早稲田大学審査学位論文
博士（スポーツ科学）

Muscle- and exercise-specific architectural plasticity of the quadriceps femoris

トレーニングによる大腿四頭筋の形状変化の筋間差とその動作依存性

２０１５年１月
早稲田大学大学院 スポーツ科学研究科

江間 諒一
EMA, Ryoichi

研究指導教員： 川上 泰雄 教授
Contents

Chapter 1 Introduction-----------------------------------------------1

1-1. Preface
1-2. Terminology
1-3. Review of literature
   1-3-1. Methods for measurement of muscle architecture
   1-3-2. Architectural and functional features of the quadriceps femoris
   1-3-3. Training-induced changes in muscle architecture of the quadriceps femoris
   1-3-4. Muscle activation levels during knee extension and leg extension exercises
1-4. Purpose

Chapter 2 Architectural changes of the quadriceps femoris induced by knee extension training-----------------------------------------------26


2-1. Introduction
2-2. Methods
2-3. Results
2-4. Discussion

Chapter 3 Architectural changes of the quadriceps femoris induced by leg extension training: Examination of quantitative profiles in sport athletes--------52

Section 1 Quantitative profiles of the quadriceps femoris in varsity oarsmen------52
Section 2 Influence of competitive cycling training on the muscle volume of the quadriceps femoris: Cross-sectional and longitudinal observations

Chapter 4 Activation of the quadriceps femoris during knee extension with or without hip extension torque

Chapter 5 Influence of exercise regimen and intensity for the activation of the quadriceps femoris: Comparison between knee extension and leg press exercises
Chapter 6 General discussion

6-1. Main findings of each chapter
6-2. Muscle- and exercise-specific architectural adaptation of the quadriceps femoris and underlying mechanisms
6-3. Possibility of generalization of the findings: Monoarticular vs. biarticular muscles
6-4. Applicability of the findings
6-5. Functional significance of RF hypertrophy
6-6. Factors that can influence interpretation of the current findings
6-7. Conclusion of the thesis

References

Acknowledgements
Chapter 1 Introduction

1-1. Preface

The quadriceps femoris, a large knee extensor group, plays important roles during lots of human movements (Thorpe et al. 1998). The muscle size of the quadriceps femoris decreases remarkably with aging, bed rest and space flight (Akima et al. 2000; Belavy et al. 2009; Frontera et al. 2000). Moreover, the quadriceps femoris size is associated with sport performance index (Rønnestad et al. 2010). To clarify what and how we should do so as to gain muscle size (hypertrophy) of the quadriceps femoris is crucial for overcoming some themes in sports and health sciences, such as improvement of sport performance, prevention of sarcopenia (decline of muscle size and strength) in elderly people and long-term stay in space. The quadriceps femoris is composed of four pennate muscles (vastus lateralis, VL; vastus medialis, VM; vastus intermedius, VI; rectus femoris, RF) with different architectural characteristics. Muscle architecture is related to athletic levels of athletes (Akima et al. 1992; Kubo et al. 2010), sport performance (Abe et al. 2000, 2001; Ikebukuro et al. 2011; Kumagai et al. 2000; Rønnestad et al. 2010), strength (Ikai and Fukunaga 1968; Alexander and Vernon 1975; Ichinose et al. 1998; Ikegawa et al. 2008), maximal shortening velocity (Bodine et al. 1982) and force-length relation (Burkholder et al. 1994) of the muscle. Such architectural attributes and their plasticity can be important parameters of muscle function.

It has been observed that the extent of decrease in muscle size (muscle atrophy) following unloading (Belavy et al. 2009) and the relations between muscle size and sport performance indices (Ikebukuro et al. 2011) differ among the four muscles of the quadriceps femoris. Nevertheless, the question of how training induces changes in
muscle architecture has often been attacked for the whole quadriceps femoris or only one of quadriceps muscles. It remains unclear therefore, whether or not training-induced changes in muscle architecture are homogeneous among the four muscles. Once this issue is clarified, one can establish useful knowledge that helps to select optimal training procedures that match distinct goals (e.g., training for the elderly). Targeting the quadriceps femoris, two major exercises, i.e., knee extension and leg extension (simultaneous extensions of knee and hip joints), have been conventionally included in the training regimens. However, it is not known also whether or not these two exercises can induce similar or different architectural responses of individual muscle of the quadriceps femoris. Furthermore, the factors influencing on architectural responses are not clear.

The purposes of this thesis are to clarify 1) how the architecture of individual muscles changes following training, 2) whether the observed changes differ among different training regimens and 3) what are the influencing factors of different architectural responses among muscles and between training regimens. To this end, a longitudinal experiment using knee extension training was firstly performed. Next, the cross-sectional and longitudinal experiments were conducted on sport athletes who had executed habitual competitive and training activities involving repetitive leg extensions. Lastly, the activation level of the quadriceps femoris was compared between conditions with knee extension and leg extension exercises.

1-2. Terminology

Quadriceps femoris
The quadriceps femoris is a skeletal muscle (hereafter muscle) group, composed of four pennate muscles (VL, VM, VI and RF). Therefore, the difference between muscles (e.g., VL and VM) indicates inter-muscle difference, and the difference within a muscle (e.g., distal region and proximal region of VL) shows intra-muscle difference.

**Muscle architecture**

In this thesis, muscle architecture represents muscle size and fascicle arrangement within a muscle. Architectural parameters show muscle volume, muscle cross-sectional area, muscle thickness, fascicle length and pennation angle.

**Muscle cross-sectional area and muscle volume**

There are two ways to determine muscle cross-sectional areas: anatomical cross-sectional area (ACSA, Fig. 1-1) and physiological cross-sectional area (PCSA). The ACSA is the area of a cross-section that is perpendicular to the long axis of a whole muscle. The PCSA is the area of a cross-section that is perpendicular to the muscle fibers. The PCSA is calculated by dividing muscle volume by the fascicle length (Fukunaga et al. 1992). In this thesis, ACSAs were determined in each Chapter. Muscle volume is determined as the sum of the product of ACSA and slice thickness along the muscle.

**Muscle thickness**

Muscle thickness is the thickness of a muscle. In a pennate muscle, superficial and deep aponeuroses constitute boundaries of a muscle in a cross-section. The
aponeuroses are seen as clear reflective echoes on a B-mode ultrasound image. In this thesis, the distance between the aponeuroses is defined as “muscle thickness”. However, the aponeuroses are not necessarily aligned parallel in the sectional image and thus the muscle thickness was determined as the mean of the distances between the two aponeuroses at both ends of each image of 60 mm image width (Fig. 1-2) (Blazevich et al. 2006; Ema et al. 2013).

**Fascicle length**

A fascicle is a bundle of dozens to hundreds of muscle fibers. On a B-mode ultrasound image, some reflective echoes are seen between the superficial and deep aponeuroses (Fig. 1-2). These are reflective echoes from the connective tissues between the fascicles, and are considered to represent the fascicle directions (Herbert and Gandevia 1995). The length of echoes is determined as the fascicle length. In this thesis, the fascicle length was calculated as the distances of intersections of the echoes and two aponeuroses.

**Pennation angle**

A pennation angle is determined as an angle of a fascicle and an aponeurosis (Kawakami et al. 1993). In this thesis, the pennation angle was calculated as the angle of the white reflective echoes and deep aponeurosis (Fig. 1-2).

**Muscle hypertrophy**
In general, muscle hypertrophy means an increase in muscle size. In this thesis, muscle hypertrophy is defined as the increase in muscle volume, ACSA or muscle thickness following training.

Training

In this thesis, the term of “training” indicates an exercise-based muscle loading. If the load induced by training exceeds a certain level, an increase in muscle size, i.e., muscle hypertrophy can occur. In general, resistance training is regarded as the most effective strategy to impose substantial load to the working muscle(s). Rowing and cycling training is often dealt with as the endurance training, but these are also considered as exercise-based muscle loading that can induce muscle hypertrophy.

Leg extension

In this thesis, leg extension means simultaneous extensions of knee and hip joints.

Muscle activation

In this thesis, muscle activation or activation level reflects the degree of recruitment of motor unit (i.e., muscle fiber) and motor unit firing rate. These are evaluated by such as surface electromyography (EMG), although it is difficult to distinguish these two factors. When one of them or both of them are greater in a muscle contraction condition compared to other condition(s), the amplitude of EMG is higher, indicating that muscle activation or activation level is high.
1-3. Review of literature

The purpose of this thesis is to examine how muscle architecture of the quadriceps femoris changes induced by training, and its dependence on training modality and factors contributing such dependence. In this section, related studies are overviewed from following four viewpoints: 1) the methods for measuring muscle architecture and those reliability, 2) architectural and functional features of the quadriceps femoris and studies that focused on the unique roles of biarticular muscles, 3) training-induced changes in muscle architecture of the quadriceps femoris, and 4) muscle activation levels during knee extension and leg extension exercises.

1-3-1. Methods for measurement of muscle architecture

Development of tissue-visualizing techniques enabled to examine the muscle size and fascicle arrangement in vivo humans (Blazevich et al. 2006; Ema et al. 2013; Fukunaga et al. 1997; Kanaeisa et al. 1998; Kawakami et al. 1993, 1995) which could be acquired directly from only a cadaver previously (Friederich and Brand 1990; Wickiewicz et al. 1983). Muscle size has been evaluated by using ultrasonography (Ikai and Fukunaga 1968), computed tomography (CT)(Schantz et al. 1983), and magnetic resonance (MR) imaging (Narici et al. 1988). In 1960’s, Ikai and Fukunaga (1968) established a method for determining anatomical cross-sectional area (ACSA) of a muscle by ultrasonography. The MR imaging and CT has been mainly used to measure ACSA rather than ultrasonography (Reeves et al. 2004a; Young et al. 1983) because of a large field of view and high resolution (Blazevich et al. 2007a; Erskine et al. 2010a, b; Fukunaga et al. 1992; Kawakami et al. 1995; Narici et al. 1988, 1989, 1996b; Schantz et al. 1983; Seynnes et al. 2007, 2009; Wakahara et al. 2012, 2013). On the other hand,
most recent studies evaluated ACSA by a new method of ultrasonography: extended-field of view (Noorkoiv et al. 2010). This new method may enable to measure ACSA easier than by the other two techniques in the future.

After the 1990’s, fascicle length and pennation angle as well as ACSA were measured during static and dynamic contractions by using ultrasonography. Fascicle length and pennation angle are determinants of muscle functions during human movements. The fascicle length is related to the maximal shortening velocity (Bodine et al. 1982) and force-length relation (Burkholder et al. 1994) of the muscle. Pennation angle is associated with the transmission efficiency of force from muscle fibers to tendon (Alexander and Vernon 1975). Measurements of the two parameters have been conducted by attaching the probe to the skin parallel along the muscle (along longitudinal axis). To our knowledge, in 1992, two studies for the first time reported the fascicle length and pennation angle in vivo humans by using ultrasonography. Henriksson-Larsen et al. (1992) measured the fascicle length and pennation angle of VL and evaluated the repeatability of measurements. The results showed that the acquisition of longitudinal image of ultrasound can be applied for measurement in humans in vivo. In the same year, Rutherford and Jones (1992) measured the pennation angle of VL and VI before and after resistance training for 3 months. They failed to find significant changes of the pennation angle in the both muscles.

Validity and repeatability of measurement of ACSA

The validity of MR imaging and CT for measuring muscle size was confirmed in a cadaver study (Mitsiopoulos et al. 1998). Regarding the repeatability of measurement of MR imaging, the coefficient of variation (CV) and intraclass
correlation coefficient (ICC) of the repeated measurements for the maximal ACSA of the triceps brachii was 2.2% and 0.979, respectively (Wakahara et al. 2013), indicating that repeatability of MR imaging is quite high. Therefore, these techniques are regarded as a gold standard for measuring muscle size, and used in many longitudinal studies.

Validity of measurement of the fascicle length and pennation angle

Validity of the fascicle length and/or pennation angle was examined thorough comparison between direct measurement value and ultrasonographic value on a cadaver. The number of validation study is limited. Kawakami et al. (1993) compared values of pennation angle of the triceps brachii obtained by ultrasonography and from a cadaver. According to the results of Kawakami et al. (1993), it is possible to measure the pennation angle of the triceps brachii in vivo with less than 1° error. By using similar methods, following studies confirmed the validation in the medial gastrocnemius (Narici et al. 1996a) and biceps femoris long head (Chleboun et al. 2001), with the error of ~8 mm for fascicle length, and ~3° for pennation angle, respectively. Recently, the validation in the semitendinosus was reported (Kellis et al. 2009). Regarding the quadriceps femoris, recent studies evaluated the validation of measurement (Ando et al. 2014; Ema et al. 2013; Engelina et al. 2014). Among the four muscles of the quadriceps femoris, the validation of RF is needed to confirm because of its complicated architecture (Blemker and Delp 2006) and difficulty in fascicle identification (Rutherford and Jones 1992). Despite bipennate architecture of RF, a probe was oriented perpendicular to the skin for identifying the fascicle (Blazevich et al. 2006). Therefore, the values of these studies should include substantial error. To overcome this issue, I adjusted the probe angle relative to the skin, and succeeded in clearly visualizing
fascicular paths of RF. As a result, validation of fascicle length and pennation angle of RF was established (Ema et al. 2013). Taken together, the validity of ultrasound measurement was confirmed at least for large limb muscles, including quadriceps femoris, under a relaxed condition with no joint motions (Kwah et al. 2013).

**Repeatability of measurement of the fascicle length and pennation angle**

Repeatability of measurement on the repeated measures had been reported in 1990’s. Henriksson-Larsen et al. (1992) measured the fascicle length and pennation angle of VL three times on the different days. They conducted an analysis of variance (ANOVA) for the measurement values and regarded as a high repeatability with no difference. The ANOVA, however, is not valid way to evaluate the variation of the repeated data. Therefore, in generally, the other index, such as the CV and ICC are mainly used to evaluate the repeatability of measurement. Rutherford and Jones (1992) measured the pennation angles of VL and VI and the CV of repeated measurement on different days was 13.5%. Narici et al. (1996a) measured the fascicle length and pennation angle of the medial gastrocnemius seven times on a subject, and they reported the CVs were 5.9% and 9.8% for the fascicle length and pennation angle, respectively. Fukunaga et al. (1997) measured VL fascicle length and pennation angle twice on different occasions and reported the CVs were 2.1% and 0.8%, respectively. Kawakami et al. (1998) showed that the CV of measurement in three times was ~2% for the fascicle length and pennation angle of the gastrocnemius and soleus. Day-to-day repeatability of VL pennation angle measurement was 3.2% (CV) and 0.821 (ICC), respectively (Gondin et al. 2005). Recent study confirmed the repeatability for the gastrocnemius during dynamic motions, such as walking (Aggeloussis et al. 2010) and
running (Giannakou et al. 2011) as well as static conditions. These results indicate that repeatability of fascicle length and pennation angle measurement is high. However, regarding the quadriceps femoris, the reports of repeatability is mainly limited to VL, although that was also examined and reported high repeatability under several knee joint angles (Mairet et al. 2006) and in various age groups (Raj et al. 2012). Most recently, I reported the repeatability of RF measurement (Ema et al. 2013) with a valid method. Before the experiment, measurement accuracy of an examiner was also confirmed. This confirmation can eliminate a possibility of high repeatability with an invalid value. The results warrant the use of ultrasonography for examining the architectural characteristics and plasticity of RF in humans in vivo. Since the extent of repeatability is various among muscles, it is needed to clarify the values for each study in detail.

1-3-2. Architectural and functional features of the quadriceps femoris

Architectural features of the quadriceps femoris

The quadriceps femoris is one of the largest muscle groups among the skeletal muscles in humans. The muscle volume of the quadriceps femoris reaches about 2000 cm$^3$ (Morse et al. 2007) and the values are more than twice as large as antagonist hamstring values (Akima et al. 2007). The VL is the largest component of the quadriceps femoris (Standring 2008), followed by VI, VM and RF (Akima et al. 2007; Morse et al. 2007).

By 1990, fascicle arrangement of the quadriceps femoris was reported in cadaver studies (Friederich and Brand 1990; Wickiewicz et al. 1983). In 1992, the fascicle arrangement in humans in vivo was reported for the first time. Henriksson-Larsen et al. (1992) measured the fascicle length and pennation angle of VL
in women. They reported that the fascicle length was from 66 mm to 155 mm, and
pennation angle was from 8.5° to 25.5°. Rutherford and Jones (1992) examined the
pennation angle of VL and VI for healthy 12 adults. On the other hand, in their study,
they did not measure VM and RF architecture because of difficulty in fascicle
identification. Fukunaga et al. (1997) measured VL fascicle length and pennation angle
at several knee joints, and showed that the fascicle length shortened (132.9 mm to 96.7
mm) and pennation angle increased (14° to 18°) from 100° to 0° of knee joint angle. In
the same year, Ichinose et al. (1997) investigated the VL fascicle length at several knee
joint angles and contraction intensities, and estimated force-length relation of VL. In
their results, the optimal length of VL fascicle was 78 mm. In 2000’s, researchers also
focused on the plasticity of the quadriceps femoris architecture. The VL fascicle length
and pennation angle was measured before and after training intervention and bed rest
(Aagaard et al. 2001; Blazevich et al. 2007a; Gondin et al. 2005; Kawakami et al. 2000,
2001; Reeves et al. 2004b, 2004c, 2009; Seynnes et al. 2007, 2009). Also, examination
for clarifying group difference of VL architecture was performed (Abe et al. 2000;
Furthermore, Kawakami et al. (2006) measured VL pennation angle in 711 subjects, and
reported that the range of VL pennation angle was 7 to 33°, suggesting the large
inter-individual variability. As mentioned above, the reports of the quadriceps femoris
architecture is mainly limited to VL, and remain unknown regarding the architecture of
other muscles.

Recently, detail information of the quadriceps femoris architecture was
provided. Blazevich et al. (2006) showed the measurement values of fascicle length and
pennation angle at the proximal, middle and distal regions for each muscle. The results
showed that the fascicle length and pennation angle differed among the four muscles and within a muscle. O'Brien et al. (2010) compared the architecture between adults and children as the same regions of Blazevich et al. (2006). They observed the longer fascicle length but similar pennation angle in adults. There are few studies which measured muscle architecture for each muscles (Blazevich et al. 2007b; Erskine et al. 2009), and the knowledge is not sufficient.

**Functional features of the quadriceps femoris**

As all the four muscles (VL, VM, VI and RF) of the quadriceps femoris cross knee joint, quadriceps femoris acts as the knee extensor. Based on the force-length characteristics (Gordon et al. 1966), knee joint angle influences the knee extension strength. Many previous studies showed that maximal voluntary isometric knee extension (MVC\_KE) torque was largest at around 70° knee joint angle (full extension = 0°) (Babault et al. 2003; Becker and Awiszus 2001; Pincivero et al. 2004; Yoon et al. 1991). This can be mainly explained by the estimation that optimal lengths of the vasti muscles are around 70° knee joint angle (Herzog et al. 1990, 1991). On the other hand, among the four muscles, only RF is a biarticular muscle which crosses knee and hip joints. Therefore, hip joint angle also affects the knee extension strength. Since Narici et al. (1992) showed that PCSA of RF reached about 24% of the total quadriceps femoris PCSA, RF substantially contributes to knee extension strength, although RF size is the smallest among the four muscles of the quadriceps femoris. At knee extended position, I showed the MVC\_KE torque was greater in hip extension position than in hip flex position with similar EMG values of the quadriceps femoris between the positions. This suggests that the difference of MVC\_KE torque is due to the difference in
length-dependent force generating capacity of RF between the two positions (Ema et al. 2010). The difference of MVC_{KE} torque reached about 20%. In addition, compared to MVC_{KE} torque at neutral pelvic position, that was smaller (about 15%) at anterior pelvic position, with the same activation level of the quadriceps femoris (Ema et al. 2012). Based on the theoretical model of force-length relation of the quadriceps femoris (Herzog et al. 1990), if hip position is extended, RF can operate optimal length even when the vasti use ascending limb of the relation (i.e., knee joint is extended). My studies support this estimation (Ema et al. 2010, 2012).

Muscle force also depends on muscle (fascicle) shortening velocity (Hill 1938). In general, isokinetic testing with various angular velocities is conducted to examine the force-velocity relation of the quadriceps femoris. It was demonstrated that concentric knee extension torque decreased with increasing angular velocity but eccentric knee extension torque was similar among different angular velocity conditions (Westing et al. 1988, 1991). Recent investigation showed that fascicle shortening velocity of a biarticular muscle is influenced by both proximal and distal joint angles which the muscle crosses. Wakahara et al. (2007) observed that fascicle shortening velocity of the biarticular medial gastrocnemius during concentric plantar flexion was higher at knee extended position than at knee flexed position. This suggests that biarticular muscles can regulate the force in terms of both force-length and force-velocity relations. Similar joint-angle dependency was also observed during eccentric plantar flexion (Wakahara et al. 2009). Moreover, during leg extensions, estimated muscle shortening velocity of RF was lower than those of the monoarticular vasti (Gregoire et al. 1984), indicating the higher velocity dependent force-generating capacity of RF compared to the vasti during
leg extensions. Considering the above studies, RF as well as the vasti can greatly contribute as a knee extensor during human movements.

**Studies that focused on the unique roles of biarticular muscles**

From various viewpoints, studies have elucidated the characteristics of biarticular muscles. In the 1980’s and 1990’s, van Ingen Schenau and colleagues conducted kinematics and kinetics analyses and showed the unique role of the biarticular muscles during leg extensions. For example, Gregoire et al. (1984) calculated joint moment and joint power during vertical jumps with the EMG data of the muscles in the lower extremity. They also estimated the shortening velocities of the muscles by using joint angular velocity and previous data of moment arm around a crossing joint. From the obtained data, it was demonstrated that mechanical energy generated by the monoarticular muscles of the hip and knee joints was transferred distally via the biarticular muscles to the ankle joints, with a low contraction velocity of the biarticular muscles. Similar findings were reported in other studies (Arakawa et al. 2013; Bobbert et al. 1986; Jacobs et al. 1996; van Soest et al. 1993). The unique role of the biarticular muscles is often called “tendon action” (Prilutsky and Zatsiorsky 1994).

Another aspect of the functional role of the biarticular muscles during leg extensions is the control of direction of an external force. In the study of Jacobs and van Ingen Schenau (1992), the subjects were asked to exert a constant force (300 N or 600 N) on the foot with various directions in sitting positions, and EMG data were obtained from the quadriceps and hamstrings muscles. The extent of activation levels of monoarticular VL and VM were linearly correlated to the knee extension moment, independent of force direction. On the other hand, a clear linear relation was observed
between the difference in the activation levels of biarticular RF and hamstrings and the
difference in net moment around the knee and hip joints. From the data, Jacobs and van
Ingen Schenau (1992) concluded that biarticular muscles control the distribution of net
moments that the muscles cross, thereby controlling the direction of the external force.
Similar observation was reported during pedaling motions (van Ingen Schenau et al.
1992). Later studies confirmed the functional role of biarticular muscles during jumping
(Jones and Caldwell 2003; Toriumi et al. 2003). The above studies hint to a notion that
training-induced architectural responses are different among biarticular RF and
monoarticular vasti, reflecting their functional roles.

Regarding the neurophysiological viewpoints, previous studies observed the
difference in activation levels of the biarticular muscles between tasks (Fujiwara and
Basmajian 1975; Yamashita 1988) and showed the difference in activation levels
between biarticular RF and monoarticular vasti during fatiguing knee extension
exercises (Akima et al. 2012; Ebenbichler et al. 1998; Kouzaki et al. 2002; Kouzaki and
Shinohara 2006). Yamashita (1988) showed that the activation level of RF at an
intensity of 20% of maximal knee extension was higher than those at simultaneous
extensions of knee and hip joints at an intensity of 20% of maximal effort, and vice
versa for VM. The similar result was obtained for the semimembranosus. Ebenbichler et
al. (1998) examined changes in the median frequency of EMG signals of VL, VM, RF
and biceps femoris long head during isometric knee extensions until exhaustion. They
found that there were differences between monoarticular and biarticular muscles:
greater decline in the EMG median frequency of RF and biceps femoris long head than
in VL and VM. Kouzaki et al. (2002) investigated the EMG amplitude during sustained
isometric contraction at an intensity of 2.5% of maximal voluntary contraction for 60
minutes. The results showed the alternation of activation levels between RF and VL or VM but not between VL and VM. The same phenomenon also existed between RF and VI (Akima et al. 2012), and the association between the frequency of alternation and the extent of muscle fatigue was shown (Kouzaki and Shinohara 2006). It is possible therefore that biarticular muscles have a different role from those of monoarticular muscles, both in single-joint and multi-joint exercises.

1-3-3. Training-induced changes in muscle architecture of the quadriceps femoris

Change in muscle size (muscle hypertrophy)

Resistance training for more than several weeks elicits an increase in muscle volume (Aagaard et al. 2001; Kubo et al. 2001a; McCarthy et al. 1997), ACSA (Ikai and Fukunaga 1970; Narici et al. 1989, 1996b; Ploutz et al. 1994; Young et al. 1983) and muscle thickness (Krotkiewski et al. 1979) of the quadriceps femoris. By using the MR imaging, it has become clear that the extent of increase in ACSA after knee extension training is not uniform among the muscles and/or along the length (Blazevich et al. 2007a; Housh et al. 1992; Narici et al. 1989, 1996b; Seynnes et al. 2007). Narici et al. (1989) observed the 8.5% increase in ACSA of the quadriceps femoris after knee extension training for 60 days. In addition, they determined the extent of increase in ACSA for each muscle along the thigh and demonstrated that muscle hypertrophy was not uniform along the length. Housh et al. (1992) indicated that a knee extension training induced the preferential increase in ACSA of RF compared to the other three muscles and the extents were 13%, 22% and 34% in the proximal, middle and distal regions, respectively. Similar findings were shown in Narici et al. (1996b). These studies indicate that muscle hypertrophy does not occur homogeneously along the
length. Regarding the hypertrophy across the muscle, only one study reported the nonuniform hypertrophy across a muscle (Wells et al. 2014), except for the current study (Chapter 2). There is room for argument on the hypertrophic adaptation of the quadriceps femoris.

Another major training regimen for the quadriceps femoris is leg extension (simultaneous extensions of knee and hip joints) training, such as squat, leg press, cycling and rowing. However, the majority of training intervention studies selected the knee extension training as a training modality, and thus architectural adaptation of the quadriceps femoris following leg extension training remains unclear. Recent training intervention studies showed that factors influencing muscle hypertrophy is training volume rather than training intensity (i.e., low intensity with high repetition training can induce substantial hypertrophy of the training muscles) (Mitchell et al. 2012). This raises a possibility that competitive sport activities induce muscle hypertrophy of the quadriceps femoris, even if the intensity of each sport motions is not so high. In fact, greater size of the quadriceps femoris compared to untrained controls are observed in some kind of athletes (Abe et al. 2000, 2001; D'Antona et al. 2006; Hoshikawa et al. 2010; Hug et al. 2006; Izquierdo et al. 2004; Kanehisa et al. 1998; Kearns et al. 2000). Detail examination of the quantitative characteristics of muscles in athletes or the longitudinal change induced by competitive training would be useful for clarifying the possible relation between muscular hypertrophy and sport-specific motions.

**Change in fascicle length**

In animal experiments, it has been shown that training induces an increase in serial sarcomere number (Butterfield et al. 2005; Lynn and Morgan 1994; Lynn et al.
For example, Lynn et al. (1998) observed that concentric and eccentric training resulted in an increase in serial sarcomere number in rat. Considering the results (Lynn et al. 1998), training can induce the increase in fascicle length in humans in vivo. After the 2000’s, some studies investigated the changes in fascicle length of the quadriceps femoris following some kind of training. Some showed the increase in fascicle length (Alegre et al. 2006; Blazevich et al. 2003, 2007a; Franchi et al. 2014; McMahon et al. 2014; Reeves et al. 2004b, 2004c, 2009; Seynnes et al. 2007), others did not (Alegre et al. 2014; Blazevich et al. 2007b; Erskine et al. 2010a, 2010b; Seynnes et al. 2009). Therefore, consensus has not been reached regarding the training-induced response of the fascicle length.

Recent studies suggested that eccentric contraction training increased the fascicle length but concentric contraction did not, or the extent of increase in fascicle length was greater after eccentric contraction training than after concentric contraction or conventional (both concentric and eccentric) training (Baroni et al. 2013; Franchi et al. 2014; Reeves et al. 2009). Most recent investigation provided an evidence of the importance of lengthening velocity of fascicle (Sharifnezhad et al. 2014). They designed four eccentric contraction training protocols: at 65%MVC \text{KE} load with 90°/s from 25° to 100° knee joint angle, at 100%MVC \text{KE} load with 90°/s from 25° to 100° knee joint angle, at 100%MVC \text{KE} load with 90°/s from 25° to 65° knee joint angle, and at 100%MVC \text{KE} load with 240°/s from 25° to 100° knee joint angle, and compared the fascicle length of VL before and after the interventions. They demonstrated that only high muscle lengthening velocity training induced the increase in VL fascicle length. However, other research groups indicated the increase in fascicle length of VL after isometric training at short and long muscle length (Noorkoiv et al. 2014) and concentric contraction training.
(Blazevich et al. 2007a). It remains unclear regarding the inconsistencies among studies. Also, it is unknown whether inter- and intra-muscle differences in the extent of increase in fascicle length exist.

**Change in pennation angle**

Training-induced increase in pennation angle is considered to be a strategy to place fascicles with training-induced diameters on a limited area of aponeurosis. Rutherford and Jones (1992) measured the pennation angle of VL and VI before and after knee extension training for 12 weeks, and they failed to find significant changes of them of the two muscles. However, since the repeatability of measurement was not so good (the CV of repeated measurements was 13.5%), that may be the reason for the no changes in pennation angle. After 2000’s, a large number of studies examined the training-induced changes in pennation angle of the quadriceps femoris. For example, Aagaard et al. (2001) examined the change in pennation angle of VL induced by several kinds of resistance training for 14 weeks. The results demonstrated the 35.5% increase in pennation angle of VL. Reeves et al. (2004b) observed 13% increase in pennation angle of VL following resistance training for 14 weeks in elderly subjects. The same research group (Reeves et al. 2004c) confirmed the at most 35% increase in pennation angle of VL after the same training program of Reeves et al. (2004b). Blazevich et al. (2007a) examined the change in pennation angle of VL induced by concentric or eccentric contraction training. They showed the 13.3% increase in concentric group and the 21.4% increase in eccentric group, but there was no difference in the extent of increase between the two groups. Similar findings are observed in other studies (Erskine et al. 2010a, 2010b; Seynnes et al. 2007). On the other hand, Reeves et al. (2009)
reported that the pennation angle of VL did not change significantly after eccentric contraction training but increased after conventional training in elderly subjects. Baroni et al. (2013) also failed to find a significant change of pennation angle in VL and RF after eccentric knee extension training for 12 weeks. Franchi et al. (2014) observed a significant increase in pennation angle of VL following concentric contraction training but not after eccentric contraction training. Note that the information of methods in Franchi et al. (2014) is not sufficient, because they did not evaluate the muscle hypertrophy at the same regions as the pennation angle measurement in the same knee joint position, and measurement regions are unclear. Considering the above findings, no consensus has been reached regarding the training-induced changes in pennation angle as well as fascicle length. Moreover, most of studies investigated the changes in pennation angle of only VL, and thus the responses in the other three muscles remain unclear.

1-3-4. Muscle activation levels during knee extension and leg extension exercises

Muscle activation levels during knee and/or leg extension exercises have been assessed through EMG, T2-weighted MR imaging and positron emission tomography (e.g., Chin et al. 2011; Endo et al. 2007; Enoescon et al. 2005; Escamilla et al. 1998, 2001; Gondoh et al. 2009; Narici et al. 1996b; Ploutz-Snyder et al. 1995; Prior et al. 2001; Richardson et al. 1998; Takahashi et al. 1994). Regarding the difference in muscle activation among the muscles of the quadriceps femoris, Ploutz-Snyder et al. (1995) found by the use of T2-weighted MR imaging that activation level during squat exercise was lower in RF than in the vasti. Lower activation of RF compared to the vasti during leg extension exercise was also shown in other studies (Chin et al. 2011; Endo et al.
2007; Escamilla et al. 1998, 2001; Gondoh et al. 2009), although the underlying mechanisms remain unclear. On the other hand, no consensus has been reached regarding the inter-muscle difference in activation levels during the knee extension exercise (Enocson et al. 2005; Escamilla et al. 1998; Richardson et al. 1998). Some showed greater activity in RF than in the vasti (Enocson et al. 2005; Richardson et al. 1998), whereas others (Escamilla et al. 1998) did not.

With respect to the difference in muscle activation among the different exercises regimens, Escamilla et al. (1998) compared the activation levels of VL, VM and RF during knee extension, squat and leg press exercises at an intensity of 12-repetition maximum. They demonstrated that the level of RF was higher in the knee extension than in the squat and leg press exercises, and vice versa for VL and VM. On the other hand, Enocson et al. (2005) assessed muscle activation during an exercise regimen which consists of 5 sets of 10 repetition maximum load. They observed that activation levels of VM, VI and RF were higher during knee extension than during leg press exercise. It should be noted that the relative exercise intensity adopted in the previous studies may not be the same among the knee extension and leg extension exercises, because the number of repetitions performed at a given percentage of one repetition maximum was higher during leg press exercise (Schoenfeld et al. 2014) than during knee extension exercise (Burd et al. 2012). Thus, it is possible that the relative intensity for the quadriceps femoris differs between the exercises, and that the difference may be involved in the previous results (Enocson et al. 2005; Escamilla et al. 1998). Moreover, although previous studies showed significant differences in activation levels among the muscles or between the different regimens, the extent of the difference is unclear. Therefore, it is difficult to discuss the relation between the architectural
response and muscle activation levels. Even if the activation level of RF is substantially low during leg extensions, it is not clear whether or not the extent is not enough to induce the hypertrophy of RF. If the influence of training regimens on the activation levels of quadriceps femoris at several exercise intensities is examined, the above points can be clarified.

1-4. Purpose

As mentioned in the review of literature, no consensus has been reached regarding the architectural changes of the quadriceps femoris induced by knee extension training. This may be related to the possible existence of inter- and intra-muscle difference in architectural changes. Furthermore, little is known about the architectural adaptation following leg extension training. The general purpose of this thesis is to examine the architectural adaptation of the individual muscles of the quadriceps femoris induced by knee extension and leg extension training.

In Chapter 2, using ultrasonography and MR imaging, architectural changes of individual muscles of the quadriceps femoris were determined. Inter- and intra-muscle differences, and relation between hypertrophic adaptation and changes in fascicle arrangement were discussed.

In Chapter 3, the influence of leg extension training on the individual size of the quadriceps femoris were examined. To this end, by using MR imaging, the quantitative characteristics of sport athletes who repeat leg extensions in their competitive and training activities were determined cross-sectionally and longitudinally.
In Chapter 4, the underlying mechanism of the difference in the results of Chapter 2 and Chapter 3 was examined through controlled experimental settings.

In Chapter 5, activation level of VL, VM and RF during knee extension and leg press were compared and examined the intensity and exercise dependence of the activation level.

In Chapter 6, the main findings of each chapter were firstly addressed. Subsequently, the following issues were discussed: 1) muscle- and exercise-specificity in architectural adaptation of the quadriceps femoris and underlying mechanisms, 2) generalization of the findings, 3) applicability of the findings, 4) functional significance of the findings, and 5) factors that might influence the interpretation of the current findings. Lastly, conclusion of the thesis was shown.
Fig. 1-1 Example of magnetic resonance images of right thigh in the coronal (left) and transverse (right) planes. White broken line indicates the anatomical cross-sectional area of the vastus lateralis (VL), vastus medialis (VM), vastus intermedius (VI) and rectus femoris (RF).
Fig. 1-2 An example of ultrasound image of the vastus lateralis. White lines show the superficial and deep aponeuroses, muscle thickness, fascicle and pennation angle.
CHAPTER 2 Architectural changes of the quadriceps femoris induced by knee extension training

2-1. Introduction

As mentioned in Chapter 1, previous results regarding training-induced changes of muscle architecture of the quadriceps femoris are controversial. Therefore, detail information of training-induced changes in muscle architecture remains unclear. The reasons for the inconsistent results among studies are unknown but may involve the differences in the regions where the architectural parameters are determined and/or the extent of muscle hypertrophy. The purpose of this study was to examine the influence of knee extension training on the individual muscle architecture of the quadriceps femoris and clarify whether or not the magnitudes of changes in architectural parameters are similar among the four muscles and within a muscle.

2-2. Methods

Subjects

Twenty-one healthy men participated in this study and were assigned to the training (n = 11) or control (n = 10) group. Quantification of the physical activity (by verbal questionnaire) revealed that they were healthy and physically active. To keep themselves fit, some of them had taken part in various recreational physical activities such as cycling, jogging or ball game once or twice a week, and others had walked or cycled when commuting. However, they had not participated in a regular resistance training program of the lower extremity for at least 1 year. Eleven men (age, 27 ± 2 yr; height, 1.73 ± 0.05 m; body mass, 68 ± 7 kg, mean ± SD) completed a resistance
training program of unilateral knee extension for 12 weeks (three days per week, i.e. 36 sessions) and ten males served as controls (age, 26 ± 4 yr; height, 1.72 ± 0.06 m; body mass, 64 ± 8 kg). An independent *t*-test revealed that the physical characteristics of the subjects (age, height, and body mass) did not differ significantly between the training and control groups. This study was approved by the Ethics Committee on Human Research of Waseda University. Prior to the execution to the experiments, the subjects were informed of the purpose and risks of the study and provided written informed consent.

**Training protocol**

The subjects sat on a bench of a training machine (Nitro S3LE, Nautilus, USA). The knee extension exercise was performed using concentric actions (for 2 s) and eccentric actions (for 2 s). The knee joint range of motion was from approximately 110° to 20° flexion. The training load was adjusted to 80% of one repetition maximum (1RM). The 1RM was determined by increasing the load until each subject was unable to lift once throughout the prescribed knee joint range of motion. One session of the resistance training consisted of five sets with eight repetitions, separated by a 90 s rest period between sets. The 1RM was measured every 2 weeks to adjust the training load throughout the training period. The training program adopted here was similar to those used in previous researches which reported more than 30% increase in ACSA of the triceps brachii (Kawakami et al. 1995; Wakahara et al. 2012). The training sessions were supervised by the experimenters.

**Ultrasonographic measurements**
Before and after the training period, the muscle thicknesses, fascicle lengths, and pennation angles of each muscle of the quadriceps femoris were measured using real-time B-mode ultrasonography (SSD-6500, ALOKA, Japan) with a 60 mm, 7.5-MHz linear-array probe. Measurements after the training period were conducted more than 3 days after the last training session. Measurements were performed at least two regions (distal and proximal) of each muscle. It has been reported inhomogeneous architecture of VL between the medial and lateral regions (Blazevich et al. 2006), and hence, in VL, measurements were also performed in the medial and lateral regions. We ensured that measurement regions and target fascicles were matched before and after the intervention. In a pilot study, I confirmed the existence of aponeurosis and little curvature in fascicles at the measurement regions. These facts were important to increase repeatability of measurement. Specifically, measurement regions were determined as follows. First, the measurement positions were assigned along the thigh length. Next, the mediolateral width of each muscle was determined over the skin surface by identifying the lateral and medial boundaries of each muscle. Along and across the muscle, the measurement regions were determined (Fig.2-1) as follows.

VL (two regions): 1) 65% of the thigh length from the greater trochanter to the popliteal crease (65% distal) - 75% of the mediolateral width from the medial boundaries (75% medial) and 2) 45% distal - 55% medial

VM (two regions): 1) 85% distal - 45% medial and 2) 65% distal - 80% medial

RF (two regions): 1) 50% distal - 80% medial and 2) 30% distal - 65% medial

VI (lateral: two regions): the same as those of VL

VI (medial: two regions): 1) 65% distal - 80% medial of the mediolateral width from the medial boundaries of RF (80% medial of RF) and 2) 45% distal - 70% medial of RF
The subjects lay supine with the legs fully extended on a bed and their muscles relaxed. Scans were taken on the right leg. The repeatability and validity of the measurements for RF has been confirmed elsewhere in my previous study (Ema et al. 2013). Previous studies observed an increase in pennation angle and a decrease in fascicle length just after the intense exercise, but the changes disappeared after 15 minutes (Csapo et al. 2011; Kubo et al. 2001b). Hence, scans were taken at least 20 minutes after the subject started to lie supine.

In the measurements after the training period, the longitudinal ultrasonographic images were taken while referring to the images acquired before the training period. Several landmarks of fat, connective tissues, and blood vessels were carefully visualized in the same manner (similar thickness, brightness, and/or position), in order to analyze the identical fascicle in the two (before and after) measurements (Fig. 2-2). Muscle thickness was determined as the mean of the distances between the deep and superficial aponeuroses (for VI, between bone and its superficial aponeurosis) measured at both ends of each image of 60 mm width (Blazevich et al. 2006). Fascicle length was determined as the distance between the intersection points of the fascicle and deep and superficial aponeuroses. Pennation angle was measured as the angle between fascicle and deep aponeurosis (in VI, fascicle and its superficial aponeurosis) (Fig. 2-2). In the distal region of VM, muscle thickness was not measured because deep and superficial aponeuroses could not be monitored on the same images due to the width limit of the ultrasound probe. The fascicle lengths were measured in the two regions for VL and in the proximal regions of VM and RF.

Ultrasound images were stored in a computer through a digital video recorder (GV-HD700, Sony, Japan). Muscle thicknesses, fascicle lengths, and pennation angles
were measured using Image J (National Institute of Health, USA). When fascicles were not visible in their entity, the fascicle length was estimated through linear extrapolation (Erskine et al. 2009) by visual observation with the straight lines. The validity of this estimation has been confirmed in a previous study (Ando et al. 2014). Trials were performed five times in each region, and three values excluding the longest and shortest fascicle lengths were averaged for further analysis. For all the images, digitization was performed two times and the mean values were used for further analysis. The CVs in the two digitization were 0.7 ± 0.6% for muscle thickness, 2.0 ± 1.3% for fascicle length, and 4.3 ± 4.3% for pennation angle. The ICCs were 0.999 for muscle thickness, 0.956 for fascicle length, and 0.984 for pennation angle.

**MR imaging measurements**

Before and after the training period, T1-weighted MR images (echo time: 10 ms, repetition time: 520 ms, matrix: 256 × 192, field of view: 240 mm, slice thickness: 10 mm) of the right thigh were obtained using MR scanner (Signa EXCITE 1.5T, GE Medical Systems, USA). Measurements after the intervention were conducted more than 3 days after the completion of the last training session. Taking into consideration fluid shifts, the subject lay supine for at least 30 minutes before MR image recordings (Berg et al. 1993). All subjects were instructed to refrain from drinking alcohol and intensive exercise on the day before MR measurements. The ACSAs were determined at the same positions as the ultrasound measurements (mean of the three nearest slices) from MR images (Fig. 2-2). The ACSAs were measured using ImageJ software (National Institute of Health, USA). Care was taken to exclude visible adipose and connective tissue incursions (Blazevich et al. 2007a). Each slice was measured two
times and the mean values were used for further analysis. The CV and ICC in the duplicate digitization data were 0.6 ± 0.7% and 0.999, respectively.

**Knee extension strength measurement**

Before and after the training period, maximal voluntary isometric knee extension torque (MVC\textsubscript{KE} torque) was measured with a dynamometer (CON-TREX, CMV AG, Switzerland). Measurements after the training period were conducted more than 3 days after the completion of the last training session. The subjects sat on a bench of the dynamometer, while securing the pelvis on the bench with a non-elastic strap and the torso on the back seat by a seat belt. Care was taken to adjust the center of rotation of the dynamometer and center of the knee joint. The hip and knee joint angles were 80° and 70° flexion, respectively. After the completion of a warm-up procedure consisting of submaximal knee extension exercises, the subjects were asked to extend the knee with maximal effort. The MVC\textsubscript{KE} torque was measured twice, and if the difference between the two trials was above 10%, the third trial was conducted, with plenty of rest between trials. The torque signals were sampled at 1 kHz with a 16-bit A/D converter (PowerLab/16SP, ADInstruments, Australia) and transferred to a computer. The MVC\textsubscript{KE} torque stood for the peak value of the knee extension torque. The higher value in the trials was used for further analysis.

**Repeatability of ultrasound measurements**

Day to day (separated by more than five days) repeatability of ultrasound measurements was examined on ten healthy men (age, 22 ± 2 years; height, 1.74 ± 0.05 m; body mass, 64 ± 10 kg) in all measurement regions. Paired \textit{t}-tests revealed no
differences of the values between the two days in any parameters in each region. The CVs were less than 3.4% for muscle thickness, 3.1% for fascicle length, and 5.3% for pennation angle, respectively. The ICCs were more than 0.860 for muscle thickness, 0.837 for fascicle length, and 0.794 for pennation angle, respectively (Table 2-1). These values were similar to or better than those of previous studies (Alegre et al. 2006; Ema et al. 2013; Erskine et al. 2009; Rutherford and Jones 1992).

**Statistical analysis**

Descriptive data are presented as means ± SDs. All the analyses were performed with statistical software (SPSS 12.0J, SPSSJapan, Japan). A two-way analysis of variance (ANOVA) with one-repeated-measurement factor and one between-group factor was used to analyze the effects of time (before, after) and groups (training, control) of the 1RM and MVC\textsubscript{KE} torque. In case of a significant interaction, paired and independent \(t\)-tests were conducted to test the difference between before and after the training period and between groups, respectively. A three-way multiple ANOVA (MANOVA) [group \(\times\) time \(\times\) region (distal, proximal)] with repeated measures was used to test the effects of group, time, and region and their interaction on the absolute values of ACSA, muscle thickness (except for VM), fascicle length (only VL), and pennation angle simultaneously in each muscle. When a significant interaction was observed, paired and independent \(t\)-tests were performed to determine whether significant differences existed between before and after the training period and between groups, respectively. A paired \(t\)-test was conducted to test the changes in the muscle thickness of VM, fascicle length of VM and RF. A three-way ANOVA [group \(\times\) muscle (VL, VM, RF and VI) \(\times\) region] with repeated measures was used to test the effects of
group, muscle, and region and their interaction on the relative change in each of ACSA, muscle thickness, and pennation angle. We did not use MANOVAs because of the difference in the number of levels of factor among the three parameters. When appropriate, additional two-way and one-way ANOVAs with repeated measures with Bonferroni test and paired t-test were performed to determine whether significant differences existed between muscles and between regions. A simple regression analysis was performed to calculate Pearson product-moment correlation coefficients for the relationships 1) between muscle thickness and pennation angle for each muscle before and after the training period (including training and control groups in all regions except for the distal region of VM) and 2) between relative changes in muscle thickness and those in pennation angle including in all regions for each muscle in the training group. For these relationships, whether each of the slope and intercept differs between before and after the training period was also examined. Significance level was set at $P < 0.05$.

2-3. Results

Knee extension strength

At baseline, there were no significant differences between the groups in the 1RM and MVC$\text{KE}$ torque. After the training period, the training group significantly increased 1RM from $68 \pm 9$ kg to $86 \pm 9$ kg ($P < 0.001$) and MVC$\text{KE}$ torque from $257 \pm 51$ Nm to $318 \pm 51$ Nm ($P < 0.001$). In the control group, there were no significant changes in the two variables (1RM, $68 \pm 15$ kg to $68 \pm 15$ kg; MVC$\text{KE}$ torque, $248 \pm 61$ Nm to $243 \pm 66$ Nm).

Absolute changes in muscle architecture
No significant differences between the two groups in any architectural parameters were found at baseline. Table 2-2 shows descriptive data on the ACSA, muscle thickness, fascicle length, and pennation angle in each region of the four muscles. The three-way MANOVAs revealed a significant group × time interaction on ACSA \((P < 0.01)\), muscle thickness \((P < 0.05)\) and pennation angle \((P < 0.01)\) in each muscle. In the training group, the ACSAs of all muscles significantly increased in all regions \((P < 0.05)\). Except for the lateral region of VI, significant increases in muscle thickness \((P < 0.05)\) and pennation angle \((P < 0.05)\) were observed in each muscle. The fascicle length did not change in any muscles. In the control group, no significant changes were observed in any architectural parameters.

**Relative changes in muscle architecture**

The three-way ANOVAs demonstrated a significant group × muscle × region interaction on ACSA \((P < 0.05)\), and a significant group × muscle interaction on muscle thickness \((P < 0.01)\) and pennation angle \((P < 0.01)\). Follow-up two-way ANOVA showed a significant interaction (muscle × region, \(P < 0.05\)) on ACSA in the training group. Follow-up one-way ANOVAs revealed that the relative increases in the ACSA \((P < 0.01)\), muscle thickness \((P < 0.05)\), and pennation angle \((P < 0.05)\) of RF were significantly greater than those of VL, VM, and VI in the training group (Fig. 2-3). In VL and RF, the relative increase in the ACSA was significantly greater (VL: \(P < 0.05\), RF: \(P < 0.01\)) in the distal region than in the proximal region (Fig. 2-4), with no differences in the other parameters. In VM, there were no regional differences in any parameters. In VI, relative increases in the muscle thickness \((P < 0.05)\) and pennation angle \((P < 0.05)\) were significantly greater in the medial region than in the lateral region.
(Fig. 2-4). On the other hand, there were no regional differences in any parameters along VI.

**Relationship between muscle thickness and pennation angle**

The relationship between muscle thickness and pennation angle is shown in Fig. 2-5. In each of the measured muscles, the muscle thickness was significantly correlated to the pennation angle both before and after the training period [VL: $r = 0.36 (P < 0.05)$ before, $r = 0.45 (P < 0.01)$ after], [VM: $r = 0.75 (P < 0.01)$ before, $r = 0.66 (P < 0.01)$ after], [RF: $r = 0.67 (P < 0.01)$ before, $r = 0.71 (P < 0.01)$ after], and [VI: $r = 0.68 (P < 0.01)$ before, $r = 0.68 (P < 0.01)$ after]. In each muscle, the slope and intercepts of the regression line for the relationship between the two variables did not significantly differ between before and after the intervention. In the training group, the relative changes of muscle thickness were significantly correlated to those of pennation angle for each muscle [VL: $r = 0.63 (P < 0.01)$, VM: $r = 0.72 (P < 0.05)$, RF: $r = 0.45 (P < 0.05)$, VI: $r = 0.49 (P < 0.01)$] (Fig. 2-6).

**2-4. Discussion**

The main findings of this study were that relative changes in ACSA, muscle thickness, and pennation angle of RF were greater than those of the three vasti, and relative changes in pennation angle and muscle size were different across as well as along the muscle. This is the first case that demonstrated inhomogeneous changes in pennation angle between muscles and within a muscle, which corresponded to the inhomogeneity of muscle hypertrophy. Moreover, the current results demonstrated significant associations between muscle thickness and pennation angle in each muscle.
in terms of both absolute values before and after the training period and their relative changes with no change in fascicle length, indicating a clear link between muscle hypertrophy and increase in pennation angle.

**Relation between changes in knee extension strength and muscle architecture**

The knee extension torque (1RM and MVC\(_{KE}\) torque) significantly increased after knee extension training for 12 weeks. In addition, ACSAs were increased in all muscles and regions, and hence muscle hypertrophy is the major factor increasing the knee extension strength. On the other hand, pennation angle influences the transmission efficiency from muscle to tendon (Alexander and Vernon 1975). Accordingly, training-induced changes in pennation angle can influence the changes in knee extension strength. This should be considered.

Neural adaptation increases the strength per ACSA. On the other hand, large pennation angle leads to small strength per ACSA (Ichinose et al. 1998; Ikegawa et al. 2008), suggesting that training-induced increase in pennation angle decrease the strength per ACSA. Therefore, it can be said that training-induced changes in the strength per ACSA are determined by the interaction of neural adaptation (positive effect) and the increase in pennation angle (negative effect). In the current study, the strength per ACSA (MVC\(_{KE}\) torque per ACSA of the quadriceps femoris) significantly increased (Fig. 2-7). Since we did not measure any indices representing neural adaptation, the magnitude of it is unknown. The subjects in the current study were untrained men, and hence neural adaptation could occur. On the other hand, pennation angles increased except for the lateral region of VI. Considering these facts, training-induced increase in the strength per ACSA indicates that the negative effect
(increase in pennation angle) does not exceed the positive effect (neural adaptation). However, potential influence of inter- and intra-muscle difference in architectural parameters to the changes in strength remains unclear. Further researches are needed to clarify this point.

**Inter-muscle differences in the changes in muscle architecture**

As observed in previous studies (Housh et al. 1992; Narici et al. 1996b; Seynnes et al. 2007), the increases in ACSA and muscle thickness were more prominent in RF than in the vasti. Two possibilities may account for the results. The first is the difference in muscle activation between muscles. Muscle activation measured by electromyography has been shown to be higher in RF than the vasti in eccentric phase during knee extension exercises (Narici et al. 1996b). Moreover, Richardson et al. (1998) reported that muscle activation during knee extension exercise determined by T2-weighted MR images was also higher in RF than the vasti. Such inter-muscle differences in muscle activation during exercises may be responsible for the observed inhomogeneous hypertrophy between RF and the vasti. The second is the difference in the muscle fiber type composition of each muscle of the quadriceps femoris. The percentage of type II fibers was slightly higher in RF than in the vasti (Johnson et al. 1973). It is known that training-induced hypertrophy is greater in type II fibers than in type I fibers (Aagaard et al. 2001). Hence, the difference in muscle fiber composition between RF and the vasti can also partly account for the current results.

Moreover, the training-induced change in the pennation angle was also greater in RF than those in the vasti. Training-induced hypertrophy is accompanied by the increase in pennation angle (Kawakami et al. 1995), because an increase in pennation
angle is considered to be a strategy to place fascicles with training-increased diameters on a limited area of aponeurosis (Kawakami et al. 2000). Thus, greater training-induced hypertrophy of RF than the vasti would result in the larger changes in pennation angles of RF. In other words, the present study indicates that the pennation angle increases in muscles that show hypertrophic responses.

Intra-muscle difference in the changes in muscle architecture

The relative increases in the ACSAs of VL and RF were greater in the distal region than in the proximal region. These results are consistent with that of Narici et al. (1996b). Moreover, although the muscle thicknesses and pennation angles of VI in the medial region increased, those in the lateral region did not. No previous studies have shown the differences in the training induced-increases of muscle thickness and pennation angle across the mediolateral direction of a muscle. These results may be linked to regional differences in muscle activation during the prescribed exercise mode. Wakahara et al. (2012) suggested that regional differences in muscle hypertrophy after resistance training could be attributable to the region specific muscle activation during the exercise. It has been observed that muscle activation in the distal region of RF during isokinetic knee extension exercise was higher than that in the proximal region (Akima et al. 2004). In addition, Akima et al. (1999) noted that knee extension training for 2 weeks resulted in the increase of muscle activation in the anterior regions of VI near RF, which corresponds to the medial region of VI in the present study. Hence, although we have no data for regional differences in muscle activation and their associations with regional differences in muscle fiber hypertrophy, the differences within a muscle in muscle activation during the knee extension training might account
for the intra-muscle differences in the training-induced changes of pennation angle and muscle size.

**Relationship between muscle thickness and pennation angle**

The regression line for the relationship between muscle thicknesses and pennation angles in each muscle was not significantly different before and after the training period. This result was in line with that of Kawakami et al. (1995) who examined the corresponding relationship for the triceps brachii. It is likely, therefore, that a pennate muscle changes its architecture in the process of hypertrophy in such a way that the relationship between muscle thickness and pennation angle shifts in an upward-right direction. Moreover, the regression lines remained constant after the training period regardless of regional differences in training-induced changes in muscle thickness and pennation angle (VI). This result indicates a pennate muscle has a generic relation between the two parameters, and that training-induced absolute changes in the both do not differ between different regions within a muscle.

**Possible reasons for the inconsistent results in training-induced changes in pennation angle**

Previous findings on the training-induced changes in the pennation angle of VL are controversial over studies; significantly increased (Aagaard et al. 2001; Blazevich et al. 2007a; Erskine et al. 2010a; Seynnes et al. 2007) and unchanged (Alegre et al. 2006; Rutherford and Jones 1992). In the present study, no regional difference in the training-induced changes was observed in the pennation angle of VL, indicating that it is difficult to explain the inconsistent results over studies in terms of the difference in
the measurement region. The extent of muscle hypertrophy observed in the present (9.8% in ACSA, and 8.4% in muscle thickness) and previous studies (11.1% in muscle volume, Blazevich et al. 2007a; 7.8% in ACSA, Seynnes et al. 2007; 10.2% in ACSA of the whole quadriceps femoris, Aagaard et al. 2001) in which pennation angle significantly increased was larger than that reported in Alegre et al. (2006) (6.9% in muscle thickness) and Rutherford and Jones (1992) (4.6% in ACSA of the whole quadriceps femoris) who failed to find significant change in pennation angle, although one exceptional result that an increase in pennation angle was induced in spite of lower extent of muscle hypertrophy (5.6% in muscle volume of the whole quadriceps femoris) has been reported by Erskine et al. (2010a). Considering these results, it seems that the existence of significant training-induced change in pennation angle would be partly attributed to the extent of hypertrophic change.

The inconsistent results regarding training-induced changes in pennation angle of VI between the present and previous studies can be explained in terms of the difference in measurement region and the magnitude of hypertrophic change in the corresponding region. In the current result, the pennation angle of VI increased in the medial region, but did not in the lateral region. On the other hand, Erskine et al. (2010b) and Rutherford and Jones (1992) observed no training-induced changes in the pennation angle of VI. The measurement regions across VI of Erskine et al. (2010b) (mid-sagittal plane of VI) and Rutherford and Jones (1992) (deeper region of VL) would correspond to the lateral region in the present study. Thus, the differences in the measurement regions across mediolateral direction of the muscle can be responsible for the disagreement in training-induced changes in pennation angle of VI between the previous and current results.
Possible reasons for the inconsistent results in training-induced changes in fascicle length

The fascicle lengths of VL, VM, and RF were unchanged in the present study. Previous findings about the training-induced changes in fascicle length are inconsistent among studies. Erskine et al. (2010b) and Seynnes et al. (2009) failed to find a significant change in the fascicle lengths of VL, VM, and RF as observed here, but others reported training-induced increase in fascicle length of VL (Alegre et al. 2006; Blazevich et al. 2007a; Seynnes et al. 2007). As mentioned in the earlier part, the differences in measurement region and/or extent of muscle hypertrophy may be possible reasons for the inconsistent results among studies. In the current result, the lengths of VL did not change in both proximal and distal regions. This denies a possibility that the inconsistent results among studies might be attributed to the difference in the measurement region. With regard to the muscle hypertrophy, those in Seynnes et al. (2007) and Alegre et al. (2006) were smaller than that in the present study. Blazevich et al. (2007a) did not provide concrete data regarding the extent of hypertrophy at the region where the fascicle length was measured. Taken together, it is difficult to explain the reason for inconsistent results in training-induced changes in fascicle length over studies in terms of the differences in measurement region and extent of muscle hypertrophy, and further research is needed to clarify the factors resulting in disagreement.

In conclusion, the current results indicate 1) hypertrophy of the quadriceps femoris is associated with a proportional increase in pennation angle but not necessarily in fascicle length, and 2) training-induced changes in muscle size and pennation do not
evenly occur among the quadriceps, along or across a muscle. The observed inter- and intra-muscle differences in the training-induced changes in pennation angle correspond to the inhomogeneous hypertrophic changes among muscles and within a muscle.
Table 2-1
The coefficient of variations (CVs) and intraclass correlation coefficients (ICCs) in between-days ultrasound measurements ($n = 10$).

<table>
<thead>
<tr>
<th></th>
<th>Muscle thickness</th>
<th></th>
<th>Fascicle length</th>
<th></th>
<th>Pennation angle</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CV (%)</td>
<td>ICC</td>
<td>CV (%)</td>
<td>ICC</td>
<td>CV (%)</td>
<td>ICC</td>
</tr>
<tr>
<td>VL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distal</td>
<td>1.5 ± 1.2</td>
<td>0.991</td>
<td>1.0 ± 0.6</td>
<td>0.966</td>
<td>2.8 ± 2.0</td>
<td>0.931</td>
</tr>
<tr>
<td>Proximal</td>
<td>2.1 ± 1.2</td>
<td>0.976</td>
<td>1.7 ± 1.3</td>
<td>0.838</td>
<td>3.8 ± 2.3</td>
<td>0.885</td>
</tr>
<tr>
<td>VM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distal</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.8 ± 2.1</td>
<td>0.940</td>
</tr>
<tr>
<td>Proximal</td>
<td>2.4 ± 1.2</td>
<td>0.861</td>
<td>3.0 ± 1.8</td>
<td>0.902</td>
<td>2.9 ± 2.7</td>
<td>0.945</td>
</tr>
<tr>
<td>RF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distal</td>
<td>2.7 ± 1.2</td>
<td>0.984</td>
<td>-</td>
<td>-</td>
<td>5.2 ± 5.2</td>
<td>0.921</td>
</tr>
<tr>
<td>Proximal</td>
<td>1.7 ± 1.2</td>
<td>0.985</td>
<td>1.6 ± 1.0</td>
<td>0.980</td>
<td>2.9 ± 2.1</td>
<td>0.921</td>
</tr>
<tr>
<td>VI (lateral)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distal</td>
<td>3.2 ± 0.8</td>
<td>0.978</td>
<td>-</td>
<td>-</td>
<td>5.0 ± 2.6</td>
<td>0.919</td>
</tr>
<tr>
<td>Proximal</td>
<td>3.3 ± 2.6</td>
<td>0.979</td>
<td>-</td>
<td>-</td>
<td>5.0 ± 3.9</td>
<td>0.943</td>
</tr>
<tr>
<td>VI (medial)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distal</td>
<td>3.2 ± 2.1</td>
<td>0.955</td>
<td>-</td>
<td>-</td>
<td>4.0 ± 3.2</td>
<td>0.948</td>
</tr>
<tr>
<td>Proximal</td>
<td>2.3 ± 1.6</td>
<td>0.951</td>
<td>-</td>
<td>-</td>
<td>5.2 ± 3.7</td>
<td>0.795</td>
</tr>
</tbody>
</table>

Data of CV are presented as mean $\pm$ SD. VL, vastus lateralis; VM, vastus medialis; RF, rectus femoris; VI, vastus intermedius.
Table 2-2 Architectural values of each muscle before and after the training period.

<table>
<thead>
<tr>
<th>Muscle Group</th>
<th>Region</th>
<th>Time</th>
<th>ACSA (cm²)</th>
<th>Muscle thickness (mm)</th>
<th>Fascicle length (mm)</th>
<th>Pennation angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Training</td>
<td>Distal</td>
<td>Before 17.9 ± 3.7</td>
<td>18.5 ± 2.3</td>
<td>60.9 ± 6.0</td>
<td>19.6 ± 2.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 19.9 ± 3.7 *</td>
<td>20.2 ± 2.2 *</td>
<td>62.2 ± 5.8</td>
<td>20.8 ± 2.6 *</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proximal</td>
<td>Before 27.1 ± 4.1</td>
<td>23.9 ± 2.1</td>
<td>72.4 ± 2.3</td>
<td>18.0 ± 1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 29.5 ± 4.0 *</td>
<td>25.9 ± 2.3 *</td>
<td>71.7 ± 2.5</td>
<td>19.9 ± 2.5 *</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Distal</td>
<td>Before 17.1 ± 5.0</td>
<td>20.0 ± 3.5</td>
<td>62.1 ± 5.1</td>
<td>19.9 ± 2.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 17.0 ± 4.9</td>
<td>20.1 ± 3.3</td>
<td>62.1 ± 4.6</td>
<td>19.7 ± 2.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proximal</td>
<td>Before 25.8 ± 5.5</td>
<td>24.0 ± 3.7</td>
<td>68.9 ± 5.6</td>
<td>18.5 ± 1.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 25.7 ± 5.7</td>
<td>23.8 ± 3.7</td>
<td>68.1 ± 5.7</td>
<td>18.7 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>Training</td>
<td>Distal</td>
<td>Before 19.9 ± 3.0</td>
<td>-</td>
<td>-</td>
<td>36.8 ± 4.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 22.4 ± 3.3 *</td>
<td>-</td>
<td>-</td>
<td>38.2 ± 4.7 *</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proximal</td>
<td>Before 21.1 ± 2.4</td>
<td>21.8 ± 2.9</td>
<td>71.9 ± 6.6</td>
<td>18.9 ± 3.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 22.9 ± 2.2 *</td>
<td>24.0 ± 2.4 *</td>
<td>72.7 ± 6.4</td>
<td>20.3 ± 2.4 *</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Distal</td>
<td>Before 20.4 ± 4.8</td>
<td>-</td>
<td>-</td>
<td>36.7 ± 2.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 20.3 ± 4.8</td>
<td>-</td>
<td>-</td>
<td>37.0 ± 2.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proximal</td>
<td>Before 21.8 ± 4.3</td>
<td>22.4 ± 3.0</td>
<td>67.5 ± 5.4</td>
<td>18.9 ± 2.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 21.8 ± 4.3</td>
<td>22.6 ± 2.7</td>
<td>68.1 ± 5.7</td>
<td>19.1 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>Training</td>
<td>Distal</td>
<td>Before 8.8 ± 2.3</td>
<td>20.0 ± 3.2</td>
<td>-</td>
<td>15.6 ± 2.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 11.2 ± 3.5 *</td>
<td>24.3 ± 3.8 *</td>
<td>-</td>
<td>18.7 ± 2.6 *</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proximal</td>
<td>Before 13.4 ± 2.1</td>
<td>23.6 ± 2.5</td>
<td>75.1 ± 4.4</td>
<td>19.7 ± 2.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 16.0 ± 2.7 *</td>
<td>27.4 ± 2.6 *</td>
<td>78.0 ± 8.2</td>
<td>22.3 ± 2.4 *</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Distal</td>
<td>Before 8.1 ± 1.8</td>
<td>19.2 ± 2.7</td>
<td>-</td>
<td>15.3 ± 2.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 8.0 ± 1.8</td>
<td>19.3 ± 2.8</td>
<td>-</td>
<td>15.0 ± 2.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proximal</td>
<td>Before 12.3 ± 3.2</td>
<td>21.4 ± 2.5</td>
<td>70.6 ± 7.1</td>
<td>19.4 ± 1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 12.3 ± 3.3</td>
<td>21.4 ± 2.6</td>
<td>71.2 ± 6.9</td>
<td>19.0 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Training</td>
<td>Distal</td>
<td>Before 17.2 ± 3.0</td>
<td>22.0 ± 3.4</td>
<td>-</td>
<td>19.3 ± 5.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 18.3 ± 2.7 *</td>
<td>22.3 ± 3.9</td>
<td>-</td>
<td>19.9 ± 5.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proximal</td>
<td>Before 24.5 ± 4.2</td>
<td>21.4 ± 4.7</td>
<td>-</td>
<td>15.7 ± 3.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 25.9 ± 4.1 *</td>
<td>21.3 ± 3.9</td>
<td>-</td>
<td>15.9 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Distal</td>
<td>Before 15.0 ± 2.5</td>
<td>21.8 ± 4.1</td>
<td>-</td>
<td>18.9 ± 2.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 14.9 ± 2.6</td>
<td>21.4 ± 3.3</td>
<td>-</td>
<td>18.5 ± 2.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proximal</td>
<td>Before 22.7 ± 3.5</td>
<td>22.8 ± 5.2</td>
<td>-</td>
<td>14.7 ± 3.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 22.6 ± 3.6</td>
<td>22.5 ± 5.2</td>
<td>-</td>
<td>15.0 ± 3.0</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. VL, vastus lateralis; VM, vastus medialis; RF, rectus femoris; VI, vastus intermedius; ACSA, anatomical cross-sectional area. The ACSA of VI is shown at the section of VI (lateral). * denotes a significant difference between before and after the training period.
Fig. 2-1 Positions of ultrasound and magnetic resonance imaging measurements. VL, vastus lateralis; VM, vastus medialis; VI, vastus intermedius; RF, rectus femoris.
Fig. 2-2 Examples of ultrasound (upper) and magnetic resonance (lower) images measured before and after the training period. Muscle thickness, fascicle length, pennation angle and anatomical cross-sectional area (ACSA) were measured as shown in the images. Adipose connective tissue and blood vessel reference marks are circled on the ultrasound image after the training period. VL, vastus lateralis; VM, vastus medialis; VI, vastus intermedius; RF, rectus femoris.
Fig. 2-3 Relative changes in ACSA (upper), muscle thickness (middle) and pennation angle (lower) induced by training program. * indicates a significant difference among the muscles. ACSA, anatomical cross-sectional area; RF, rectus femoris; VL, vastus lateralis; VM, vastus medialis; VI, vastus intermedius.
Fig. 2-4 Relative changes in ACSA of VL (upper left) and RF (upper right) in the distal and proximal regions, and in muscle thickness (lower left) and pennation angle (lower right) of VI in the medial and lateral regions induced by training program. * indicates a significant difference between the regions. ACSA, anatomical cross-sectional area; VL, vastus lateralis; RF, rectus femoris; VI, vastus intermedius.
Fig. 2-5 Relationship between muscle thickness and pennation angle of the vastus lateralis (upper left), vastus medialis (lower left), rectus femoris (upper right) and vastus intermedius (lower right) before and after (including training and control groups in all regions except for the distal region of the vastus medialis) the training period.
Fig. 2-6 Relationship between relative changes in muscle thickness and pennation angle of the vastus lateralis (upper left), vastus medialis (lower left), rectus femoris (upper right) and vastus intermedius (lower right) in the training group.
Fig. 2-7 Maximal voluntary knee extension torque per anatomical cross-sectional area (ACSA) before (white bar) and after (black bar) the training period. The ACSA was the mean of all muscles and measurement regions. * indicates a significant difference between before and after the training period.
CHAPTER 3 Architectural changes of the quadriceps femoris induced by leg extension training: Examination of quantitative profiles in sport athletes

Section 1 Quantitative profiles of the quadriceps femoris in varsity oarsmen

3-1-1. Introduction

In Chapter 2, the architectural response following knee extension training was greater in RF compared to the other three muscles (VL, VM and VI). Moreover, the results of Chapter 2 indicate that hypertrophic changes represent the changes in fascicle arrangement. Therefore, in this Chapter, quantitative adaptations of the quadriceps femoris were focused. Regarding another major training modality, leg extension training, how the training modality changes the quadriceps femoris architecture is unclear. The purpose of this chapter is to examine the influence of leg extension training on the individual muscle volume of the quadriceps femoris. To this end, the muscularity was examined in oarsmen who repeat leg extensions in their competitive and training activities.

3-1-2. Methods

Subjects

Fourteen varsity oarsmen (age, 20 ± 1 years; height, 1.77 ± 0.06 m; body mass, 74 ± 8 kg; ergometer all-out 2000 m rowing time, 6 min 44 s ± 13 s; mean ± SD) and 19 untrained men (age, 25 ± 2 years; height, 1.72 ± 0.05 m; body mass, 65 ± 8 kg) participated in this study. Of the oarsmen six had rowed in stroke side and six in bow side, and the remainders (n = 2) were scullers. All oarsmen had been training daily and approximately 18 hours per week on-water rowing and/or ergometer on the ground.
Their rowing experience was more than four years (6.3 ± 1.2 years). Except for one oarsman, they had won a prize in national college competitive meets in Japan. In addition to the rowing training, although the oarsmen conducted resistance training twice (approximately 1.5 hours per each time) per week, the training modality was combination of knee extension and hip extension (e.g., squat, power clean), and the training frequency was lower than that of rowing training. Thus, it is likely that the majority of the training-induced muscle adaptations were from their rowing training. All untrained men were physically fit, but had not conducted conventional sport activities for several years before the experiment. This study was approved by the Ethics Committee on Human Research of Waseda University. Prior to the execution to the experiments, the subjects were informed of the purpose and risks of the study and provided written informed consent.

Data acquisition and data analysis

T1-weighted MR images (echo time: 10 ms, repetition time: 520 ms, matrix: 256 × 192, field of view: 24 cm, slice thickness: 1.0 cm) of the whole thigh were obtained. To take into consideration fluid shifts by the change in posture, the subject lay supine for at least 20 minutes before MR image recordings (Berg et al. 1993). All the subjects were instructed to refrain from drinking alcohol the day before MR measurement. The subjects lay spine with their legs fully extended and muscles relaxed in the magnet bore (Signa 1.5T, GE, USA). Scans were taken on the right leg. The ACSAs of VL, VM, VI, and RF were determined from MR images (Fig. 3-1-1). The ACSAs were measured using ImageJ software (National Institute of Health, USA). Care was taken to exclude visible adipose and connective tissue incursions. Each image was
digitized two times and the mean values were used for further analysis. The coefficient of variation (CV) and intraclass correlation coefficient (ICC) in the two digitization were 0.8 ± 0.7% and 1.000, respectively. The ACSA in 30% (proximal), 50% (middle), and 70% (distal) of muscle length of each muscle was determined as mean of the three nearest slices, respectively, and was divided by the two-thirds power of body mass (ACSA-to-body mass$^{2/3}$) (Åstrand 2003). The muscle volume of each of the four muscles was determined by summing the ACSA times the slice thickness (1.0 cm), and the muscle volume relative to body mass was calculated. The percentage of individual muscle to the total quadriceps femoris in volume was also determined.

**Statistical analysis**

Values are presented as means ± SDs. An independent $t$-test was used to test the significant difference between the two groups in each of the variables. If the assumption of equal group variances was violated, a Welch’s $t$-test was performed. A two-way ANOVA with one-repeated-measurement factor and one between-group factor was used to analyze the effects of region (3 regions) of the ACSA-to-body mass$^{2/3}$ in each muscle and groups (oarsmen and untrained men). In the case with a significant interaction, an independent $t$-test was conducted to test the difference between the two groups in ACSA-to-body mass$^{2/3}$. Significance level was set at $P < 0.05$. All analyses were performed with a statistical software (SPSS 12.0J, SPSSJapan, Japan).

**3-1-3. Results**

**ACSA**

Fig. 3-1-2 shows the ACSA-to-body mass$^{2/3}$ at 30% (proximal), 50% (middle),
and 70% (distal) of each muscle length. Significant main effects of group (VL, \(P < 0.001\); VI, \(P < 0.01\)) and region on the ACSA-to-body mass\(^{2/3}\) with no significant interaction between the two factors were found in VL and VI. Significant main effects of group (\(P < 0.001\)) and region on the ACSA-to-body mass\(^{2/3}\) with a significant interaction (\(P < 0.01\)) between the two factors were found in VM. The ACSA-to-body mass\(^{2/3}\) values of VM in all three regions (proximal, \(P < 0.05\); middle, \(P < 0.001\); distal, \(P < 0.01\)) were significantly greater in the oarsmen than in the untrained men. No significant main effect of group or interaction between the two factors was found in the ACSA-to-body mass\(^{2/3}\) for RF.

**Muscle volume**

The muscle volume and muscle volume relative to body mass are shown for each muscle in Fig. 3-1-3. The absolute values of the total quadriceps femoris, VL, VM, and VI (\(P < 0.001\)) were significantly greater in the oarsmen than in the untrained men. The muscle volume of RF did not significantly differ between the two groups. Although the body mass was significantly greater in the oarsmen than in the untrained men, relative values to body mass results were greater in ROW for the vasti (VL, \(P < 0.001\); VM and VI, \(P < 0.01\)) and not for RF.

**Muscle volume constituents**

Descriptive data on the percentages of each muscle volume to the total quadriceps femoris volume are presented in Fig. 3-1-4. The percentage of RF volume was significantly lower in the oarsmen (13.0 ± 1.0%) than in the untrained men (14.8 ± 1.7%, \(P < 0.01\)), whereas that of VL was significantly higher in the oarsmen (34.3 ±
2.0%) than in the untrained men (32.6 ± 2.3%, \( P < 0.05 \)). The percentage of VM and VI did not differ significantly between the two groups.

3-1-4. Discussion

The current results showed that the muscle size of VL, VM, and VI were greater in the oarsmen than in the untrained men, but the corresponding difference in RF was not significant. Moreover, the percentage of the volume of VL to that of the quadriceps femoris was higher in the oarsmen than in the untrained men. These results give support to our hypothesis that oarsmen demonstrate selective hypertrophy among the four muscles of the quadriceps femoris, and suggest that rowing training induce preferential hypertrophy of VL among the four muscles. The ACSA of the whole quadriceps femoris has been reported for young oarsmen (Hoshikawa et al. 2010; Tachibana et al. 2007), but no information on the muscle volume and the ACSA of each of the four muscles is available from the literature. To our knowledge, the present research is the first case that indicated the quantitative profile of the quadriceps femoris and its constituents in oarsmen.

The lack of hypertrophy of RF in rowers may be related to muscle activation that is unique to this muscle during rowing. Janshen et al. (2009) and Turpin et al. (2011) who examined the electromyographic patterns during rowing motions, and indicated that the muscle activation levels of VL, VM, and RF during a drive phase (leg extension) were clearly higher than those during a recovery phase. This finding indicates that the quadriceps femoris are active mainly in the drive phase compared to the recovery phase, although in their results, the inter-muscle differences in muscle activation levels within each phase were not mentioned. Guével et al. (2011) reported
that the muscle activation level during rowing motions normalized to those recorded
during maximal voluntary isometric knee extension was less than 20% in RF, and >40% in VL and VM. This study clearly indicates that the activation level in RF during rowing motions is substantially low, which would provide little stimulus to bring about a sizable hypertrophic response in this muscle. Regarding VI, no information on the electromyographic pattern and activation level during rowing motions is available to date for the activation level of VI during rowing motions from the previous literatures. But the present study demonstrated that, in addition to VL and VM, the muscle volume of VI was also greater in the oarsmen than in the untrained men. This finding provides suggestion that the activation level of VI is similarly activated to those of VL and VM during rowing motions. Moreover, the muscle activation level during cycling (involving similar joint actions to rowing), determined using electromyography and T2-weighted MR imaging, has been shown to be lower in RF than in the vasti (Chin et al. 2011; Endo et al. 2007; Reid et al. 2001). Besides, in other previous studies (Akima et al. 2005; Richardson et al. 1998), the highest MR T2-based muscle activation level during cycling (involving similar joint actions to rowing) was observed in VL among the four muscles during cycling. These findings clearly support an assumption that the inter-muscle difference in the muscularity of the quadriceps femoris in oarsmen is attributable to the inter-muscle differences in the activation level during rowing motions. One of the candidates for the difference in activation might be the shortening velocity of RF that is lower than the vasti during a leg extension owing to its biarticular nature and hence higher force-producing potential (Gregoire et al. 1984). Others might include a difference between RF and vasti in the functional roles (transfer of mechanical energy vs. work generation) during a leg extension (Prilutsky and Zatsiorsky 1994; van Ingen
Schenau et al. 1995), but this cannot readily explain the inferiority of RF musculature in oarsmen.

In conclusion, the present results indicate inferior muscularity of the rectus femoris compared to the vasti in oarsmen. This may be due to muscle-specific adaptation to the rowing exercise.

Section 2 Influence of competitive cycling training on the muscle volume of the quadriceps femoris: Cross-sectional and longitudinal observations

3-2-1. Introduction

If the cross-sectional observation (great size of the vasti but not of RF) in oarsmen (Chapter 3, section 1) is due to muscle-specific adaptation to the rowing motions, which include repetitive leg extensions, this notion can be generalized to competitive cyclists because similar motions are involved during pedaling motions. The purpose of the current section is to test the hypothesis that muscle-specificity in the hypertrophic response of the quadriceps femoris to the competitive cycling training exists, leading to muscular profiles in experienced cyclists. To this end, serial MR images of the thigh were obtained from experienced cyclists (experience: > 4 years) (experiment 1) and before and after regular training of competitive cycling for 6 months (experiment 2) and analyzed.

3-2-2. Methods

Experiment 1: Cross-sectional approach

Subjects
Eight varsity male cyclists (age, 20.3 ± 1.4 yr; height, 1.73 ± 0.06 m; body mass, 64.2 ± 6.6 kg; mean ± SD) and 10 untrained men (age, 21.3 ± 1.5 yr; height, 1.72 ± 0.04 m; body mass, 65.2 ± 5.5 kg) participated in this study. Independent t-tests revealed that there was no significant difference in the age, height or body mass between the two groups. All cyclists had participated in both track and road races. The cyclists experienced competitive cycling for more than 4 years (7.0 ± 3.1 yr). The records of a 200 m and 1000 m time trials in the latest season were 11.5 ± 0.2 s and 69.6 ± 1.4 s, respectively. Each had won a prize or participated in national college competitive meets in Japan. The untrained men were sedentary or recreationally active, but none had conducted conventional sport activities or resistance training for at least 2 years. Prior to the experiment, the subjects were informed of the purpose and risks of the study and provided written informed consent. The studies including experiment 2 were approved by the Ethics Committee on Human Research of Waseda University.

**MR imaging and data analysis**

T1-weighted MR images of the whole right thigh (echo time: 10 ms, repetition time: 520 ms, matrix: 256 × 192, field of view: 240 mm, slice thickness: 10 mm) were obtained using an MR scanner (Signa EXCITE 1.5T, GE Medical Systems, USA). The subjects lay supine with their legs fully extended and muscles relaxed in the magnet bore. Taking into consideration fluid shifts of the lower extremity, the images were acquired after 15-20 minutes rest in the supine position. The ACSAs of each muscle of the quadriceps femoris (VL, VM, VI and RF) were measured by using ImageJ software (National Institute of Health, USA). Care was taken to exclude visible adipose and connective tissue incursions. Each image was digitized twice, and the mean of the two
values were used for further analysis. The coefficient of variation (CV) of the two measurements by a tester was $0.9 \pm 0.7\%$. The intraclass correlation coefficient (ICC) of the two measurements was 1.000. Moreover, the inter-tester (two testers) repeatability of ACSA measurements was evaluated ($n = 6$; at 50% of thigh length). The CV was $2.8 \pm 2.0\%$ and the ICC was 0.997, respectively. The muscle volume of each muscle was determined by summing ACSA times the slice thickness (1.0 cm). The ACSA at 30% (proximal), 50% (middle) and 70% (distal) of muscle length of each muscle was determined from the mean of the 3 nearest slices, respectively. In addition, percentage of each muscle volume to the total quadriceps femoris was calculated.

**Experiment 2: Longitudinal approach**

**Subjects**

Twelve varsity cyclists (10 males and two females; age, $20.0 \pm 1.2$ yr; height, $1.70 \pm 0.08$ m, body mass, $62.6 \pm 8.3$ kg) and 10 untrained men as controls (age, $21.5 \pm 1.7$ yr; height, $1.73 \pm 0.06$ m; body mass, $65.6 \pm 5.0$ kg) participated in this study. All the cyclists had participated in both track and road races. Their competitive experience was $4.1 \pm 2.9$ yr (range: 0.1 - 10.7 yr) at the beginning of the experiment. During the observation period, the cyclists practiced cycling training for $15 \pm 5$ hours per week and training distance was $280 \pm 125$ km per week. Most of the competitive trainings were conducted on the road. The first and second measurements were made at the early and last stages of a season, respectively. Among the cyclists, three had conducted resistance training (squat at an intensity of 70% of one repetition maximum) twice per week. The training modality was a combination of knee extension and hip extension, and training frequency was lower than that of cycling training. We confirmed similar hypertrophic
adaptation of the three cyclists to others and therefore all data were pooled. The untrained men were requested to continue usual life style and not to perform intensive exercises. Some of them had taken part in various recreational physical activities such as jogging or ball games once or twice a week and others had walked or cycled when commuting. Prior to the experiment, the subjects were informed of the purpose and risks of the study and provided written informed consent.

**MR imaging**

The same measurement in the experiment 1 was carried out before and after the observation period for 6 months. To investigate the regional difference in hypertrophy, ACSAs at 30% (proximal), 50% (middle) and 70% (distal) along the length of each muscle was determined from the mean of the three nearest slices, respectively. In addition, the muscle volume was determined for each muscle.

**Leg extension power**

Maximal voluntary bilateral leg extension power was measured with a leg extension machine (Anaeropress 3500, Combi Co., Tokyo, Japan). The load of leg extension was set according to subjects’ body mass. The subjects sat on a bench of the machine with the pelvis and the foot secured to the bench and the plate by non-elastic straps, respectively. After several warming up trials, the subjects were asked to extend the legs as fast as possible with the maximal effort. The leg extension power was measured five times. The mean of the highest and second highest values in the trials was used for further analysis. The CV and ICC of the two values was 2.9 ± 2.7% and 0.967, respectively.
Statistical analysis

Descriptive data are presented as means ± SDs. All the analyses were performed with a statistical software (IBM SPSS 22.0, IBM, Japan). In the experiment 1, analysis of the muscle volume was performed by a two-way analysis of variance (ANOVA) with one within-group factor (VL, VM, VI, RF) and one between-group factor (experienced cyclists, untrained men). A two-way ANOVA was conducted to test the difference in percentage of individual muscle volume to the total quadriceps femoris. In the experiment 2, a paired \( t \)-test was used to test the significance of the difference in body mass and leg extension power before and after the observation period. A two-way ANOVA with repeated measures was used to analyze the effects of time (before, after) and muscle on the muscle volume. A two-way ANOVA was conducted to test the significance of the effects of time and regions (proximal, middle, and distal) on ACSA for each muscle. A one-way ANOVA with repeated measures was performed to determine whether relative changes differed among the four muscles. The ANOVAs were followed by post hoc tests with Bonferroni correction. Pearson product-moment correlation coefficients for the relationship between the extent of increase in muscle volume and 1) the year of competitive experience and 2) muscle volume before measurement were determined. Significance level was set at \( P < 0.05 \).

3-2-3. Results

Experiment 1

ACSA

The ACSA distributions along the thigh are illustrated in Fig. 3-2-1. The
two-way ANOVA showed a significant main effect of group for VL ($P < 0.01$), VM ($P < 0.05$) and VI ($P < 0.05$) with no significant interaction of group $\times$ region. The ACSAs of the three muscles were significantly greater in the experienced cyclists than in the untrained men. On the other hand, there was no significant main effect of group or interaction of group $\times$ region for RF.

**Muscle volume**

Descriptive data of each muscle’s volume is shown in Fig. 3-2-2. The two-way ANOVA indicated a significant main effect of group ($P < 0.05$) with a significant interaction of group $\times$ muscle ($P < 0.001$). The volumes of VL ($P < 0.01$), VM ($P < 0.05$) and VI ($P < 0.05$) were significantly greater in the experienced cyclists than in the untrained men. On the other hand, the muscle volume of RF did not differ significantly between the two groups. These results were consistent even when each muscle volume was normalized by body mass.

**Relative muscle volume**

The percentage of the muscle volume of each muscle to that of the total quadriceps femoris is presented in Fig. 3-2-3. The two-way ANOVA showed a significant interaction of group $\times$ muscle ($P < 0.01$). The percentage of RF volume was significantly lower in the experienced cyclists ($12.0 \pm 1.1\%$) than in the untrained men ($15.4 \pm 1.5\%$) ($P < 0.001$). The percentage of VL volume was significantly higher in the experienced cyclists ($33.9 \pm 1.6\%$) than in the untrained men ($31.5 \pm 2.1\%$) ($P < 0.05$). The corresponding values for VM and VI did not differ significantly between the two groups.
Experiment 2

Body mass and leg extension power

Paired t-tests indicated no changes in body mass (cyclists: before, 62.6 ± 8.3 kg, after, 61.8 ± 8.8 kg; controls: before, 65.6 ± 5.0 kg, after, 65.4 ± 5.7 kg) or leg extension power (cyclists: before, 1477 ± 399 W, after, 1522 ± 408 W; controls: before, 2040 ± 357 kg, after, 1985 ± 298 W) for both groups.

ACSA

In the control group, no main effect of time or interaction of time × region was observed in any muscles. In the cyclists, there was a significant main effect of time (VL, $P < 0.05$; VM, $P < 0.01$) with a significant interaction of time × region (VL, $P < 0.01$; VM, $P < 0.05$) of VL and VM. The ACSAs of the two muscles in the middle (VL, $P < 0.05$; VM, $P < 0.05$) and distal (VL, $P < 0.001$; VM, $P < 0.01$) regions increased significantly, whereas those in the proximal region did not (Fig. 3-2-4). For VI, a main effect of time ($P < 0.05$) with no significant interaction of time × region was observed. For RF, there was no main effect of time or interaction of time × region. Regarding the relative changes in ACSA, one-way ANOVA showed a main effect of region in VL ($P < 0.001$) but not in VM, VI, or RF. Relative change in ACSA of VL was significantly greater in the middle and distal ($P < 0.01$) regions than in the proximal region.

Muscle volume

In the control group, no main effect of time or interaction of time × muscle was observed. In the cyclists, the two-way ANOVA demonstrated significant main effects of
time ($P < 0.001$) and muscle ($P < 0.001$) with a significant interaction of the two factors ($P < 0.001$). The volumes of VL ($P < 0.001$), VM ($P < 0.001$) and VI ($P < 0.01$) increased significantly (Fig. 3-2-5). On the other hand, the volume of RF did not change significantly. The one-way ANOVA revealed a significant main effect of muscle for the relative change in muscle volume ($P < 0.001$). The relative changes in the muscle volume of VL, VM ($P < 0.001$) and VI ($P < 0.01$) were significantly higher than that of RF.

**Relationship between variables**

The relationships between the extent of increases in muscle volume of each muscle or total quadriceps femoris and 1) the year of competitive experience or 2) muscle volume before measurement did not reach statistical significance in any muscles, although a tendency toward a negative correlation was observed in VI ($r = -0.544, P = 0.067$) and total quadriceps femoris ($r = -0.508, P = 0.091$) with the year of competitive experience.

**3-2-4. Discussion**

To the best of our knowledge, this is the first study to examine the influence of regular training of competitive cycling on the individual muscle volumes of the quadriceps femoris cross-sectionally and longitudinally. It was demonstrated that 1) the volumes of VL, VM and VI were greater in the experienced cyclists than in the untrained men, but that of RF was similar between the two groups, and 2) the increases in the volumes of VL, VM and VI were observed after the competitive cycling training for 6 months, but did not for RF. Namely, the results of longitudinal study (experiment
2) substantiated those of cross-sectional study (experiment 1). These cross-sectional and longitudinal findings suggest that the unique muscularity in the experienced cyclists are due to regular training of competitive cycling rather than being inherited, and that longitudinal results are due to the specificity of cycling-induced hypertrophy, not due to the time course difference in hypertrophic response between RF and vasti.

The current cross-sectional and longitudinal findings demonstrated that competitive cyclists have large quadriceps femoris and cycling training induces hypertrophy of the quadriceps femoris. These were consistent with the previous studies (cross-sectional study, Hug et al. 2006; longitudinal study, Harber et al. 2012; Linossier et al. 1997; McPhee et al. 2010). On the other hand, some cross-sectional (Izquierdo et al. 2004) and longitudinal studies (Farup et al. 2012; Rønnestad et al. 2010) failed to find a significant difference in the quadriceps femoris size compared to the controls or a significant change following cycling training. However, these studies evaluated only single ACSA of the total quadriceps femoris. The present findings indicated that competitive cycling training elicited uneven hypertrophy among the muscles and over the regions within a muscle: preferential hypertrophy of the vasti, middle and distal regions in VL and VM in particular. Rønnestad et al. (2010) and Farup et al. (2012) evaluated ACSA of the total quadriceps femoris at the proximal regions where RF ACSA was comparatively large. Specificity of hypertrophic response among the muscles and among different regions may also have been existed in those studies (Izquierdo et al. 2004; Rønnestad et al. 2010; Farup et al. 2012). In any case, the current approach (muscle volume evaluation) provides clearer evidence for the greater size of the quadriceps femoris in experienced cyclists compared to untrained controls, together with their hypertrophic response.
The quantitative profiles of the quadriceps in the cyclists were consistent with the finding in oarsmen (Chapter 3, section 1). The current results strongly suggest that such muscle-specificity is generalized to athletes who mainly perform repetitive leg extensions in their sport activities. A possible explanation for the lack of hypertrophy of RF is low activation during pedaling motions. The activation level during pedaling motions determined by several methodologies (EMG, T2-weighted MR imaging or positron emission tomography) was lower for RF than the vasti (Chin et al. 2011; Endo et al. 2007; Gondoh et al. 2009). Another possible factor that account for the lack of hypertrophy of RF is a less trainability of this muscle. However, more prominent hypertrophy of RF compared to the vasti has been shown after knee extension training (Chapter 2), thereby discarding this possibility. Taken together, the difference in muscle activation between the vasti and RF during pedaling motions may account for the current results.

Generally, training-induced muscle hypertrophy is accompanied by a greater extent of increases in muscle strength (e.g., Ikai and Fukunaga 1970). In the current study, however, there was no change in leg extension power of the cyclists, despite significant increases in the volumes of the quadriceps femoris. These results are partly in line with the previous reports showing no change (Sale et al. 1992) or a decrease (Akima et al. 1997) in strength after training with a significant increase in muscle size, when the strength was measured in a different task from the one used in the training. Although pedaling motions are repetitive unilateral leg extensions, the current study evaluated bilateral leg extension power. It is known that strength of maximal voluntary bilateral actions is less than that of the sum of the unilateral actions (bilateral deficit, Vandervoort et al. 1984). Trained cyclists showed a similar degree of bilateral deficit of
leg extension strength compared to untrained controls (Secher et al. 1988), suggesting that competitive cycling training does not improve the extent of the deficit. Moreover, Taniguchi (1997) showed that the unilateral leg extension training increased the extent of bilateral deficit (3.9%). Therefore, the difference in the task (unilateral versus bilateral) may be related to the result that the bilateral leg extension power was not changed following 6 month of competitive cycling training.

In conclusion, the current results indicate that regular training of competitive cycling induces muscle-specific hypertrophy of the quadriceps femoris, leading to quantitative muscular profiles in experienced cyclists.
Fig. 3-1-1 An example of the magnetic resonance image of the right thigh in the experienced oarsmen (left) and untrained men (right). VL, vastus lateralis; VM, vastus medialis; VI, vastus intermedius; RF, rectus femoris.
Fig. 3-1-2 ACSA divided by the two-thirds power of body mass (ACSA-to-body mass$^{2/3}$) at 30% (proximal), 50% (middle) and 70% (distal) of each muscle length in the vastus lateralis (upper left), vastus medialis (lower left), vastus intermedius (upper right) and rectus femoris (lower right). * indicates a significant difference between the oarsmen (black circle) and untrained men (white circle). ACSA, anatomical cross-sectional area.
Fig. 3-1-3 Muscle volume (upper) and muscle volume relative to body mass (lower) of the total quadriceps femoris and individual muscles. * denotes a significant difference between the oarsmen (black bar) and untrained men (white bar). QF, total quadriceps femoris; VL, vastus lateralis; VM, vastus medialis; VI, vastus intermedius; RF, rectus femoris.
Fig. 3-1-4 The percentage of each muscle volume to the total quadriceps femoris volume. * denotes a significant difference between the oarsmen (left bar) and untrained men (right bar). VL, vastus lateralis; VM, vastus medialis; VI, vastus intermedius; RF, rectus femoris.
Fig. 3-2-1 Distribution of the anatomical cross-sectional area of each muscle of the quadriceps femoris in the experienced cyclists (black circle) and untrained men (white circle). The origin (0) of the horizontal axis means the center of thigh.
Fig. 3-2-2 The volume of each muscle of the quadriceps femoris of experienced cyclists (black bar) and untrained men (white bar). * indicates a significant difference between the two groups. VL, vastus lateralis; VM, vastus medialis; VI, vastus intermedius; RF, rectus femoris.
Fig. 3-2-3 The percentage of each muscle volume to the total quadriceps femoris volume. * denotes a significant difference between the experienced cyclists (left bar) and untrained men (right bar). VL, vastus lateralis; VM, vastus medialis; VI, vastus intermedius; RF, rectus femoris.
Fig. 3-2-4 Relative changes in anatomical cross-sectional area (ACSA) of the vastus lateralis (upper) and vastus medialis (lower) at 30% (proximal), 50% (middle), and 70% (distal) of each muscle length of the cyclists. # indicates a significant change as a result of regular training of competitive cycling for 6 months. * denotes a significant difference between the regions.
Fig. 3-2-5 Relative changes in muscle volume of each muscle of the quadriceps femoris of the cyclists (upper) and controls (lower). # indicates a significant change as a result of regular training of competitive cycling for 6 months. * denotes a significant difference between the muscles. VL, vastus lateralis; VM, vastus medialis; VI, vastus intermedius; RF, rectus femoris.
Chapter 4 Activation of the quadriceps femoris during knee extension with or without hip extension torque

4-1. Introduction

Hypertrophic response of RF differed between Chapter 2 and Chapter 3. In Chapter 2, knee extension training elicited the greatest increase in ACSA of RF among the four muscles of the quadriceps femoris. On the other hand, competitive cycling training induced the increases in muscle volume of the vasti but not of RF. The reasons for the inconsistency of RF response between Chapters are unclear, but may involve the difference in training modality; knee joint raining vs. leg extension training. The major difference between the two training regimens is whether knee extension is accompanied by hip extension or not. It is hypothesized that the activation level of RF during knee extension is decreased with additional hip extension. The purpose of this chapter was to test the hypothesis through controlled experimental settings.

4-2. Methods

Subjects

Eleven healthy adults (9 males, 2 females, age, 23 ± 2 yr; height, 1.68 ± 0.06 m; body mass, 64 ± 7 kg; mean ± SD) participated in this study. This study was approved by the Ethics Committee of Human Research of Waseda University. Prior to the execution to the experiments, the subjects were informed of the purpose and risks of the study and provided written informed consent.

Experimental setup
EMG measurement

Surface EMG signals were obtained from VL, VM, distal and proximal regions of RF (RF<sub>distal</sub>, RF<sub>proximal</sub>), and biceps femoris (BF). Using real-time B-mode ultrasonography (SSD-6500, ALOKA, Japan), muscle berry and fascicle direction were confirmed with a measurement posture (i.e., 90° of knee and hip joints, full extension = 0°). Previous studies have shown that regional difference in muscle activation of RF during knee extension (Akima et al. 2004; Watanabe et al. 2012) and hip flexion (Miyamoto et al. 2012; Watanabe et al. 2012) and thus the EMG data from the two regions of RF were acquired. After skin shaving, rubbing with sandpaper and cleaning with alcohol, pre-amplified bipolar surface electrodes (1 × 10 mm, 10mm inter-electrode distance) with band-pass filtering between 20 and 450 Hz (Bagnoli 8 EMG System, DELSYS, Boston, MA, USA) were placed over at the level of 90% (VM), 70% (RF<sub>distal</sub>), 50% (VL, BF), 30% (RF<sub>proximal</sub>) of the thigh length between the greater trochanter and popliteal crease. The reference electrode was placed over the right patella for all EMG measurements.

Experimental procedure

First, maximal voluntary isometric knee extension (MVC<sub>KE</sub>) and flexion (MVC<sub>KF</sub>) torque was measured. The Subjects sat on a bench of a dynamometer (CON-TREX, CMV AG, Switzerland), while securing the pelvis on the bench with a non-elastic strap and torso on the back seat by a seat belt. Care was taken to adjust the center of rotation of the dynamometer and center of the knee joint. The hip and knee joint angles were 90° flexion, respectively. After the completion of a warm-up procedure consisting of submaximal knee extension and flexion exercises, the subjects
were asked to extend or flex the knees with maximal effort. Next, maximal voluntary isometric hip extension (MVC_{HE}) and flexion (MVC_{HF}) torque was measured. The subjects lay spine on the bench of the dynamometer, while securing the pelvis and torso on the bench with a non-elastic strap. Care was taken to adjust the center of rotation of the dynamometer and center of the hip joint. The hip and knee joint angles were 90° flexion, respectively. After the completion of a warm-up procedure consisting of submaximal hip extension and flexion exercises, the subjects were asked to extend or flex the hip with maximal effort. Each MVC measurement was conducted twice, and if the difference of MVC value between the two trials was above 10%, the third measurement was conducted. The two values were averaged and used for further analysis.

Then, two types of trials were conducted in random order (Fig. 4-1). The one is “constant isometric hip extension torque condition”. Under lying spine position with hip at 90°, the subjects were asked to extend the knee to full extension from 90° in 5 seconds, while maintaining constant at an intensity of either 0, 20, or 50% of MVC_{HE}. The other is “ramp isometric hip extension torque condition”. While exerting constant isometric knee extension torque, the subjects were requested to perform a ramp isometric hip extension by increasing the torque from relaxation to the maximum in 5 seconds. In both conditions, a weight (equivalent to 10% of maximal voluntary knee extension at 90° knee joint angle) was attached to the lower leg. The weight was selected so as to control a steady or ramp hip extension torque easily, and in a pilot study, we confirmed a substantial activation of VL, VM, and RF for attaining the present purpose. Three to five measurements for each trial were performed and data were averaged. The knee joint angle was measured by an electronic goniometer (SG150,
Biometrics, UK). The data were simultaneously stored on a computer after A/D conversion (PowerLab/16SP, ADInstruments, Sydney, Australia) at 1kHz of sampling frequency for EMG, torque and joint angle data.

Data analysis

In the constant isometric hip extension torque condition, the root mean square values of EMG signals (RMS-EMG) were calculated in the range from 90˚ to 30˚ of knee joint angle. This range was selected because some subjects could not extend their knee to < 30˚, and because around the knee joint of full extension, co-contraction of the quadriceps femoris and hamstrings were observed in some subjects, which were not suited for the current purpose. Each RMS-EMG value was normalized to those over 1s period during MVC\textsubscript{KE} (VL, VM and RF) or MVC\textsubscript{HE} (BF) tasks. In the ramp isometric hip extension torque condition, RMS-EMG were calculated in the following four windows: (1) 0%MVC\textsubscript{HE} (for 1 second), (2) from 0%MVC\textsubscript{HE} to 20%MVC\textsubscript{HE}, (3) from 20%MVC\textsubscript{HE} to 40%MVC\textsubscript{HE}, (4) from 40%MVC\textsubscript{HE} to 60%MVC\textsubscript{HE}. Each RMS-EMG value was normalized to that of window (1).

Statistical analysis

Descriptive data are presented as means ± SDs. All the analyses were performed with a statistical software (IBMSPSS 22.0, IBM, Japan). In the constant isometric hip extension torque condition, a one-way ANOVA with repeated measures followed by Bonferroni test was performed to determine whether RMS-EMG differed among the intensity (0, 20, and 50% of MVC\textsubscript{HE}) for each muscle and region. In the ramp isometric hip extension torque condition, a one-sample t-test with Bonferroni
adjustment was conducted on the differences of RMS-EMG between the window (1) and the windows (2), (3), or (4) for each muscle. A one-way ANOVA with repeated measures with Bonferroni test was used to test the difference of RMS-EMG among the windows (2) to (4). Significance level was set at $P < 0.05$, and for the one-sample $t$-test, significance level was set at $P < 0.0167 (= 0.05/3)$.

4-3. Results

**Constant isometric hip extension torque condition**

Descriptive data of RMS-EMG for each muscle is shown in Fig. 4-2. The one-way ANOVA indicated a significant main effect of intensity for each muscle (VL, VM, RF$_{\text{distal}}$ and RF$_{\text{proximal}}$, $P < 0.001$; BF, $P < 0.01$). In VL and VM, the RMS-EMG value was highest in 50%MVC$_{\text{HE}}$ (vs. 20%MVC$_{\text{HE}}$, $P < 0.01$; vs. 0%MVC$_{\text{HE}}$, $P < 0.001$) condition, and lower in 20%MVC$_{\text{HE}}$ (vs. 0%MVC$_{\text{HE}}$, $P < 0.01$) and 0%MVC$_{\text{HE}}$ conditions in this order. In RF$_{\text{distal}}$ and RF$_{\text{proximal}}$, the RMS-EMG was significantly higher in 0%MVC$_{\text{HE}}$ condition than in 20%MVC$_{\text{HE}}$ and 50%MVC$_{\text{HE}}$ conditions ($P < 0.01$). For BF, the RMS-EMG was significantly higher in 50%MVC$_{\text{HE}}$ condition than in 0%MVC$_{\text{HE}}$ ($P < 0.01$) and 20%MVC$_{\text{HE}}$ ($P < 0.05$) conditions. The results that greater activation of VL, VM, BF and smaller activation of RF with greater intensity of hip extension torque were observed in all the subjects.

**Ramp isometric hip extension torque condition**

The percentage of RMS-EMG to that of window (1) for each muscle is presented in Fig. 4-3. The one-sample $t$-tests indicated the significant differences between the window (1) and windows (2), (3) or (4) ($P < 0.001 \sim 0.01$). The one-way
ANOVA demonstrated a significant main effect of intensity for each muscle (VL, VM, \( R_{\text{distal}} \) and \( R_{\text{proximal}} \), \( P < 0.001 \); BF, \( P < 0.01 \)). The RMS-EMG values of VL, VM and BF (\( P < 0.01 \sim 0.05 \)) significantly increased with the increase of hip extension torque. The RMS-EMG values of \( R_{\text{distal}} \) and \( R_{\text{proximal}} \) significantly decreased with the increase of hip extension torque, except for between the windows (3) and (4). The tendency was observed in all subjects.

4-4. Discussion

The greater hip extension torque or its increase was associated with a smaller RMS-EMG of \( R_{\text{distal}} \) and \( R_{\text{proximal}} \) or their decrease, and vice versa for VL and VM. The current results demonstrated that the activation of RF during knee extension decreases with additional hip extension despite regions along the length, and vice versa for VL and VM. The results suggest that in human movements, contribution of each of the quadriceps femoris to knee joint torque depends on hip joint kinetics. The current results were consistent with some previous findings (Fujiwara and Basmajian 1975; Yamashita 1988). Yamashita (1988) showed that the activation level of RF during 20%MVC\(_{\text{KE}}\) was higher than those during 20%MVC\(_{\text{KE}}\) with 20%MVC\(_{\text{HE}}\), and vice versa for VM. The current results support this notion. Moreover, the present findings indicate that the magnitude of hip extension torque affects the activation level of VL, VM and RF, and that can be applied for dynamic (concentric) knee extension contractions as well as static (isometric) knee extension contractions.

A possible reason for the decrease in RF activation is the reciprocal Ia inhibition between antagonist biarticular muscles, as evidenced by an animal experiment (Eccles and Lundberg 1958). When the Ia afferents from the muscle spindle
of an agonist are activated, they then inhibit the motoneurons of an antagonist acting on the same joint through the inhibitory interneurons (Schmidt et al. 1985). This can be considered as a mechanism for smooth joint actions during human motions. The current results suggest that this Ia inhibition influences the only RF activation among the quadriceps femoris.

Regarding the increase in activation level of VL and VM with additional hip extension, two possibilities should account for the results. The first is the compensation for the decrease of RF activation under exerting constant knee extension torque. Previous studies showed the compensation of activation each other among the quadriceps femoris to complete a knee extension task (Akima et al. 2002; Kouzaki et al. 2002). For example, prior VL fatigue induced the increases in activation of VM and RF during a submaximal knee extension task (Akima et al. 2002). Such compensation among the quadriceps femoris should be responsible for the observed results. The second is the biarticular nature of the hamstrings. The biceps femoris long head, semitendinosus and semimembranosus are biarticular muscles among the hamstrings, and thus exerting hip extension torque results in exerting knee flexion torque at the same time. Therefore, an increase in the hip extension torque would be accompanied by the increase in the force by the quadriceps femoris to keep a constant net knee extension torque (10% of MVCKE torque), resulting in the increase in VL and VM activation.

In conclusion, the current results indicate that the activation of the rectus femoris during knee extension decreases with additional hip extension, and vice versa for the vastus lateralis and vastus medialis.
Fig. 4-1 Schematic drawings of the constant isometric hip extension torque condition (upper) and ramp isometric hip extension torque condition (lower). Upper: The subject extended the knee to full knee extension from 90° knee flexion in 5 s while exerting constant hip extension torque at an intensity of 0, 20 or 50% of the maximal voluntary contraction. Lower: The subject exerted gradual increase in isometric hip extension torque to maximum in 5 s.
Fig. 4-2 Root mean square (RMS) of electromyogram (EMG) of the vastus lateralis, vastus medialis, rectus femoris (distal and proximal regions) and biceps femoris long head during constant isometric hip extension torque condition. RMS-EMG was determined in the range form 90° to 30° of knee joint angle and normalized by that at MVC\textsubscript{KE} (vastus lateralis, vastus medialis and rectus femoris) or MVC\textsubscript{HE} (biceps femoris). * indicates a significant difference between the intensities. MVC\textsubscript{KE}, maximal voluntary isometric knee extension; MVC\textsubscript{HE} maximal voluntary isometric hip extension.
RMS-EMG [%\((1)\)]

- **Vastus lateralis**
- **Vastus medialis**
- **Rectus femoris (distal)**
- **Rectus femoris (proximal)**
- **Biceps femoris**

**Fig. 4-3** Root mean square (RMS) of electromyogram (EMG) of the vastus lateralis (●), vastus medialis (○), rectus femoris (distal, ▲; proximal, △) and biceps femoris long head (◇) during ramp isometric hip extension torque condition. RMS-EMG was calculated in the following windows. (1): 0% of maximal voluntary hip extension torque (MVC\(_{\text{HE}}\)) for 1 s; (2): From 0% MVC\(_{\text{HE}}\) to 20% MVC\(_{\text{HE}}\); (3): From 20% MVC\(_{\text{HE}}\) to 40% MVC\(_{\text{HE}}\); (4): From 40% MVC\(_{\text{HE}}\) to 60% MVC\(_{\text{HE}}\) and was normalized by that of (1) for each muscle. The number in parenthesis denotes the windows, where a significant difference was found.
Chapter 5 Influence of exercise regimen and intensity for the activation of the quadriceps femoris: Comparison between knee extension and leg press exercises

5-1. Introduction

The results of Chapter 4 indicated that additional hip extension torque decreased RF activation during knee extension. This was observed under the constant hip joint angle (i.e., isometric contraction). On the other hand, hip joint angle changes during actual training procedure of leg extension including rowing and pedaling motions (Chapter 3). It is unclear whether the finding of Chapter 4 is applied for dynamic leg extension. Moreover, in Chapter 4, the intensity of hip joint torque affected RF activation during knee extension. Therefore, even when high intensity of dynamic leg extension is performed, it is possible that the activation level of RF is substantially low. The purpose of this study was to examine the activation level of the quadriceps femoris during knee extension and leg press and compared them between the two exercise regimens, and to examine the influence of exercise intensity on the activation level of the quadriceps femoris.

5-2. Methods

Subjects

Fifteen healthy men (age, 23 ± 2 yr; height, 1.73 ± 0.07 m; body mass, 64 ± 6 kg; mean ± SD) participated in this study. This study was approved by the Ethics Committee of Human Research of Waseda University. Prior to the execution to the experiments, the subjects were informed of the purpose and risks of the study and provided written informed consent.
Experimental setup

EMG measurement

Surface EMG signals were obtained from VL, VM and RF. Using real-time B-mode ultrasonography (SSD-900, ALOKA, Japan), muscle berry and fascicle direction were confirmed. After skin shaving, rubbing with sandpaper and cleaning with alcohol, pre-amplified bipolar surface electrodes (1 × 10 mm, 10-mm inter-electrode distance) with band-pass filtering between 20 and 450 Hz (Bagnoli 8 EMG System, DELSYS, Boston, MA, USA) were placed over at the level of 50% (VL), 90% (VM), 30% (RF) of the thigh length between the greater trochanter and popliteal crease. The reference electrode was placed over the left patella for all EMG measurements.

Experimental procedure

Prior to EMG testing, 1RM was determined for the both exercises. The 1RM was determined by increasing the load until each subject was unable to lift once throughout the prescribed knee joint range of motion. The subjects sat on a bench of a knee extension training machine (Nitro S3LE, Nautilus, USA) or leg press machine (Nitro S3LP, Nautilus, USA). Thereafter, the unilateral (right leg) knee extension exercise or leg press exercise was performed using concentric actions (for 3 s) and eccentric actions (for 3 s) (Akima and Saito 2013) in random order. Care was taken to match the initial knee joint angle between the two exercises (110°, full extension = 0°). The initial hip joint angle was 60° (full extension = 0°) for knee extension and 130° for leg press exercises, respectively. The hip joint angle difference could not be avoided because of the correspondence of initial knee joint angle (110°) between the exercises.
The exercise load was adjusted to 20%, 40%, 60% or 80% of 1RM. Each exercise was consisted of five repetitions. Knee joint angle was measured by an electronic goniometer (SG150, Biometrics, UK). The data were simultaneously stored on a computer after A/D conversion (PowerLab/16SP, ADInstruments, Sydney, Australia) at 1kHz of sampling frequency for EMG and joint angle data.

**Data analysis**

RMS-EMG at each repetition was calculated separately in the concentric and eccentric phases of the exercises, which were determined from the knee joint angle and averaged them of five repetitions. Each RMS-EMG was normalized by that during 1RM of the knee extension trial.

**Statistical analysis**

Descriptive data are presented as means ± SDs. All the analyses were performed with statistical software (IBMSPSS 22.0, IBM, Japan). A two-way ANOVA with repeated measures was conducted to test the significance of the effects of exercise (knee extension and leg extension) and intensity (20%, 40%, 60% and 80% of 1RM) on normalized RMS-EMG for each muscle. When a significant interaction or both of the two main effects were observed, an additional one-way ANOVA with repeated measures with Bonferroni test was performed to determine whether significant differences existed among all (i.e., eight) the conditions. A two-way ANOVA with repeated measures was used to test the effects of muscle and exercise on the normalized RMS-EMG at 80% of 1RM. When appropriate, an additional one-way ANOVA with repeated measures
followed by Bonferroni test was performed to determine significant differences among
the muscles for each exercise. Significance level was set at $P < 0.05$.

5-3. Results

Difference between knee extension and leg press exercises

Descriptive data on normalized RMS-EMG of each muscle is shown in Fig. 5-1. The two-way ANOVAs demonstrated a significant main effect of intensity on VL and VM ($P < 0.001$) with no significant main effect of exercise or interaction of exercise × intensity. The normalized RMS-EMGs were highest in 80% of 1RM condition, and lower 60% of 1RM, 40% of 1RM and 20% of 1RM conditions in this order for both muscles ($P < 0.001 \sim 0.05$). Regarding the normalized RMS-EMG of RF, the two-way ANOVA showed the main effects of exercise ($P < 0.001$) and intensity ($P < 0.001$), and an interaction of the two factors ($P < 0.01$). The normalized RMS-EMG was significantly higher during knee extension exercise than during leg press exercise at all intensity (20% of 1RM, $P < 0.05$; 40% of 1RM, $P < 0.001$; 60% of 1RM, $P < 0.001$; 80% of 1RM, $P < 0.001$). The normalized RMS-EMG of RF at 80% of 1RM during leg press exercise was significantly lower than those of RF at 40, 60 and 80% of 1RM during knee extension exercise. There were no differences in normalized RMS-EMG of RF among that of 20% of 1RM during knee extension exercise and those of 40%, 60% and 80% of 1RM during leg press exercise.

Difference among the muscles

The two-way ANOVA showed that there were significant main effects of muscle ($P < 0.01$) and exercise ($P < 0.01$) and interaction of the two factors ($P < 0.001$).
The normalized RMS-EMG of RF during leg press exercise was significantly lower than those of VL and VM ($P < 0.001$). There was no significant difference in RMS-EMG among the three muscles during knee extension exercise.

5-4. Discussion

The main findings of the current study were that the activation level of RF was higher during knee extension exercise than during leg press exercise regardless of exercise intensity but those of VL and VM were similar between the two exercises. Moreover, the higher exercise intensity was, the higher activation level of all muscles were in both exercises. However, there was no statistical difference between the activation level of RF at 80% of 1RM of leg press exercise and at 20% of 1RM of knee extension exercise, and those were lower than that at 40% of 1RM of knee extension exercise. These results indicate that the activation level of RF during leg extension is substantially low at high intensity (80% of 1RM) which can induce muscle hypertrophy (American College of Sports Medicine 2009).

The result of lower activation level of RF during leg press exercise compared to that of during knee extension exercise was consistent with the previous findings (Enocson et al. 2005; Escamilla et al. 1998). By using surface EMG, Escamilla et al. (1998) determined the activation level of the quadriceps femoris during leg press, squat and knee extension exercises at an intensity of 12-repetition maximum corresponding to 67% of 1RM (Baechle and Earle 2008). They demonstrated that the activation level of RF during knee extension exercise was approximately 45% higher than those during squat and leg press exercises. This was consistent with the current results at an intensity of 60% of 1RM (= 53% difference between the two exercises). Moreover, the current
results added the evidence the lower activity of RF during leg press than during knee extension regardless of the exercise intensity.

One of the possible reasons for the difference in RF activation between the two exercises is explained by the results of Chapter 4. In Chapter 4, additional hip extension torque decreased RF activation during knee extension. Leg press exercise requires hip extension torque. Therefore, whether or not knee extension is accompanied by hip extension should account for the difference in RF activation between the knee extension and leg press exercises. Another possible factor is related to the force-length and force-velocity characteristics of RF during exercises. It is difficult to control these factors between the two types of exercises, and thus these are limitations of the current study. My previous studies (Ema et al. 2010, 2012) showed that hip joint angle did not affect RF activation during isometric knee extension with maximal effort. Although the contraction mode and intensity are different between the previous (Ema et al. 2010, 2012) and current studies, it can be considered that the effect of difference in the regions of the force-length relationship during the two exercises is small. To acquire further implications of influence of these factors, the effect of hip joint angle and angular velocity during knee extensions were examined. Twelve healthy men conducted isometric (i.e., angular velocity of knee joint is 0°/s) and concentric knee extension (30°/s) with two hip joint angles (0° and 80°, full extension = 0°). Surface EMG signal was recorded from VL, VM and RF, and RMS-EMGs during knee extensions were determined. The two-way ANOVA demonstrated that there were no significant main effects of hip joint angle and angular velocity or interactions of the two factors on RMS-EMG for each muscle. This suggests that the effects of difference in the region of force-length relationship and difference in fascicle shortening velocity of RF on the
difference in activation level of RF between knee extension and leg press exercises are not large.

Inter-muscle difference in activation level during leg extension exercise was in line with the previous reports (Escamilla et al. 1998, 2001; Ploutz-Snyder et al. 1995). Ploutz-Snyder et al. (1995) evaluated the activation level of RF and vasti during squat exercise by transverse relaxation time (T2) of MR images. They showed that the T2 increase following squat exercise was higher in the vasti than in RF. This should be related to the results of Chapter 4. The additional hip extension torque decreased only RF activation among the quadriceps femoris. This suggests that inhibition induced by hip extension torque to the activation during knee extension is muscle-specific among the quadriceps femoris. Therefore, the activation level during leg press exercise would be different among the vasti (VL and VM) and RF in the current and previous studies.

In conclusion, the present results indicate that 1) the activation level of RF is lower during leg extension exercise than during knee extension exercise, 2) the activation level of RF is lower than those of VL and VM during leg extension exercise, and 3) even when at high intensity, the activation level of RF is substantially low during leg extension exercise. These suggest that leg extension training is unlikely to induce architectural adaptation of RF regardless of its training intensity.
Fig. 5-1 Root mean square (RMS) of electromyogram (EMG) of the vastus lateralis (upper), vastus medialis (middle), and rectus femoris (lower) during knee extension (white circle) and leg press (black circle) exercises at 20%, 40%, 60% and 80% of 1 repetition maximum (1RM). Each RMS-EMG was normalized by that determined at 1RM trial of knee extension. * indicates a significant difference between the intensities. # denotes a significant difference between the two exercises at each intensity. $ means a significant difference with the 80% of 1RM during leg press exercise.
Fig. 5-2 Root mean square (RMS) of electromyogram (EMG) of the vastus lateralis (white bar), vastus medialis (gray bar), and rectus femoris (black bar) during leg press (left) and knee extension (right) exercises at 80% of 1 repetition maximum (1RM). Each RMS-EMG was normalized by that determined at 1RM trial of knee extension. * indicates a significant difference between the muscles. VL, vastus lateralis; VM, vastus medialis; RF, rectus femoris.
General discussion

6-1. Main findings of each chapter

The main findings of each chapter are as follows.

Chapter 2

1. Knee extension training for 12 weeks induced architectural changes in each muscle. The extents of hypertrophy and increase in pennation angle of RF were greater than those of VL, VM and VI.
2. The extent of hypertrophy was not uniform over VL, VI and RF.
3. Correlations between the magnitudes of muscle hypertrophy and changes in pennation angle were confirmed.
4. Inconsistent results regarding training-induced changes in pennation angle can be explained by the difference in the extent of hypertrophy or difference in measurement regions among studies.

Chapter 3

1. Compared to the untrained controls, competitive athletes who repeat leg extension (simultaneous extensions of knee and hip joints) in their competitive and training activities had greater muscle volumes of the vasti (VL, VM and VI) but not of RF.
2. Competitive cycling training for 6 months induced muscle-specific hypertrophy: hypertrophy of VL, VM and VI but not of RF.
3. The muscular profiles of the quadriceps femoris in athletes were consistent with the longitudinal changes in muscle volume induced by competitive training.

Chapter 4
1. The activation level of RF during knee extension decreased with additional hip 
extension torque, whereas those of VL and VM increased. The changes depended on 
the intensity of hip extension torque.

Chapter 5

1. The activation level of RF was higher during the knee extension than that during leg 
extension exercise regardless of exercise intensity, but those of VL and VM were 
similar between the two exercises at the same intensity.
2. The activation level of RF at high intensity (80% of 1RM) of leg extension which is 
normally used for the purpose to induce muscle hypertrophy was substantially low.
3. The activation level of RF during the leg extension exercise was lower than those of 
VL and VM.

6-2. Muscle- and exercise-specific architectural adaptation of the quadriceps 
femoris and underlying mechanisms

Based on the results of Chapter 2 and Chapter 3, it was made clear that 
architectural adaptations of the quadriceps femoris differ among each muscle of the 
quadriceps femoris, especially between the monoarticular vasti and biarticular RF. This 
muscle specificity was exercise-dependent. Single-joint knee extension training induced 
greatest architectural response in RF (Chapter 2), whereas multi-joint leg extension 
training elicited hypertrophy of the vasti but not of RF (Chapter 3). Cross-sectional data 
of Chapter 3 (experienced oarsmen and cyclists) discard the possibility of time course 
difference in hypertrophic response between RF and the vasti. These findings indicate 
that architectural adaptation of the quadriceps femoris is muscle- and exercise-specific.
The results of Chapter 5 showed substantial activations of VL, VM and RF during knee extension exercise at 80% of 1RM, supporting the architectural response of the muscles following knee extension training in Chapter 2. However, inter-muscle difference in architectural responses in Chapter 2 (i.e., greater change in RF than in the vasti) cannot be explained by the difference in muscle activation during knee extension exercise. Therefore, it is possible that there are other factors rather than the difference in activation levels among muscles that are responsible for the observed findings. These factors might include the difference in fiber type composition or protein synthesis. On the other hand, the results of Chapter 4 and Chapter 5 would account for the exercise dependence of RF response. The major difference of training regimens between Chapter 2 and Chapter 3 is whether knee extension is accompanied by hip extension or not. The results of Chapter 4 indicated that additional hip extension torque decreased the activation of RF during knee extension. In fact, the activation level of RF during a leg extension exercise was lower than those of VL and VM, and lower than that during knee extension exercise (Chapter 5). Furthermore, the activation level of RF at a high intensity (80% of 1RM) of leg extension was substantially low (similar to that during 20% of 1RM of knee extension, Chapter 5), being insufficient stimulus to bring about a hypertrophic response of RF induced by leg extension training regardless of the training intensity. Taken together, it can be said that whether hip extension is involved in exercises is attributable to the differences in muscle activation levels among muscles and between exercises, which are the major factors for the muscle- and exercise-specific adaptation of the quadriceps femoris.

6-3. Possibility of generalization of the findings: Monoarticular vs. biarticular
The results of Chapter 2 and Chapter 3 raise a possibility that single-joint training induces prominent hypertrophy of biarticular muscles whereas multi-joint training does not. If so, similar observations should be obtained in other thigh muscles (e.g., hamstrings and adductors). Regarding the single-joint training, Housh et al. (1992) showed that knee flexion training for 8 weeks induced increases in ACSA of the biarticular hamstrings (biceps femoris long head, semitendinosus and semimembranosus). However, they did not examine the monoarticular biceps femoris short head. To our knowledge, no information on inter-muscle difference in hypertrophic response of hamstrings is available from the literature. Therefore, it is unclear whether the results of Chapter 2 can be generalized in terms of anatomical features, although single-joint training can induce hypertrophy of the biarticular muscles.

With respect to multi-joint training, to examine the above notion, the same measurements were conducted for the subjects (cyclists) in the Chapter 3, section 2, because hamstrings and adductors act as agonists (knee flexors and hip extensors) during pedaling motions (Dostal et al. 1986; Richardson et al. 1998; Endo et al. 2007; Gondoh et al. 2009). Briefly, from the MR images, muscle volumes of each muscle of the hamstrings (biceps femoris short head, biceps femoris long head, semitendinosus, semimembranosus), gracilis and sartorius were obtained. Regarding the adductors (adductor magnus, adductor longus, adductor brevis, pectineus, they are monoarticular muscles), it was difficult to separate individual muscles along the thigh. Therefore the data of total adductors were obtained. As a result, the volumes of the biceps femoris short head, semitendinosus and total adductors were significantly greater in the
experienced cyclists than those in the untrained controls, and significantly increased following competitive cycling training for 6 months. On the other hand, the volumes of the biceps femoris long head, semimembranosus, gracilis and sartorius were similar between the two groups and did not change by competitive cycling training for 6 months (Fig. 6-1). In the control group, there was no change in any muscles. These results demonstrate that competitive cycling training induces increases in volumes of monoarticular muscles but not of biarticular muscles except for the semitendinosus. It is difficult to refer to the reasons for the difference in hypertrophic response among the semitendinosus and other biarticular muscles, but may be related to the difference in the function during knee flexion or hip extension exercises among the hamstrings (Ono et al. 2010, 2011).

6-4. Applicability of the findings

Implications for the training regimen for recovering from muscle atrophy

The current findings demonstrate that hypertrophic response of the quadriceps femoris following training is muscle- and exercise-specific, especially between RF and the vasti. If atrophic response is also muscle-specific, the present results can hint to the selection of effective training regimen against muscle atrophy. Regarding this issue, some previous studies showed that unloading resulted in nonuniform atrophy of the quadriceps femoris. For example, Belavy et al. (2009) observed 15.9% decrease in muscle volume of the vasti after bed rest for 56 days. Although the relative change of RF volume was -5.1%, it was not statistically significant. The results of this thesis suggest that leg extension training rather than knee extension training can be useful for the recovery from atrophy of each muscle of the quadriceps femoris after unloading.
Note that if we conduct only knee extension training after unloading condition, the muscle volume distributions within the quadriceps femoris would change greatly, because unloading can change all muscle in the quadriceps whereas knee extension training can change RF size greatly. Possibly, inter-individual variability of muscle volume constituents in untrained men might be related to this possibility.

Another factor for inducing muscle atrophy is aging (Frontera et al. 2000). It has been shown or suggested that aging induces prominent atrophy of the quadriceps femoris compared to other muscle groups (Frontera et al. 2008; Ogawa et al. 2012). It was shown that the extent of atrophy was similar among the four muscles of the quadriceps femoris (Trappe et al. 2001). However, the determination of muscle size in the previous study (Trappe et al. 2001) was insufficient, because although they reported muscle volume for each muscle, they did not include the proximal regions of the thigh where RF ACSA was comparatively large. Therefore, there is a possibility that their finding lacks complete accuracy. To clarify this issue, the volume of each muscle of the quadriceps femoris should be measured incorporating all the cross-sectional information from origin to insertion. This was done by the author’s group, and comparison was made between young men and elderly men. Briefly, MR images of the right thigh were obtained from health young (26 ± 3 yr, n = 26) and elderly men (71 ± 4 yr, n = 22). From the MR images, the volume of each muscle and the relative volume to the total quadriceps femoris were determined. The two-way ANOVA showed a significant interaction of group × muscle (P < 0.001). The volumes of all four muscles were significantly smaller in the elderly men than in the young men. The difference between the two groups reached 38% for RF, 27% for VL, 21% for VM and 26% for VI, respectively. The percentage of RF volume was significantly lower in the elderly men.
than in the young men, and vice versa for VM. (Fig. 6-2). These results suggest that atrophy induced by aging is more prominent in RF than in the other three muscles of the quadriceps femoris. Possible reasons for the inter-muscle difference in the extent of atrophy related to aging can be explained by 1) the difference in fiber type composition among the muscles and/or 2) the findings from Chapter 4 and 5. It was suggested that aging-related atrophy was prominent in type II than in type I fibers (Lexell et al. 1988; Nilwik et al. 2013). The percentages of type II fibers are 65% for RF, 60% for VL and 48% for VM (mean of surface and deep, Johnson et al. 1973). Thus, higher percentage of type II fiber in RF and lower in VM may account for the above results. In addition, the results of Chapter 4 and 5 showed that the activation level of RF was substantially low during leg extensions. For ordinary individuals who do not have regular exercise habit in daily life, physical activities of daily living can be stimulus to the muscles that otherwise are liable to develop atrophy by aging. Daily physical activities often include leg extensions but not so much for knee extensions alone. Therefore, activities of daily living cannot be major stimulus to RF. Taking into account the results of Chapter 2 and Chapter 3, knee extension training should be conducted as a training modality for the elderly people, at least for maintaining quadriceps femoris size and its constituents.

**Implications for the research field**

The present study puts forward serious reconsideration of methodology for evaluation of muscle size of the quadriceps femoris. In order to assess the quadriceps femoris size, numerous studies have measured muscle thickness (sum of RF and VL thickness) at 50% of the thigh length (e.g., Abe et al. 1994, 2014; Sanada et al. 2006). Based on the results of Chapter 2 and Chapter 3, I can say that, at least for the rowers,
cyclists and elderly people, the muscle thickness (sum of RF and VI) can underestimate the whole quadriceps femoris size. In addition, the magnitude of exercise-induced change in the size of the quadriceps femoris can be erroneously evaluated. For example, Kubo et al. (2003) who examined the effect of body mass-based squat training in middle-aged women showed a significant increase in knee extension strength but no change in muscle thickness (sum of VL, VI and RF) at 50% of the thigh length. The findings of Chapter 3 and Chapter 5 suggest that leg extension training does not induce an increase RF size. Also, the results of Chapter 2 and Wells et al. (2014) demonstrated the nonuniform hypertrophy of VL and VI across the muscle. Thus, it is difficult to draw a conclusion that squat training in Kubo et al. (2003) did not improve muscle size of the quadriceps femoris. The findings of this thesis indicate the need for taking into account the possibility of inhomogeneous response of muscle architecture following training.

6-5. Functional significance of RF hypertrophy

The current findings suggest that the role of RF during leg extensions is trivial at most. Some unique roles of the biarticular muscles during leg extensions have been proposed, such as tendon action (transfer of mechanical energy, Prilutsky and Zatsiorsky 1994) and control of the direction of the force (van Ingen Schenau et al. 1992). Theoretically, these actions should not necessarily need high activations during motions. Thus, it is possible that RF does not need to be hypertrophied to achieve human movements. However, owing to its biarticular nature, RF can show a large force-generating potential at some joint positions where those of monoarticular vasti are small. As mentioned in the review of literature, when the hip is extended, RF operates at its optimal length even when the vasti are on the ascending limb of the force-length
curves (i.e., knee joint is extended, Herzog et al. 1990). This was supported by my previous studies (Ema et al. 2010, 2012). These studies suggest that RF has a significant length-dependent force-generating potential at the knee and hip extended positions. In addition, estimated shortening velocity of biarticular muscles is lower than that of monoarticular muscles during multi-joint leg extensions (Gregoire et al. 1984; van Ingen Schenau et al. 1992). This indicates that velocity-dependent force-generating potential of RF is larger compared to the monoarticular vasti during multi-joint leg extensions. Namely, the contribution of RF to the total knee extension moment can be considerably large at knee and hip joints extended positions. These joint positions are frequently observed during sport activities and human movements (e.g., push off phase during walking and running, ball kicking in soccer and rugby). In these situations, a hypertrophic change of RF might therefore increase the ground reaction force on the push off phase and ball speed after kicking. Notably, it is possible that RF hypertrophy enables the elderly people with smaller RF size (results of Chapter 6) and smaller step length (Himann et al. 1988) to increase the stride length during walking and running.

An increase in RF size may be significant in terms of attenuation of muscle fatigue during exercises. Muscle hypertrophy is accompanied by the increase in force-generating potential of muscle fibers. This can reduce the number of muscle fiber recruitments so as to exert a given force. The unique roles of the biarticular muscles mentioned above would be associated with lower activation level compared with the vasti. Repetitive leg extension exercises including rowing and cycling can also result in substantial RF fatigue, but hypertrophied RF might contribute to attenuate RF fatigue, leading to maintain a performance during leg extension exercises.
6-6. Factors that can influence interpretation of the current findings

Event-related profiles in athletes

It is possible that the results of Chapter 3 are not specific to athletes who repeat leg extensions during sport activities but common to other athletes (such as field sport athletes). To attack this issue, the volume of RF was determined in lacrosse players \(n = 13\) and sprinters \(n = 9\) and compared to that in untrained men \(n = 10\). A one-way factorial ANOVA followed by Bonferroni test demonstrated that the volume of RF in the lacrosse players \(P < 0.05\) and sprinters \(P < 0.05\) was significantly greater in the untrained men. This result was consistent even when the muscle volume was normalized by body mass (Fig. 6-3). Field sports as well as sprint running involve repeated sprint activities (Spencer et al. 2005). The RF is activated during swing phase as a hip flexor (Simonsen et al. 1985), especially at high running speed (Kyrolainen et al. 2005). These facts may be related to the greater RF in the lacrosse players and sprinters than in the untrained men. Hip (leg) flexion is involved in also rowing and cycling, but the current results showed non-responsiveness of RF in oarsmen and cyclists. This suggests that the extent of RF activation during leg flexion phase is not high enough to induce RF hypertrophy. In fact, the level of RF activity during leg flexion phase on rowing was smaller than that during leg extension phase (Guével et al. 2011; Turpin et al. 2011). Taken together, it is highly likely that inferior musculature of RF is a common feature only among athletes repeating leg extensions with involving small RF activity as the hip extensor (oarsmen and cyclists).

6-7. Conclusion of the thesis
The general purpose of this thesis is to examine the architectural adaptation of the individual muscles of the quadriceps femoris induced by the different type of joint motions. The results indicate that training-induced architectural changes of the quadriceps femoris differ among the four muscles of the quadriceps femoris and between exercises: knee extension and leg extension. Further studies led to a conclusion that this is due to the different magnitude of activation levels during training, depending in part on the difference in the number of joints that the muscles cross.
Fig. 6-1 The volume of each muscle (upper) of the hamstrings, total adductors, gracilis and sartorius of the experienced cyclists (black bar) and untrained men (white bar), and relative changes in muscle volume after competitive cycling training for 6 months (lower). * indicates a significant difference between the two groups. # indicates a significant change as a result of regular training of competitive cycling for 6 months. $BF_{long}$, biceps femoris long head; $BF_{short}$, biceps femoris short head; ST, semitendinosus; SM, semimembranosus; $ADD_{total}$, total adductors, Gr, gracilis; Sar, sartorius.
Fig. 6-2 The volume of each muscle of the quadriceps femoris (upper) of the young men (black bar) and elderly men (white bar), and the percentage of each muscle volume to the total quadriceps femoris volume (lower). * indicates a significant difference between the two groups. RF, rectus femoris; VL, vastus lateralis; VM, vastus medialis; VI, vastus intermedius.
Fig. 6-3 The absolute volume (upper) and volume relative to body mass (lower) of the rectus femoris (RF) in the lacrosse players (black bar), sprinters (gray bar) and untrained men (white bar). * indicates a significant difference between the groups.
References


output and work done by the human triceps surae muscle-tendon complex in jumping. J Biomech 19: 899-906


59. Franchi MV, Atherton PJ, Reeves ND, Flück M, Williams J, Mitchell WK, Selby A,


84. Ikai M, Fukunaga T (1968) Calculation of muscle strength per unit cross-sectional area of human muscle by means of ultrasonic measurement. Int Z Angew Physiol 26: 26-32


144. Raj IS, Bird SR, Shield AJ (2012) Reliability of ultrasonographic measurement of
the architecture of the vastus lateralis and gastrocnemius medialis muscles in older adults. Clin Physiol Funct Imaging 32: 65-70


Acknowledgements

I would like to express my gratitude to everyone who supported me. In particular, my supervisor, Dr. Yasuo Kawakami gave me a lot of wonderful experience on my research life at Waseda University. I had a fateful encounter with him when I was a second year undergraduate student. This thesis would not have been possible without his support and encouragement.

I would also like to express my thanks to the following people:

Dr. Toshimasa Yanai, for his advice and useful suggestions for interpretation of my study. I learned the approaches to the science from him.

Dr. Kazuyuki Kanosue, for his valuable comments for this thesis. His suggestions helped me to expand my scope in sport sciences.

Dr. Hiroaki Kanehisa (National Institute of Fitness and Sports in Kanoya), for his thoughtful comments and encouragements. I would not complete the thesis as well as my published studies without his guidance.

Dr. Taku Wakahara (Doshisha University), for his guidance and helpful comments. All of my researches would not exist without his support. I learned a lot of things to write articles and attitude to the sciences from him.

Dr. Toshihiko Komatsu (Osaka University), Dr. Naokazu Miyamoto (National Institute of Fitness and Sports in Kanoya), Dr. Masanori Sakaguchi (University of Calgary), Mr. Yasuyoshi Mogi, Mr. Kosuke Hirata (National Institute of Fitness and Sports in Kanoya) and Mr. Takuya Yanaka, for their helps on my previous and current experiments.

My deepest appreciation goes to all members of the Biomechanics Laboratory for their scientific advices and suggestions.
Finally, I would like to show my greatest appreciation to my family and friends. Since they have watched my research life affectionately, I can finish my research career as a Ph.D. candidate.

This thesis was supported in part by the Grant-in-Aid for JSPS Fellows (no. 25-2723), Grant-in-Aid for Scientific Research (no. 24300209; 21700630) from Japan Society for the Promotion of Science, and Waseda University Global COE program, “Sport Sciences for the Promotion of Active Life”. 