.2	12.1±11.8	21.1±12.0	29.8±18.1*	20.3±13.5	8.6±1.2
ST	[mm]	8.4±11.7	10.9±11.0	25.4±15.9*	35.6±23.2*	24.0±14.6*	13.4±11.1
SM	[mm]	8.8±9.5	11.6±11.3	17.1±13.0	28.9±22.0	23.4±16.5	15.0±16.9

Values are mean ± SD.

BFl biceps femoris long head muscle, *ST* semitendinosus muscle, *SM* seimembranosus muscle

*P < 0.05 vs. pre

MRI

Typical T2-weightened MR images of the right thigh before and following exercise are presented in Fig. 3-3. The CSA of the ST was increased on the third day (Fig. 3-4 lower left). The brightness of hamstring muscles increased immediately after exercise. On the third day after exercise, the ST showed a conspicuous increase in brightness (Fig. 3-5 lower left).

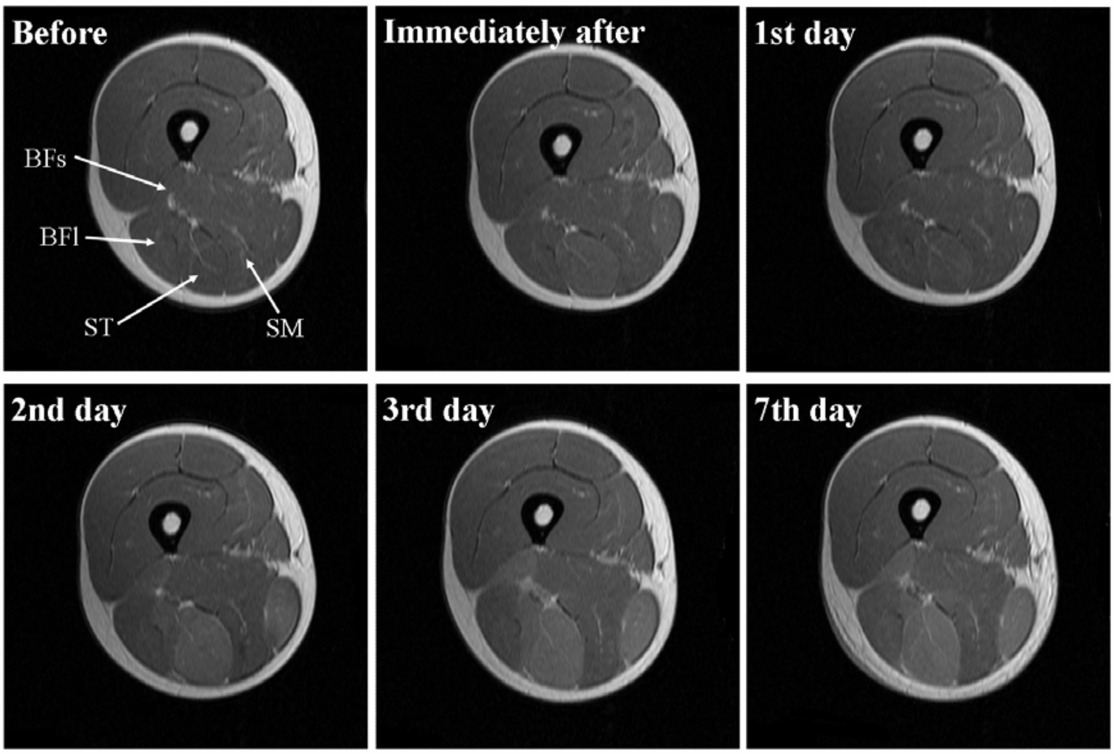

Fig. 3-4 Representative T2-weightened magnetic resonance images of the middle region (50% of thigh length) from one subject before, immediately after, and on the 1st, 2nd, 3rd, and 7th days following eccentric exercise. *BFs* biceps femoris short head muscle, *BFl* biceps femoris long head muscle, *ST* semitendinosus muscle, *SM* semimembranosus muscle.

CSA

The time course of the changes in CSA of the hamstring muscles is shown in Fig. 3-4. As shown in this figure, the BFs showed a significant main effect for the muscle region ($F_{1,11} = 30.0$; $P < 0.001$) and day ($F_{5,55} = 6.9$; $P < 0.001$), but no muscle region by day interaction effects occurred. The CSA of the BFs distal region on the second and seventh day was significantly higher than that before exercise ($P < 0.003$). On the third day, the CSA showed a trend toward higher values than pre-values. The ST showed a significant main effect for muscle region ($F_{2,22} = 84.2$; $P < 0.001$) and day ($F_{5,55} = 15.0$; $P < 0.001$), and muscle region by day interaction effects ($F_{10,110} = 6.8$; $P < 0.001$) were observed for the ST CSA. The CSA of the proximal region on the third day was significantly higher than that before exercise and remained so until at least the seventh day ($P < 0.003$). The CSA of the middle region in ST was higher immediately after exercise, on the second, third and seventh days ($P < 0.003$) compared with the level before exercise. The BFl and SM showed a significant main effect for muscle region ($F_{2,22} = 23.9$; $P < 0.001$, $F_{2,22} = 100.2$; $P < 0.001$), but no main effect for muscle region and interaction effect between muscle regions and days occurred.

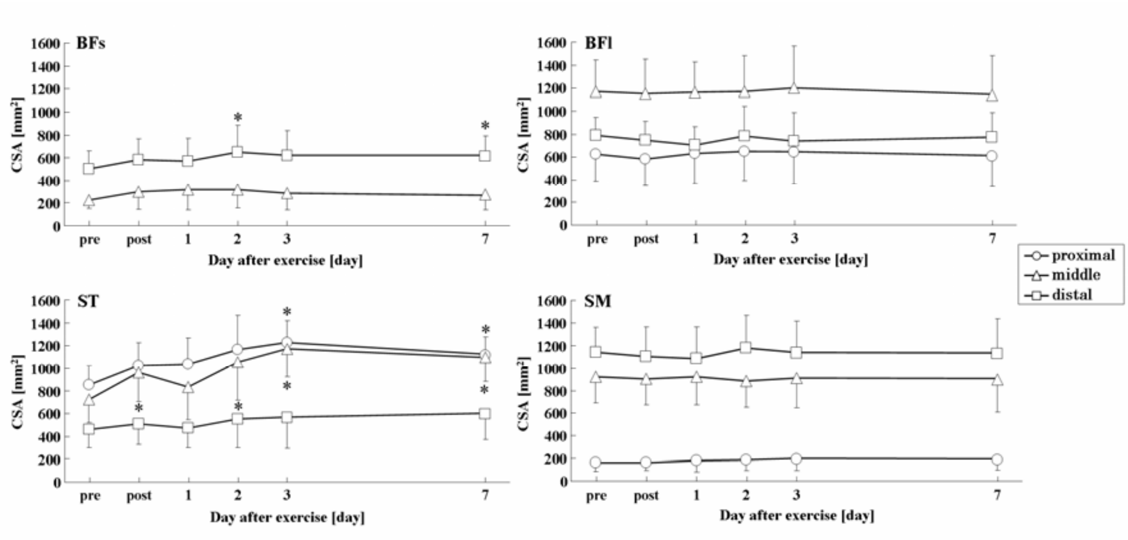

Fig. 3-5 Change in the cross-sectional area (*CSA*) of the 30% (*proximal*), 50% (*middle*) and 70% (*distal*) of thigh length in the biceps femoris short head muscle (*BFs*) and long head muscle (*BFl*), semitendinosus muscle (*ST*), and semimembranosus muscle (*SM*) before (pre), immediately after (post), and on the 1st, 2nd, 3rd, and 7th days following eccentric exercise. Asterisks indicate significant differences from the pre value (*$P < 0.003$).

T2

Figure 3-5 presents changes in T2 values of the hamstring muscles. A significant main effect for muscle region ($F_{1,11} = 37.6$; $P < 0.001$) and day ($F_{5,55} = 4.4$; $P < 0.001$), and interaction effect between muscle regions and days ($F_{5,55} = 4.2$; $P < 0.01$) were observed for the T2 values in the BFs. Both the middle and distal regions of the BFs showed elevated T2 values immediately after exercise ($P < 0.003$). The T2 values immediately after and on the third day after exercise were significantly different between the middle and distal regions ($P < 0.01$). The ST T2 values showed a significant main effect for muscle region ($F_{2,22} = 17.4$; $P < 0.001$) and day ($F_{5,55} = 13.1$;

$P < 0.001$), and interaction effect between muscle regions and days ($F_{10,110} = 3.9$; $P < 0.001$) for the ST T2 values. T2 values were obtained around 40 ms as pre-values, i.e. in the proximal region (38.7±1.4 ms), middle region (37.5±2.3 ms) and distal region (37.4±1.8 ms) of the ST. The T2 values on the ST proximal region were elevated immediately after (51.1±5.7 ms), and on the second day (65.1±28.1 ms), the third day (71.2±23.7 ms), and seventh day (75.5±28.1 ms) after exercise ($P<0.003$). In the middle and distal regions in the ST, the T2 values increased immediately after (52.3±5.9 ms; 50.9±8.5 ms, respectively), and were significantly higher on the third day (67.5±23.8%; 60.8±2.7 ms, respectively), and on the seventh day (71.4±26.2 ms; 68.6±27.0 ms, respectively). The proximal T2 value was lower than the middle T2 value immediately after exercise ($P < 0.01$), and was higher than the distal T2 value on the second and third day after exercise ($P < 0.01$). In the BFl, a significant day effect ($F_{5,55} = 3.8$; $P < 0.01$) was observed for the T2 values, but no day effect and muscle region by day interaction effect occurred. The T2 values immediately after exercise were significantly elevated on the middle and distal regions ($P < 0.01$). No T2 value changes were apparent in any regions of the SM following exercise.

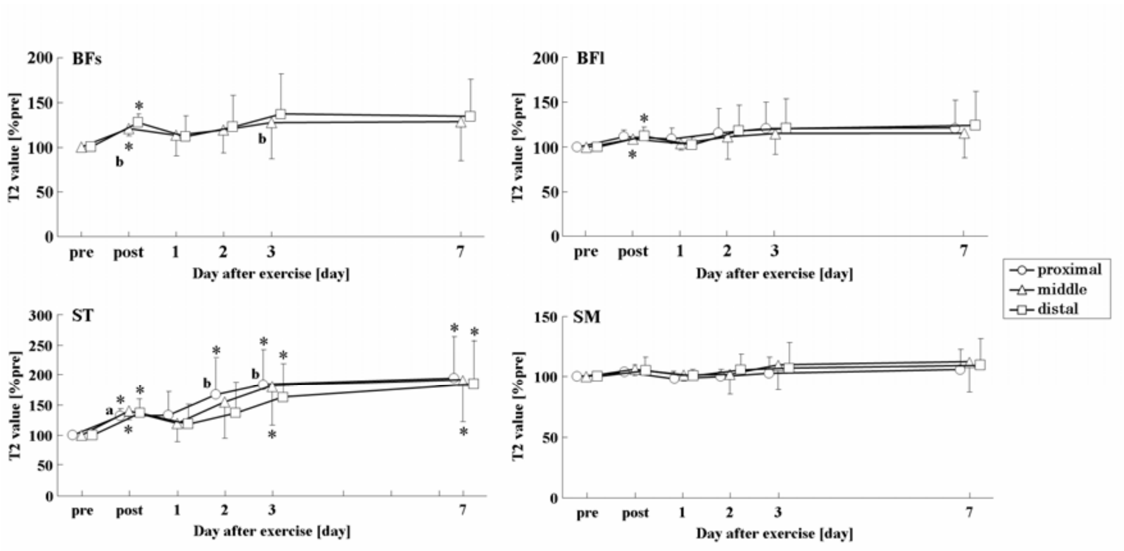

Fig. 3-6 Change in the transverse relaxation time (T2 value) of the 30% (proximal), 50% (middle) and 70% (distal) of thigh length in the biceps femoris short head muscle (*BFs*) and long head muscle (*BFl*), semitendinosus muscle (*ST*), and semimembranosus muscle (*SM*) before (pre), immediately after (post), and on the 1st, 2nd, 3rd, and 7th days following eccentric exercise. All values are given as a percentage of the pre value. Asterisks indicate significant differences from the pre value (*P < 0.003) and alphabets indicate significant differences between regions (^{a}P <0.017 vs. the middle region, ^{b}P <0.017 vs. the distal region).

3-4. Discussion

This study examined differences of changes in MR measurements, represented as CSA and T2 values, among hamstring muscles. Results showed that almost all the hamstring muscles exhibited a T2 increase immediately after intensive eccentric exercise. Moreover, the ST presented an increase of CSA and T2 values following the exercise, along with increases in the regional differences of the T2 values immediately after exercise, and on the second and third days after exercise.

It is generally considered that the hamstring muscles were similarly activated in knee-flexion exercise. However, this study showed that the ST muscle especially changed the MR measurements following the intensive eccentric exercise. This result suggested that the response of the hamstring muscles following the exercise were different among each hamstring muscle, especially the ST was damaged. The changes in MR measurements of the ST relate to the architectural characteristics. The ST is a fusiform, thin, and biarticular long muscle, whereas the BFl and SM are pennate and bulky muscles. The results of previous studies demonstrated that the greater the degree of passive extension to failure in the pennate muscle tended to be greater than that in the fusiform muscle (Garrett et al. 1988). For this reason, the fusiform muscle is potentially more easily injured than the pennate muscle. The results of this study showed the increase of T2 values in the ST. Moreover, the ST has a TI at about the mid-point of the muscle (Lee et al. 1988; Woodley and Mercer 2005). The TI divides the ST muscle into proximal and distal neurovascular compartments, with each region innervated by a different nerve branch. These neuroanatomical characteristics are associated mainly with the notable changes of MR measurements in the ST.

This region-specific difference of changes in MR measurements among the proximal, middle and distal regions in the ST suggests that these regions might have different functional roles. It is interesting that the proximal and middle regions show CSA increase and higher T2 changes compared as the distal region in the ST. A possible cause of the regional differences is the presence of the TI, which is contained in the proximal and middle regions whereas that is not contained in the distal region. The ending architecture of the muscle fibers near the TI is similar to the muscle-tendon junction (MTJ) architecture. The MTJ or muscle fibers near the MTJ were more damaged within a muscle when muscle-tendon units were intensively loaded (Tidball et al. 1993). Consequently, the MR measurements in the proximal and middle ST regions more changed than that in the distal region. Moreover, the TI induced the regional difference of the EMG activity, work distribution, and metabolic state. Consequently, as previous studies suggested (Adams et al. 1992; Jenner et al. 1994; Kinugasa et al. 2006;

Vandenborne et al. 2000), the regional difference of the T2 value might reflect the muscle-cell metabolism and fluid uptake, represented as the regional difference of the neuromuscular and/or metabolic activities.

In this study, the ST especially showed a conspicuous increase of the CSA and T2 value. The delayed increase of muscle-injury markers, such as muscle-swelling, muscle soreness, and plasma CK activity, following the exercise that subjects performed in the present study, represented a similar time course to that of the previous studies (Harrison et al. 2001; Jayaraman et al. 2004; Nosaka and Sakamoto 2001). However, compared with those previous studies, the degree of the reduction of maximal isometric knee-flexion torque was lower, and the plasma CK activity was higher. In addition, the MR images showed the delayed onset muscle damage signs only in the ST. The eccentric knee-flexion exercise using a prone leg-curl machine with 120%MVC loads can cause damage especially to the ST in the hamstring muscles. Biomechanical analyses of the intensive eccentric knee-flexion exercise, such as the force contribution of the individual muscles and the three-dimensional knee-joint kinematics, would be necessary to resolve the issue of why only the ST showed the T2 and CSA changes following intensive eccentric knee-flexion exercise.

The changing mechanism of the increased T2 values and CSA remains unknown. The exercise-induced T2 increases in skeletal muscle involve not only the osmotic movement of muscle water into the myofibrillar space, but also rather the vascular dilation or increase of blood flow. In this study, although any muscles and regions showed a T2 increase immediately after exercise, the increase of T2 values was shown only in the ST on the second day following exercise. The relationship between the changes of the plasma CK activity and of the T2 value of the ST was not statistically significant only immediately after exercise: it became statistically significant from the second day after exercise (data not shown). The plasma CK activity indicates much skeletal muscle damage, and reflects a delayed increase after unaccustomed eccentric exercise. Therefore, it is conceivable that the T2 increase immediately after exercise reflects the increased blood flow, and that the increase after the second day after exercise reflects severe muscle damage.

Recently, Baczkowski et al. (2006) examined thigh-muscle activations using MR imaging method before and after 100 Australian Rules football kicks. The ST muscle showed great increases of the signal intensity in the kicking and stance legs, whereas the BFl, which is the most commonly injured muscle among football players (Askling et al. 2007; Connell et al. 2004; Koulouris and Connel 2006), showed minimal change in both legs. The hamstring muscles of both legs can act eccentrically during and after the ball contact. Compared with the SM and BF, the increases of the ST signal intensity were shown in an actual kicking performance, indicating the important contribution of the ST. However, the workload of the hamstring muscles might be lower than that of the quadriceps femoris muscles during the kicking performance. Consequently, each hamstring muscle might have different sensitivities to eccentric stimulation. The ST might sensitively respond to the eccentric contraction. Further studies are necessary to clarify the pathomechanics and relationships between the injury factors and the observed injury rate in the hamstring muscles.

In summary, the present study has shown that the changes of the CSAs and T2 values following the intensive knee eccentric flexion exercise were different among hamstring muscles. This result suggested that the hamstring muscles, which were activated similarly in knee-flexion tasks, were respectively different the degree of response following the exercise. Moreover, the ST showed the regional-specific difference of the MR measurements following the exercise, which affected the anatomical characteristics of the ST muscle, especially the presence of the TI.

CHAPTER 4:

NEUROMUSCULAR PROPERTIES OF THE SEMITENDINOSUS MUSCLE DEPENDING ON FORCE LEVEL AND JOINT POSITION

4-1. Introduction

It is well known that changing joint position in isometric contractions (i.e. changing muscle length) is a determining factor on the maximal voluntary muscle contraction strength (Hansen et al. 2003; Linnamo et al. 2006; Onishi et al. 2002). However, it is not clear that changing muscle length affect to the motorneuron activity such as recruitment and/or rate-coding strategies. Previous studies investigated the relationships between muscle length and electromyographic (EMG) activity in the biceps brachii muscle (Linnamo et al. 2006), the knee extensor muscles (Babault et al., 2003; Kubo et al., 2004), the knee flexor muscles (Onishi et al. 2002; Mohamed et al. 2002), the ankle dorsi flexor muscles (Desbrosses et al. 2006), and the ankle planter flexor muscles (Kennedy and Cresswell 2001). These authors suggested that the muscle length-EMG activity relationship was inherent in the muscle and that depended on the joint angle. It is also possible that the muscle length changing alter the relationships between force and EMG activity.

Previous studies have investigated the relationships between muscle length and EMG activity of the hamstring muscles (Onishi et al. 2002; Mohamed et al. 2002). The hamstring was composed of four muscles, i.e. the biceps femoris long (BFl) and short head (BFs) muscles, semitendinosus (ST) muscle and semimembranosus (SM) muscle. The hamstring muscles have been often examined as one functional muscle group. Therefore, the EMG activities in each hamstring muscle have not so investigated comprehensively. The investigations of the effect of hip and knee joints' position on the knee flexion torque, EMG activity and neuromuscular efficiency (i.e. the ratio between torque output and EMG activity) are important for applying to the clinical practice.

Moreover, the ST muscle has unique architectural characteristics. A tendinous intersection (TI) is present within the muscle belly, and the TI separates the ST into proximal and distal distinct compartments (Lee et al. 1988; Woodley and Mercer 2005). Moreover, the two compartments are respectively innervated via two branches from the tibial part of the sciatic nerve: proximal and distal compartments of the intersection (Romans 1986; Woodburne and Barkel 1994; Woodley and Mercer 2005). Hutchison et al. assessed the cat ST muscle EMG activations of the proximal and distal compartments, and demonstrated that the EMG activities between the two compartments are different for some tasks. However, to our knowledge, the comparison of the EMG activities between proximal and distal compartments in human ST muscle has never shown. And to elucidate the normal relationships among muscle length, knee-flexion strength and EMG activity in the ST muscle is important, which could be applied to the clinical case in the rehabilitation process after injured or operated.

In light of these considerations, we hypothesized that the hip and knee joints position affect the EMG activity of the hamstring muscles and that the ST proximal and distal compartments would showed the different EMG activities because the TI divided the ST muscle into two distinct compartments with each compartments innervated by a different nerve branch. The purpose of this study was (1) to investigate the effect of joint position on the torque and intramuscular EMG activities of the hamstring muscles in the experiment 1 and (2) to examine the difference of the EMG activities between proximal and distal compartments of the ST muscle in the experiment 2.

4-2. Methods

Subjects

The authors examined eight healthy male volunteers in the experiment 1 (age 22.7±1.2 years, height 172.8±4.8 cm, weight 69.8±8.7 kg) and seven healthy male volunteers in the experiment 2 (age 22.3±1.1 years, height 172.7±4.7 cm, weight 70.3±9.6 kg) with no history of neuromuscular or orthopedic disease of their thigh muscle or hip and knee joints. None were participating in any regular exercise regime. This study was approved by the Human Research Ethics Committee of the School of Sport Sciences of Waseda University and is consistent with their requirements for human experimentation. This study conforms to the Declaration of Helsinki. Written informed consent statements were obtained after participants had read the volunteer information sheet and questions pertaining to the study had been answered to their satisfaction.

Fine-wire electrode placement

Bipolar urethane-coated stainless steel fine-wire electrodes were used to record intramuscular EMG detection. Each electrode's diameter was 25 μm; each electrode tip was bared for about 2 mm. The inter-tip distance was set as 5 mm (Kaneko et al. 2003;

Onishi et al. 2002). Two wires of each pair of electrodes were mutually connected using nontoxic adhesives to prevent changes in the inter-tip distance.

The insertion placement of fine-wire electrodes was determined the muscle belly of the BFl, ST and SM muscles in the experiment 1. In the experiment 2, the electrodes were inserted the proximal and distal compartments of the TI. The actual location of the TI was clarified using an ultrasonographic apparatus (SonoSite 180PLUS; SonoSite, WA, USA). For each electrode location, the surrounding area was shaved using a disposable razor and then cleaned by rubbing the area with cotton wool soaked in alcohol. Fine-wire electrodes were inserted into the each muscle point of the right side hamstring muscles using a medical disposable 23-gauge needle, which was withdrawn leaving the electrode in place. The electrodes were confirmed as set in the targeting muscle using electrical stimulations (SEM-4201; Nihon Kohden, Tokyo, Japan). A rectangular pulse train with frequency of 1 Hz was used for stimulation. The surface ground electrode was placed on the lateral malleolus. Wire electrodes were fixed to the skin and a 2-cm loop was formed to avoid pulling on the intramuscular site during muscle contraction and/or hip and knee angle transition (Onishi et al. 2002). The wires were then taped to the skin to avoid accidental dislodgement and were then inserted into coiled springs where the signal was amplified prior to recording. The coiled springs were taped to the leg to minimize movement.

Procedures

Before the main testing session, the subjects performing submaximal contractions a few times were familiarize them to these conditions, with the inserted fine-wire electrodes and the dynamometer. Simultaneously, the electrode positions and EMG recordings were checked to detect errors.

A dynamometer (Cybex Norm; Lumex, Ronkonkoma, NY, USA) was used to obtain quantitative measurements of isometric knee-flexion strength. Torque and EMG data were collected for a series of four combinations of hip and knee positions. The hip and knee angles were defined using degree of flexion. The maximal isometric knee-flexion was performed with three joint positions in the experiment 1; hip at 90 degrees and knee at 0 degrees (90-0), the hip at 0 degrees and knee at 0 degrees (0-0), and the hip at 0 degrees and knee at 90 degrees (0-90), and with four joint positions in the experiment 2; the experiment 1 positions and the hip at 90 degrees and knee at 90 degrees (90-90). The hip joint was fixed to the table with a strap, the knee joint was fixed to the lever arm of a dynamometer and the tibia was maintained in a neutral position of rotation. The center of knee-joint rotation was aligned visually with the axis of the dynamometer's rotation (Fig. 4-1).

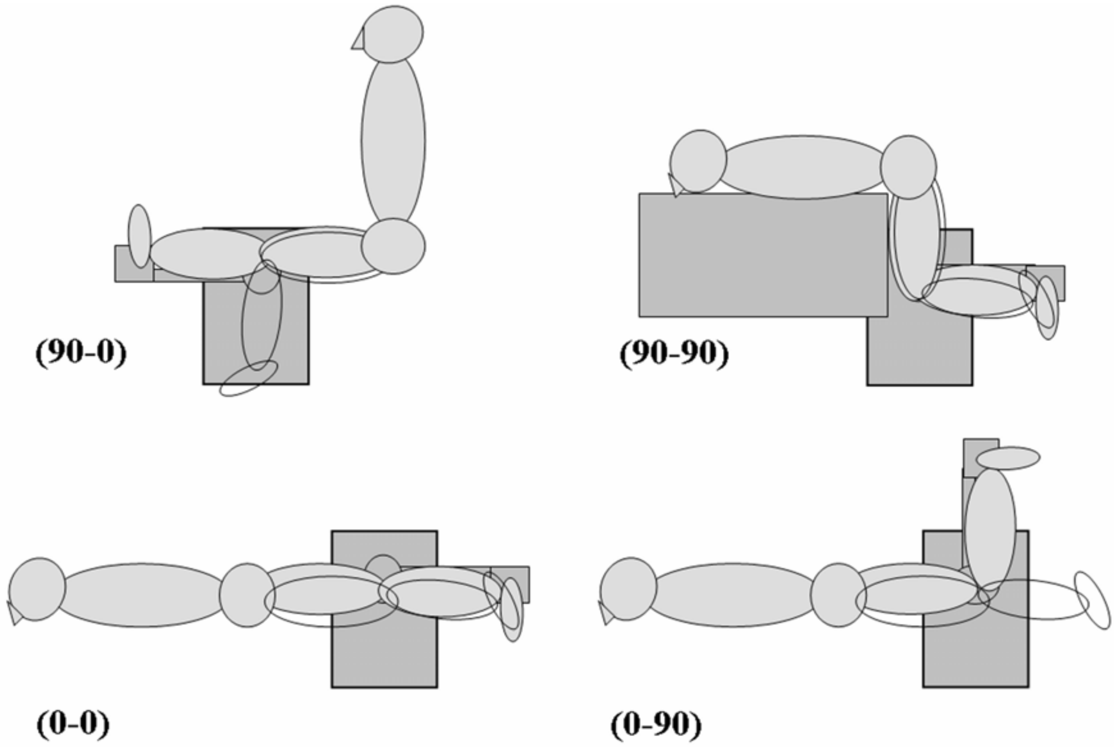

Fig. 4-1 The test positions. (upper, left) hip at 90 degrees and knee at 0 degrees (90-0), (upper, right) hip at 90 degrees and knee at 90 degrees (90-90), (lower, left) hip at 0 degrees and knee at 0 degrees (0-0), (lower, right) hip at 0 degrees and knee at 90 degrees (0-90). The experiment 1 was examined the three (90-0, 0-0 and 0-90) positions, and the experiment 2 was examined the all positions.

Subjects performed an isometric knee-flexion task by gradually exerting a force from a relaxed state to a maximal voluntary contraction (MVC) within 5 s, while torque data were displayed on a screen throughout the task. Before testing, the subjects performed a standardized warm-up and submaximal contractions to accustom themselves to the testing procedure. The subjects repeated the task two or three times with at least 2-min rest between trials.

Data acquisition and analysis

The EMG signals were connected to a differential preamplifier (Neurotop; Nihon Kohden, Tokyo, Japan). The EMG and torque signals were analog-to-digital converted using a 2 kHz sampling rate (NI SCXI 1000; National Instrument, Austin, TX, USA). The torque signals excluded the effects of gravity. All data were subsequently recorded on a personal computer.

The fine-wire EMG data channels were band-pass filtered between 10 and 500 Hz. All EMG data were full-wave rectified, then low-pass filtered using a Butterworth filter with a cut-off frequency of 25 Hz. The average rectified value (ARV) was then computed at their center equal to the torque levels: 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% MVC (Fig. 4-2).

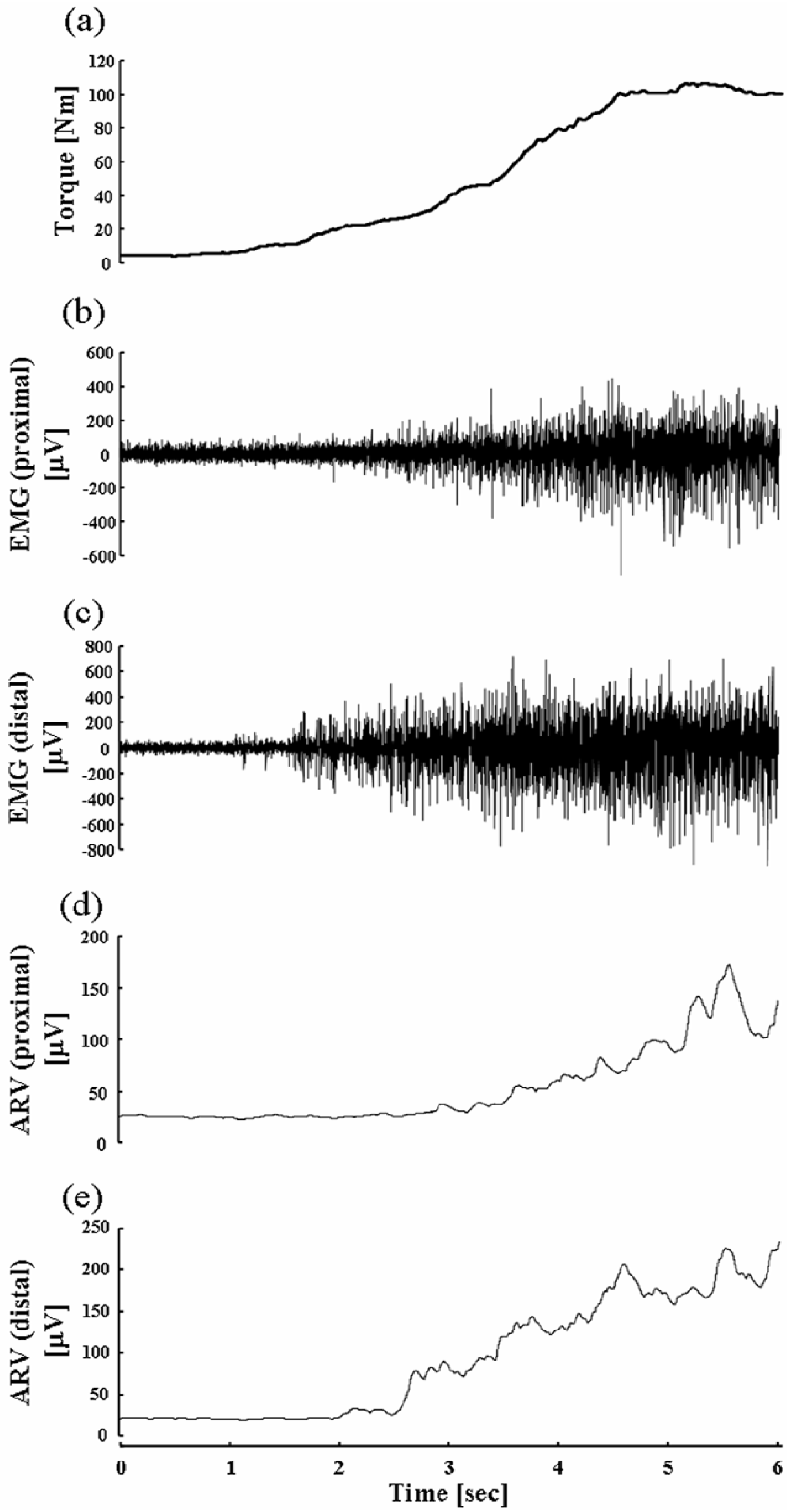

Fig. 4-2 Typical example of isometric knee-flexion torque (a), raw EMG at the proximal region (b), and the distal region (c). Average rectified value (ARV) of the proximal (d) and distal (e) compartments in the experiment 2.

Statistical analysis

Significant differences of the maximal knee-flexion torque, the maximal ARV and the torque/ARV ratio with combinations of hip and knee joint angles were determined using one-way analysis of variance (ANOVA) with repeated measured. In the experiment 2, significant differences of the EMG increasing behaviors between proximal and distal compartments were determined using two-way ANOVA with repeated measurements (compartments ×torque levels). Post-hoc analyses were conducted by LSD (experiment 1) and Bonferroni's (experiment 2) tests if the ANOVA showed statistical significant main effects or interaction effects. The statistical significance was set at $P < 0.05$.

4-3. Results

Experiment 1

Maximal isometric knee-flexion torque

The differences of the maximal isometric knee-flexion torque with changing joint positions are summarized in Table 4-1. Joint position was observed to have a significant effect on the MVC torque ($F_{2,14}$=12.2, P<0.01). The maximal torque at 0-0 position was significantly higher than that at the 90-0 and 0-90 positions, and that at the 90-0 position was tended to show higher torque than that at the 0-90 position.

Table 4-1 Maximal isometric knee flexion torque in the experiment 1

	90-0	0-0	0-90	Significant multiple comparison
Maximum torque [Nm]	66.9±24.4	93.5±23.4	53.7±14.6	0-0 vs. 90-0, 0-90

Maximal ARV and torque/ARV ratio

The differences of the normalized maximal ARV and torque/ARV ratio with changing joint positions are summarized in Table 4-2. The maximal ARV showed a significant main effect for the joint position in the BFl ($F_{2,14}$=4.1, P<0.05) and ST ($F_{2,14}$=6.0, P<0.05) muscles. The 90-0 position was significantly lower maximal ARV than the 0-0 and 0-90 positions in the ST. In the BFl, the 0-0 position was significantly higher maximal ARV than 0-90 position.

The torque/ARV ratio showed a significant main effect for the joint position in the BFl ($F_{2,14}$=4.9, P<0.05), ST ($F_{2,14}$=8.4, P<0.01) and SM ($F_{2,14}$=3.6, P<0.05). In the all three muscles, the 90-0 position was significantly higher torque/ARV ratio than the 0-90 position.

Table 4-2 Maximal ARV and torque/ARV ratio during maximal isometric knee flexion in the experiment 1.

		90-0	0-0	0-90	Significant multiple comparison
	BF	255.4±169.7	375.5±188.4	283.2±182.6	0-0 vs. 0-90
Maximal ARV [μV]	ST	120.8±65.1	300.1±123.1	316.3±238.7	0-0 vs. 90-0
	SM	176.8±227.4	261.4±217.0	288.2±259.1	n.s.
	BF	469.4±265.5	296.1±134.6	259.6±158.2	90-0 vs. 0-90
Torque/ARV ratio [N/mV]	ST	713.6±272.1	471.4±318.7	240.6±115.3	90-0 vs. 0-90
	SM	976.8±570.9	767.7±979.3	435.4±584.1	90-0 vs. 0-90

Experiment 2

Differences of maximal torque among positions

The differences of the maximal torque with changing joint positions are summarized in Table 4-3. Joint position was observed to have a significant effect on the MVC torque ($F_{3,18}$=7.9, P<0.01). The respective maximal torques at two positions (90-90, 0-0) were significantly higher than that at the 0-90 position, and that at the 90-90 position was significantly higher than that at the 90-0 position.

Table 4-3 Maximal isometric knee flexion torque in the experiment 2

	90-0	90-90	0-0	0-90	Significant multiple comparison
Maximum torque [Nm]	63.5±24.2	86.2±26.8	93.5±25.3	54.9±16.1	0-90 vs. 90-90, 0-0 90-0 vs. 90-90

Differences of maximal ARV and torque/ARV ratio among joint positions

The relationship between the torque and ARV at each joint position are shown in Fig. 4-3. Maximal ARVs and torque/ARV ratios were normalized with the values the value at 0-0 position and expressed as a percent. The differences of the normalized maximal ARV and torque/ARV ratio with changing joint positions are summarized in Table 4-4. The maximal ARV showed a significant main effect for the joint position ($F_{3,18}$=15.8, P<0.001). In the proximal compartment, the 90-0 position was significantly lower maximal ARV than the 90-90, 0-0 and 0-90 positions. In the distal compartment, the 90-0 position showed significantly higher ARV than the 90-90 and 0-0 positions and tended to display higher ARV than the 0-90 position.

The torque/ARV ratio showed a significant main effect for the joint position ($F_{3,18}$=9.9, P<0.001). In the proximal compartment, the 0-90 position was significantly lower torque/ARV ratio than the 90-0, 90-90 and 0-0 positions. In the distal compartment, the 90-0 position tended to display higher torque/ARV ratio than the 0-90 position.

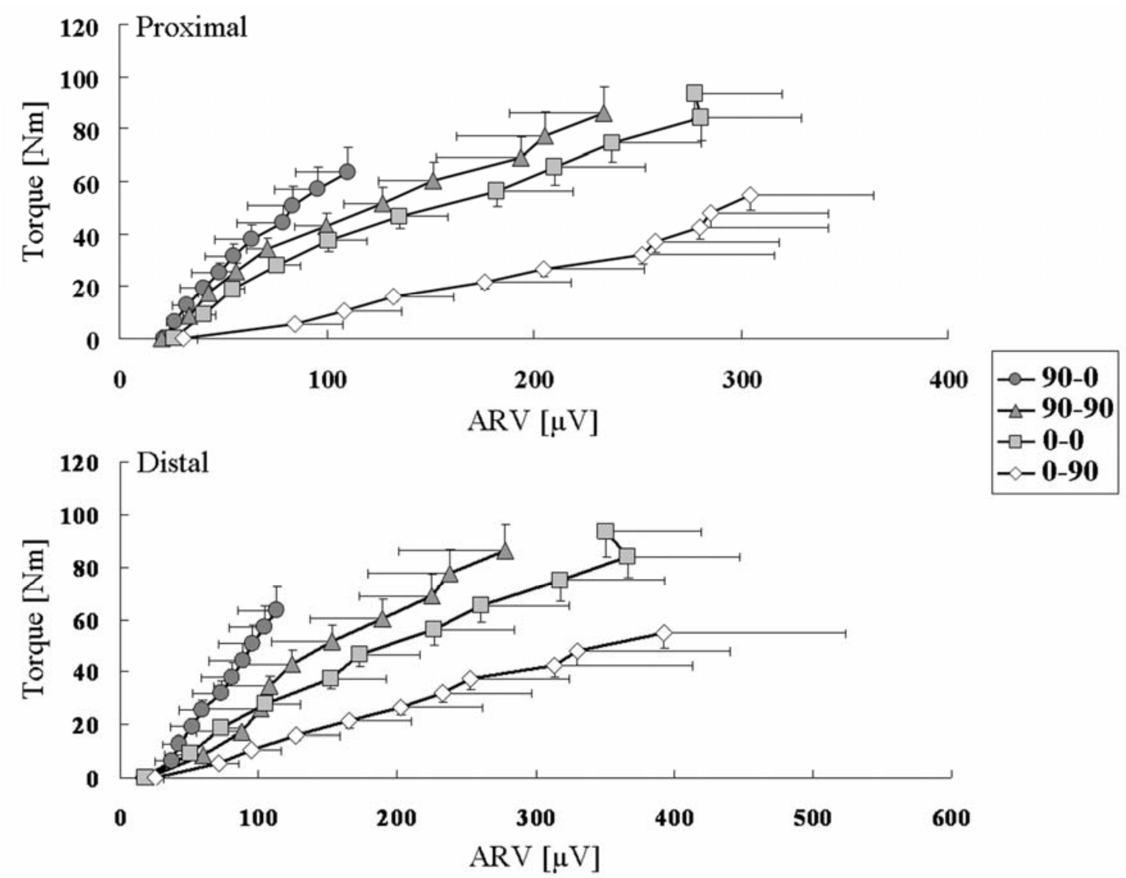

Fig. 4-3 Relationships of the isometric knee-flexion torque and ARV on the proximal (upper) and distal (lower) compartments.

Table 4-4 Normalized maximal ARV and torque/ARV ratio during maximal isometric knee flexion

		90-0	90-90	0-0	0-90	Significant multiple comparison
Maximal ARV [%]	proximal	43.4±21.1	88.2±27.7	100	115.6±25.1	90-0 vs. 90-90, 0-0, 0-90
	distal	41.0±32.8	88.0±42.9	100	106.4±51.1	90-0 vs. 90-90, 0-0
Torque/ARV ratio [%]	proximal	191.7±96.9	116.4±46.3	100	53.9±20.6	0-90 vs. 90-0, 90-90, 0-0
	distal	264.4±177.7	121.6±35.0	100	69.1±37.6	n.s.

Difference of EMG behaviors between proximal and distal compartments in ST

Figure 4-4 shows the EMG behaviors of the proximal and distal compartments during increasing isometric knee-flexion torque. Both compartments indicated a linear increment with increasing isometric knee-flexion torque in each of the four positions. The 90-0 position showed a significant main effect for the compartment ($F_{1,7}$=12.2, P<0.05) and compartment by torque level interaction effect ($F_{10,70}$=2.2, P<0.05). The ST distal ARV was higher than the ST proximal ARV at 60%, 80%, or 90% MVC level. The 0-90 position showed a significant main effect for the compartment ($F_{1,7}$=8.0, P<0.05) and compartment by torque level interaction effect ($F_{10,70}$=2.5, P<0.05). The ST proximal ARV was higher than the ST distal ARV at 50% MVC with the 0-90 position; an identical tendency was apparent at 40%, 60% and 70% MVC, although no statistically significant difference was found.

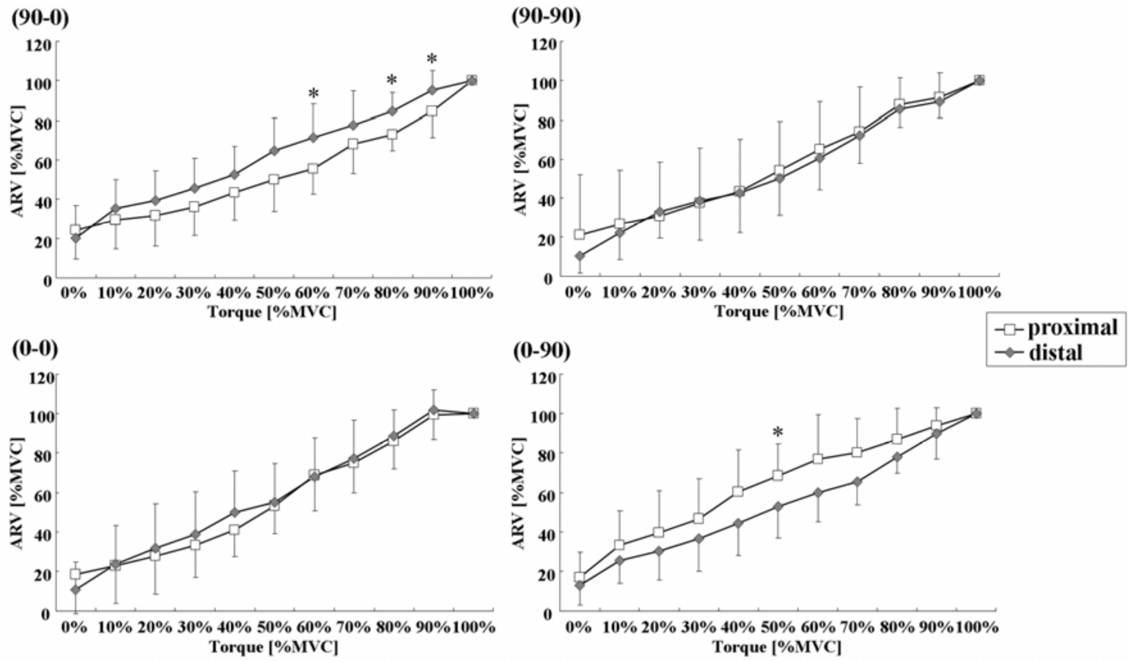

Fig. 4-4 Relationships of the knee-flexion torque level and the ARV level in proximal and distal compartments with the hip at 90 degrees and knee at 0 degrees position (90-0, upper-left), the hip at 90 degrees and knee at 90 degrees (90-90, upper-right), the hip at 0 degrees and knee at 0 degrees (0-0, lower-left) and the hip at 0 degrees and knee at 90 degrees (0-90, lower-right). Asterisks indicate significantly different between joint positions.

4-4. Discussion

This study was examined the effect of joint position on the torque, intramuscular EMG activities and the torque/EMG ratio of the hamstring muscles and of the proximal and distal compartments of the ST muscle.

One of the main findings of the present study was that the maximal ARV and maximal neuromuscular efficiency (the knee-flexion torque/ARV ratio) depends on the muscle length. In the most lengthened (the hip at 90 degrees, knee at 0 degrees) or shortened (the hip at 0 degrees, knee at 90 degrees) joint positions in this study, the MVC torque was less than other two positions (90-90, 0-0) in the experiment 1 and 2. On the other hand, the maximal ARV and neuromuscular efficiency presented a tendency varied concomitant with change in the hamstring muscles' length. The shorter muscle length tended to show higher maximal ARV and lower maximal torque/ARV ratio. The significant effect of muscle length on the maximal ARV and neuromuscular efficiency of the muscles could act as the compensative for the muscle-mechanical disadvantage. Previous study suggested that the EMG increasing with shortening muscle length was a compensation method for the muscle mechanical disadvantage (Babault et al. 2003). Moreover, the joint positions could induce the changes of three-dimensional morphology and elastic properties of the muscle-tendon complex in the hamstring muscles. Therefore, the EMG activity could exert compensatory and/or facilitatory effects on these muscle length changes.

Moreover, other finding was that the different EMG increasing behaviors between the two compartments of the ST muscle were observed at the longest (90-0) and shortest (0-90) muscle positions in this study, which was observed for contractions greater than the 50%MVC level. These findings suggest that, while the two distinct compartments of the ST muscle showed equivalent activity in two mid-range of muscle length, the lengthened or shortened muscle conditions could induce the region-specific difference of the EMG increasing behavior between the proximal and distal compartments. The ST has two anatomical distinct compartments separated by TI, but it is not clarify whether the separation of the ST muscle into proximal and distal compartments is functionally important in human. Considering that fibers in each compartment are innervated respectively by a single motorneuron although the motorneuron pools of the proximal and distal compartments are largely overlapped (Nelson and Mendell 1978), the potential for independent recruitment could exist for the two compartments with ST muscle condition. Previous study demonstrated that the excitation levels were different between the ST proximal and distal compartments for some tasks, which might relate to the mechanical effect (Hutchison et al. 1989).

Consequently, the regionally different EMG behavior in human ST muscle showed in the lengthened and shortened positions might be affected by the mechanical efficiency.

In lengthened position (90-0), the distal ARV was higher than the proximal ARV during 60% to 90% of MVC. In contrast, the proximal ARV was higher than the distal ARV during 50% to 80% of MVC in the most shortened position (0-90). However, previous studies suggested that the physiological cross-sectional areas and muscle fiber lengths were almost same in both the proximal and distal compartments of the ST muscle in not only cat (Bodine et al. 1982) but also human (Woodley and Mercer 2005). It is possible that not so difference of sarcomere number between the proximal and distal compartments is apparent in the ST muscle. Consequently, the rationale of the regional EMG difference is not for the compensation for the regional difference of the mechanical disadvantage, i.e., sarcomere lengthened or shortening beyond the optimal actin-myosin overlap. It is possible that the location of the TI is different along with changing joint positions, which changes the ratio of the fiber lengths for the proximal and distal compartments. The functional properties of the ST proximal and distal compartments could be differing depending on muscle length and/or contraction strategy. Therefore, analyses of the factors of the regional EMG difference occurrence with changing joint positions are necessary for future studies.

One possible for the regional EMG difference is that the other hamstring muscles' contraction behaviors, especially the semimembranosus (SM) muscle, might affect to the ST muscle contraction behavior. In the lengthened position (90-0), the ST and SM muscle-tendon units lengthened, and the increase of tightness could occur in these muscles. The SM muscle belly locates under the ST distal region because of the long proximal tendon of the SM (Woodley and Mercer 2005). In addition, the EMG values increased in the same manner as intramuscular pressure for voluntary isometric contractions (Maton et al. 2006; Sjogaard et al. 2004). Consequently, the SM muscle contraction might increase the ST intramuscular pressure, which leads to the distal higher EMG activity. Moreover, the nerve inserted to the proximal compartment is frequently also supplying the BFl and that inserted to the distal compartment arise in common with the nerve to the SM (Woodburne and Burkel 1994). It could be possible that the neuromuscular activities of the proximal and distal compartment related to the BFl and SM muscles, respectively.

In this study, the clearly relationships among the muscle length, force and EMG activity showed especially at the proximal compartment, which is possibly because of the number of subjects. The more number of subjects could show the statistically significance showed as the proximal region. Moreover, the posture at the 90-90, 0-0 and

0-90 positions are based on the prone position, where as that at the 0-90 position is based on the sitting position. Therefore, analyses of the effects of the lower limb and trunk orientation are necessary for future studies.

In summary, we used wire electrodes to examine the functional behaviors of the ST proximal and distal compartments, as represented by the intramuscular EMG activity and neuromuscular efficiency. Results of this study demonstrated that the increasing behaviors of the ST EMG activity during isometric knee flexion were different between proximal and distal compartments in the lengthened and shortened muscle positions, and that the longer ST muscle length tended to show higher torque/ARV relationship. These results could be affected by the EMG compensative mechanism for the muscle-mechanical disadvantages.

CHAPTER 5:

NON-UNIFORM CHANGES IN THE SEMITENDINOSUS MUSCLE ARCHITECTURE DURING ISOMETRIC KNEE FLEXION

5-1. Introduction

The human skeletal muscle has a complicated organization that includes muscle fibers and connective-tissue networks, such as the regional difference of fascicle length, the internal aponeurosis and/or external fascia. It is assumed that the muscle architecture complexity could induce a non-uniform muscle-shortening behavior, and such inhomogeneous shortenings have functional importance (Pappas et al. 2002; Blemker et al. 2005). Pappas et al. (2002) observed that shortening of the anterior fascicles of the biceps brachii muscle is uniform and that of the centerline fascicles is non-uniform during elbow flexion with low loads. Blemker et al. (2005) indicated that the complex geometric arrangement of muscle fascicles is the determining factor contributing to non-uniform behaviors and their associated muscle contraction mechanics. In addition, connective tissues transmit contractile forces effectively from muscle fibers. They transmit force not only in the long-axis direction, but also in a lateral direction from the center (Monti et al. 1999; Maas et al. 2001). Therefore, the non-uniform muscle contraction behavior would be shown not only the long-axis direction but also the short-axis direction of muscle. *In-vivo* measurement of human muscle contraction behaviors using magnetic resonance imaging or ultrasonography is necessary to elucidate the complicated skeletal-muscle contraction behaviors.

The semitendinosus (ST) muscle is one of the hamstring muscles, which is a parallel-fibered, bi-articular muscle. The ST has unique architectural characteristics; a tendinous intersection (TI) is present within the muscle belly. The TI is a complex tendinous structure; it is V-shaped on the muscle superficial (Wickiewicz et al. 1983; Lee et al. 1988) and on the muscle sagittal plane, which is visible with using ultrasonography. It separates the ST into proximal and distal distinct regions. The fibers, which in the two regions are relatively equal in length (Wickiewicz et al. 1983; Woodley and Mercer 2005), are connected in series by the TI. Two regions are respectively innervated via two branches from the tibial part of the sciatic nerve: one proximal and one distal regions of the intersection (Woodley and Mercer 2005). Consequently, the TI would be important to transmit contractile forces effectively from the muscle fibers to the tendon during muscle activation. However, to our knowledge, it is not clear whether the separation of the ST muscle by the TI is functionally important in human.

Moreover, the ST distal tendon is commonly used to replace the ruptured anterior cruciate ligament (ACL). After being harvested for use as an ACL graft, the ST tendon can regenerate with a morphological similarity to the native tendon. However, morphological changes, such as atrophy or shortening of the ST muscle belly, have been confirmed and deep knee-flexion torque has considerably decreased in patients with ACL reconstruction with the ST tendon (Makihara et al. 2006; Nishino et al. 2006). Therefore, to elucidate the normal ST muscle contraction behavior could apply to evaluate the recovery of the ST muscle function after ACL reconstruction surgery.

In light of these considerations, we hypothesized that the ST muscle contraction behaviors (thickening and shortening) would be non-uniform between the superficial, the middle, and the deep layers around the TI and the non-uniform muscle contraction behaviors could be examine by using ultrasonography. The purpose of this study was to investigate (1) the changes of the ST muscle architecture including the muscle thickness and TI shape and (2) the magnitude of the TI displacement, which indicated the ST muscle fibers' shortening behaviors during isometric knee flexion.

5-2. Methods

Subjects

This study examined 15 healthy young male volunteers with no history of neuromuscular or orthopedic disease (age 22.0±1.6 years, height 173.9±3.2 cm, weight 63.3±5.9 kg). None were participating in any regular exercise regime. This study was approved by the Human Research Ethics Committee of the School of Sport Sciences of Waseda University and consistent with their requirements for human experimentation. This study conforms to the Declaration of Helsinki. Written informed consent statements were obtained after participants had read the volunteer information sheet and questions pertaining to the study had been answered to their satisfaction.

Procedures

Participants performed an isometric knee-flexion task by gradually exerting a force from a relaxed state to a maximal voluntary contraction (MVC) within 5 s, while torque data were displayed on a screen throughout the task. Before the test, they performed a standardized warm-up and submaximal contractions to accustom themselves to the testing procedure. The subjects repeated the task two or three times with at least a 2-min rest between trials.

Force measurement

Each subject was instructed to lie prone on a bed. The subjects' left leg was fixed to the lever arm of a dynamometer (Cybex Ⅱ; Cybex International, USA) with the hip joint and the knee joint at 0 deg of flexion. The center of knee-joint rotation was aligned visually with the axis of the dynamometer's rotation.

Torque signals were analog-to-digital converted at a 1 kHz sampling rate (Power-Lab 16/sp; AD Instruments, Japan); data were subsequently recorded on a personal computer.

The ST muscle architecture measurements

Real-time B-mode ultrasonography (SSD-1000; Aloka, Japan) using an electronic linear array probe (7.5 MHz wave frequency; Aloka, Japan) obtained the longitudinal ultrasonographic images. The probe was placed over the ST muscle belly, and the longitudinal section around the TI in ST was imaged (Fig. 5-1(A)). Ultrasound gel was used to fill the gap between the probe and the skin to maintain good coupling during the test. To evaluate the ST muscle thickness and the TI architecture, images were displayed on a real-time basis and recorded on digital videotape at 30 Hz. Then they were synchronized with a clock timer for subsequent analyses. The ST muscle thickness was defined as the distance between superficial fascia and deep fascia (Fig. 5-1(C)). We also measured the change in architecture of TI as three angles: the superficial angle was defined as the angle between the superficial fascia and the short leg of TI; the apex angle was that between the short leg and the long leg; and the deep angle was that between the long leg and the deep fascia (Fig. 5-1(C)).

We also measured the magnitude of the TI displacement during isometric knee flexion. The point at which the TI was attached to the superficial fascia (superficial point) and the apex point of TI (apex point) were observed (Fig. 5-1(C)); their displacement magnitudes were compared.

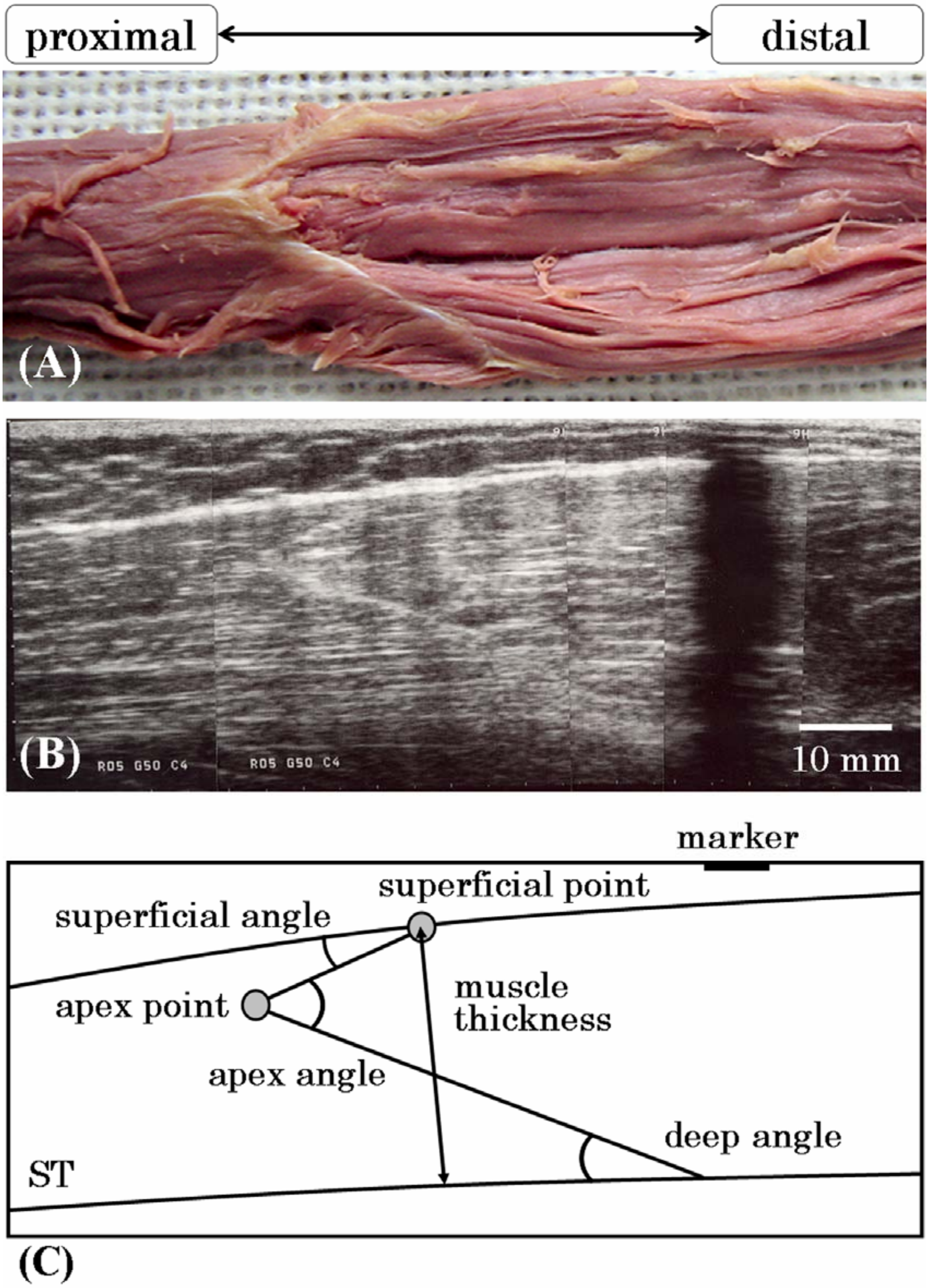

Fig. 5-1 Photograph, ultrasonographic image, and schematic illustration of the region around the tendinous intersection (TI) in the semitendinosus muscle (ST). (A) Photograph of the ST incised sagittal plane. (B) Ultrasonographic image of the TI. (C) Schematic of the ultrasonographic image.

Statistics

Descriptive data are expressed as means and SD. Significant differences during isometric knee flexion were determined using Student’s *t*-test. Pearson’s correlation analysis was used to assess the relationships between maximal knee-flexion torque and muscle thickness, and between maximal torque and the three angles (superficial angle, apex angle, deep angle). Significance was inferred for $P < 0.05$. All statistical analyses were conducted using a statistical analysis software program.

5-3. Results

ST muscle thickness and TI architecture

The thickness of the ST increased significantly during MVC ($P<0.001$); 19.1±2.4 mm at the relaxed state compared to 22.7±3.1 mm at the MVC state (Fig. 5-2). Results of regression analysis showed that the muscle thickness measured during MVC was correlated significantly to the maximal knee-flexion torque.

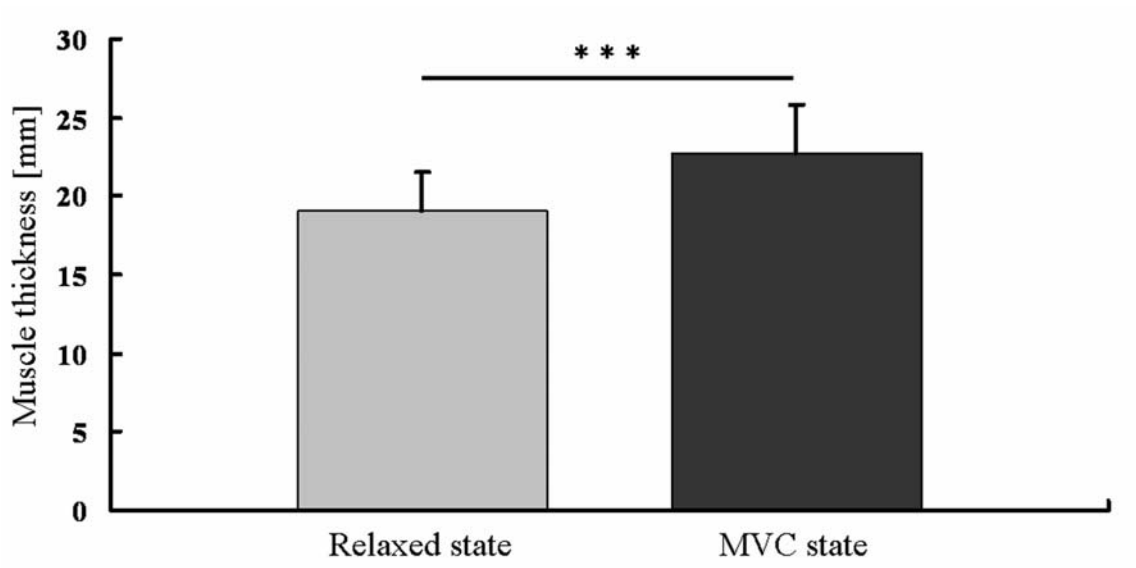

Fig. 5-2 ST muscle thickness measured in relaxed and MVC states. The asterisk indicates significantly increased muscle thickness ($P<0.001$).

The superficial angle and the apex angle of the TI increased significantly with increasing isometric knee-flexion torque. However no significant changes were observed in the deep angle (Fig. 5-3). The superficial angle, 26.8±9.1 deg at the relaxed state, increased to 30.7±7.6 deg at the MVC state. The respective apex angles were 52.5±13.3 deg and 58.7±12.6 deg at the relaxed and MVC states. The maximal knee-flexion torque was correlated significantly to the superficial and the apex angle ($P<0.01$), but no significant relationship was apparent between the maximal torque and the deep angle (Fig. 5-4).

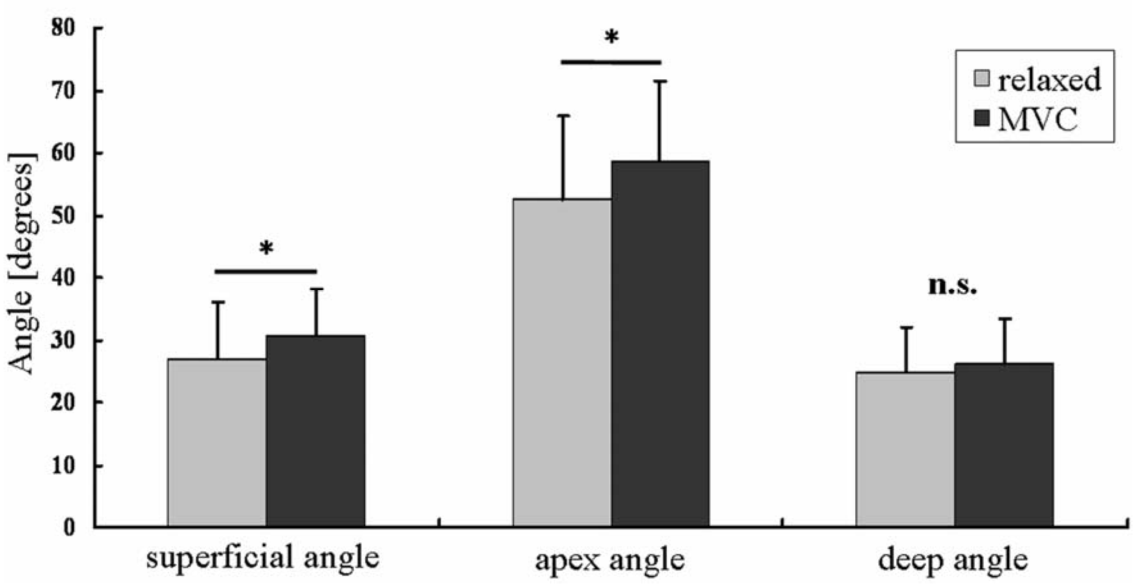

Fig. 5-3 TI angle changes measured in relaxed and MVC states. Superficial angle: between the superficial fascia and the short leg of TI. Apex angle: between the short and long leg of the TI. Deep angle: between the long leg of TI and the deep fascia. Asterisks indicates significantly increased angles ($P<0.05$).

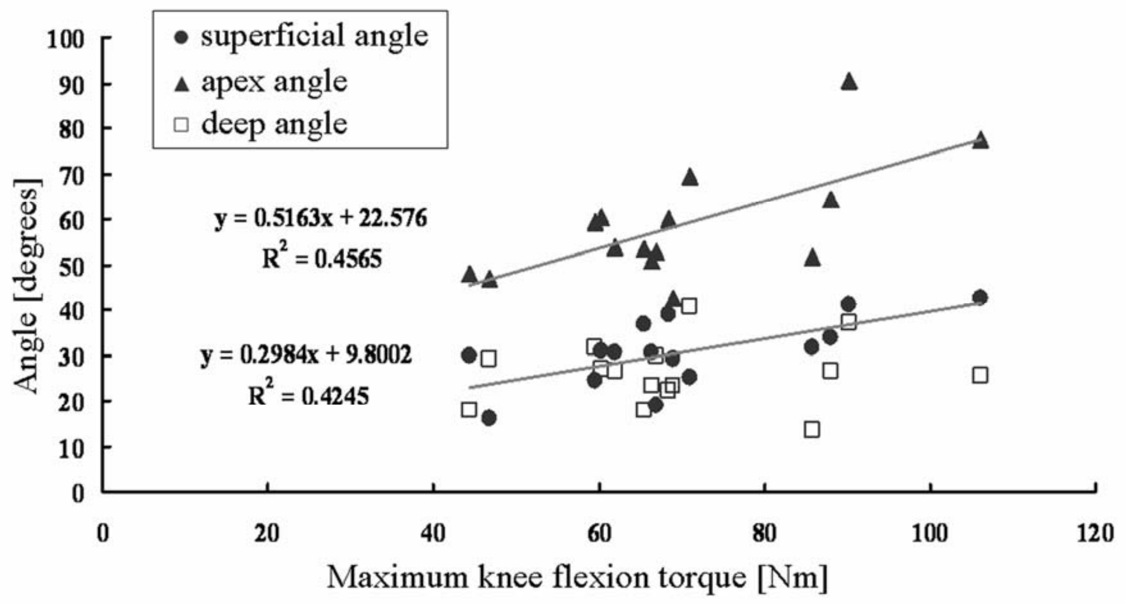

Fig. 5-4 Relationships between maximum isometric knee flexion torque and TI angles. At the superficial and apex angle, the maximum torque was correlated significantly to these angles ($P = 0.046$; 0.027, respectively).

TI displacement

Results showed that the magnitude of displacement of the superficial point at the MVC state was significantly greater (P<0.01) than that of the apex point. The superficial point was displaced 8.3±3.6 mm and the apex point was displaced 6.1±3.2 mm compared to those respective values for the relaxed state (Fig. 5-5).

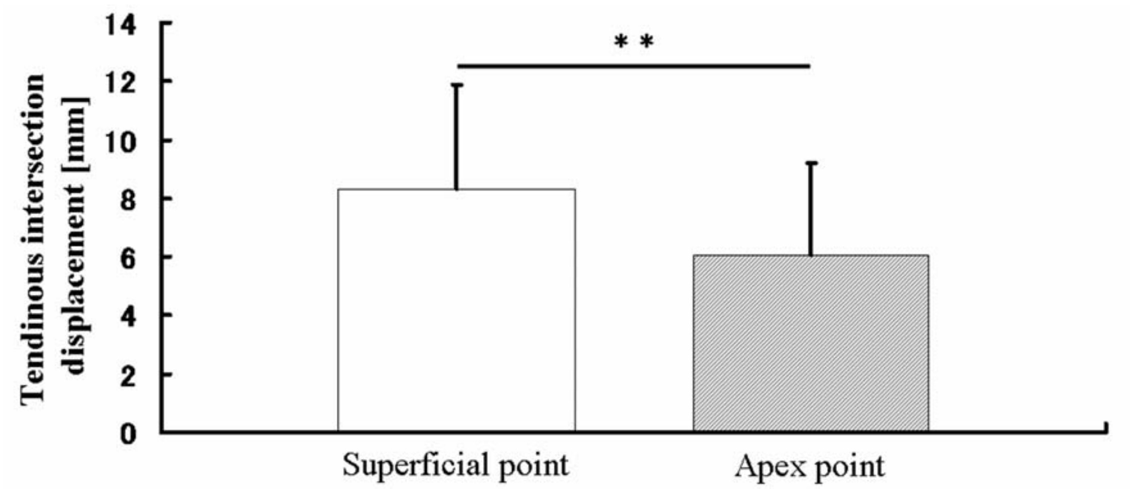

Fig. 5-5　TI displacement at the superficial point and apex point. Asterisk indicate significantly smaller displacement of apex point than that of the superficial point (P<0.01).

5-4. Discussion

This study was examined the ST muscle contraction behaviors in the superficial, middle and deep layer by using ultrasonography. One of the main findings was that the muscle contraction behaviors of ST muscle fascicles differed at the superficial and middle layers during isometric knee flexion. The moving of the ST muscle belly to proximal was agreed with the previous study reported by Hioki et al. (2003). Comparison of the magnitude of displacements at two points, the superficial point and apex point, showed that the magnitude of the superficial point displacement was greater than that of the apex point displacement. This result supports our hypothesis that the shortening behavior of the ST muscle fascicles is non-uniform. The rationale of this result would be the morphological characteristics of the ST muscle-tendon complex. The proximal fascicles of the ST muscle arose from three locations and the proximal tendon was relatively short, whereas the distal tendon long and thin (Woodley and Mercer 2005). Moreover, the internal aponeurosis is located along the distal centerline of the muscle (Fig. 5-6). The presence of aponeurosis, which is less compliant than passive muscle tissue and more compliant than tendon (van Bavel et al. 1996), would strongly affect to the ST muscle contraction behavior. Therefore, the different stiffness of tendon, aponeurosis and muscle could be a determining factor of the non-uniformity of contraction behaviors in the superficial and middle layers in this study. Additionally, other factors may influence of the regional difference of muscle shortening such as the sarcomere lengths (van Eijden and Raadsheer 1992), muscle fiber composition (Gardiner et al. 1991; Pernus and Erzen 1991), connective tissue and vascularization (Sjostrom et al. 1992; Korfage and van Eijden 1999), and intramuscular fluid pressures (Ebersole et al. 1999).

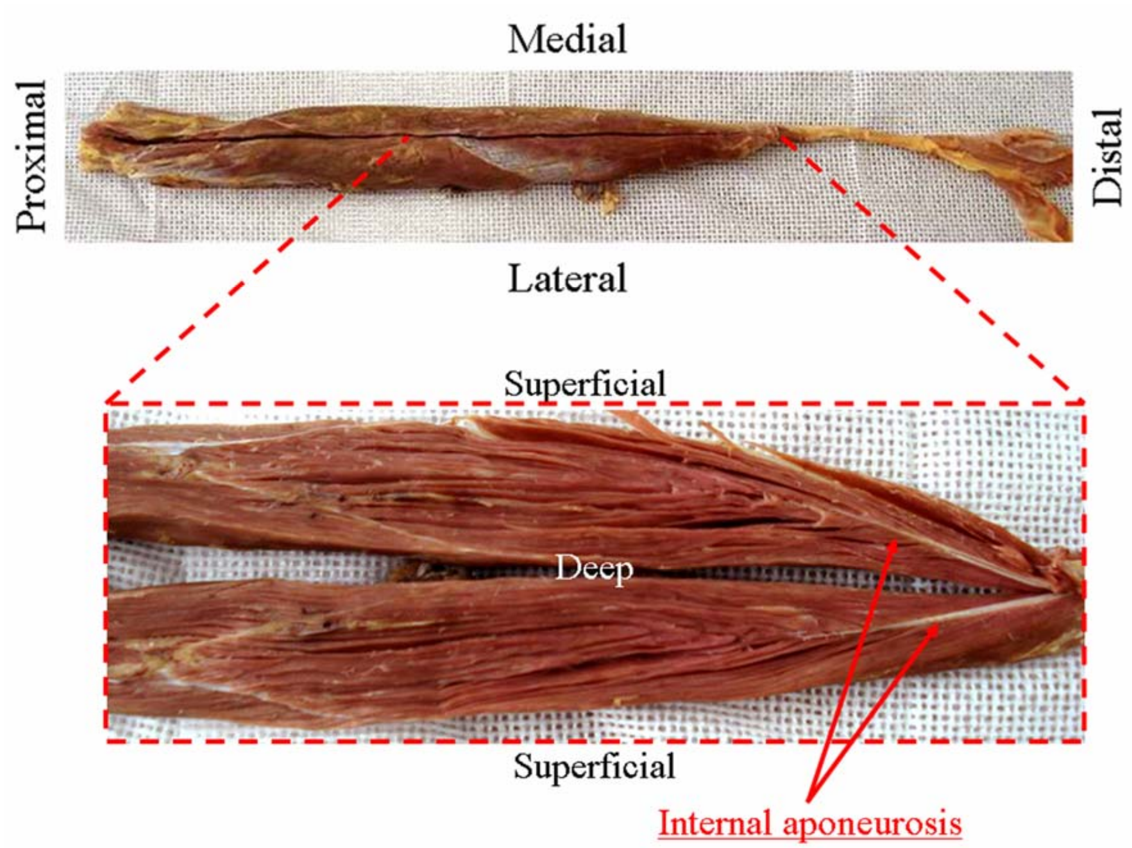

Fig. 5-6 The left semitendinosus muscle of posterior view (upper) and saggital plane (lower).

Other findings of this study were that the two angles around the TI, the superficial angle and apex angle, showed an increase at the MVC state, whereas the deep angle was not. This result indicates that the increased ST muscle thickness during isometric knee flexion was non-uniform among the superficial, middle, and deep layers. Numerous precedent studies have shown that muscle thickness increases with exertion of static contraction force (Woittiez et al. 1984; Bakke et al. 1992; Herbert and Gandevia 1995; Maganaris et al. 1998; Chi-Fishman et al. 2004; McMeeken et al. 2004). However, to our knowledge, no studies have reported non-uniform changes of muscle thickness during force exertion. Not all human muscle thickness increases with exerting force (Aratow et al. 1993; Maganaris et al. 1998). A probable factor affecting the non-uniform thickening behavior of the ST muscle is the interaction of contraction behaviors of the neighboring pennate muscles: the biceps femoris muscle and semimembranosus muscle. An exercise task generally activates more than one muscle. To elucidate the skeletal-muscle function entirely, the investigation should be performed not only of the objective muscle but also of the interaction of the surrounding musculoskeletal systems.

Results of the present study are helpful to clarify the functional importance of the presence of TI. Blemker et al. reported that non-uniform muscle fascicle strains were affected by the complex geometric arrangement of muscle fascicle lengths or fascicle curvatures (Pappas et al. 2002). Moreover, the ST is a parallel-fibered muscle with long muscle fibers (Wickiewicz et al. 1983; Friederich and Brand 1990); the mode of muscle-tendon insertion is therefore complicated. The ST has a heterogeneous geometric arrangement, fascicle lengths or fascicle curvatures. Consequently, it is possible that the TI of ST has a role in buffering site-specific force differences and in sharing strain behavior. Results of recent studies have indicated that contractile force transmission from muscle fibers is not attributable solely to the muscle tendon junction (MTJ), but also to lateral fibers within the muscle (Monti et al. 1999; Maas et al. 2001). Huijing reported that this characterization of the sharing behavior among fibers is important because it influences the potential for fibers to transmit force laterally via intramuscular connective tissue (Huijing 1999). The ending architecture of the muscle fibers near the TI is similar to the MTJ architecture. Consequently, the function of TI is to participate in lateral force transmission. In view of these facts, strain sharing behavior by the TI may be a characteristic of lateral force transmission of ST.

Precedent studies have investigated ST muscle contraction behaviors through functional evaluation of the ST muscle tendon complex in a clinical setting (Hioki et al. 2003; Makihara et al. 2006). The ST muscle is one of the hamstring muscles, in which muscle strain injury occurs frequently. In many cases of the ACL reconstruction surgery, the ST distal tendon is used as an autograft. Rehabilitation after muscle injury or ACL reconstruction surgery is important for prevention of re-injury or early return to sports activity. Evaluation of the ST muscle contraction behaviors is necessary for rehabilitation processes and is helpful to change the emphasis of the rehabilitation programs. The relationship between the knee flexion strength and the muscle architectural changes are not simple because the causes affecting to the relationships include various factors. Advances of knowledge related to force and excursion ability would contribute to our understanding of musculoskeletal systems and would facilitate rehabilitation.

One shortcoming of this study is that the evaluation of longitudinal sagittal images alone is insufficient for full assessment of ST muscle contraction behavior and for indicating the TI function. For this study, we used an ultrasonographic apparatus, which is a two-dimension application of the technique, and investigated the short-axis and the long-axis of the ST muscle in the sagittal plane, not to the medial-lateral direction. Results suggested that the muscle architecture might be changing in three-dimensions during muscle contraction. Future studies should adapt to the three-dimensional ultrasonography, MRI or CT for assessing the three-dimensional characteristics of the muscle function. However, evaluating using ultrasonography is better for real-time evaluation of muscle contraction behavior. For that reason, the results of this study are useful for revealing non-uniform contraction behaviors to the superficial-deep and proximal-distal directions.

In summary, this study measured the changes of the ST muscle architecture and ST muscle contraction behavior during isometric knee flexion by using ultrasonography. The muscle thickness around the superficial and the middle layers increased significantly with increasing isometric torque, but no significant change was observed around the deep layer. The degree of the muscle fascicle shortening of the superficial layer at the MVC state was significantly greater than that of the middle layer. These results showed that the ST muscle contraction behaviors are non-uniform around the superficial, middle, and deep layers in a direction to the short- and long-axes. From a clinical viewpoint, results of this study indicate that evaluation of the ST muscle function should be based on non-uniform muscle contraction behaviors.

CHAPTER 6:

GENERAL DISCUSSION

The purpose of this dissertation was to investigate the morphological and functional characteristics of the semitendinosus (ST) muscle by using magnetic resonance (MR) imaging in the Chapter 3, electromyography (EMG) in the Chapter 4 and ultrasonography in the Chapter 5. In Chapter 3, it was examined the responses of MR measurements (CSA, T2 value) in the hamstring muscles following eccentric knee-flexion exercise. It was interesting that the proximal and middle regions of the ST muscle show CSA increase and higher T2 changes compared with the distal region. Chapter 4 examined the regional difference of the EMG activities, and it is found that the EMG increasing behaviors were different between the proximal and distal compartments at the longest (hip at 90 degrees and knee at 0 degrees) and shortest (hip at 0 degrees and knee at 90 degrees) muscle positions in this study. The difference of ST muscle contraction behaviors was examined in Chapter 5. The results was found that the shortening of muscle fibers and increasing muscle thickness was non-uniform in the superficial, middle, and deep layers in the ST muscle.

These regional differences between proximal and distal regions in the long axis direction or among superficial, middle and deep layers in the short axis direction would be contributed the presence of a tendinous intersection (TI) within the ST muscle belly. The proximal and distal regions divided by the TI are receiving each partitioned innervations from one muscle nerve or the primary branch of the nerve, which one nerve enters above the level of the TI and the other enters below (Woodley and Mercer 2005). Previous studies showed that the fascicular lengths of the two regions in the human ST muscle were almost equal (Wickiewicz et al. 1983; Woodley and Mercer 2005). And our examination of the sarcomere length in the ST muscle dissected from four embalmed human cadaveric lower limbs indicated that the two regions have almost the same length of sarcomere. The ST has an internal aponeurosis in the distal centerline. However, the TI formulates the “V” shape in saggital plane, which would contribute to eliminate the differences of muscle fascicle length in the distal region. Therefore, the TI would present because of affecting to the uniformity of the muscle fascicle arrangement in the ST muscle.

The TI would work functionally during the hamstring muscles’ contractions such as knee flexion or hip extension movements. The Chapter 5 showed that the degrees of shortening or thickening of the superficial, middle and deep layers on the ST muscle belly were different. From these results, it could be indicated that the TI worked to buffer the strain among muscle fascicles and to transmit the contractile force to the longitudinal and lateral directions effectively. The fascicles of the proximal regions do not originate from a single point, but arise form three distinct areas. The distal fascicles inserted to the internal aponeurosis. Considering that the ST is a parallel-fiberd and bi-articular muscle, the absence of the TI might make the unfocused muscle contraction and the strain of the muscle fascicles. Therefore, the presence of the "V-shaped" TI would contribute to the effective contractions of the ST muscle.

However, regional differences of the neurophysiological activity may contribute to the effective ST muscle contraction for compensating the muscle mechanical disadvantage depending on the muscle length. In Chapter 4, the EMG increasing behaviors in the proximal and distal regions were almost equal in the mid-range of muscle length, but in the longest or shortest muscle length conditions the EMG behaviors were not the same during isometric knee flexion. The non-uniformity of these neurophysiological activities may be a compensation mechanism for the ST muscle mechanical disadvantage. Taking into account that muscle fibers in the proximal and distal regions are respectively innervated by a single nerve and which arise in common with the nerve to the biceps femoris long head (BFl) and semimembranosus

(SM) muscles, the potential for independent EMG control could exist for the two regions.

One of the functional properties of the ST muscle in the hamstring muscles would be the knee-joint motor control. Whereas the BFl or SM muscles are pennate muscles, the ST muscle is a parallel-fibered muscle. The pennate muscles, which have a large number of sarcomeres lying in parallel, excels in generating high force, by contrast the parallel-fibered muscle, which have more sarcomeres in series, excels in generating force over a wide range of motion. For the four hamstring muscles, the ST is the only muscle for which the proximal and distal regions divided by the TI could be defined on the basis of both architecture and innervations. The presence of the TI may contribute to the less of non-uniformity of the ST muscle fascicle arrangement, which could lead to the slight control of the knee joint.

Other functional properties of the ST muscle would provide for protection mechanism to the damage of hamstring muscles. In Chapter 3, the intensive eccentric exercise led the heavy damage to the ST muscle, but not so heavy to the BFl and SM muscles. It is true that the parallel-fibered muscle architecture is structurally weaker than the pennate muscle. The damage to the ST muscle may lead the protection of the

BFl or SM muscles' damages. Therefore, it may be contributed to keep the function of the BF or SM muscles to produce high force.

There were shortcomings in this dissertation. For example, despite the ST muscle was a two-joint muscle, it was investigated only about the knee flexion tasks, not about the hip extension tasks. Examining of the ST muscle contraction behaviors in the hip extension and knee flexion exercises could be clarify the ST muscle function comprehensively. Moreover, findings of these experiments could not directly explain the clinical applications, such as pathomechanisms or injury prevention methods. Therefore, the relations between the anatomical and physiological information of the ST muscle and the clinical findings are necessary for future studies.

This dissertation provided morphological and functional characteristics of the semitendinosus muscle in the hamstring muscles. The structure-function relationship in skeletal-muscle have been described and examined for over a century. However, the relationships of hamstring muscles have not been clarified. The ST muscle has interesting architectural characteristics, which would present for not-producing the non-uniform muscle fascicle arrangements. And the EMG properties of the ST muscle would compensate for the muscle mechanical disadvantages. These functional properties of the ST muscle could be recruited for the knee-joint movement control and/or the severe damage protection of the ST muscle or other hamstring muscles. The findings of this dissertation will contribute to future studies in sports science or sports medicine.

RELATED ARTICLES

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