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博士（スポーツ健康科学）

Acute and chronic effects of resistance training on passive stiffness of the hamstring muscles

レジスタンストレーニングがハムストリングスの受動的な硬さに及ぼす急性的・慢性的影響

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Chapter 1. General introduction

1.1. Preface

Resistance training is an effective way to increase muscular strength and size, and thus is widely prescribed in sports and rehabilitation fields. Meanwhile, no clear consensus has been reached regarding the effects of resistance training on passive muscle stiffness. For example, some studies reported an acute increase in muscle stiffness after resistance exercise (Green et al., 2011; Lacourpaille et al., 2014; Xu et al. 2018; Ema et al., 2021), while others reported its acute decrease (Andonian et al. 2016; Morales-Artacho et al. 2016; Hotfied et al. 2017). Passive muscle stiffness is reported to be one of the factors that limit the joint range of motion (ROM [Miyamoto et al. 2018; Hirata et al. 2020]). The limitation of joint ROM was suggested to negatively affect the athletic performance of some sporting events (García-Pinillos et al. 2015; Panoutsakopoulos et al. 2021) and increase the risk to sustain a musculoskeletal injury (Witvrouw et al. 2003; Watsford et al. 2010; Kumagai et al. 2019). Hence, an understanding of the training-induced changes in passive muscle stiffness is important in terms of physical performance enhancement and injury risk reduction.

Passive muscle stiffness has been shown to be decreased by stretching. For example, the passive muscle stiffness was immediately decreased by the passive muscle lengthening (Nakao et al. 2018). Moreover, the magnitude of immediate decrease in passive muscle stiffness was influenced by muscle lengths (Freitas et al. 2015) and duration (Caliskan et al. 2019) during static stretching. Thus, it is possible that the program variables of resistance exercise (contraction mode, exercise ROM, muscle length, and exercise duration) influence the magnitude of decrease in the stiffness. Nevertheless, it remains unclear about the acute effects of resistance exercise with different program variables on passive muscle stiffness. Moreover, only limited studies have investigated the chronic changes in muscle stiffness by resistance training.

The general purpose of this thesis is to clarify the acute and chronic effects of resistance training on the passive stiffness of the hamstring muscles. To this end, I investigated the acute changes in passive stiffness of the biarticular hamstring muscles (biceps femoris long head, BFlh; semitendinosus, ST; semimembranosus, SM) after resistance exercise with different combinations of contraction modes and exercise ROMs in Chapter 2, and different combinations of muscle lengths and exercise durations in Chapter 3. Through the experiments in Chapters 2 to 3, the program variables that can acutely decrease muscle stiffness are selected for clarifying the chronic effects of resistance training on muscle stiffness. In Chapter 4, I examined the chronic changes in passive stiffness of the biarticular hamstring muscles after resistance training with the combination of selected program variables.

1.2. Terminology

Resistance exercise

Resistance exercise is an exercise that applies resistance to muscles. This term does not include running and cycling exercises in the present thesis.

Resistance training

Resistance training is a series of resistance-exercise sessions performed over a period of time (more than 4 weeks according to Ichihashi et al. [2016]).

Young's modulus

Young's modulus is a ratio of the longitudinal stress (applied force per unit area of a material) to the corresponding longitudinal strain (ratio of a deformation of the material to its original lengths). This term represents the tendency of the material to deform axially when forces opposite and parallel to this axis are applied.

Shear modulus

Shear modulus is a ratio of the shear stress (applied shear force per unit area of a material) to the corresponding shear strain (ratio of a deformation of the material to its original shape). This term represents the tendency of the material to change its shape in order to keep a constant volume.

Passive muscle stiffness

Passive muscle stiffness is one of the representative indicators of muscle mechanical properties during passive muscle lengthening. Specifically, this term indicates the magnitude of a force required for a given change in muscle length along the longitudinal axis during passive muscle lengthening (Leonard et al. 2004). In the present thesis, muscle shear modulus measured parallel to muscle fascicle with an ultrasound shear wave elastography is used as a measure of passive muscle stiffness based on previous studies (Hirata et al. 2016; Miyamoto et al. 2017). Meanwhile, muscle may contract involuntarily during passive muscle lengthening, referred to as muscle tone (DeMott and Flinn, 2020). However, a previous study observed that electromyographic (EMG) activity was very low (less than 10 μV) during passive muscle lengthening in healthy female volunteers (Magnusson et al.1996). Additionally, EMG activity during the passive lengthening did not differ between the participants with low and high levels of joint flexibility (evaluated by toe-touch test) in healthy male volunteers (Magnusson et al. 1997). Thus, the muscle tone seems to have a negligible effect on passive muscle stiffness in healthy individuals.

Joint ROM

Joint ROM is generally defined as the extent of a joint movement without restriction or pain. Traditionally, the joint ROM has been used as a surrogate index of passive muscle stiffness. In the present thesis, this term is defined as the difference in joint angles between the initial position and the final position that the participants started to feel discomfort or pain (Miyamoto et al. 2017).

Stretch tolerance

In general, stretch tolerance is defined as a threshold for starting to feel discomfort during passive muscle lengthening. In the present thesis, stretch tolerance was assessed from passive torque at the final position (Magnusson et al. 1997).

Acute change

Resistance exercise-induced changes in the muscle shear modulus were reported to last for 72 h (Ema et al. 2021). Thus, the acute change in muscle shear modulus is defined as the changes occurring within 72 h in the present thesis. In particular, the changes within 3 min are defined as the immediate change.

Chronic change

Changes in the muscle shear modulus were observed for at least after 4 weeks of static-stretching intervention (Ichihashi et al. 2016). Therefore, the chronic change in muscle shear modulus is defined as the changes that occur after intervention of 4 weeks or longer in the present thesis.

1.3. Review of literature

The purpose of this thesis is to investigate the acute and chronic effects of resistance training on the passive stiffness of the hamstring muscles. In this section, related studies are overviewed from the following viewpoints: 1) determinants of passive muscle stiffness, 2) Methodology for quantifying passive muscle stiffness, 3) the acute and chronic effects of stretching on passive muscle stiffness, and 4) the acute and chronic effects of resistance training on passive muscle stiffness. The second viewpoint was included to gain insight into possible program variables of resistance exercise that acutely influence the muscle stiffness and the association between the acute and chronic changes in the stiffness.

1.3.1. Determinants of passive muscle stiffness

One of the remarkable properties of **s**keletal muscle is its contractility. Hence, a number of physiologists have studied the characteristics of muscle contraction (Wilkie, 1949, 1953; Rassier et al. 1999; Fukunaga et al. 2002). In contrast to such great attention to mechanical properties of muscle in the contracted state, that in the relaxed state has not been relatively studied.

Passive muscle stiffness has been suggested to be influenced by the stable binding of myosin to actin filaments (Hill, 1968), titin (Freiburg et al. 2000), and muscle connective tissue (Gajdosik et al. 2001). Firstly, the passive muscle stiffness is likely to be influenced by the stable binding (cross-bridges) of myosin to actin filaments, known as the main protein filaments of a sarcomere. It is well known that the myosin heads bind to the actin filaments through an increase in the intracellular Ca^{2+} concentration followed by a structural change in troponin during muscle contraction (Huxley and Taylor, 1958). Meanwhile, Hill (1968) stated that some cross-bridges exist stably in the relaxed state, and generate a passive tension to stretching of a sarcomere. The number of stable cross-bridges was suggested to increase under certain conditions (Proske and Morgan 1999; Whitehead et al. 2001). For example, Whitehead et al. (2001) mentioned that the number of stable cross-bridges increased after high-load eccentric exercise that induces severe muscle damage, possibly due to the acute increase in the intramuscular Ca^{2+} concentration (Chen et al. 2007). In addition, Proske and Morgan (1999) stated that the number of stable cross-bridges increased with muscle elongation, possibly due to an increase in the sensitivity of muscle fibers to Ca^{2+} (Stephenson and Wendt 1984; Balnave and Allen 1996; Claflin et al. 1998). Collectively, increasing the number of stable cross-bridges appears to increase passive muscle stiffness after high-load eccentric exercise and/or at long muscle lengths. Secondly, the passive muscle stiffness is affected by the titin, an elastic giant protein that spans half of the sarcomere from the Z-disc to the M-line regions (Fig. 1-1). The titin acts as a spring to maintain the central position of the myosin filament within the sarcomere and develops a passive tension to stretching of the sarcomere (Freiburg et al. 2000). The I-band region of the titin has two spring elements consisting of Ig and PEVK segments. These segments are extensible, whereas the binding region of the titin and Z-disc is inextensible (Trombitás et al. 1998). Previous studies observed that the passive tension of a sarcomere to its stretching was greatly reduced after the titin was selectively degraded by low doses of ionizing radiation (Horowits et al. 1986) and trypsin (Yoshioka et al. 1986). Thus, titin is a determinant of passive muscle stiffness. Finally, the passive muscle stiffness is influenced by the connective tissue comprising the muscle. The muscle connective tissue consists of epimysium, perimysium, and endomysium, which surround the muscle, fascicle, and fiber, respectively. The muscle connective tissue contains abundant collagen with fibrous networks. In the resting state, the collagen fibers have a wavy shape, called crimp (Woo et al. 1994). During the early phase of muscle elongation, the collagen fibers are straightened and then elongated with the development of a passive tension during the late phase of muscle elongation. Thus, the muscle connective tissue consisting of the abundant collagen fibers generates a passive tension to stretching of the muscle, fascicle, and fiber. Johns and Wright (1962) reported that the muscle connective tissue accounts for more than 30% of muscle weight. Moreover, Light et al. (1985) observed that the proportion of dry material to total dry muscle was substantially larger in the perimysium (mean value: 4.1%) than in the epimysium (1.8%) and endomysium (0.3%) in six bovine muscles. Based on these findings, the muscle connective tissue, especially the perimysium, would influence the passive muscle stiffness.

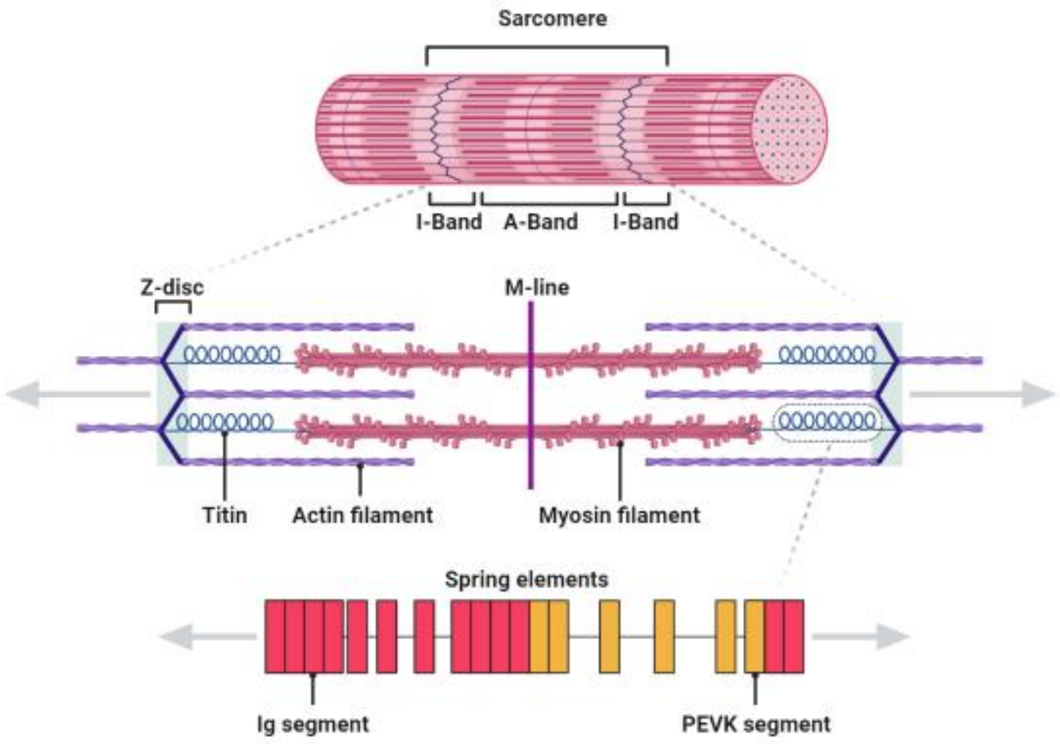

Fig. 1-1 Structure of titin during sarcomere stretching

1.3.2. Methodology for quantifying passive muscle stiffness

In human experiments, the passive mechanical properties of muscle have been evaluated by some approaches. A traditional approach for evaluating the passive mechanical properties of muscle *in vivo* is to measure the relationship between joint angle and passive joint torque (Magnusson et al. 1996; Magnusson 1997; Gajdosik 2001; Kataura et al. 2017; Takeuchi and Nakamura, 2021; Fukaya et al. 2021; Takeuchi et al. 2021). The slope of this relationship is defined as joint stiffness (Magnusson et al. 1997). The slope represents the passive joint torque for a given change in joint angle. Theoretically, the passive joint torque increases as increasing the amount of tissue elongation with changes in joint angle. This implies an increase in tissue stiffness. Previous studies reported that the joint stiffness was immediately changed by stretching (Noardez et al. 1998; Kataura et al. 2017; Takeuchi and Nakamura, 2021; Takeuchi et al. 2021; Fukaya et al. 2021) and a session of resistance exercise (Whitehead et al. 2001; Hoang et al. 2007). Additionally, the joint stiffness was reported to be changed by resistance training (Klinge et al. 1997), long-term stretching intervention (Magnusson et al. 1996), muscle hypertonia (Broberg and Grimby, 1983), and hemiparetic stroke (Given et al. 1995). It should be noted that the joint torque results from a composite of contractile (i.e., synergists and antagonists) and non-contractile (e.g., tendon, skin, ligament, nerve, and articular structure) tissues. Thus, the joint stiffness is not suitable for evaluating the stiffness of the individual muscles.

Another approach for evaluating passive mechanical properties of muscle *in vivo* is to measure a change in architectural parameters during passive muscle lengthening using B-mode ultrasound apparatus (Herbert et al. 2002; Kawakami et al. 2008; Morse et al. 2008; Abellaneda et al. 2009; Nakamura et al. 2011; Blazevich et al. 2012; Nakamura et al. 2013). For example, the displacement of the muscle-tendon junction has been used as a measure of the degree of muscle extensibility during passive muscle lengthening (Kawakami et al. 2008). Nakamura et al. (2011, 2013) revealed the behaviors of muscle-tendon unit during passive muscle lengthening were immediately and chronically changed after stretching. However, the displacement of the muscle-tendon junction reflects not only the mechanical properties of the muscle but also that of the tendon. A major drawback of this method is that it does not assess a force applied to the muscle-tendon junction during passive muscle lengthening. Thus, the passive muscle stiffness cannot be calculated. An alternative approach is required to evaluate the stiffness of the individual muscles.

To quantitatively assess the passive mechanical properties of the individual muscles *in vivo*, ultrasound elastography has been recently used (Freitas et al., 2015; Maeda et al. 2017; Miyamoto et al. 2017; Lacourpaille et al. 2017; Ema et al., 2021). The ultrasound elastography is classified into some methods (e.g., strain elastography and shear wave elastography) based on the physical quantities related to tissue elasticity and methods that excite a tissue. In strain elastography, an operator applies vertical manual pressure to the body surface with the ultrasound probe (the rhythmical compression-relaxation cycles). Subsequently, an elastographic image is obtained based on the principle that softer tissue deforms to a greater degree (larger strain) compared to harder tissue. The ratio between the strain of a region of interest in the target tissue and that of a reference material (e.g., acoustic coupler) is calculated and defined as muscle hardness. Some researchers observed acute changes in the muscle hardness after stretching (Maeda et al. 2017) and resistance exercise (Yanagisawa et al. 2015; Murayama et al. 2022). However, the reproducibility of strain elastographic measurement is substantially influenced by the patterns of the rhythmical compression-relaxation cycles (Bamber et al. 2013). Moreover, the strain ratio quantified by strain elastography does not represent the absolute value of muscle stiffness.

The ultrasound shear wave elastography can measure a localized tissue stiffness based on the following principle (Fig. 1-2). First, an ultrasound probe generates an acoustic radiation force impulse that perturbs peripheral tissue. Second, the perturbation of the peripheral tissue results in the propagation of a transient shear wave. Finally, the shear wave propagation velocity along the principal axis of the probe is measured by an ultrafast echographic imaging and can be used to calculate the shear modulus, which is accepted as an index of passive stiffness (Eby et al. 2013). The shear modulus is calculated from the shear wave velocity as follows:

$$\mu = \rho Vs^2$$

where μ is the shear modulus (Pa), ρ is the muscle density (1,055 kg/m^3 [Ward and Lieber, 2005]), and Vs is the shear wave speed (m/s).

If the soft tissue is assumed to be linear elasticity, homogeneous density, and isotropic, Young's modulus can be calculated by the following equation:

$$E = 3\mu$$

where E is Young's modulus (Pa).

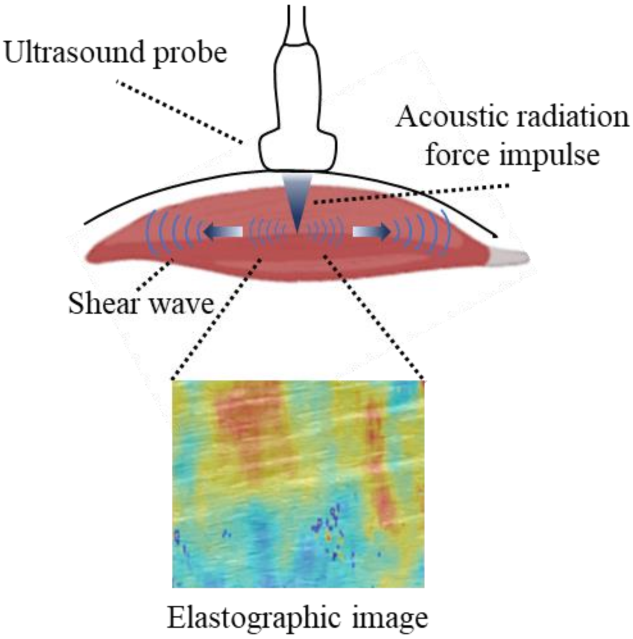

Fig. 1-2 Basic principle of ultrasound shear wave elastography

However, this assumption does not hold for skeletal muscle due to its inhomogeneous and anisotropic mechanical properties (Gennisson et al. 2003). Eby et al. (2013) reported that the muscle shear modulus obtained via ultrasound shear wave elastography was highly correlated with Young's modulus of the brachialis obtained via a traditional tensile test. Furthermore, the reproducibility of this method has been confirmed in the biarticular hamstring muscles (intraclass correlation coefficients [ICC] = 0.97–0.99 [Miyamoto et al. 2017]) and the triceps surae muscles (ICC = 0.97–1.00 [Hirata et al., 2016]). Collectively, the ultrasound shear wave elastography is a promising approach to evaluate the stiffness of the individual muscles. Several researchers have used this approach to investigate the stiffness of the individual muscles during passive (Le Sant et al. 2015; Xu et al. 2018) and active conditions (Yoshitake et al. 2014: Evangelidis et al. 2021). Besides, the ultrasound shear wave elastography was also used to examine the effects of musculoskeletal injuries (Taş et al, 2018; Zhou et al. 2018; Kumagai et al. 2019) and diseases (Eby et al. 2017; Wu et al. 2017; Le Sant et al. 2019) on the muscle stiffness and the effects of the muscle stiffness on athletic performance (Miyamoto et al. 2019; Ema et al. 2022; Yamazaki et al. 2022) and the risk of injury (Kumagai et al. 2019). Other studies investigated the sporting event-specific differences in the muscle stiffness with the ultrasound shear wave elastography (Miyamoto et al. 2019; Avrillon et al. 2020). Moreover, recent studies also investigated the acute and chronic changes in muscle stiffness after stretching and resistance training as reviewed below.

1.3.3. Acute and chronic effects of stretching on passive muscle stiffness

Since the 2010s, several researchers have investigated the acute effects of static stretching on passive muscle stiffness using ultrasound shear wave elastography (Table 1-1). Umegaki et al. (2015) showed that the shear moduli of the biarticular hamstring muscles decreased immediately after 300 s (five repetitions of 60 s per repetition) of static stretching at the knee extended position with the hip flexed. They also observed that the magnitude of the decrease in the shear modulus was the largest in SM among the hamstrings. Hirata et al. (2016) also reported that the shear modulus of the medial gastrocnemius decreased immediately after 300 s (one repetition of 300 s per repetition) of static stretching at the dorsiflexed position with the knee extended, whereas the shear modulus of the lateral gastrocnemius and soleus was not significantly changed. Miyamoto et al. (2017) revealed that the static stretching at the hip flexed position with the knee extended for 450 s (five repetitions of 90 s per repetition) immediately decreased the shear moduli of ST and SM, whereas the static stretching at the knee extended position with the hip flexed for 450 s (five repetitions of 90 s per repetition) immediately decreased the shear moduli of BFlh, ST, and SM. A similar observation was reported in a previous study (Nakao et al. 2018), which showed an immediate decrease in the shear moduli of BFlh, ST, and SM after 150 s (five repetitions of 30 s per repetition) of passive knee extension stretching. Additionally, Hirata et al. (2020) investigated the immediate effects of 450 s-static stretching (five repetitions of 90 s per repetition) at dorsiflexed position with the knee extended on muscle shear modulus of the triceps surae (the medial and lateral gastrocnemii and soleus) in younger and older men. They observed that the shear moduli of the middle and distal regions within the medial gastrocnemius and the lateral gastrocnemius immediately decreased in younger men, and the shear modulus of the distal region within the medial gastrocnemius immediately decreased in older men. Nakao et al. (2018) also showed that the shear moduli of BFlh, ST, and SM decreased immediately after 150 s (five repetitions of 30 s per repetition) of dynamic knee extension stretching. Additionally, Pamboris et al. (2018) reported an immediate decrease in the shear wave velocity of the medial gastrocnemius after dynamic dorsiflexion stretching (three sets of 20 repetitions [a duration per repetition was not described]). These studies showed that passive muscle stiffness is immediately decreased by static and dynamic stretching.

Some studies have investigated the effects of program variables of stretching on passive muscle stiffness. For example, Freitas et al. (2015) investigated the immediate effects of static stretching at the dorsiflexed position with the knee extended for 600 s at different joint ROMs (40%, 60% and 80% of maximal joint ROM) on the shear modulus of medial gastrocnemius. They observed an immediate decrease in the shear modulus only after static stretching at 80% of maximal joint ROM. Nakamura et al. (2021) demonstrated that the shear modulus of the rectus femoris decreased immediately after 180 s of the static stretching (three repetitions of 60 s per repetition) at the knee flexed position with the hip extended at 100% of maximal knee joint ROM, but not after the stretching at 80% or 120% of maximal knee joint ROM. Meanwhile, Caliskan et al. (2019) examined the immediate effects of static stretching at the knee flexed position with the hip extended with different durations (120 s and 300 s) on the shear modulus of the rectus femoris. The results showed that the shear modulus decreased immediately after 300 s of the static stretching, but not after 120 s of the stretching. Fukaya et al. (2020) investigated the immediate effects of static stretching at the dorsiflexed position with the knee extended with a wide joint ROM (120% of maximal joint ROM) and a short duration (100 s) or a narrow joint ROM (50% of maximal joint ROM) and a long duration (240 s) on the shear modulus of the medial gastrocnemius. They revealed that the shear modulus was greatly decreased immediately after the stretching with a wide joint ROM and a short duration than after the stretching with a narrow joint ROM and a long duration. These findings imply that passive muscle stiffness is immediately influenced by the program variables of static stretching, such as joint ROM (muscle lengths) and duration. Specifically, static stretching with a wide ROM (a long muscle length) and a long duration appears to greatly decrease muscle stiffness.

Table 1-1. Program variables and main findings of the previous studies investigating the acute changes in muscle stiffness after stretching

Author Year	Stretching type	Position	Repetition	Duration per repetition (s)	Muscle	Measurement point	Result
Freitas et al. 2015	Static dorsiflexion stretching with full knee extension	40% of maximal dorsiflexion ROM	1	600	MG	Pre Immediately after	Not change
		60% of maximal dorsiflexion ROM					Not change
		80% of maximal dorsiflexion ROM					Decrease
Umegaki et al. 2015	Static knee extension stretching with 90° hip flexion	Maximal knee extension ROM	5	60	BFlh	Pre Immediately after	Decrease
					ST		Decrease
					SM		Decrease
Hirata et al. 2016	Static dorsiflexion stretching with full knee extension	Maximal dorsiflexion ROM	1	300	MG	Pre Immediately after	Decrease
					LG		Not change
					SOL		Not change
Miyamoto et al. 2017	Static knee extension stretching with 120° hip flexion	Maximal knee extension ROM	5	90	BFlh	Pre Immediately after	Decrease
					ST		Decrease
					SM		Decrease
	Static hip flexion stretching with 0° knee extension	Maximal hip flexion ROM			BFlh		Decrease
					ST		Decrease
					SM		Decrease
Nakao et al. 2018	Passive knee extension stretching with 90° hip flexion	90° knee flexion to maximal knee extention ROM	5	30	BFlh	Pre Immediately after	Decrease
					ST		Decrease
					SM		Decrease
	Dynamic knee extension stretching with 90° hip flexion				BFlh		Decrease
					ST		Decrease
					SM		Decrease
Pamboris et al. 2018	Dynamic dorsiflexion stretching with full knee extention	Full plantarflexion to full dorsiflexon	60	-	MG	Immediately after	Increase
Caliskan et al. 2019	Static knee flexion stretching with full hip extension	Not controlled	2	30	RF	Pre Immediately after	Not change
			5				Decrease
Fukaya et al. 2020	Static dorsiflexion stretching with full knee extension	50% of maximal dorsiflexion ROM	1	240	MG	Pre Immediately after	Decrease
		120% of maximal dorsiflexion ROM		100			Decrease
Hirata et al. 2020	Static dorsiflexion stretching with full knee extension	Maximal dorsiflexion ROM	5	90	MG (middle)	Pre Immediately after	Decrease (younger)
					MG (distal)		Decrease (younger, older)
					LG		Decrease (younger)
					SOL		Not change (both group)

Table 1-1. Program variables and main findings of the previous studies investigating the acute changes in passive muscle stiffness after stretching (continued)

Author Year	Stretching type	Position	Repetition	Duration per repetition (s)	Muscle	Measurement point	Result
Nakamura et al. 2020	Static knee flexion stretching with 90° hip flexion	80% of maximal knee flexion ROM	3	60	RF	Pre Immediately after	Not change
					VL		Not change
					VM		Not change
		100% of maximal knee flexion ROM			RF		Decrease
					VL		Not change
					VM		Not change
		120% of maximal knee flexion ROM			RF		Not change
					VL		Not change
					VM		Not change
Sato et al. 2020	Static dorsiflexion stretching with full knee extension	Maximal dorsiflexion ROM	1	20	MG	Pre Immediately 5 min 10 min after	Not change
Takeuchi et al. 2021	Static knee flexion stretching with 90° hip flexion	120% of maximal knee flexion ROM	3	20	RF	Pre Immediately after	Not change
					VL		Not change
					VM		Not change
				60	RF		Not change
					VL		Not change
					VM		Not change
		110% of maximal knee flexion ROM		60	RF		Decrease
					VL		Not change
					VM		Not change

Chronic effects of stretching intervention on the passive muscle stiffness have been examined in limited studies (Table 1-2). Ichihashi et al. (2016) investigated the chronic effects of 4 weeks (three times per week) of intervention of 300 s-static stretching (one repetition of 300 s per repetition) at the knee extended position with the hip flexed on the shear moduli of BFlh, ST, and SM. After the stretching intervention, the shear moduli of the biarticular hamstring muscles decreased, and the magnitude of the decrease in muscle shear modulus was largest in SM among the biarticular hamstring muscles. Additionally, Andrade et al. (2020) investigated the chronic effects of 12 weeks (five times per week) of intervention of 225 s-static stretching (five repetitions of 45 s per repetition) at the dorsiflexed position with the knee extended on the shear wave velocities in the proximal, middle, and distal regions of the medial and lateral gastrocnemius and the proximal and distal regions of the soleus. They reported a decrease in the shear moduli of all regions of the muscles, except for the proximal region of the lateral gastrocnemius after the stretching intervention.

It is interesting to note that the results of Ichihashi et al. (2016) are consistent with those of Umegaki et al. (2015) who investigated the immediate changes in the shear modulus of BFlh, ST, and SM after a static knee extension stretching. Specifically, both studies reported that the magnitude of the decrease in shear modulus was the largest in SM among the biarticular hamstring muscles after static stretching (Umegaki et al. 2015; Ichihashi et al. 2016), suggesting that the chronic changes in muscle stiffness correspond to their immediate changes.

Table 1-2. Program variables and main findings of the previous studies investigating the chronic changes in passive muscle stiffness after stretching

Author Year	Stretching type	Position	Repetition	Duration per repetition (s)	Frequency per week (day)	Intervention period (week)	Muscle	Measurement point	Result
Ichihashi et al. 2016	Static knee extension stretching with 90° hip flexion	Maximal knee extension ROM	1	300	3	4	BFlh	Pre 4 week after	Decrease
							ST		Decrease
							SM		Decrease
Andrage et al. 2019	Static dorsiflexion stretching with 20° knee flexion	Maximal plantar flexion ROM	5	45	5	12	MG (proximal)	Pre 12 week after	Decrease
							MG (middle)		Decrease
							MG (distal)		Decrease
							LG (proxomai)		Decrease
							LG (middle)		Decrease
							LG (distal)		Decrease
							SOL (proximal)		Not change
							SOL (distal)		Decrease

BFlh, biceps femoris long head; ST, semitendinosus; SM, semimembranosus; MG, medial gastrocnemius; LG, lateral gastrocnemius; SOL, soleus; ROM, range of motion

1.3.4. Acute and chronic effects of resistance training on passive muscle stiffness

Several studies have shown that passive muscle stiffness is acutely changed by resistance exercise (Table 1-3). To the best of my knowledge, Lacourpaille et al. (2014) is the first study to examine the changes in passive muscle stiffness after resistance exercise using ultrasound shear wave elastography. They showed that the shear moduli of the biceps brachii (proximal, middle, and distal regions) and brachialis (middle region) increased at 1 and 48 h after 30 maximal voluntary eccentric contractions of elbow flexion. Agten et al. (2017) also demonstrated that the shear wave velocity of the brachialis increased at 15 min (in male participants) and 24 h (in female participants) after 36 eccentric elbow flexions with a load of 90% of one repetition maximum (RM). Pournot et al. (2016) showed an increase in the shear modulus of the biceps brachii immediately and at 5 min after 40 concentric and eccentric elbow flexions with a load of 70% of 1RM. Lacourpaille et al. (2017) revealed that the shear moduli of elbow flexors (pooled values of the biceps brachii and brachialis) increased at 30 min after 30 or 60 maximal voluntary eccentric contractions of elbow flexion. They also observed increases in the shear moduli of the knee extensors (pooled values of the rectus femoris, vastus lateralis, and vastus medialis) at 30 min after 75 or 150 maximal voluntary eccentric contractions of knee extension. Similarly, Xu et al. (2018) reported an increase in the shear modulus of the rectus femoris immediately and at 48 h after 75 maximal voluntary eccentric contractions of knee extension. Ema et al. (2021) showed increases in the shear moduli in the proximal and distal regions of the rectus femoris at 24 to 72 h after 100 maximal voluntary eccentric contractions of knee extension at short (seated position) and long (supine position) muscle lengths. They also demonstrated that the magnitude of the increase in the shear modulus of the proximal region in the rectus femoris was greater at long muscle lengths than the exercise at short muscle lengths at 24 h after the exercise. More recently, Goreau et al. (2022) reported increases in the shear moduli of BFlh and ST at 30 min after 75 maximal voluntary eccentric contractions of knee flexion without the corresponding change in SM. Similarly, Voglar et al. (2022) reported that the shear moduli of BFlh and ST increased immediately after combined eccentric knee flexions (30 maximal voluntary eccentric contractions of knee flexion and 18 repetitions of Nordic hamstring exercise with a body weight load), whereas the stiffness of SM was not significantly changed.

In contrast to the above findings, some studies have demonstrated an acute decrease in muscle stiffness after resistance exercise. Xu et al. (2018) observed a decrease in the shear modulus of the vastus lateralis immediately after 75 maximal eccentric contractions of knee extension without a change in the shear modulus of the vastus medialis oblique. Chalchat et al. (2020) reported a decrease in the shear modulus of the vastus lateralis immediately and at 24 h after 60 maximal isometric contractions of knee extension. Kisilewicz et al. (2020) showed a decrease in the shear modulus of the upper trapezius at 24 h after 50 maximal voluntary eccentric contractions of shoulder elevation. More recently, Zhi et al. (2022) found that the shear modulus of BFlh decreased immediately after maximal voluntary eccentric contractions of knee flexion with low repetitions (five repetitions) and a relatively long duration (4.5 s per repetition), whereas the shear modulus did not significantly change after its concentric contractions.

Taken together, no consensus has been reached regarding the acute effects of resistance exercise on muscle stiffness. The inconsistent results may be attributed to different program variables (contraction mode, exercise ROM [muscle length], duration, and load) of resistance exercise in the previous studies. As reviewed above, the acute increase in muscle stiffness was observed in most studies that adopted eccentric resistance exercise with a combination of high-load and high repetitions, except for a few studies (Xu et al. 2018; Kisliewicz et al. 2020).

Table 1-3. Program variables and main findings of the previous studies investigating the acute changes in passive muscle stiffness after resistance exercise

Author Year	Exercise	Contraction mode	Load	Exercise ROM	Duration per repetition (s)	Repetition	Set	Muscle	Measurement point	Result
Lacourpaille et al. 2014	Elbow flexion	Eccentric	Maximal voluntary contraction	120° elbow flexion to 5° elbow flexion	About 1	10	3	BB (proximal)	Pre 1 h 48 h 3 week after	Increase (Pre- 1 h after, pre-48 h after, pre-3 week after)
								BB (middle)		Increase (Pre- 1 h after, pre-48 h after, pre-3 week after)
								BB (distal)		Increase (Pre- 1 h after, pre-48 h after, pre-3 week after)
								BRA (middle)		Increase (Pre- 1 h after, pre-48 h after, pre-3 week after)
Agten et al. 2016	Elbow flexion	Eccentric	90% RM	Full elbow flexion to its full extension	3-5	12	3	BRA	Pre 15 min 12 h 1 day 2day 3 day 1 week after	Increase (Pre-15 min, men) Increase (Pre-1 day after, women)
Pournot et al. 2016	Elbow flexion	Concentric Eccentric	Maximal voluntary contraction	Full elbow flexion to its full extension	1-2 s (Concentric phase) 5 s (Eccentric phase)	10	4	BB	Pre immdiately 5 min after	Increase (Pre-immediately after Pre-5 min after)

Table 1-3. Program variables and main findings of the previous studies investigating the acute changes in passive muscle stiffness after resistance exercise (continued)

Author Year	Exercise	Contraction mode	Load	Exercise ROM	Duration per repetition (s)	Repetition	Set	Muscle	Measurement point	Result
Hotfiel et al. 2017	Heel raise	Concentric Eccentric	Body weight	a slant plate of -35°	1 (concentric phase) 3 (eccentric	15	2	MG	Pre 60 h after	Decrease
								SOL		Not change
Lacourpaille et al. 2017	Knee extension	Eccentric	Maximal voluntary contraction	10°knee flexion to 110° knee flexion	About 1.6	15	5	Knee extensors (pooled value of RF, VL, and VM)	Pre 30 min after	Increase (at 110° knee flexion)
						30	5			Increase (at 90° and 110° knee flexion)
	Elbow flexion	Eccentric		120° elbow flexion to 5° elbow flexion	About 1	10	3	Elbow flexors (pooled values of BB and BRA)		Increase (at 5° elbow flexion)
						10	6			
		Concentric				10	3			Not change
Xu et al. 2018	Knee extension	Eccentric	Maximal voluntary contraction	30° knee flexion to 110° knee flexion	About 6	75	1	RF	Pre immediately 48 h after	Increase (Pre-immediately after, Pre-48 h after, at 90° knee flexion)
								VL		Decrease (Pre-immediately after, at 30° and 60° knee flexion)
								VMO		Not change
Chalchat et al. 2020	Knee extension	Isometric	Maximal voluntary contraction	90° knee flexion	5	10	6	VL	Pre immediately 20 min after	Decrease (Pre-immediately after, Pre-20 min after)

Table 1-3. Program variables and main findings of the previous studies investigating the acute changes in passive muscle stiffness after resistance exercise (continued)

Author Year	Exercise	Contraction mode	Load	Exercise ROM	Duration per repetition (s)	Repetition	Set	Muscle	Measurement point	Result
Kisliewicz et al. 2020	Shoulder elevation	Eccentric	Maximal voluntary contraction	Lowest position to highest position of shoulder joint	-	10	5	up TRAP (four regions)	Pre 24 h after	Decrease (in all regions)
Ema et al. 2021	Knee extension (at long muscle lengths)	Eccentric	Maximal voluntary contraction	40° knee flexion to 110° knee flexion	About 1	10	10	RF (proximal)	Pre 1 day 2 day 3 day after	Increase (Pre-1 day after, Pre-2 day after, Pre-3 day after)
								RF (distal)		Increase (Pre-1 day after, Pre-2 day after)
								VL		Increase (Pre-1 day after, Pre-2 day after, Pre-3 day after)
								VM		Increase (Pre-1 day after, Pre-2 day after, Pre-3 day after)
	Knee extension (at short muscle lengths)							RF (proximal)		Increase (Pre-1 day after)
								RF (distal)		Increase (Pre-1 day after, Pre-2 day after)
								VL		Increase (Pre-1 day after, Pre-2 day after, Pre-3 day after)
								VM		Increase (Pre-1 day after, Pre-2 day after, Pre-3 day after)

Table 1-3. Program variables and main findings of the previous studies investigating the acute changes in passive muscle stiffness after resistance exercise (continued)

Author Year	Exercise	Contraction mode	Load	Exercise ROM	Duration per repetition (s)	Repetition	Set	Muscle	Measurement point	Result
Gooreau et al. 2022	Knee flexion	Eccentric	Maximal voluntary contraction	10° knee flexion to 90° knee flexion	About 2.6	15	5	BFlh	Pre 30 min after	Increase
								ST		Increase
								SM		Not change
Zhi et al. 2022	Knne flexion	Concentric	Maximal voluntary contraction	0° knee flexion to 90° knee flexion	4.5	5	1	BFlh	Pre 30 s 60 s 90 s 120 s after	Not change (Pre-30 s after Pre-60 s after Pre 90 s after Pre-120 s after)
		Eccentric								Decrease (Pre-30 s after Pre-60 s after Pre 90 s after Pre-120 s after)
Voglar et al. 2022	Knee flexion	Eccentric	Maximal voluntary contraction	-	-	10	3	BFlh	Pre immediately 1 h 24 h 48 h after	Increase (Pre-immediately after)
	Nordic hamstring	Eccentric	Body weight	About 90° knee flexion to maximal knee extension ROM	-	6	3	ST		Increase (pre-immediately after, Pre-1 h after, Pre-48 h after)
								SM		Not change

BB, biceps brachii; BRA, brachialis; MG, medial gastrocnemius; SOL, soleus; RF, rectus femoris; VL, vastus lateralis; VM, vastus medialis; VMO, vastus medialis oblique; up TRAP, trapezius (upper region); BFlh, biceps femoris long head; ST, semitendinosus; SM, semimembranosus

Several studies have investigated the chronic effects of resistance training on passive muscle stiffness (Table 1-4). Akagi et al. (2016) examined the effects of a 6-week elbow extension training with concentric and eccentric contractions with a load of 80% 1RM on the shear modulus of the triceps brachii. They reported that the shear modulus was not significantly changed after the resistance training. Seymore et al. (2017) also found no significant change in the shear modulus of BFlh after 6 weeks of eccentric knee flexion training (Nordic hamstring exercise with a load of body mass). A similar finding was shown in Ochi et al. (2018), who reported no significant changes in the shear moduli of the vastus lateralis, rectus femoris, and vastus medialis after an 11-week knee extension training including concentric and eccentric contractions with a load of 67% 1RM. Additionally, Vatovec et al. (2021) reported that the shear modulus of BFlh was comparable before and after a 6-week progressive eccentric training (Nordic hamstring exercise at the hip flexed with a load of 5 to 10 kg and glider exercise with a load of 8 to 20 kg). In contrast, Mannarino et al. (2019) demonstrated that the shear modulus of the vastus lateralis was increased after 8 weeks of free-weight squat and knee extension training comprised of concentric and eccentric contractions with a load of 10 RM. However, Dankel and Razzano (2021) concluded in their systematic review and meta-analysis that passive muscle stiffness is not changed after a long-term resistance training intervention (6 to 11 weeks), although the previous review included only four studies (Akagi et al. 2016; Seymore et al. 2017; Ochi et al. 2018; Mannarino et al. 2019). Taken together, resistance training is unlikely to chronically change muscle stiffness. However, resistance training is implemented with various combinations of the program variables (contraction mode, exercise ROM, muscle lengths, and exercise duration) in actual sports and clinical settings. Thus, more investigation is warranted to conclude the chronic effects of resistance training on the passive muscle stiffness.

Table 1-4. Program variables and main findings of the previous studies investigating the chronic changes in passive muscle stiffness after resistance training

Author Year	Exercise	Contraction mode	Load	Exercise ROM	Duration per repetition (s)	Repetition	Set	Frequency per week (day)	Intervention period (week)	Muscle	Measurement point	Result
Akagi et al. 2016	Lying elbow extension exercise	Concentric Eccentric	80% RM	Full elbow flexion to its full extension with 90° shoulder flexion	2 (each contraction phase)	8	5	3	6	TB (long head)	Pre, 6 week after	Not change
Seymore et al. 2017	Nordic hamstring	Eccentric	Body weight	Approximetely 90° knee flexion to maximal knee extension ROM	-	5 6 6-8 8-10 8-12	2 (week 1-2) 3 (week 3-6)	1 2 3	6	BFlh	Pre, 6 week after	Not change
Ochi et al. 2018	Knee extension	Concentric Eccentric	67% RM	90° knee flexion to maximal knee extension ROM	1 (each contraction phse)	12	6	1	11	RF	Pre, 3, 6, 9, 11 week after	Not change
										VL		Not change
										VM		Not change
						12	2	3		RF		Not change
										VL		Not change
										VM		Not change
	Detraining	-	-	-	-	-	-	-	6	RF	11, 14, 17 after	Not change
										VL		Not change
										VM		Not change
										RF		Not change
										VL		Not change
										VM		Not change

Table 1-4. Program variables and main findings of the previous studies investigating the chronic changes in passive muscle stiffness after resistance training (continued)

Author Year	Exercise	Contraction mode	Load	Exercise ROM	Duration per repetition (s)	Repetition	Set	Frequency per week (day)	Intervention period (week)	Muscle	Measurement point	Result
Mannarino et al. 2019	**Free-weight Squats** **Knee extensions**	**Concentric** **Eccentric**	**10 RM**	**Full hip extensino to its full flexion** **Full knee flexion to its full extension**	**-**	**8-12**	**4 (week 1-4)** **6 (week 5-8)**	**2**	**8**	**VL**	**Pre, 8 week after**	**Increase**
Vatovec et al. 2021	**Modified Nordic hamstring**	**Eccentric**	**5-10 kg**	**Approximetely 90° knee flexion to maximal knee extension ROM at 75° hip flexion**	**-**	**5-6**	**2 (week 1-2)**	**2**	**6**	**BFlh**	**Pre, 6 week after**	**Not change**
	Glider		**8-20 kg**	**Slight hip flexion to maximal hip flexion ROM at slight knee flexion**		**6-8**	**3 (week 3-6)**					

TB, triceps brachii; BFlh, biceps femoris long head; RF, rectus femoris; VL, vastus lateralis; VM, vastus medialis; RM, repetition maximum; ROM, range of motion

1.4. Summary and unresolved issues of the previous studies

As mentioned in the review of literature, passive muscle stiffness could be acutely and chronically decreased by static stretching. However, a recent study has indicated that 10 sessions (for approximately one month) of a static stretching program chronically decreased eccentric strength (Barbosa et al. 2020). Low levels of eccentric strength have been suggested to negatively influence athletic performance (Booysen et al. 2015) and increase the risk of musculoskeletal injury (Opar et al. 2015). Thus, an alternative approach to static stretching is essential to decrease muscle stiffness while maintaining muscle strength. Meanwhile, some studies reported that passive muscle stiffness was acutely decreased by resistance exercise (Andonian et al. 2016; Morales-Artacho et al. 2016; Hotfied et al. 2017). However, the other studies observed an acute increase in muscle stiffness after resistance exercise (Green et al., 2011; Lacourpaille et al., 2014; Xu et al. 2018; Ema et al., 2021). Thus, no consensus has been reached regarding the resistance exercise-induced acute changes in muscle stiffness.

Possible factors that could determine the change in passive muscle stiffness are program variables of resistance exercise. Previous stretching studies showed that the muscle stiffness was immediately decreased after passive muscle lengthening (Nakao et al. 2018). Moreover, muscle stiffness was greatly decreased by one session of static stretching with a wide ROM (long muscle lengths [Freitas et al. 2015]) and a long duration (Caliskan et al. 2019). These findings suggest a possibility that passive muscle stiffness is acutely decreased by resistance exercise with the combination of lengthening (eccentric) contraction with a wide exercise ROM (long muscle lengths) and a long duration. However, it remains unclear as to the acute effects of program variables (contraction mode, exercise ROM, muscle lengths, and exercise duration) of resistance exercise on muscle stiffness.

Previous stretching studies (Umegaki et al. 2015; Ichihashi et al. 2016) suggested that the immediate changes in muscle stiffness corresponded to its chronic changes. Hence, it is possible that resistance training that immediately changes muscle stiffness also chronically influences the stiffness. However, only limited studies have investigated the chronic changes in muscle stiffness after resistance training.

1.5. Purpose

The general purpose of this thesis is to investigate the acute and chronic effects of resistance training on passive stiffness of the hamstring muscles.

In Chapter 2, I examined the acute effects of resistance exercise with different contraction modes and exercise ROMs on the passive stiffness of the biarticular hamstring muscles using an ultrasound shear wave elastography.

In Chapter 3, I also investigated the acute effects of resistance exercise with different muscle lengths and exercise durations on the passive stiffness of the biarticular hamstring muscles. Through Chapters 2 to 3, program variables that can acutely decrease the passive muscle stiffness are selected for investigating the chronic effects of resistance training on the stiffness in Chapter 4.

In Chapter 4, I examined the chronic changes in the passive stiffness of the biarticular hamstring muscles after resistance training with the combination of selected program variables.

In Chapter 5, I firstly addressed the main findings of each chapter. Subsequently, the following points were discussed: 1) determinants of passive muscle stiffness, 2) intermuscular differences, and 3) limitations and future directions. Lastly, I made the conclusion of the thesis.

Chapter 2. Acute changes in passive stiffness of the biarticular hamstring muscles induced by resistance exercise: effects of contraction mode and range of motion

2.1. Introduction

Studies have examined the acute changes in muscle stiffness following resistance exercise. Some studies reported the acute increase in muscle stiffness after resistance exercise (Green et al. 2011; Lacourpaille et al. 2017; Xu et al. 2018; Ema et al. 2021), whereas others observed the opposite results (Morales-Artacho et al. 2016; Andonian et al. 2016; Hotfied et al. 2017). Thus, no consensus has been reached regarding the acute changes in muscle stiffness after resistance exercise.

Possible factors that could determine the change in muscle stiffness are program variables of resistance exercise (e.g., contraction mode and exercise ROM) based on the following results of the stretching studies (Nakao et al. 2018; Fukaya et al. 2020). Nakao et al. (2018) reported that muscle stiffness was immediately decreased after passive muscle lengthening. In addition, Fukaya et al. (2020) showed that muscle stiffness was greatly decreased after static stretching exercise with a wide ROM (120% of maximal joint ROM) and short-duration (100 s) compared to that with a narrow ROM (50% of maximal joint ROM) and long duration (240 s). These findings suggest that a large extent of muscle lengthening induced by exercise with a wide exercise ROM may be an important factor for decreasing muscle stiffness. Therefore, it is possible that resistance exercise comprised of lengthening (eccentric) contractions with a wide exercise ROM rather than resistance exercise comprised of isometric (no change in muscle length) or shortening (concentric) contractions with a narrow exercise ROM can acutely decrease muscle stiffness.

The aim of the present chapter was to investigate the acute changes in stiffness of the biarticular hamstring muscles after resistance exercises with different combinations of contraction modes and exercise ROMs. I hypothesized that muscle stiffness would be greatly decreased immediately after eccentric-only resistance exercise with a wide ROM as compared to eccentric-only exercise with a narrow ROM and concentric-only exercise with a wide ROM. Additionally, a previous study found that the stretching effect was greatest in SM among the biarticular hamstring muscles (Umegaki et al. 2015). Thus, I also hypothesized that the magnitude of the changes in stiffness immediately after exercise would be greater in SM than in the other hamstring muscles. Part of this chapter has been published elsewhere (Kawama et al. 2022).

2.2. Methods

Participants

Thirteen healthy young males (age, 24.1 ± 2.9 years; height, 169.3 ± 5.5 cm; body mass, 61.7 ± 3.3 kg; mean ± SD) participated in this experiment. The number of participants was determined by a priori power analysis for one-way analysis of variance with a power of 80%, an α error of 0.05, and an effect size (partial η^2) of 0.40 using G*Power 3.1 software (Heinrich Heine University, Dusseldorf, Germany). The effect size of 0.40 was selected based on a previous study that found a significant interaction of the shear modulus between exercise session and time (partial $\eta^2 = 0.45$ [Morales-Artacho et al., 2017]). The results of the power analysis demonstrated that the minimum sample size was 12. Considering possible attrition, 13 participants were recruited for this study. None of the participants had a history of severe neuromuscular diseases or musculoskeletal injuries of the right lower extremity. They refrained from strenuous exercise and alcohol consumption for 48 h before each test. Some of them participated in recreational sports activities, such as baseball, football, and long-distance running. Throughout the experiment, the participants were asked to maintain their daily activities and not perform additional interventions such as stretching and resistance exercises. All participants completed an informed consent form after the procedures, purposes, and possible risks of this study were explained. This study was approved by the Doshisha University Research Ethics Committee (no. 21006).

Experimental design

In the present study, I examined the acute effects of three resistance exercise sessions with different contraction modes and exercise ROMs on the shear moduli of the biarticular hamstring muscles (Fig. 2-1). The participants visited the laboratory on four occasions with an interval of >5 days. It has been demonstrated that the magnitude of muscle damage induced by a session of eccentric contractions is greater in the first occasion than in the subsequent occasions, which is referred to as the repeated bout effect (Nosaka et al. 2001). To minimize this effect on the results of the muscle shear modulus, I conducted a familiarization session when the participants first visited my laboratory. On the subsequent 3 days, the participants completed one of the three resistance exercise sessions that consisted of stiff-leg deadlift (SDL) with different contraction modes and exercise ROMs: (1) eccentric contractions with a wide exercise ROM (EW); (2) eccentric contractions with a narrow exercise ROM (EN); and (3) concentric contractions with a wide exercise ROM (CW). Three sessions of resistance exercises were performed on separate days in a randomized order across participants. Maximal joint ROM, passive torque, shear moduli of the biarticular hamstring muscles, and maximal isometric torque of knee flexion were measured before and 3 min, 30 min, and 60 min after completion of the SDL session in the order as described above.

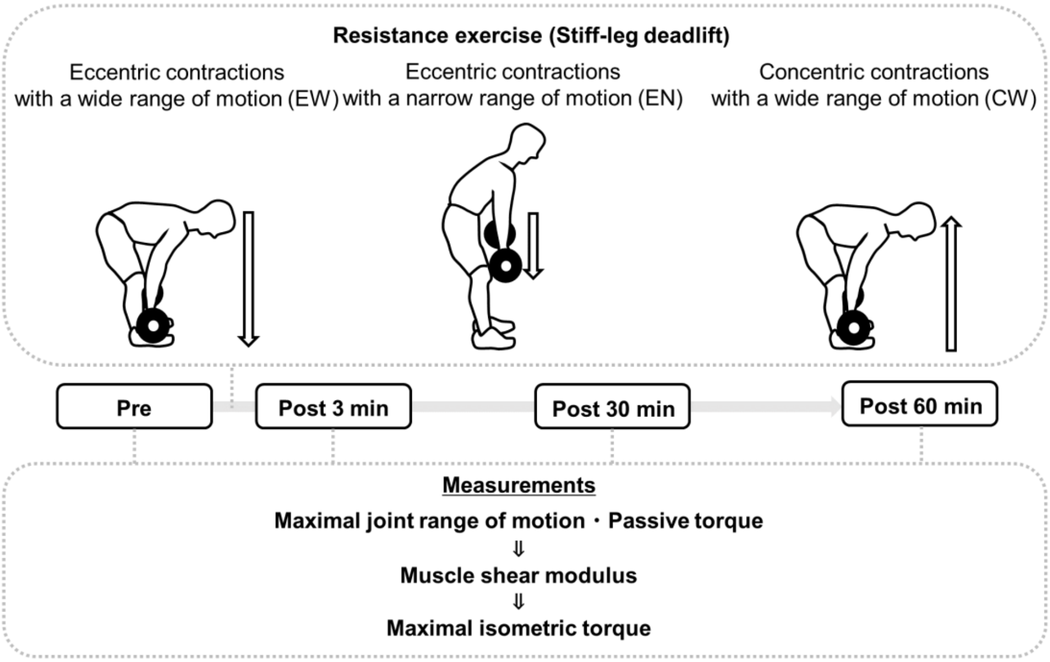

Fig. 2-1 Overview of the study design. Participants performed three sessions of resistance exercise across 3 days in each experiment. Maximal knee joint range of motion, passive torque, muscle shear modulus, and maximal isometric torque were measured before, and 3 min, 30 min, and 60 min after completing one of the sessions of resistance exercise.

Resistance exercise

The SDL was selected in this study based on the following three reasons. First, this exercise is widely used by strength and conditioning professionals to enhance the muscular function of the hamstring muscles. Second, a decrease in shear modulus could be expected because the biarticular hamstring muscles are substantially stretched by flexing the hip joint while keeping knee extension during the exercise (Hooren et al. 2022). Finally, I could easily manipulate program variables (contraction modes, exercise ROM, load, and duration per repetition) to explore their effects on muscle shear modulus. On the first occasion, participants performed three sets of 10 repetitions of SDL with a load of 60% of the participants' body mass as a familiarization session. The SDL was started from the upright position with a barbell placed in front of the thighs. The participants lowered the barbell (eccentric phase) with the knee and elbow joints extended until they could not tolerate and then they lifted the barbell (concentric phase) from the lowest position to the initial position. Each of the concentric and eccentric phases of SDL was completed in 2 s with a metronome set to 60 bpm. It has been reported that the hip joint positions (adduction-abduction and internal-external rotation) during SDL could affect EMG activities of biarticular hamstring muscles (Kawama et al. 2021). Thus, the feet were placed parallel to the distance between the middle of the heels, which was set at 20% of their height. During the first occasion, all participants were carefully instructed about the proper techniques of SDL by a Certified Strength and Conditioning Specialist. Additionally, the maximal ROM of SDL was determined using a wire-type displacement transducer (DTPA-A-2K, KYOWA, Tokyo, Japan) attached to the midpoint of the barbell. The barbell height was sampled with an A/D converter (PowerLab 16SP, AD Instruments, New South Wales, Australia) at 1 kHz and transferred to a computer. The ROM of SDL was defined as the difference between barbell heights in the upright position and the lowest position that each participant could reach. The average value of ROM across all repetitions on the first occasion was used to determine the narrow and wide ROMs of SDL for each participant.

Measurement and procedures

Maximal joint ROM and passive torque

The participants lay supine on a bench and the right hip joint was flexed at 120° with 90° of knee flexion (full knee extension = 0°, Fig. 2-2). The rotation axis of the right knee joint was matched to that of a dynamometer (Biodex System4, Biodex Medical Systems, Shirley, USA), and the right leg was attached to the lever arm. To avoid hip flexion movement during passive knee extension, a form pad was placed in front of the right thigh. The trunk and pelvis were firmly fixed to the bench using nonelastic straps. The knee joint was passively extended at an angular velocity of 2°/s from the initial position (90° of knee flexion) to the final position where the participants started to feel discomfort or pain (Miyamoto et al. 2017, 2020). The participants were asked to relax completely during passive knee extension. Subsequently, the knee joint was immediately returned to its initial position. The passive knee extension test was performed only once at each time point because passive muscle lengthening may influence the shear moduli of the muscles (Miyamoto et al. 2017). The torque and joint angle were sampled using the A/D converter (PowerLab 16SP, AD Instruments, New South Wales, Australia) at 1 kHz and then transferred to a computer. The difference in the knee joint angle between the initial and final positions was defined as the maximal joint ROM. Additionally, passive knee joint torque at the final position was used as a measure of stretch tolerance (Magnusson et al. 1997).

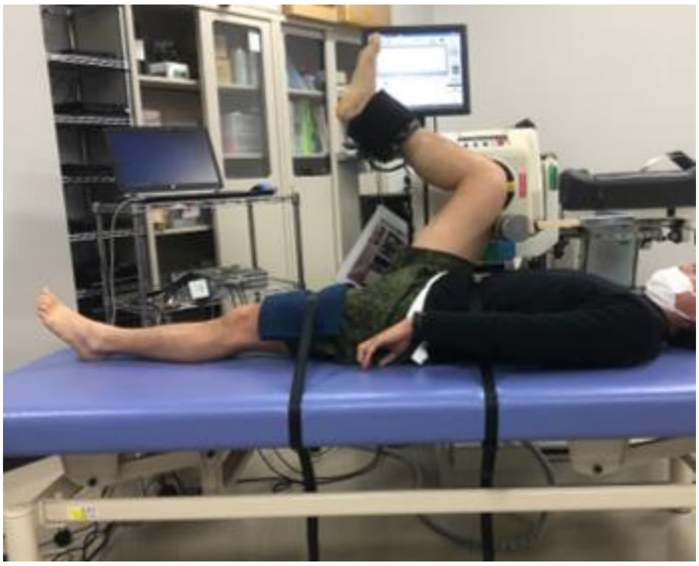

Fig. 2-2 Experimental setup in the passive knee extension test.

Muscle shear modulus

To quantify the passive stiffness of the biarticular hamstring muscles, a real-time ultrasound scanner (Aixplorer Ver. 8, Supersonic Imagine, Aix-en-Provence, France) coupled with a linear array transducer (4 to 15 MHz; SuperLinear 15 to 4; Vermon, Tours, France) was used in the shear wave elastography mode (musculoskeletal preset). The shear moduli of the biarticular hamstring muscles were measured at 80% (0% = 90°) of the maximal joint ROM evaluated before the resistance exercise on the second occasion according to the previous two findings (Lacourpaille et al. 2017; Miyamoto et al. 2017). First, a previous study detected a significant change in the muscle shear modulus, especially at a longer muscle length following eccentric exercises (Lacourpaille et al. 2017). Second, another study revealed that maintaining a muscle at an excessively long length during measurements can decrease the shear modulus of the muscle (Miyamoto et al. 2017). In this position, the muscle fascicle orientations of BFlh, ST, and SM were identified within B-mode images obtained at 50%, 40%, and 60% of the thigh length (the distance between the greater trochanter [0%] and the popliteal crease [100%]), respectively (Fig. 2-3). These measurement regions were determined for the following two reasons: First, the shear moduli of the biarticular hamstring muscles were shown to be higher in the middle regions than in the proximal region (Miyamoto et al. 2020), and a large stretching effect was observed in a region with a relatively high shear modulus within the muscle (Hirata et al. 2020). Second, the corresponding regions have enough area to minimize the crosstalk from adjacent muscles in EMG recordings (described below). The probe position was marked with a permanent marker pen, when several fascicles of the individual muscles were carefully identified. Before the first measurement, at least 10 min of rest was provided in a supine position to minimize any effects of the previous basic activity (e.g., walking) and to avoid possible effects of fluid shift on the obtained images.

At each time point, the static images of BFlh, ST, and SM were obtained twice within 2 min in a random order across participants after the probe was aligned with fascicles of the individual muscles.

In each image, the border of the corresponding muscle was manually outlined as large as possible with care taken to exclude artifacts (saturated area), missing areas (unfilled region within the elasticity map), fascia, aponeurosis, and bone. The spatial average of Young's modulus in the region of interest was computed using software of the ultrasound apparatus. The obtained Young's modulus was divided by 3 to calculate the muscle shear modulus (Nakamura et al. 2014), and two measured values of the individual muscles were averaged for further analysis.

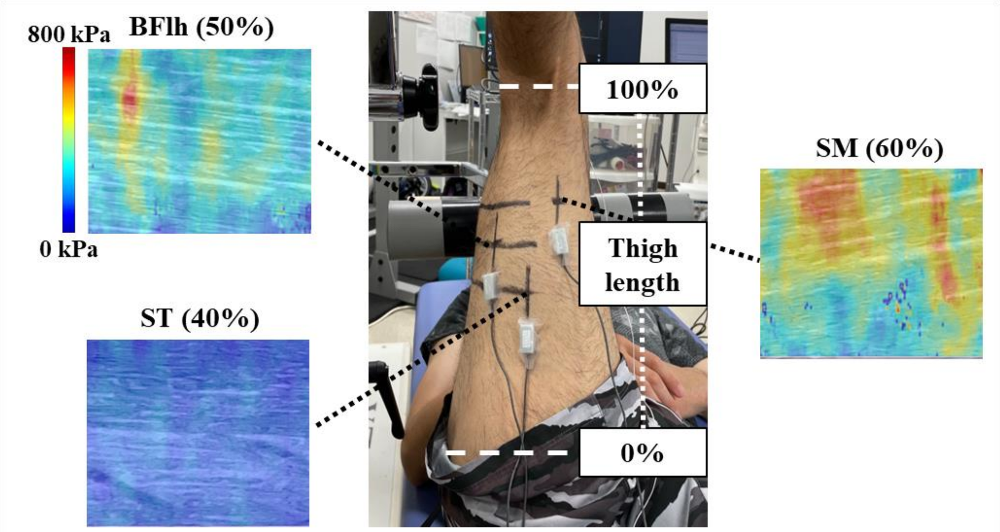

Fig. 2-3 Typical elastographic images of the hamstring muscles. The colored region represents the shear modulus map of each muscle. BFlh, biceps femoris long head; ST, semitendinosus; SM, semimembranosus.

Maximal isometric torque

The maximal isometric torque of knee flexion was measured at 80% of the maximal joint ROM. Prior to the main tests, the participants performed five submaximal isometric contractions of knee flexion as a warm-up. The maximal isometric torque (3 s) was measured only once at each time point because multiple isometric contractions may have affected the muscle shear modulus (Chalchat et al. 2020). During the test, the participants were asked to grasp the bench with their hands and concentrate only on performing knee flexion without performing hip extension. Vigorous encouragement was given to the participants to perform maximal voluntary contraction. The peak value of the knee flexion torque was determined as the maximal isometric torque.

EMG activity

The EMG activities of BFlh, ST, and SM were obtained during the measurement of shear modulus and maximal voluntary contraction of knee flexion. Pairs of surface electrodes (DL-141, S&ME, Tokyo, Japan, inter-electrode distance: 12 mm) were placed at 45% of the thigh length in BFlh, 35% in ST, and 55% in SM to avoid contact with the ultrasound scanner probe. The electrodes were fixed with surgical tape after careful preparation of the skin by shaving the hair, rubbing with sandpaper, and cleaning with alcohol. A reference electrode was attached to the right patella. The EMG signals were pre-amplified and simultaneously sampled with the torque, and angle at 1 kHz. The signals were band-pass filtered between 5 and 500 Hz using computer software (LabChart ver.8, AD Instruments, New South Wales, Australia). The root-mean-square value of EMG data (RMS-EMG) of each muscle was averaged between two measurements of the shear modulus at each time point. The RMS-EMG during maximal voluntary contraction (MVC) of knee flexion was determined for 1.0 s around the peak torque at each time point. The RMS-EMG during shear moduli measurements were normalized to that during MVC before the resistance exercise as % MVC.

Reliability of measurements

Ten healthy young males (age, 23.9 ± 2.8 years; height, 170.1 ± 5.6 cm; body mass, 65.4 ± 12.8 kg) were recruited to test the reliability of the maximal joint ROM, passive torque, and shear moduli of the biarticular hamstring muscles. These variables were measured twice with an interval of 10 min between each measurement on the same day, and the two measured values were used to calculate ICC (1, 2) and coefficient of variation (CV).

Statistical analysis

The Shapiro-Wilk normality test was used to assess the distribution of the shear moduli of the biarticular hamstring muscles. As a result, some of the data were non-Gaussian ($p = 0.006$ to 0.997). Thus, the Friedman test was adopted to determine the significant effects of time (time points) on the change in maximal joint ROM, passive torque, muscle shear moduli, and maximal isometric torque in each session. When a significant main effect of time or session type was detected, the Wilcoxon signed-rank test was performed to identify significant differences in the aforementioned variables. To test the association between the change in maximal joint ROM and the change in the other measured variables at 3 min post-exercise, the Spearman's rank correlation analysis was performed in each session. The significance level was set at $p < 0.05$. For multiple tests, the p-value was corrected using the Benjamini and Hochberg method (Glickman et al. 2014) at a cut-off of a false discovery rate of < 0.05. The effect size (r) was calculated for all the tests in this study. The results of this study are presented as median value (interquartile range). All statistical analyses were conducted using a statistical software package (IBM SPSS Statistics, version 27.0, IBM Corporation, Armonk, USA).

2.3. Results

Reliability of maximal knee joint ROM and shear modulus

The ICCs (1, 2) for the maximal joint ROM and passive torque were 0.95 and 0.98, respectively. The CVs for the maximal joint ROM and passive torque were 11.7% and 2.7%, respectively. The ICCs (1, 2) for the shear moduli of BFlh, ST, and SM were 0.95, 0.81, and 0.94, respectively. The CVs for the shear moduli of BFlh, ST, and SM were 2.6%, 1.9%, and 2.9%, respectively.

Maximal knee joint ROM & passive torque

Friedman test revealed a significant main effect of time on the maximal joint ROM in EN ($p = 0.002$, $r = 0.87$) and CW ($p = 0.026$, $r = 0.62$), but not in EW ($p = 0.250$, $r = 0.32$, Fig. 2-4). The post hoc test showed that the maximal joint ROM was significantly larger at 3 min post-exercise (44.2° [37.6 to 47.5°]) than at pre-exercise (40.0° [36.0 to 44.6°], corrected $p = 0.022$, $r = 0.52$), 30 min post-exercise (40.3° [33.1 to 42.7°], corrected $p = 0.015$, $r = 0.59$), and 60 min post-exercise (40.7° [31.1 to 42.3°], corrected $p = 0.006$, $r = 0.72$) in EN. In CW, the maximal joint ROM was significantly smaller at 30 min post-exercise (45.4° [40.1 to 47.7°], corrected $p = 0.034$, $r = 0.47$) and 60 min post-exercise (43.2° [35.3 to 46.4°], corrected $p = 0.034$, $r = 0.49$) than at pre-exercise (46.3° [40.8 to 49.1°]). The maximal joint ROM at 60 min post-exercise (corrected $p = 0.034$, $r = 0.47$) was also significantly smaller than that at 3 min post-exercise (48.2° [37.8 to 52.4°]) in CW. A significant main effect of time on passive torque was observed in EN ($p = 0.020$, $r = 0.65$), but not in EW ($p = 0.232$, $r = 0.33$) or CW ($p = 0.157$, $r = 0.39$). However, there were no significant differences in passive torque between any time points in EN (corrected $p = 0.092$ to 0.807, $r = 0.08$ to 0.44).

Maximal isometric torque

There was a significant main effect of time on the maximal isometric torque in EW ($p = 0.005$, $r = 0.77$), but not in EN ($p = 0.656$, $r = 0.12$) or CW ($p = 0.577$, $r = 0.16$). The maximal isometric torque at 60 min post-exercise (76.1 Nm [60.2 to 90.0 Nm]) was significantly lower than at pre-exercise (78.8 Nm [68.2 to 101.3 Nm], corrected $p = 0.024$, $r = 0.62$), 3 min post-exercise (75.9 Nm [67.6 to 90.8 Nm], corrected $p = 0.026$, $r = 0.52$), and 30 min post-exercise (79.5 Nm [64.8 to 93.1 Nm], corrected $p = 0.026$, $r = 0.50$) in EW.

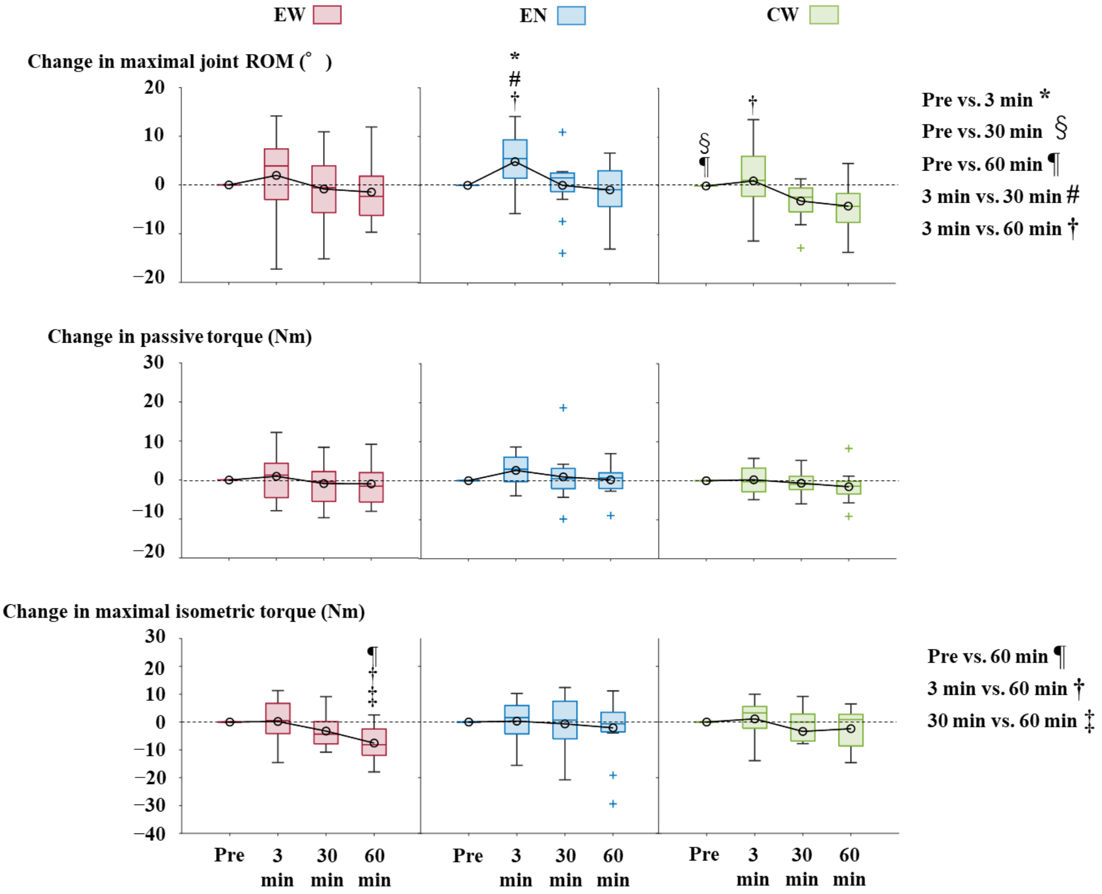

Fig. 2-4 Boxplots of the time-course changes in maximal joint range of motion (ROM), passive torque, and maximal isometric torque following the resistance exercise sessions. EW, eccentric contractions with a wide range of motion (red box); EN, eccentric contractions with a narrow range of motion (blue box); CW, concentric contractions with a wide range of motion (green box). *: pre-exercise versus 3 min post-exercise. §: pre-exercise versus 30 min post-exercise. ¶: pre-exercise versus 60 min post-exercise. #: 3 min post-exercise versus 30 min post-exercise. †: 3 min post-exercise versus 60 min post-exercise. ‡: 30 min post-exercise versus 60 min post-exercise. ○: the group mean value in each measured variable. +: outlier value in each measured variable.

Muscle shear modulus

The RMS-EMG during the shear moduli measurement was 1.0 to 1.6% MVC in BFlh, 0.8 to 1.3% MVC in ST, and 0.5 to 1.1% MVC in SM across all exercise sessions.

In BFlh, there was no significant main effect of time on the shear modulus in EW ($p = 0.564$, $r = 0.16$), EN ($p = 0.130$, $r = 0.42$), or CW ($p = 0.461$, $r = 0.21$, Fig. 2-5). Similarly, no significant main effect of time on the shear modulus of ST was detected in EW ($p = 0.418$, $r = 0.22$), EN ($p = 0.165$, $r = 0.39$), or CW ($p = 0.993$, $r < 0.01$). However, there was a significant main effect of time on the shear modulus of SM in EW ($p = 0.001$, $r = 0.90$), but not in EN ($p = 0.092$, $r = 0.47$) or CW ($p =$

0.632, $r = 0.13$). The shear modulus of SM was significantly lower at 3 min post-exercise (120.2 kPa [114.9 to 133.0 kPa]) than at pre-exercise (130.3 kPa [123.7 to 142.8 kPa], corrected $p = 0.041$, $r = 0.45$), 30 min post-exercise (126.2 kPa [124.2 to 143.1 kPa], corrected $p = 0.015$, $r = 0.55$), and 60 min post-exercise (133.6 kPa [115.78 to 146.5 kPa], corrected $p = 0.012$, $r = 0.62$) in EW.

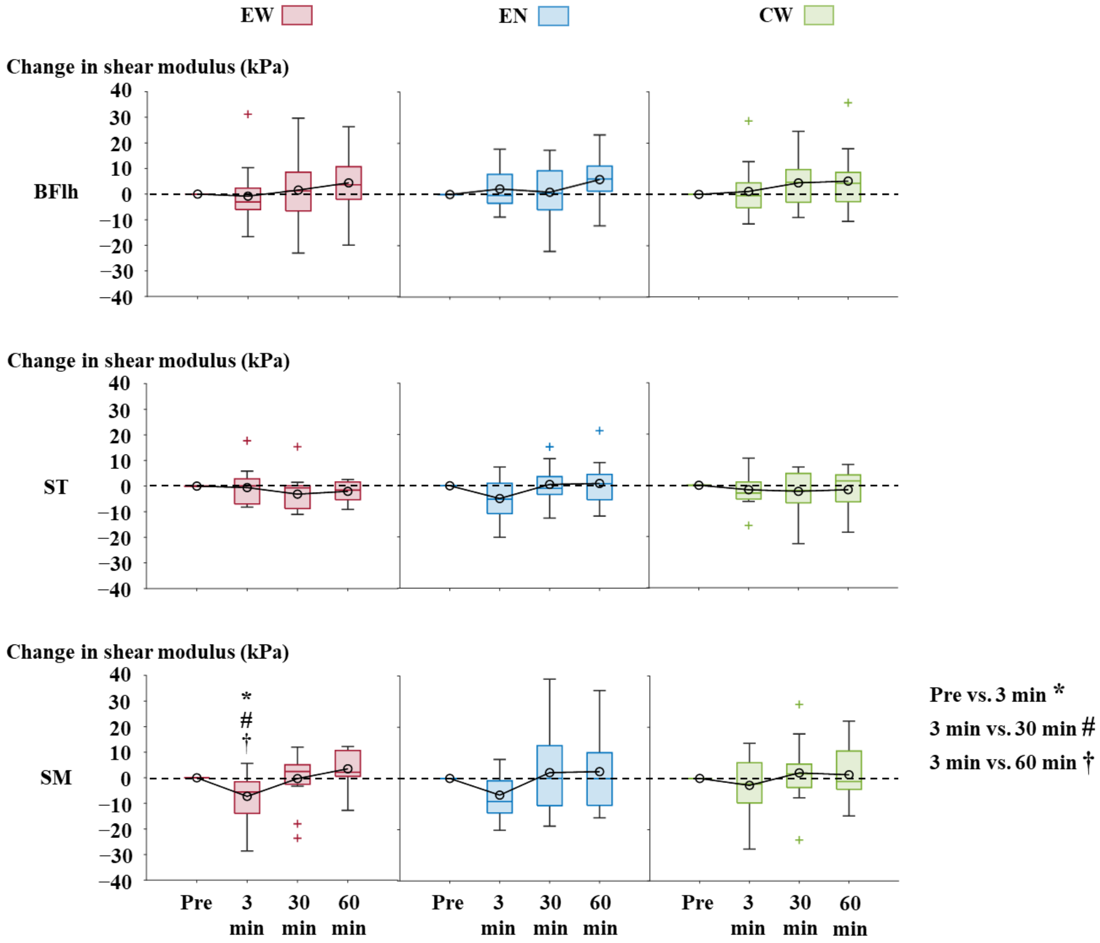

Fig. 2-5 Boxplots of the time-course changes in the shear moduli of the biarticular hamstrings following the resistance exercise sessions. EW, eccentric contractions with a wide range of motion (red box); EN, eccentric contractions with a narrow range of motion (blue box); CW, concentric contractions with a wide range of motion (green box); BFlh, biceps femoris long head; ST, semitendinosus; SM, semimembranosus. *: pre-exercise versus 3 min post-exercise. #: 3 min post-exercise versus 30 min post-exercise. †: 3 min post-exercise versus 60 min post-exercise. ○: the group mean value in each measured variable. +: outlier value in each measured variable.

Association between changes in maximal joint ROM and the other measured variables at 3 min post-exercise

There was a significant correlation between the change in maximal joint ROM and the change in passive torque at 3 min post-exercise in EW ($\rho = 0.846$, corrected $p < 0.001$), EN ($\rho = 0.841$, corrected $p < 0.001$), and CW ($\rho = 0.830$, corrected $p < 0.001$, [Table 2-1]). No significant correlation was found between the change in maximal joint ROM and the change in shear moduli of BFlh (ρ = 0.170 to 0.516, corrected p = 0.213 to 0.957), ST (ρ = −0.192 to 0.275, all corrected $p = 0.529$), or SM (ρ = −0.302 to 0.456, corrected p = 0.351 to 0.426) at 3 min post-exercise in any combinations.

Table 2-1 Association between changes in maximal joint ROM and the other measured variables at 3 min post-exercise

	Passive torque		**BFlh**		**ST**		**SM**	
	ρ	*Corrected p*	*ρ*	*Corrected p*	*ρ*	*Corrected p*	*ρ*	*Corrected p*
EW	0.846	< 0.001*	−0.170	0.578	−0.192	0.529	0.456	0.117
EN	0.841	< 0.001*	−0.016	0.957	0.275	0.364	−0.242	0.426
CW	0.830	< 0.001*	0.516	0.071	−0.203	0.505	−0.302	0.316

*Significant correlation between the change in maximal joint range of motion and the change in passive torque ($p < 0.05$). ROM, range of motion; EW, eccentric contraction with a wide range of motion; EN, eccentric contraction with a narrow range of motion; CW, concentric contraction with a wide range of motion; BFlh, biceps femoris long head; ST, semitendinosus; SM, semimembranosus

2.4. Discussion

This chapter aimed to investigate the acute changes in passive stiffness of biarticular hamstring muscles after resistance exercise with different combinations of contraction modes and exercise ROMs. The main findings of the present chapter were that the shear modulus of SM decreased immediately after SDL with EW. Meanwhile, no changes in the shear moduli of BFlh or ST were observed at any time points in any exercise sessions. These results partly support the first hypothesis that muscle stiffness would be greatly decreased immediately after resistance exercise with EW and support the second hypothesis that the magnitude of change in stiffness immediately after the exercise would be greater in SM than in the other hamstring muscles. Several studies have examined the acute effects of resistance exercise on muscle stiffness (Lacourpaille et al. 2014; Pournot et al. 2016; Morales-Artacho et al. 2017; Lacourpaille et al. 2017; Xu et al. 2019; Chalchat et al. 2020; Ema et al., 2021; Kisliewicz et al. 2020). However, the findings of these studies are inconsistent, and less information is available regarding the possible effects of program variables of resistance exercise on passive muscle stiffness. In the present study, SDL with EW immediately decreased the shear modulus of SM, but that with EN or CW did not affect the shear modulus of any of the examined muscles. This suggests that the combination of eccentric contraction with a wide ROM of resistance exercise is important to immediately decrease the stiffness of a specific muscle.

In the present study, the shear modulus of SM decreased with EW, while that of BFlh and ST did not change in any exercise sessions at 3 min post-exercise. Additionally, the shear moduli of the biarticular hamstring muscles did not change at 30 min post-exercise in any sessions. These results are not consistent with those of previous studies that reported an increase in the muscle shear modulus (biceps brachii and rectus femoris) immediately (Xu et al. 2019) and 30 min (Lacourpaille et al. 2014, 2017) after eccentric exercise. This discrepancy may be explained by the difference in the exercise load between the present and previous studies (Lacourpaille et al. 2014, 2017; Xu et al. 2019). In the previous studies, the eccentric exercise was performed with a higher load (MVC [Lacourpaille et al. 2014, 2017; Xu et al. 2019]) to induce severe muscle damage than in the present study (a load of 60% of body mass). It has been reported that eccentric exercise at a high load induced greater muscle damage (assessed by changes in maximal voluntary torque and joint ROM) than exercise at low to moderate loads (Chen et al. 2007). In fact, the change in maximal voluntary torque immediately after exercise was larger in the previous study (−16.8% [mean value, Xu et al. 2019]) than in SDL with EW in the present study (+0.3% [mean value]). Muscle damage induced by eccentric exercise was shown to acutely increase the shear modulus, possibly due to an increase the number of stable cross-bridges followed by rapid calcium homeostasis perturbation (Lacourpaille et al. 2014). Hence, the higher load exercise in the previous studies (Lacourpaille et al. 2014, 2017; Xu et al. 2019) might cause an acute increase in muscle stiffness due to severe muscle damage. The present results suggest that low to moderate-load eccentric exercise with a wide ROM may result in an acute decrease in muscle stiffness.

In the present study, there were large individual differences in the change in shear modulus after resistance exercise. For example, two participants showed an immediate increase in shear modulus of SM, whereas four participants showed an immediate decrease by more than 10 kPa at 3 min post-exercise in EW. To identify the underlying factor(s) responsible for the individual differences, I examined the correlation between the shear modulus of SM at pre-exercise and its change at 3 min post-exercise in EW. However, the correlation was not statistically significant ($r = -0.478$, $p = 0.098$, data not shown). Thus, it appears that the individual difference in the shear modulus at pre-exercise was not a factor for the difference in the exercise-induced change in shear modulus. Other factors, such as fascicular lengths and/or strain during SDL, might be related to the inter-individual variability in the immediate changes in shear modulus. Meanwhile, the maximal joint ROM immediately increased after SDL with EN. This result is consistent with those of previous studies that reported an acute increase in maximal joint ROM after eccentric exercise (Nelson et al. 2006; Aune et al. 2019). However, the underlying factors have not been clarified in the previous studies. My correlation analysis showed that the change in the maximal joint ROM was positively correlated with the change in passive torque, but not with the change in the shear moduli of the biarticular hamstring muscles in

SDL with EN. Although the change in passive torque at 3 min post-exercise did not reach statistical significance, the results of the correlation analysis suggest that the acute increase in the maximal joint ROM following SDL with EN is attributable to an increase in stretch tolerance rather than the change in the mechanical properties of the muscles. Inconsistent with the present finding of SDL with EN and previous findings (Nelson et al. 2006; Aune et al. 2018), the maximal joint ROM did not immediately change after SDL with EW. It is difficult to explain this result, but it might be related to differences in movement velocity and/or muscle activation levels. The participants performed SDL with EW (100% of maximal ROM) and EN (50% of maximal ROM) at the same cadence (2 s). This means that the movement velocity per repetition was different between EW and EN. Such a difference between EW and EN might have influenced immediate changes in the joint ROM.

In summary, the present study investigated the acute changes in passive stiffness of biarticular hamstring muscles after resistance exercise with different combinations of contraction modes and exercise ROMs. The results showed that the shear modulus of SM decreased immediately after SDL with EW, but did not change after SDL with EN or CW. Additionally, no changes in the shear moduli of BFlh or ST were observed at any time points in any exercise sessions. Collectively, the present results suggest that the combination of eccentric contraction with a wide exercise ROM during resistance exercise has the potential to immediately decrease the passive stiffness (shear modulus) of a specific muscle.

Chapter 3. Acute changes in passive stiffness of the biarticular hamstring muscles induced by resistance exercise: effects of muscle length and exercise duration

3.1. Introduction

In Chapter 2, resistance exercise with eccentric contractions with a wide ROM immediately decreased the shear modulus of SM, whereas exercise with eccentric contractions with a narrow ROM and exercise with concentric contractions with a wide ROM did not change the shear modulus of any of the biarticular hamstring muscles. These results suggest that the combination of eccentric contractions with a wide ROM during resistance exercise is important to immediately decrease the stiffness of a specific muscle. However, the magnitude of the decrease in SM shear modulus in Chapter 2 (−7.2 kPa [mean value]) was not so large when compared to previous studies that reported the stretching-induced immediate decrease in SM shear modulus (−12.8 kPa [mean value, Nakao et al. 2018]; −34.0 kPa [Umegaki et al. 2015]). It is therefore necessary to explore further approaches to induce a large immediate decrease in muscle stiffness by resistance exercise.

It has been shown that static stretching at a long muscle length (80% of maximal joint ROM) induced a greater immediate decrease in muscle shear modulus than the stretching at a short muscle length (40% of maximal joint ROM [Freitas et al. 2015]). Furthermore, the magnitude of the immediate decrease in muscle shear modulus was greater after static stretching with a long duration (300 s) than after static stretching with a short duration (120 s [Caliskan et al. 2019]). These findings suggest the possibility that resistance exercise at long muscle lengths with a long duration could induce a large immediate decrease in muscle stiffness. Meanwhile, the duration that muscle was kept at its maximum lengths in each repetition of the exercise was shorter in EW of Chapter 2 (0 to 100% of maximal exercise ROM) than in the previous stretching study that reported a large decrease in the hamstring muscle stiffness (consistently 100% of maximal joint ROM [Umegaki et al. 2015]). Moreover, the exercise duration (duration per repetition × the total number of repetitions) was also shorter in EW of Chapter 2 (60 s) than in the stretching study (300 s [Umegaki et al. 2015]). Therefore, the acute effects of resistance exercises with different muscle lengths and exercise durations on muscle stiffness needs to be further examined.

The aim of the present chapter was to examine the acute changes in stiffness of the biarticular hamstring muscles after resistance exercises with different combinations of muscle lengths and exercise durations. Throughout the present chapter, eccentric-only SDL was adopted based on the results in Chapter 2. I hypothesized that muscle stiffness would be greatly decreased immediately after eccentric-only resistance exercise with long muscle lengths and a long duration as compared to an exercise with long muscle lengths and a short duration and an exercise with short muscle lengths and a short duration. Moreover, it was also hypothesized that the magnitude of the change in stiffness observed immediately after the eccentric-only exercise would be the greatest in SM among the biarticular hamstring muscles similar to the findings in Chapter 2. Part of this chapter has been published elsewhere (Kawama et al. 2023).

3.2. Methods

Participants

Thirteen healthy young males (age: 23.9 ± 2.9 years; height: 170.0 ± 5.7 cm; body mass: 63.3 ± 5.1 kg) were recruited for this study. A priori power analysis for time-course changes in shear moduli of the biarticular hamstring muscles was performed to determine the number of participants for one-way analysis of variance with a power of 80%, an α error of 0.05, and an effect size (partial η^2) of 0.40 using G*Power 3.1 software (Heinrich Heine University, Dusseldorf, Germany). The effect size of 0.40 was adopted based on a previous study that showed a significant interaction between exercise session and time (partial $\eta^2 = 0.45$ [Morales-Artacho et al. 2017]) for the shear modulus of the hamstrings. The power analysis showed a minimum sample size of 12, and therefore 13 participants were recruited. None of the participants reported a previous history of neuromuscular diseases or musculoskeletal injuries specific to the right lower extremity. They were asked to refrain from strenuous exercise 48 h before each session. Throughout the experiment, the participants were instructed to keep their daily activities and not to engage in additional interventions, such as stretching and resistance exercises. Each participant provided written informed consent after being informed of the purpose, procedures, and possible risks related to this study. This study was approved by the Doshisha University Research Ethics Committee (no. 21006).

Experimental design

I examined the acute effects of three resistance exercise sessions with different muscle lengths and exercise durations on the shear modulus of the biarticular hamstring muscles (Fig. 3-1). This study comprised four sessions, including a familiarization session and three sessions of resistance exercise. When the participants first visited my laboratory, they conducted the familiarization session. On the subsequent 3 days, they performed three sessions of eccentric-only resistance exercise that comprised SDL with different muscle lengths and exercise durations. The sessions were: (1) short muscle lengths with a short duration (SS); (2) long muscle lengths with a short duration (LS); and (3) long muscle lengths with a long duration (LL). Three sessions of resistance exercise were performed in a randomized order among participants on separate days with an interval of >5 days. Before, and at 3, 30, and 60 min after each session of SDL, maximal joint ROM, passive torque, shear moduli of the biarticular hamstring muscles, and maximal isometric torque of knee flexion were measured in the order described above. Additionally, EMG activities of the biarticular hamstring muscles were obtained during the measurements of shear modulus and maximal isometric torque. The measurement procedures of the aforementioned variables were the same as those in Chapter 2.

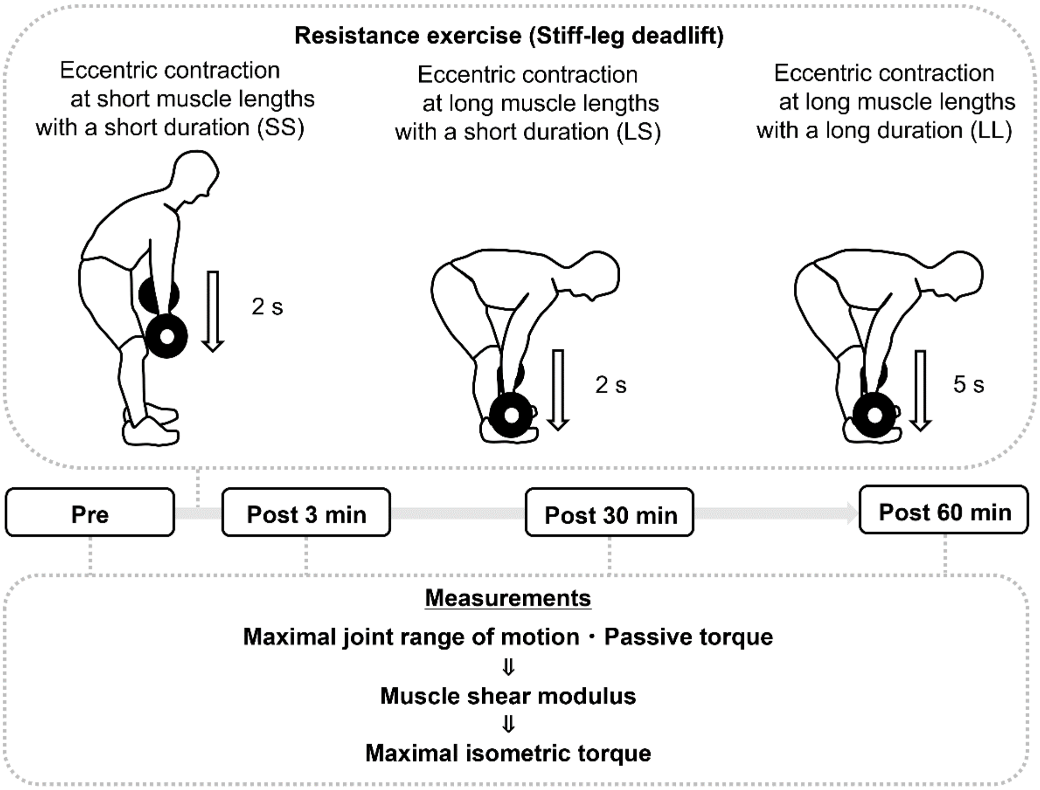

Fig. 3-1 Overview of study design

Participants performed three sessions of resistance exercise over 3 days in each experiment. Maximal knee joint range of motion, passive torque, muscle shear modulus, and maximal isometric torque were measured before, and at 3, 30, and 60 min after completing each session of resistance exercise.

Resistance exercise

Before three sessions of resistance exercise, participants underwent a familiarization session comprising three sets of 10 repetitions of eccentric-only SDL with a load of 60% of their body mass. During the concentric phase of SDL, the barbell was elevated by an examinator to achieve eccentric-only SDL. The eccentric phase of SDL was completed in 2 s with a metronome set to 60 bpm. The feet were placed parallel with the distance between the middle of the heels set at 20% of the participant's height. The maximal ROM of SDL was defined as the difference in the barbell heights between the upright position (0% of the maximal exercise ROM) and the lowest position (100% of the maximal exercise ROM) that the participants could achieve. The maximal ROM of SDL across all repetitions during the first session was averaged, and used to determine the exercise ROM of each session for the individual participants.

During the subsequent three sessions, the participants conducted one of three resistance exercise sessions after a warm-up protocol, comprising five repetitions of eccentric-only SDL with a load of 60% of their body mass. In each session, the participants performed three sets of 10 repetitions of eccentric-only SDL with the same load and foot position as in the familiarization session (Table 3-1). Short and long muscle lengths were defined as 0 to 50% and 50 to 100% of the maximal ROM of SDL, respectively. Short and long exercise durations were defined as 60 s (2 s per repetition × 30 repetitions) and 150 s (5 s per repetition × 30 repetitions), respectively. Sufficient rest (2 min) was allowed between sets.

Table 3-1 Resistance exercise sessions

Session	Contraction mode	Range of motion of stiff-leg deadlift (%)	Load (%)	Sets	Repetitions	Duration per repetition (s)	Exercise duration (s)
SS	Eccentric	0 to 50	60	3	10	2	60
LS	Eccentric	50 to 100	60	3	10	2	60
LL	Eccentric	50 to 100	60	3	10	5	150

SS, eccentric contractions at short muscle lengths with a short duration; LS, eccentric contractions at long muscle lengths with a short duration; LL, eccentric contractions at long muscle lengths with a long duration

[a] Range of motion of stiff-leg deadlift and load were presented as relative values of maximal range of motion of the exercise (0% = upright position) and body mass, respectively

Statistical analysis

The Shapiro-Wilk normality test showed that the data of the shear moduli of the biarticular hamstring muscles were partly non-Gaussian (p = 0.007 to 0.991), and therefore the Friedman test was used to determine significant effects of time (time points) on the change in maximal joint ROM, passive torque, muscle shear modulus, and maximal isometric torque during each session. If a significant main effect of time was observed, the Wilcoxon signed-rank test was performed to identify significant differences in the aforementioned variables. The Spearman's rank correlation analysis was performed to clarify the association between the change in maximal joint ROM and the change in the other measured variables at 3 min post-exercise in each session. For multiple tests, the p-values were corrected using Benjamini–Hochberg method (Glickman et al. 2014) at a cut-off of a false discovery rate of < 0.05. The effect size (r) was calculated for all the tests. The results of this study are presented as median value (interquartile range). All statistical analyses were performed by using the statistical software package (IBM SPSS Statistics, version 27.0, IBM Corporation, Armonk, USA) and the web application (Langtest, Atsushi Mizumoto, Kansai University, Japan [Mizumoto and Plonsky, 2016]).

3.3. Results

Maximal joint ROM & passive torque

The Friedman test revealed a significant main effect of time on the maximal joint ROM in SS ($p = 0.022$, $r = 0.64$) and LL ($p = 0.016$, $r = 0.67$), but not in LS ($p = 0.520$, $r = 0.18$ [Fig. 3-2]). In LL, the maximal joint ROM was significantly larger at 3 min post-exercise (58.0° [51.4 to 75.5°], corrected $p = 0.019$, $r = 0.54$), 30 min post-exercise (52.3° [45.7 to 66.1°], corrected $p = 0.019$, $r = 0.54$), and 60 min post-exercise (50.2° [45.6 to 66.6°], corrected $p = 0.043$, $r = 0.45$) than at pre-exercise (49.9° [46.0 to 63.2°]). Meanwhile, no significant differences in maximal joint ROM were found between any time points in SS (corrected $p = 0.096$ to 0.305, $r = 0.20$ to 0.40). There was no significant main effect of time on passive torque in SS ($p = 0.076$, $r = 0.49$), LS ($p = 0.392$, $r = 0.24$), or LL ($p = 0.237$, $r = 0.33$).

Maximal isometric torque

There was a significant main effect of time on the maximal isometric torque in SS ($p = 0.035$, $r = 0.59$), but not in LS ($p = 0.338$, $r = 0.27$) or LL ($p = 0.128$, $r = 0.42$). In SS, the maximal isometric torque at 60 min post-exercise (71.0 Nm [55.6 to 83.8 Nm]) was significantly lower than that at pre-exercise (80.6 Nm [58.6 to 88.9 Nm], corrected $p = 0.043$, $r = 0.47$), 3 min post-exercise (75.9 Nm [66.7 to 88.3 Nm], corrected $p = 0.043$, $r = 0.45$), and 30 min post-exercise (72.6 Nm [60.5 to 86.0 Nm], corrected $p = 0.043$, $r = 0.47$).

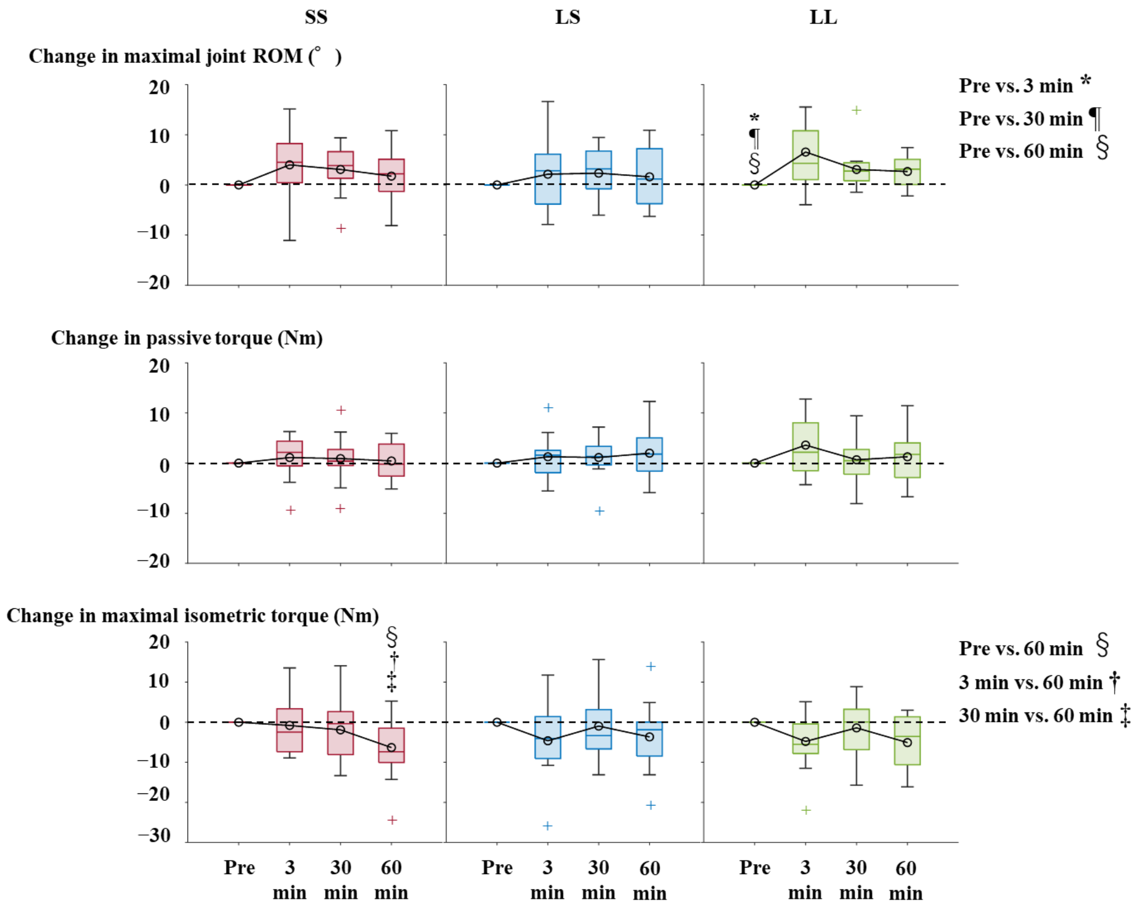

Fig. 3-2 Boxplots of the time-course changes in maximal joint range of motion (ROM), passive torque, and maximal isometric torque following the resistance exercise sessions. SS, short muscle lengths with a short duration (red box); LS, long muscle lengths with a short duration (blue box); LL, long muscle lengths with a long duration (green box). *: pre-exercise vs. 3 min post-exercise; ¶: pre-exercise vs. 30 min post-exercise; §: pre-exercise vs. 60 min post-exercise; †: 3 min post-exercise vs. 60 min post-exercise; ‡: 30 min post-exercise vs. 60 min post-exercise; ○: group mean value for each measured variable. +: outlier value for each measured variable.

Muscle shear modulus

During the shear moduli measurements, the RMS-EMGs were 0.1 to 1.3% MVC, 0.3 to 0.5% MVC, and 0.1 to 1.1% MVC for BFlh, ST, and SM, respectively across all exercise sessions.

In BFlh, there was a significant main effect of time on the shear modulus in LL ($p = 0.046$, $r = 0.55$), but not in SS ($p = 0.917$, $r = 0.03$) or LS ($p = 0.576$, $r = 0.16$ [Fig. 3-3]). However, no significant differences in shear modulus of BFlh were observed between any time points in LL (corrected $p = 0.080$ to 0.787, $r = 0.05$ to 0.49). In ST, a significant main effect of time on the shear modulus was found in LS ($p = 0.007$, $r = 0.75$) and LL ($p = 0.012$, $r = 0.70$), but not in SS ($p = 0.089$,

$r = 0.41$). In LL, the shear modulus of ST at 30 min-post exercise (41.7 kPa [37.2 to 51.9 kPa]) was significantly lower than that at pre-exercise (48.2 kPa [39.3 to 58.0 kPa], corrected $p = 0.001$, $r = 0.72$). Meanwhile, no significant differences in shear modulus of ST were found between any time points in LS (corrected $p = 0.064$ to 0.839, $r = 0.04$ to 0.47). In SM, there was a significant main effect of time on shear modulus in SS ($p = 0.031$, $r = 0.60$) and LL ($p = 0.002$, $r = 0.86$), but not in LS ($p = 0.595$, $r = 0.15$). In LL, the shear modulus of SM at 3 min post-exercise (128.1 kPa [120.2 to 136.1 kPa]) was significantly lower than that at pre-exercise (135.2 kPa [129.0 to 140.5 kPa], corrected $p = 0.009$, $r = 0.56$) and 60 min post-exercise (142.7 kPa [137.5 to 155.2 kPa], corrected $p = 0.004$, $r = 0.66$). Meanwhile, no significant differences in the shear modulus of SM were observed at any time points in SS (corrected $p = 0.119$ to 0.999, $r = 0.01$ to 0.44).

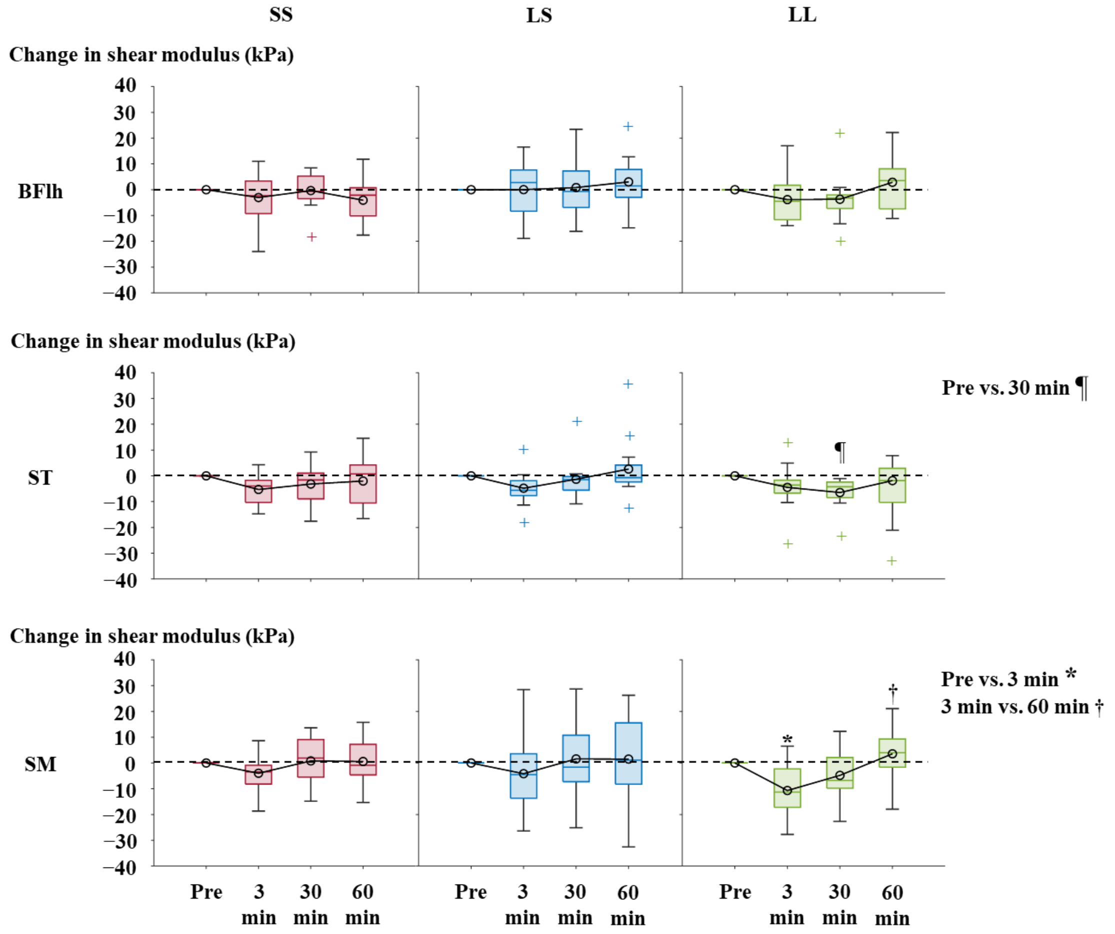

Fig. 3-3 Boxplots of the time-course changes in the shear moduli of the biarticular hamstrings following the resistance exercise sessions. SS, short muscle lengths with a short duration (red box); LS, long muscle lengths with a short duration (blue box); LL, long muscle lengths with a long duration (green box); BFlh, biceps femoris long head; ST, semitendinosus; SM, semimembranosus. *: pre-

exercise vs. 3 min post-exercise; ¶: pre-exercise vs. 30 min post-exercise; †: 3 min post-exercise vs. 60 min post-exercise; ○: the group mean value in each measured variable. +: outlier value in each measured variable.

Association between changes in maximal joint ROM and the other measured variables at 3 min post-exercise

There was a significant correlation between the change in maximal joint ROM and the change in passive torque at 3 min post-exercise in SS ($\rho = 0.720$, corrected $p = 0.028$) and LL ($\rho = 0.808$, corrected $p = 0.004$), but not in LS ($\rho = 0.544$, corrected $p = 0.116$, [Table 3-2]). There was no significant correlation between the change in maximal joint ROM and the change in the shear moduli of BFlh, ST, or SM at 3 min post-exercise in SS ($\rho = -0.525$ to -0.181, corrected $p = 0.130$ to 0.554), LS ($\rho = -0.428$ to -0.054, corrected $p = 0.290$ to 0.862), or LL ($\rho = -0.571$ to -0.291, corrected $p = 0.090$ to 0.383).

Table 3-2 Association between the changes in maximal joint ROM and the other measured variables at 3 min post-exercise

	Passive torque		**BFlh**		**ST**		**SM**	
	ρ	*Corrected p*	*ρ*	*Corrected p*	*ρ*	*Corrected p*	*ρ*	*Corrected p*
SS	0.720	0.028*	−0.412	0.326	−0.346	0.329	−0.016	0.964
LS	0.544	0.116	−0.342	0.373	−0.115	0.710	−0.582	0.116
LL	0.808	0.004*	−0.291	0.383	−0.264	0.383	−0.571	0.090

*Significant correlation between the change in maximal joint ROM and the change in passive torque ($p < 0.05$). ROM, range of motion; SS, short muscle lengths with a short duration; LS, long muscle lengths with a short duration; LL, long muscle lengths with a long duration; BFlh, biceps femoris long head; ST, semitendinosus; SM, semimembranosus

3.4. Discussion

The present study showed that the shear modulus of SM decreased immediately after eccentric-only SDL with LL, whereas the shear moduli of BFlh or ST did not change immediately in any of exercise sessions. These results partially support my hypotheses that (1) muscle stiffness is greatly decreased immediately after eccentric-only resistance exercise with LL, and that (2) the magnitude of change in stiffness immediately after the eccentric-only exercise is the greatest in SM among the examined hamstring muscles. In the present study, SM shear modulus decreased immediately after SDL with LL, but not after SDL with LS. These results indicate that a long exercise duration during eccentric-only resistance exercise is essential to immediately decrease the muscle stiffness. Therefore, my results suggest that the combination of long muscle lengths and a long duration during eccentric-only resistance exercise is important to immediately decrease the stiffness of a specific muscle.

In this study, the shear modulus of SM decreased immediately after eccentric-only SDL with LL, but not after that with LS. These results could be interpreted by the difference in exercise duration between LL and LS. The exercise duration was 2.5 times longer in LL (150 s) than in LS (60 s) in this study, whereas the other program variables, such as load and exercise ROM, were the same in the two sessions. It has been reported that static stretching with a long duration (300 s) immediately decreased muscle shear modulus to a greater extent than stretching with a short duration (120 s [Caliskan et al. 2019]). Thus, the longer exercise duration in LL may have accounted for the substantial decrease in SM shear modulus in the present study. Meanwhile, the stiffness of the biarticular hamstring muscles was not immediately decreased at any time points in LS as well as in SS. These results are inconsistent with a previous finding that static stretching at a long muscle length (80% of maximal joint ROM) substantially decreased muscle shear modulus as compared to that at a short muscle length (40% of maximal joint ROM [Freitas et al. 2015]). It is difficult to explain this discrepancy between the previous (Freitas et al. 2015) and present studies; however, the involvement of muscle contraction might have an effect on the magnitude of change in muscle stiffness. Static stretching is performed under passive conditions (without any muscle contractions), whereas the eccentric-only resistance exercise is performed with muscle contraction. The muscle contraction could limit the lengthening of muscle fascicles; thus, the eccentric-only exercise at long muscle lengths alone may not be sufficient to immediately decrease the stiffness.

In contrast to the present study, previous studies showed an increase in muscle shear modulus immediately after eccentric-only resistance exercise (Xu et al. 2019; Voglar et al. 2022). These inconsistent results among studies might be caused by the different exercise loads and/or the number of repetitions. In the previous reports (Xu et al. 2019; Voglar et al. 2022), the load during eccentric exercise was higher (MVC) than in my study (a load of 60% of body mass). Moreover, the total number of repetitions was also higher in the previous studies (48 repetitions [Voglar et al. 2022], 75 repetitions [Xu et al. 2019]) than in my study (30 repetitions). Previous studies reported that the eccentric exercise with high-load (Chen et al. 2007) and/or high repetitions (Nosaka et al. 2003) caused severe muscle damage (evaluated by a change in maximal voluntary torque) as compared to that with low load and/or low repetitions. In fact, the magnitude of the immediate decrease in maximal voluntary torque was greater after the eccentric exercise in Xu et al. (2019 [−16.8%, mean value]) than after the eccentric-only SDL with LL in my study (−6.0%, mean value). Unfortunately, Voglar et al. (2022) did not measure the maximal voluntary torque. Muscle damage following eccentric exercise was suggested to acutely increase the muscle shear modulus, possibly due to an increase in rapid perturbation of calcium homeostasis (Lacourpaille et al. 2014). Therefore, the higher load and greater number of repetitions of eccentric exercise in the previous studies (Xu et al. 2019; Voglar et al. 2022) might have caused severe muscle damage, thereby indirectly increasing the muscle shear modulus.

Resistance exercise with low-to-moderate load and repetitions may be recommended for an immediate decrease in muscle stiffness.

The ST shear modulus decreased only at 30 min post-exercise in SDL with LL. A previous study reported that ST has the longest fascicle length among the hamstring muscles (Woodley and Mercer 2005). In this case, the fascicular strain of ST could be smaller than those of BFlh and SM during the eccentric-only exercise. Thus, factor(s) other than the fascicular strain would contribute to the decrease in ST stiffness after SDL with LL. Although the underlying mechanism(s) regarding this result are not identified, the change in muscle shear modulus was suggested to be influenced by the viscosities of titin (Kellermayer et al. 1997, 2001; Mártonfalvi et al. 2014) and muscle connective tissue (Chytil et al. 2010; Stecco et al. 2021 [see general discussion]). Since these variables were not measured in the present study, further studies are warranted to examine the underlying factor(s) responsible for the exercise-induced changes in muscle shear modulus.

The maximal joint ROM increased immediately after SDL with LL, which is in accordance with the results of a previous study that observed an immediate increase in the maximal joint ROM after eccentric exercise (Nelson 2006). To clarify the underlying factor(s), I performed correlation analyses and revealed that the change in maximal joint ROM at 3 min post-exercise had a positive correlation with the change in passive torque in LL. This suggests that an increase in stretch tolerance is associated with the immediate increase in the maximal joint ROM after SDL with LL.

There were some inconsistent results between the experiments in Chapters 2 and 3. For example, the maximal joint ROM was not changed at any time points after SDL with SS in the present study, whereas that was increased at 3 min after SDL with a combination of eccentric-only contractions and a narrow exercise ROM (EN: equal to SS) in Chapter 2. These inconsistent results may be partly explained by the different magnitudes of the change in passive torque. The magnitude of the change in passive torque at 3 min post-exercise was slightly smaller in SS (2.2 Nm [−0.4 to 4.3 Nm]) than in EN (2.9 Nm [0.1 to 5.9 Nm]). The present and previous results of correlation analyses showed that the change in maximal joint ROM at 3 min post-exercise was positively correlated with the change in passive torque at 3 min post-exercise in SS and EW. These results suggest that the change in maximal joint ROM is partly determined by the change in passive torque in both SS and EN. Collectively, the slightly smaller change in the passive torque in SS may be related to the present result of the lack of significant immediate increase in maximal joint ROM in SS.

As another inconsistent result between Chapters 2 to 3, the maximal isometric torque was decreased at 60 min post-exercise in SS of the present chapter, while that was not changed at any time points in EN of Chapter 2. To identify the underlying factor(s) for the decrease in the maximal isometric torque at 60 min post-exercise in SS, I examined the time-course changes in RMS-EMG of the biarticular hamstring muscles during the maximal voluntary isometric contractions in SS and EN. In SS, RMS-EMG of ST at 60 min post-exercise was significantly lower than that at pre-exercise (corrected *p* = 0.020, *r* = 0.52) and 3 min post-exercise (corrected *p* = 0.020, *r* = 0.49), whereas no significant differences in RMS-EMG of BFlh or SM were observed between any time points. Meanwhile, there were no significant time-course changes in RMS-EMG of any of the biarticular hamstring muscles in EN (corrected *p* = 0.417 to 0.916, *r* = 0.02 to 0.35). Thus, the changes in RMS-EMG of ST after SDL may be, at least in part, responsible for the inconsistent results in the maximal isometric torque between Chapters 2 to 3.

There is a limitation that should be addressed here. The statistical power in some variables might not be large to detect significant paired differences between time points, possibly due to the use of the nonparametric statistical test and small sample size. Prior to this study, the ideal number of participants (n = 12) was determined by a priori power analysis for one-way analysis of variance with a power of 80%, an α error of 0.05, and an effect size (partial η^2) of 0.40 using G*power. Thus, 13 participants were recruited for the present study. Meanwhile, the Wilcoxon signed-rank test (matched pair) was eventually used to identify significant differences in the measured variables because some of the data were non-Gaussian. To examine the actual statistical powers, I performed a post-hoc power analysis with an actual effect size (*r*), α error, and sample size for the Wilcoxon signed-rank test (matched pair). The results showed that the actual statistical powers were not large in some variables (e.g., SM shear modulus between pre and 3 min post-exercise in LS [calculated statistical power = 52%, actual α error = 0.216, *r* = 0.24, sample size = 13]). As the relatively low statistical power may increase the risk of type I error, future studies are warranted with more large sample size, especially when using nonparametric statistical tests to investigate the time-course changes in muscle shear modulus after resistance exercise.

In summary, the results of this chapter revealed that SM shear modulus decreased immediately after SDL with LL, but not after SDL with SS or LS. No immediate changes in shear moduli of BFlh or ST were observed in any exercise sessions. Taken together, the present results suggest that the combination of long muscle lengths and a long duration during eccentric-only resistance exercise is important to immediately decrease the stiffness of a specific muscle.

Chapter 4. Chronic effects of resistance training on passive stiffness of the hamstring muscles

4.1. Introduction

In Chapter 3, eccentric-only resistance exercise with LL immediately decreased the passive stiffness of SM. The magnitude of the immediate decrease in SM stiffness was greater after SDL with LL (−11.3 kPa [−15.2 to −2.6 kPa]) in Chapter 3 than after SDL with EW (−5.5 kPa [−13.3 to −1.9 kPa]) in Chapter 2. These results imply that eccentric-only resistance exercise with LL is more effective in immediately decreasing the stiffness of a specific muscle than the exercise with EW. Meanwhile, the acute decrease in passive muscle stiffness induced by passive knee extension stretching was reported to be greater in SM than in BFlh and ST (Umegaki et al. 2015). Similarly, the chronic decrease in passive muscle stiffness was also greater in SM than in BFlh and ST after a 4-week stretching intervention (Ichihashi et al. 2016) using the aforementioned stretching maneuver (Umegaki et al. 2015). These findings suggest that the immediate changes in passive muscle stiffness correspond to the chronic changes in stiffness. Thus, it is possible that eccentric-only resistance training with LL also chronically decreases SM stiffness. This possibility has not been investigated yet, although several studies examined the effects of resistance training on passive muscle stiffness (Akagi et al. 2016; Seymore et al. 2017; Ochi et al. 2018; Mannarino et al. 2019; Vatovec et al. 2021).

The purpose of the present chapter was to clarify the chronic effects of eccentric-only resistance training with LL on passive stiffness of the biarticular hamstring muscles (BFlh, ST, and SM). It was hypothesized that eccentric-only resistance training with LL chronically decreases SM stiffness.

4.2. Methods

Experimental design

The present study consisted of screening, testing, and training sessions (Fig. 4-1). Initially, the participants took part in the screening session including flexibility test and practice of resistance exercise. Through the screening session, the participants were selected for the testing and training sessions (described in the following section). One month after the screening session, the testing session was started to measure the maximal joint ROM, passive torque, shear moduli of the biarticular hamstring muscles, maximal isometric torque of knee flexion, and muscle volume of the biarticular hamstring muscles. These measurements were conducted across two experimental days. The muscle volumes were measured on the first day of the testing session and the other variables (maximal joint ROM, passive torque, shear modulus, and maximal isometric torque) were measured on the second day. One week after the testing session, the training session was started. In the training session, the participants in the training group performed three sets of 10 repetitions of eccentric-only SDL with LL for 10 weeks (two sessions per week). Meanwhile, the participants in the control group did not perform any resistance training or stretching for the lower limb muscles throughout the intervention period. Three to 7 days after completion of the final training session, the aforementioned variables were measured again.

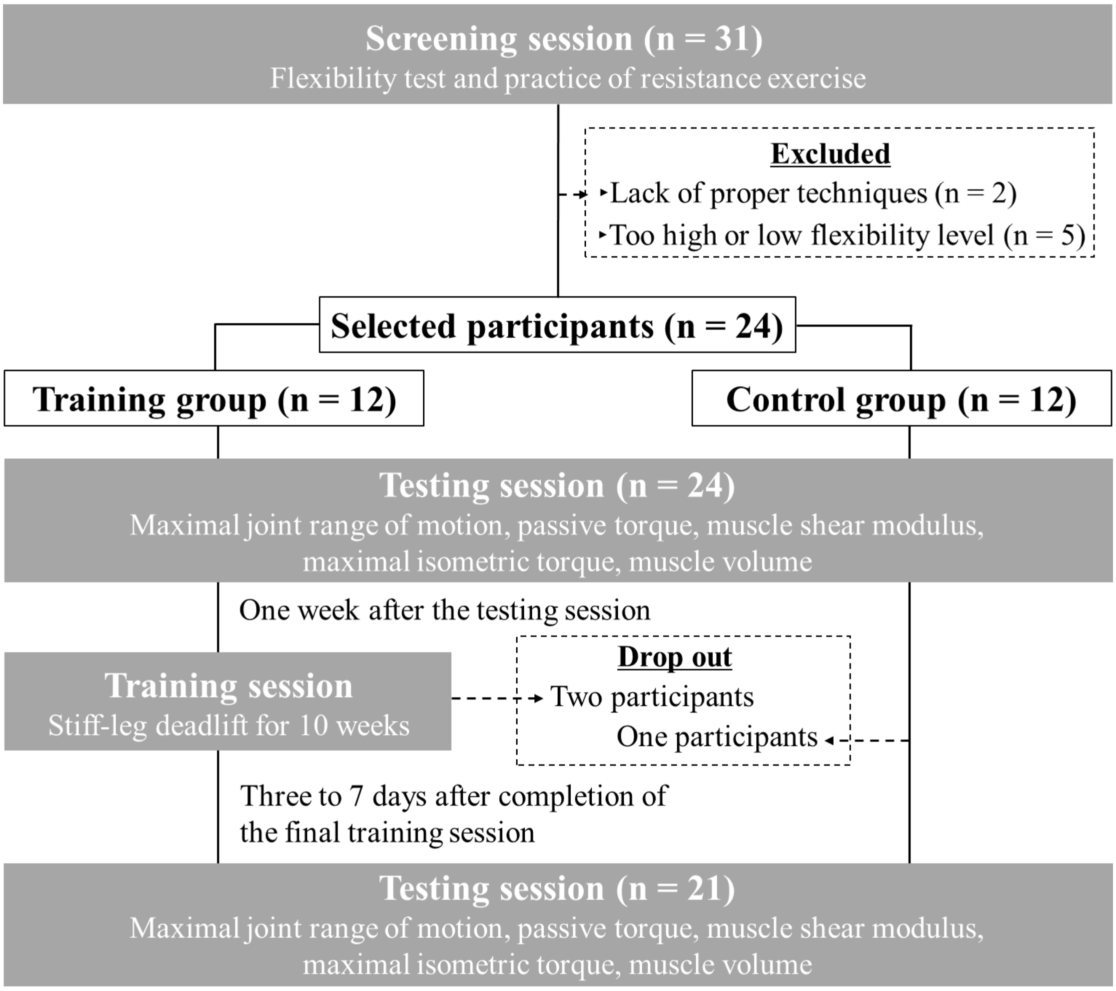

Fig. 4-1 Flow chart of the study design.

Participants

The number of participants was determined using a priori power analysis for paired t-test with a power of 80%, an α error of 0.05, and an effect size (Cohen's *d*) of 0.95 using G*Power 3.1 software (Heinrich Heine University, Dusseldorf, Germany). The effect size of 0.95 was chosen based on a previous study that reported a significant decrease in the shear modulus of BFlh immediately after eccentric-only knee flexion (Zhi et al. 2022). The results of the power analysis showed the minimal sample size of 11 in each experimental group. Initially, 31 healthy young males were recruited for the screening session. Thereafter, 24 participants were selected for the testing and training sessions considering possible attrition during the intervention period. They were randomly assigned to either a training (n = 12; age, 20.7 ± 1.6 years; height, 171.6 ± 6.7 cm; body mass, 62.7 ± 4.8 kg) or a control group (n = 12; age, 20.0 ± 2.3 years; height, 172.3 ± 4.4 cm; body mass, 63.2 ± 5.7 kg). None of the participants had performed any types of resistance training for at least 1 year prior to this study, and had histories of musculoskeletal injuries of the right lower extremity in the past 5 years. All participants completed an informed consent form after a careful explanation of the purpose, procedures, and possible risks of this study. The present study was approved by the Doshisha University Research Ethics Committee (no. 21603).

Screening session

The stand and reach test was used to assess the flexibility level of the participants before the intervention period. The participants started this testing from the upright position with legs fully extended on a bed. Subsequently, they slowly lowered their both arms along the legs until their maximum tolerances. The vertical distance between the surface of the bed and the middle fingertips of both hands was measured with a steel tape and defined as flexibility level. The flexibility level was evaluated as positive and negative values when the middle fingertips were located below and above the surface of the bed, respectively.

After the flexibility test, the participants performed three sets of 10 repetitions of SDL with a load of 60% of their body mass. The SDL included concentric and eccentric phases, and each phase was completed in 2 s with a metronome set to 60 bpm. The feet were placed parallel to the distance between the middle of the heels, which was set at 20% of the participant's height. Before the start of the practice, a Certified Strength and Conditioning Specialist carefully instructed the proper techniques of SDL to the participants. During the practice, the specialist checked whether the participants could perform the exercise with the proper techniques throughout all repetitions. The barbell height was determined with a wire-type displacement transducer (DTPA-A-2K, KYOWA, Tokyo, Japan) attached to the midpoint of the barbell. The barbell height signal was sampled with an A/D converter (PowerLab 16SP, AD Instruments, New South Wales, Australia) at 1 kHz and transferred to a computer. The ROM of SDL was defined as the difference between barbell heights in the upright position and the lowest position that each participant could reach. The mean value of ROM of SDL across all repetitions was calculated and used to determine exercise ROM for the individual participants.

The flexibility level before the intervention period may partly determine the lengthening of hamstring muscles during SDL, thereby affecting the changes in passive muscle stiffness during intervention period. To detect the effects of long muscle lengths during SDL on passive muscle stiffness, it is desirable that the flexibility level is comparable among the participants. Thus, two participants with the highest flexibility levels and three participants with the lowest flexibility levels were excluded from the testing and training sessions. Another two participants could not perform some repetitions of SDL with the proper techniques, and thus were excluded from the subsequent sessions.

Testing session

Maximal joint ROM and passive torque

The measurement procedures of maximal joint ROM and passive torque were the same as those in Chapters 2 and 3.

Muscle shear modulus and electromyographic activity

The measurement procedures of muscle shear modulus and electromyographic activity were the same as those in Chapters 2 and 3. For shear modulus, the measurement region within each hamstring muscle was carefully matched before and after the intervention period based on some anatomical landmarks (e.g., thigh lengths and muscle widths). The shear modulus was measured in the identical position (80% of maximal joint ROM measured before the intervention period) before and after the intervention period. To test the inter-day reliability of shear moduli of the biarticular hamstring muscles, eight healthy young males (age, 23.0 ± 2.2 years; height, 169.6 ± 6.2 cm; body mass, 66.5 ± 14.3 kg) were recruited. The shear modulus of each muscle was measured twice across 2 days with an interval of a week, and the two measured values were averaged for each day. The mean of two values were used to calculate ICC (1, 2) and CV. The ICCs (1, 2) for the shear moduli of BFlh, ST, and SM were 0.94, 0.83, and 0.71, respectively. The CVs for the shear moduli of BFlh, ST, and SM were 5.4%, 6.4%, and 16.1%, respectively.

Maximal voluntary torque

The participants were seated on a dynamometer (Biodex System 4, Biodex Medical Systems, Shirley, USA) with the hips flexed at 85° and the right knee flexed at 30°. The trunk, pelvis, and left thigh were tightly secured to the dynamometer with non-elastic straps. The rotation axis of the dynamometer was aligned with that of the right knee joint, and the lever arm of the dynamometer was attached to the right lower leg. Before the main trials, the participants performed five submaximal voluntary isometric contractions of knee flexion with the arms crossed over the chest as a warm-up. Subsequently, they performed two maximal voluntary isometric contractions (3 s) of knee flexion. Vigorous encouragement was given to the participants to perform maximal voluntary contraction. A rest period of 2 min was provided between trials. The torque was sampled at 1 kHz, and then transferred to the computer with the A/D converter (PowerLab 16SP, AD Instruments, New South Wales, Australia). The peak torque was determined for each trial, and the higher value was used for subsequent analysis. All of the analysis for the maximal isometric torque was performed using computer software (LabChart ver.8, AD Instruments, New South Wales, Australia).

Muscle volume

T1-weighted magnetic resonance (MR) images of the right thigh were obtained using body array and spine coils (Body 18 and CP Spine Array Coil, Siemens Healthineers, Germany) with the following parameters: field of view, 260 × 260 mm; matrix, 512 × 512; slice thickness, 5 mm; pixel size, 0.51 × 0.51 mm; repetition time, 472 ms; echo time, 14 ms; gap, 5 mm; the number of slices, 18 × 3 to 4 blocks. The participants lay supine with their legs extended and muscles relaxed in a 3.0 T magnet bore (MAGNETOM Skyra, Siemens Healthineers, Germany). To avoid contact between the bed of the bore and the hamstring muscles, cushions were placed under the upper body, the upper region of the gluteus (the iliac crests to ischial tuberosity), and the right lower leg. Additionally, the right foot was placed into a handmade pad to minimize the hip joint rotation. Before scanning, the participants lay supine for at least 20 min to reduce the effect of fluid shift by the postural change on cross-sectional areas of the thigh muscles (Berg et al. 1993). In the coronal plane, the most proximal slice was set slightly above the ischial tuberosity (the origin of the biarticular hamstring muscles), and then three to four blocks of images were taken to cover the whole hamstring muscles.

The MR images were analyzed with semi-automatic image analysis software (SASHIMI segmentation, Bartbols). Specifically, the outline of each hamstring muscle was semi-automatically traced from their origins to insertions to assess the anatomical cross-sectional areas of each muscle. When errors and overlaps between adjacent muscle cross-sections were observed, the outline of the muscle was manually traced. In the traced area, the anatomical cross-sectional area of each muscle was calculated as the product of pixel size and the number of pixels with MATLAB 2021b (MathWorks inc., USA). The total volumes of the individual hamstring muscles were determined by summing the anatomical cross-sectional areas of each image times 1 cm (the sum of the slice thickness and interslice gap). The sum of the volume of four hamstring muscles was defined as the volume of the whole hamstring (WHAM). To test the inter-day reliability of the muscle volume analysis, 10 healthy young male participants were recruited on another day. The muscle volume was measured twice, and the two measured values were used to calculate ICC (1, 2) and CV. The ICCs (1, 2) were 0.94, 0.82, 0.94, and 0.91 for BFlh, biceps femoris short head (BFsh), ST, and SM, respectively. The CVs were 1.9%, 1.9%, 2.3%, and 2.3% for BFlh, BFsh, ST, and SM, respectively.

Training session

In each training session, the participants of the training group performed five repetitions of eccentric-only SDL with LL as a practice to check the exercise techniques. The LL during SDL was defined as 50% to 100% of the exercise ROM and 5 s per repetition in the same way as Chapter 3. The exercise ROM was adjusted for each participant with safety bars attached to a power rack and lashing belts for the highest and lowest positions, respectively. To accomplish eccentric-only SDL, the barbell was elevated by an experimenter. After the practice, the participants performed three sets of 10 repetitions of eccentric-only SDL with LL in the same foot position as the screening session. The training loads were 60%, 65%, and 70% of body mass at weeks 1 to 4, 5 to 7, and 8 to 10, respectively. A rest of 2 min was allowed between sets to maintain the proper techniques of SDL. The training session was repeated twice a week on nonconsecutive days for 10 weeks. The participants in both groups were asked to maintain their daily physical activities and not to perform additional resistance training or stretching throughout the intervention period. They were also required to maintain their dietary habits and not to take any supplements.

Seven participants were unable to attend the training session twice within a week due to schedule conflicts on several occasions during the intervention period. In such a case, they attended an additional session in the previous or next week and thereby completed 20 sessions within 10 weeks.

Statistical analysis

The Shapiro-Wilk normality test was performed to assess the distribution of the shear moduli of the biarticular hamstring muscles. The results showed that some of the data were non-Gaussian ($p = 0.001$ to 0.917). Thus, non-parametric tests were used for all of the data in the present chapter. The Mann-Whitney U test was used to compare the measured variables (age, body height, body mass, maximal joint ROM, passive torque, shear modulus, maximal isometric torque, muscle volume, and RMS-EMG) between the training and control groups before the intervention period. The Wilcoxon signed-rank test was performed to identify the differences in the measured variables (except for age, body mass, and body height) before and after the intervention period in each group. Based on the previous findings, I expected that changes in shear moduli of the biarticular hamstring muscles were influenced by shear modulus before the intervention (Hirata et al. 2020) and barbell height at the lowest position during SDL (Freitas et al. 2015 [see discussion section]). Thus, Spearman's rank correlation test was used to examine the relationship between the aforementioned variables and changes in shear moduli of the biarticular hamstring muscles. The significance level was set at $p < 0.05$. For multiple tests, the p-value was corrected using the Benjamini and Hochberg method (Glickman et al. 2014) at a false discovery rate of < 0.05. Specifically, the p-value was corrected based on the number of experimental groups (2) when the measured variables were compared before and after the intervention period. For all the tests in this chapter, the effect size (r) was calculated. The results of this study are presented as median value (interquartile range). All statistical analyses were performed using a statistical software package (IBM SPSS Statistics, version 28.0, IBM Corporation, Armonk, USA) and the web application (Langtest, Atsushi Mizumoto, Kansai University, Japan [Mizumoto and Plonsky, 2016]).

4.3. Results

During the intervention period, two participants in the training group and one participant in the control group dropped out of this experiment, due to COVID-19 infection and a schedule conflict. Thus, the subsequent analyses were performed for 10 and 11 participants in the training and control groups, respectively.

Comparison of the measured variables between groups before the intervention period

There were no significant differences in age (*p* = 0.241, *r* = 0.24), height (*p* = 0.954, *r* = 0.01), or body mass (*p* = 0.977, *r* = 0.01) between the training and control groups. Similarly, no significant differences were found in maximal joint ROM (*p* = 0.526, *r* = 0.14) or passive torque (*p* = 0.888, *r* = 0.03) between the two groups. Additionally, there were no significant differences in the shear modulus of BFlh (*p* = 0.944, *r* = 0.02), ST (*p* = 0.725, *r* = 0.08), or SM (*p* = 0.725, *r* = 0.08) between the two groups. There were no significant between-group differences in maximal isometric torque (*p* = 0.398, *r* = 0.18) or muscle volume of BFlh (*p* = 0.121, *r* = 0.34), BFsh (*p* = 0.260, *r* = 0.25), ST (*p* = 0.067, *r* = 0.40) or SM (*p* = 0.260, *r* = 0.25).

Changes in the measured variables before and after the intervention period

In the training group, maximal isometric torque of knee flexion was significantly increased after the intervention period (corrected *p* = 0.004, *r* = 0.69 [Fig. 4-2]). There was no significant change in maximal isometric torque after the intervention period in the control group (corrected *p* = 0.520, *r* = 0.14).

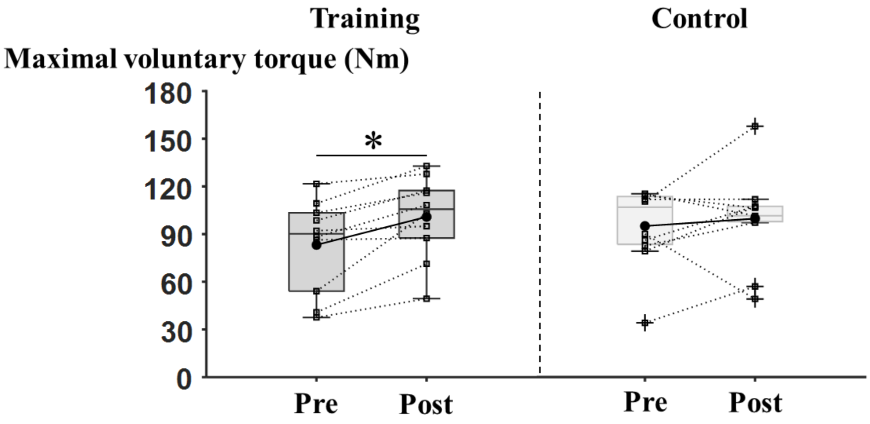

Fig. 4-2 Boxplots of the maximal voluntary torque before and after the intervention period. *: a significant difference before and after the intervention period.

In the training group, muscle volumes of BFlh (corrected *p* = 0.012, *r* = 0.62), ST (corrected *p* = 0.012, *r* = 0.62), SM (corrected *p* = 0.004, *r* = 0.69), and WHAM (corrected *p* = 0.004, *r* = 0.69) were significantly increased after the intervention period (Fig. 4-3). There was no significant change in BFsh volume after the intervention period in the training group (corrected *p* = 0.625, *r* = 0.11). In the control group, there were no significant changes in muscle volumes of BFlh (corrected *p* = 0.278, *r* = 0.23), BFsh (corrected *p* = 0.296, *r* = 0.31), ST (corrected *p* = 0.278, *r* = 0.23), SM (corrected *p* = 0.123, *r* = 0.33), or WHAM (corrected *p* = 0.102, *r* = 0.35) after the intervention period.

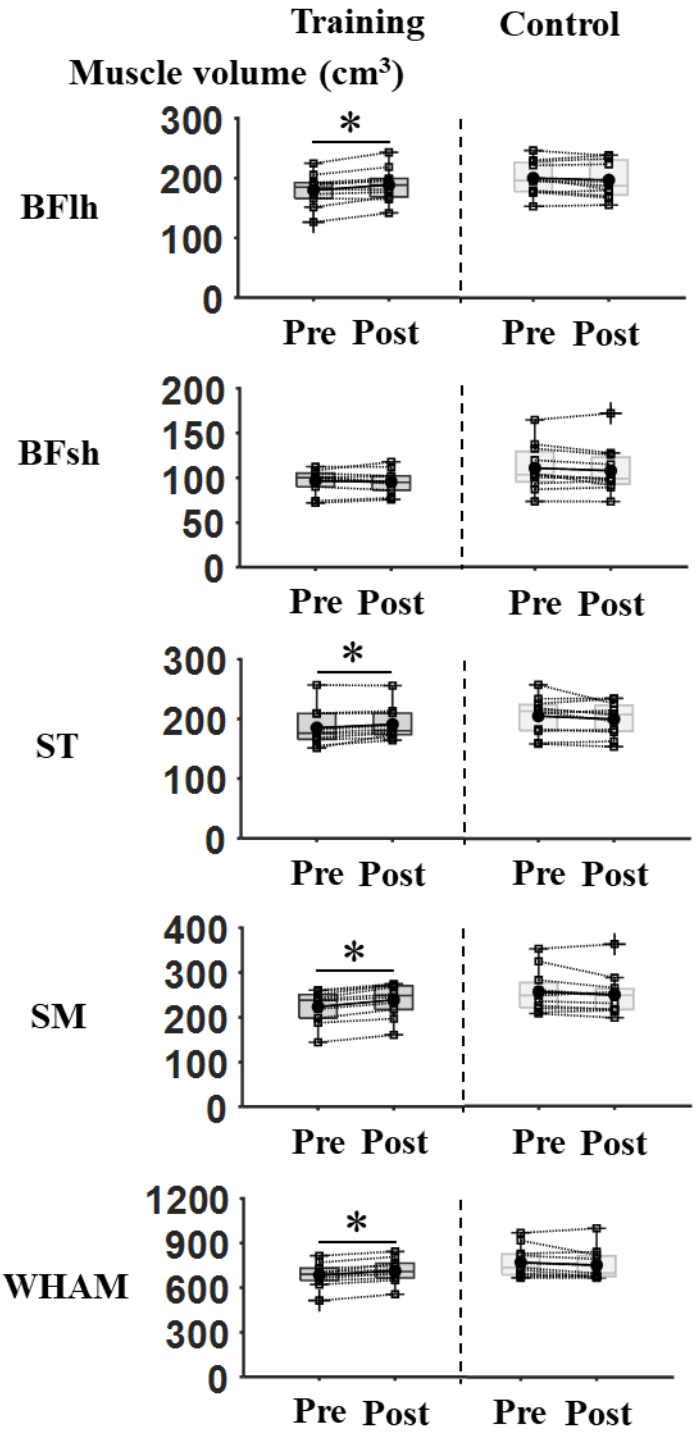

Fig. 4-3 Boxplots of the muscle volume of the individual hamstring muscles before and after the intervention period. BFlh, biceps femoris long head; BFsh, biceps femoris short head; ST, semitendinosus; SM, semimembranosus; WHAM, whole hamstring. *: a significant difference before and after the intervention period.

Maximal joint ROM was not significantly changed after the intervention period in the training (corrected $p = 0.464$, $r = 0.27$) or control (corrected $p = 0.966$, $r = 0.01$) groups (Fig. 4-4). Passive torque was also not significantly changed after the intervention period in the training (corrected $p = 0.262$, $r = 0.34$) or control (corrected $p = 0.721$, $r = 0.08$) groups.

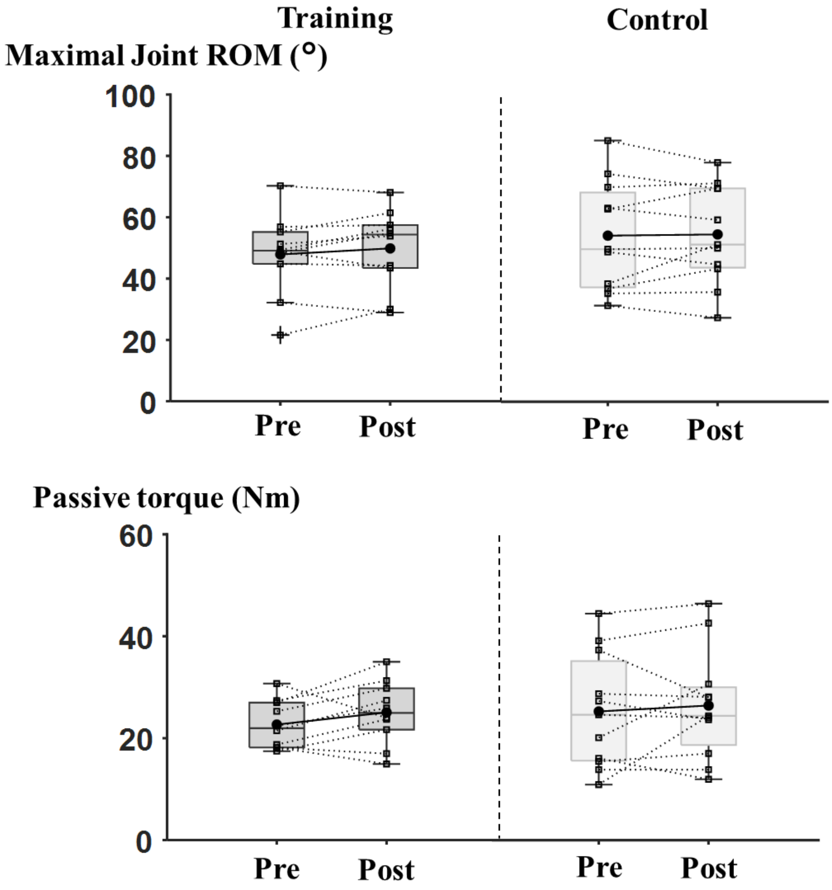

Fig. 4-4 Boxplots of the maximal joint ROM and passive torque before and after the intervention period. ROM, range of motion.

There was no significant change in the shear modulus of BFlh after the intervention period in the training (corrected $p = 0.846$, $r = 0.04$) or control (corrected $p = 0.480$, $r = 0.25$) groups (Fig. 4-5). The shear modulus of ST was not significantly changed after the intervention period in the training (corrected $p = 0.922$, $r = 0.02$) or control (corrected $p = 0.640$, $r = 0.25$) groups. In SM, the shear modulus was not significantly changed after the intervention period in the training (corrected $p = 0.375$, $r = 0.20$) or control (corrected $p = 0.375$, $r = 0.19$) groups.

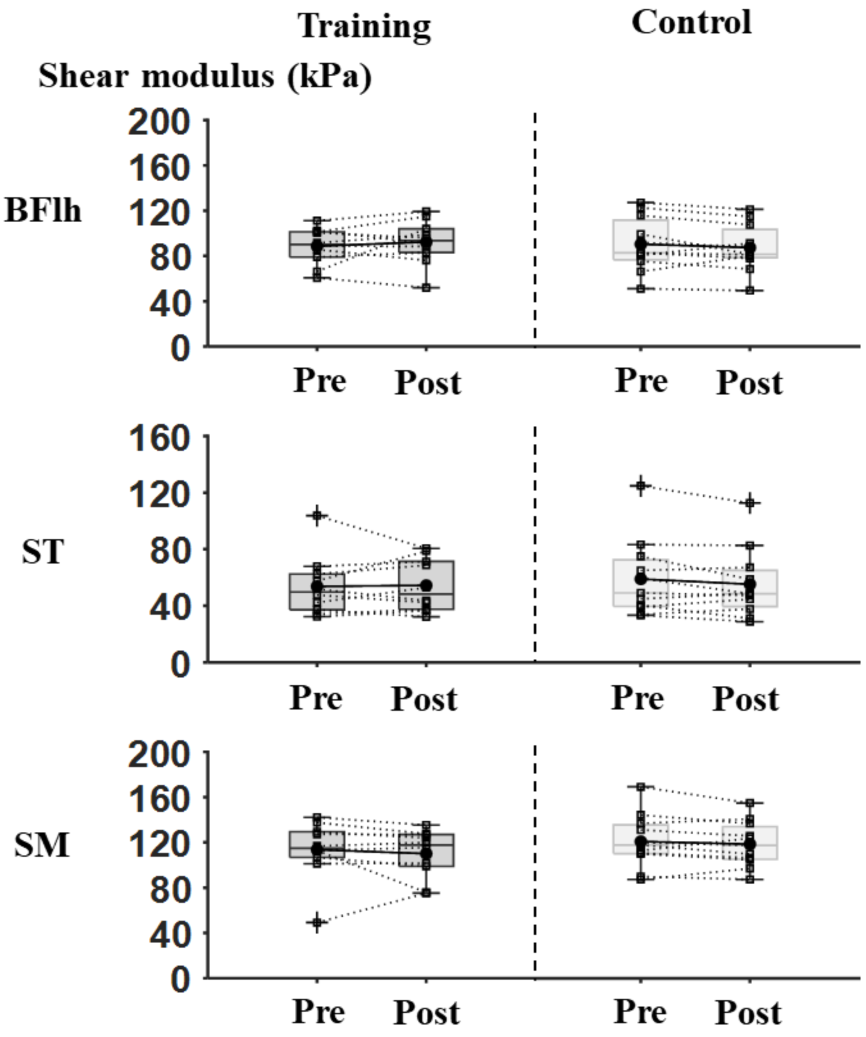

Fig. 4-5 Boxplots of the shear moduli of the biarticular hamstring muscles before and after the intervention period. BFlh, biceps femoris long head; ST, semitendinosus; SM, semimembranosus.

In the training group, there were no significant differences in RMS-EMGs during shear modulus measurement before and after intervention in BFlh (corrected $p = 0.625$, $r = 0.17$), ST (corrected $p = 0.875$, $r = 0.06$), or SM (corrected $p = 0.875$, $r = 0.06$, [Table 4-1]). In the control group, no significant differences in RMS-EMG during shear modulus measurement were observed before and after the intervention period in BFlh (corrected $p = 0.250$, $r = 0.41$), ST (corrected $p = 0.375$, $r = 0.31$), or SM (corrected $p = 0.625$, $r = 0.17$).

Table 4-1 RMS-EMGs (% MVC) of the biarticular hamstring muscles during shear modulus measurement before and after the intervention period

	Training group		Control group	
	Before	**After**	**Before**	**After**
BFlh	3.8 (2.3 to 3.8)	4.2 (2.5 to 4.5)	3.1 (1.6 to 3.8)	2.6 (1.7 to 3.2)
ST	2.5 (1.7 to 3.7)	2.4 (2.4 to 3.4)	3.6 (2.0 to 3.8)	2.4 (2.2 to 3.8)
SM	2.9 (2.6 to 3.2)	3.5 (3.3 to 3.8)	2.4 (2.3 to 4.2)	3.3 (2.5 to 3.3)

All data are presented as median value (interquartile range). RMS-EMG, root-mean-square value of electromyographic data; MVC, maximal voluntary contraction; BFlh, biceps femoris long head; ST, semitendinosus; SM, semimembranosus

Correlations between the measured variables in the training group

No significant correlations were found between shear modulus before the intervention period and changes in shear modulus in BFlh ($p = 0.946, \rho = 0.03$), ST ($p = 0.682, \rho = -0.15$), or SM ($p = 0.279$, $\rho = -0.38$). Similarly, the barbell height at the lowest position during SDL was not significantly correlated with changes in shear moduli of BFlh ($p = 0.584, \rho = 0.20$), ST ($p = 0.707, \rho = -0.14$), or SM ($p = 0.865, \rho = 0.07$).

4.4. Discussion

The present study aimed to clarify the chronic effects of eccentric-only resistance training with LL on passive stiffness of the biarticular hamstring muscles. The results revealed that shear moduli of BFlh, ST, or SM were not significantly changed after the intervention period in either training or control groups. Meanwhile, maximal isometric torque and muscle volumes of BFlh, ST, SM, and WHAM were significantly increased after the intervention period in the training group. These results suggest that the passive stiffness of the biarticular hamstring muscles does not change even after eccentric-only resistance training with LL.

The present study showed no significant changes in shear moduli of BFlh, ST, or SM during the intervention period in the training group. These results did not support my hypothesis that eccentric-only resistance training with LL chronically decreases SM stiffness. Meanwhile, the results are consistent with the previous studies reporting insignificant changes in muscle shear modulus after resistance training (Akagi et al. 2016; Seymore et al. 2017; Ochi et al. 2018; Vatovec et al. 2021). The present and previous studies suggest that it is not easy to chronically change passive muscle stiffness through resistance training. There are two possible reasons for the lack of significant changes in passive stiffness of the biarticular hamstring muscles in the present study. First, muscle lengths during resistance exercise and/or training frequency per week might be insufficient to chronically decrease passive muscle stiffness. In the present study, eccentric-only resistance training was performed at 50 to 100% of maximal exercise ROM at a frequency of two sessions per week for 10 weeks. Meanwhile, Ichihashi et al. (2016) reported chronic decreases in the passive stiffness of the biarticular hamstring muscles after the intervention of static stretching at maximal muscle lengths with three sessions per week for 4 weeks. Thus, the fact that the hamstring muscles were not maintained at the maximal length during resistance training and/or relatively low training frequency may be related to the insignificant chronic changes in passive stiffness of the muscles. Second, the statistical power may not be sufficient to detect a significant change in SM shear modulus after the intervention period, possibly due to the use of the nonparametric statistical test and small sample size. Before starting this study, the ideal number of participants (n = 11) was calculated by a priori power analysis for paired t-test with a power of 80%, an α error of 0.05, and an effect size (Cohen's *d*) of 0.95 using G*power. Thus, 12 participants were recruited in each of the training and control group for the present study. Meanwhile, the Wilcoxon signed-rank test (matched pair) was used to detect significant differences in the measured variables as some of the data were non-Gaussian. Moreover, the final number of participants in the training group did not reach the required sample size of 11 after the intervention period, due to the drop-out of two participants. I performed a post-hoc power analysis with the actual effect size (*r*), α error, and sample size (10) for the Wilcoxon signed-rank test (matched pairs) using G*power. The results showed that the actual statistical power was particularly low in SM (62%) compared to BFlh (88%) and ST (93%). Thus, the relatively low statistical power may result in the insignificant change in SM stiffness.

There were large individual differences in the training-induced changes in the shear moduli of the biarticular hamstring muscles in the present study. For example, the changes in the shear modulus ranged from −11.5 to 37.7 kPa in BFlh, from −23.2 to 21.1 kPa in ST, and from −37.0 to 26.0 kPa in SM. To identify the factor(s) for such individual differences, two correlation analyses were performed for the training group. First, I examined the relationship between the shear moduli of the biarticular hamstring muscles before the intervention period and the changes in those shear moduli after the intervention period. This is because the muscle shear modulus before one session of static stretching was reported to be negatively correlated to the stretching-induced change in the shear modulus (Hirata et al. 2020). As a result, these relationships were not statistically significant in the present study. Second, I examined the relationship between the barbell height at the lowest position during SDL and the changes in the shear moduli of the biarticular hamstring muscles. This examination is based on the finding that a long muscle length during static stretching was reported to be important for acutely decreasing muscle stiffness (Freitas et al. 2015). However, there were no significant correlations between the corresponding variables. The results of two correlation analyses suggest that changes in passive muscle stiffness induced by resistance training are not significantly affected by shear modulus before the intervention or barbell height at the lowest position during SDL. Passive muscle stiffness was reported to be influenced by the number of stable cross-bridges (Hill, 1968) and the amounts of titin (Freiburg et al. 2000) and intramuscular connective tissue (Gajdosil et al. 2001 [see general discussion]). Changes in the intracellular structures and intramuscular components, if any, might be related to the individual differences in the training-induced changes in passive muscle stiffness.

The maximal joint ROM did not significantly change after the intervention period in the training group. This result was inconsistent with the previous results showing an increase in maximal ROM of the hip joint after eccentric-only resistance training with body weight (Nordic hamstring, single-leg SDL, slide leg, and Askling's glider) for 6 weeks (Delvaux et al. 2020). These inconsistent results between the present and previous studies may be associated with the large differences in training volume during the intervention period and/or the differences in the joint examined for maximal joint ROM. In the present study, the total training volume (repetitions per set × number of sets per session × number of sessions) was 600 repetitions, whereas that in the previous study (Delvaux et al. 2020) was 1464 repetitions (366 repetitions × four exercises). It was shown that the magnitude of acute increases in maximal joint ROM was greater after static stretching with high volume than the stretching with low volume (Kamandulis et al. 2013), although no study examined the chronic effects of stretching volume on the maximal joint ROM. Based on the finding (Kamandulis et al. 2013), the relatively low volume of the resistance training in the present study may be related to the lack of chronic changes in maximal joint ROM. Meanwhile, the maximal ROM of the knee joint was measured after the eccentric-only SDL (hip joint motion) in the present study, whereas that of the hip joint was measured after eccentric resistance training of the hip joint motions in the previous study (Delvaux et al. 2020). Such a difference in the joint examined for the maximal joint ROM may also be related to the inconsistent results between the previous (Delvaux et al. 2020) and present studies.

The maximal voluntary isometric torque significantly increased after the intervention in the training group. This result is supported by previous results of an increase in maximal voluntary isometric torque after eccentric-only resistance training (Pavone and Moffat, 1985; Sato et al. 2022). Additionally, the present study also showed increases in volumes of BFlh, ST, and SM after the intervention period in the training group. Similar results were observed in a previous study that the volumes of the biarticular hamstring muscles increased after 10 weeks of hip extension training (Bourne et al. 2017). However, the magnitudes of the increases in volumes of the individual hamstring muscles were smaller in the present study (3.3 to 6.5% [mean values]) than in the previous study (over 10% [mean value, Bourne et al. 2017]). These inconsistent results could be explained by the difference in training load between the two studies. In the present study, the training load was 60 to 70% of the body mass throughout the intervention period, whereas that was approximately 60 to 80% of 1RM at weeks 1 to 3 and 100% of 1RM in the remaining weeks. Meanwhile, the total training volume throughout 10 weeks was comparable between the two studies (approximately 600 repetitions). A systematic review suggested that resistance training with a high load (> 60% 1 RM) is more effective for muscle hypertrophy than the training with a low load (≤ 60% 1RM [Schoenfeld et al. 2017]). Hence, the low training load in the present study may be linked to the smaller magnitude of the increase in muscle volume than the previous study (Bourne et al. 2017).

This study has several limitations that should be addressed. As mentioned earlier, the final number of participants after the intervention period did not reach the required sample size of 11 in the training group. In future studies, a larger number of participants should be recruited to ensure the statistical power after the intervention period, especially when nonparametric statistical tests are adopted. Meanwhile, seven participants of the training group in the present study could not maintain the weekly training frequency throughout the period. These inconsistent training frequencies may affect the chronic changes in passive muscle stiffness, which warrants further study. In this chapter, I adopted only a combination of program variables (LL) in resistance training. Therefore, future studies are needed to investigate the chronic effects of resistance training with different combinations of program variables on passive muscle stiffness.

In the present chapter, I investigated the chronic effects of eccentric-only resistance training with LL on passive stiffness of the biarticular hamstring muscles. The results showed that shear moduli of BFlh, ST, and SM were not significantly changed after the intervention period in the training or control groups. Meanwhile, maximal isometric torque and muscle volumes of BFlh, ST, SM, and WHAM were significantly increased after the intervention period in the training group. These results suggest that the passive stiffness of the biarticular hamstring muscles does not change even after eccentric-only resistance training with LL.

Chapter 5. General discussion

5.1. Main findings of each chapter

The purpose of this thesis was to clarify the acute and chronic effects of resistance training on the passive stiffness of the hamstring muscles. To this end, I investigated the acute changes in the shear moduli of the biarticular hamstring muscles after a session of resistance exercise of SDL with different combinations of contraction modes and exercise ROMs in Chapter 2, and different combinations of muscle lengths and exercise durations in Chapter 3. Through the experiments in Chapters 2 to 3, I selected the combination of program variables (LL) that induced a marked decreases in SM shear modulus immediately after a session of resistance exercise. In Chapter 4, I investigated the chronic changes in the shear moduli of the biarticular hamstring muscles after resistance training of SDL with LL. Main findings of each chapter are summarized as follows:

Chapter 2

The resistance exercise of SDL with EW immediately decreased the shear modulus of SM, whereas that with EN or CW did not acutely change the shear moduli of any of the biarticular hamstring muscles.

Chapter 3

The resistance exercise of eccentric-only SDL with LL immediately decreased SM shear modulus, whereas that with SS or LS did not induce acute changes in the shear moduli of any of the biarticular hamstring muscles. Moreover, the magnitude of the immediate decrease in SM shear modulus was greater after eccentric-only SDL with LL (−11.3 kPa [−15.2 to −2.6 kPa]) in Chapter 3 than after eccentric-only SDL with EW (−5.5 kPa [−13.3 to −1.9 kPa]) in Chapter 2.

Chapter 4

A 10-week training intervention using eccentric-only SDL with LL did not change the shear moduli of any of the biarticular hamstring muscles, whereas that increased maximal isometric torque of knee flexion and volumes of the individual hamstring muscles.

In this chapter, I discuss the acute and chronic changes in passive muscle stiffness in Chapters 2 to 4 in terms of three points: 1) determinants of passive muscle stiffness, 2) intermuscular differences, and 3) limitations and future directions.

5.2. Determinants of passive muscle stiffness

In this section, I firstly describe some factors (the number of sarcomeres in series within a muscle fascicle, sarcomere length, and fascicle length) that could influence the determinants of passive muscle stiffness (the stable cross-bridges, titin, and muscle connective tissue). Subsequently, the acute and chronic changes in passive muscle stiffness in Chapters 2 to 4 are interpreted in terms of each of the determinants.

Factors influencing the determinants of passive muscle stiffness

The determinants of passive muscle stiffness are influenced by several factors, such as the number of sarcomeres in series within a muscle fascicle, sarcomere length, and fascicle length. It has been proposed that some sarcomeres within a muscle fascicle pop when elongated beyond their optimal length in the contracted state (Morgan and Proske, 2004). In Chapters 2 and 3, the biarticular hamstring muscles were supposed to exceed their optimal length (Guex et al. 2013), especially at 100% of exercise ROM, during SDL with EW and LL. Thus, some sarcomeres may have popped during these exercise sessions due to their significant elongations. In this situation, the decrease in the number of sarcomeres in series within a muscle fascicle would result in a high elongation of remaining sarcomeres for a given amount of muscle-tendon unit elongation. When a sarcomere is highly elongated, the sensitivity of the sarcomere to Ca^{2+} was reported to increase (Stephenson and Williams 1983; Stephenson and Wendt 1984; Balnave and Allen 1996). As a result, the number of stable cross-bridges may also increase, inducing an increase in their passive tensions to stretching of the sarcomere (Whitehead et al. 2001). Meanwhile, the high elongation of a sarcomere elongates titin and develops its passive tension. Collectively, the passive muscle stiffness would be increased with increases in the passive tensions of the stable cross-bridges and titin if the sarcomere popping occurred during SDL with EW and LL in Chapters 2 and 3, respectively. However, the passive stiffness of SM decreased immediately after those sessions. Thus, the exercise-induced reduction in the number of sarcomeres in series within a muscle fascicle, if any, had no significant effect on the passive tension of stable cross-bridges and titin. Meanwhile, if the number of sarcomeres in series within a muscle fascicle did not change after SDL with EW and LL, the magnitude of a sarcomere elongation for a given amount of muscle-tendon unit elongation would not have changed before and after the exercises. In this case, the passive tension of the stable cross-bridges and titin would remain unchanged, which could lead to a lack of change in the passive muscle stiffness. However, the results in Chapters 2 and 3 showed the immediate decrease in SM stiffness after SDL with EW and LL. These results may be related to changes in architectural and mechanical properties of the determinants of passive muscle stiffness (e.g., a decrease in the stiffness of titin and muscle connective tissue).

Some studies have reported chronic changes in the serial sarcomere number within a muscle fascicle (Butterfield et al. 2005), sarcomere length (Pincheira et al. 2021), and fascicle length (Guex et al. 2016; Bourne et al. 2017; Pincheira et al. 2021) after eccentric-only resistance training. For example, an animal study reported that the number of serial sarcomeres within a muscle fiber of the vastus lateralis of rats increased after 10 days of eccentric-based downhill training (Butterfield et al. 2005). Additionally, a human study observed an increase in fascicle length of BFlh after 3 weeks of eccentric knee flexion training (Guex et al. 2016). Another human study also reported an increase in BFlh fascicle length after 10 weeks of Nordic hamstring (Bourne et al. 2017). They speculated that the increase in fascicle lengths after the resistance training was mainly attributed to an increase in the number of serial sarcomeres within a muscle fascicle (Guex et al. 2016; Bourne et al. 2017). Meanwhile, a recent study showed increases in the fascicle lengths and sarcomere lengths without an addition of sarcomeres in series within muscle fibers in the distal region of BFlh after 3 weeks of Nordic hamstring training (Pincheira et al. 2022). Based on this finding, sarcomere and fascicle lengths may also have increased after 10 weeks of eccentric-only SDL with LL in Chapter 4. An increase in a sarcomere length could elevate its sensitivity to Ca^{2+} (Stephenson and Wendt 1984; Balnave and Allen 1996; Claflin et al. 1998), and then increase passive muscle stiffness by increasing the number of stable cross-bridges (Whitehead et al. 2001). Besides, the increase in a sarcomere length could also increase the passive tension of titin with its elongation. Moreover, an increase in fascicle length could also increase passive muscle stiffness through elongation of the perimysium surrounding the fascicles. Taken together, the passive tensions of the stable cross-bridges, titin, and perimysium would have increased if there had been increases in the sarcomere and fascicle lengths. Meanwhile, the passive stiffness of the biarticular hamstring muscles did not change after 10 weeks of eccentric-only resistance training of SDL with LL in Chapter 4. Thus, the increases in sarcomere and fascicle lengths, if any, would not have significantly influenced the determinants of passive muscle stiffness. Meanwhile, it is possible that the resistance training including eccentric-only SDL with LL did not change the sarcomere and fascicle lengths, thereby not influencing the passive tensions of the determinants of passive muscle stiffness. This possibility was partially supported by a previous finding showing insignificant changes in fascicle lengths of BFlh after 6 weeks of Nordic hamstring training (Seymore et al. 2017).

Stable cross-bridges

Stable cross-bridges within a sarcomere are one of the determinants of passive muscle stiffness. The number of stable cross-bridges has been suggested to increase under two conditions (Proske and Morgan 1999; Whitehead et al. 2001). First, the number of stable cross-bridges was discussed to increase, due to severe muscle damage after a high-load eccentric exercise (Whitehead et al. 2001). However, SDL with EW and LL in Chapters 2 and 3 was performed at the moderate load (60% of the participant's body mass) and did not immediately decrease the maximal joint ROM or maximal isometric torque (indices of muscle damage [Nosaka et al. 2003]). Thus, SDL with EW and

LL is unlikely to induce severe muscle damage. Second, the number of stable cross-bridges was also described to increase with muscle elongation (Proske and Morgan 1999). In Chapters 2 and 3, the shear moduli of the biarticular hamstring muscles were measured in an identical joint position (80% of maximal joint ROM measured before exercise) before and after SDL with EW and LL. Hence, the lengths of the biarticular hamstring muscles would not change during shear modulus measurements before and after SDL with EW or LL. Considering the magnitude of muscle damage and muscle lengths during the measurements in the Chapters 2 and 3, the number of stable cross-bridges would not be different before and after those exercise sessions.

To the best of my knowledge, less is known about the chronic effects of resistance training on the number of stable cross-bridges. Meanwhile, a few studies examined the effects of resistance training on the factors that regulate the intramuscular Ca^{2+} concentration (Klitgaard et al. 1989; Green et al. 1995). A cross-sectional study showed that Ca^{2+} ATPase content did not differ between the elderly people who had performed daily resistance training and the elderly people without habitual physical activity (Klitgaard et al. 1989). In addition, another study showed that Ca^{2+} ATPase activity did not change after 12 weeks of heavy resistance training consisting of squat, leg press, and leg extension (Green et al. 1995). These findings allow me to speculate that resistance training does not substantially change the intramuscular Ca^{2+} concentration. Thus, 10 weeks of the eccentric-only resistance training of SDL with LL in Chapter 4 did not seem to change the number of stable cross-bridges, which may be one reason for the lack of chronic changes in passive stiffness of the biarticular hamstring muscles.

Titin

Titin is one of the determinants of passive muscle stiffness. It has been suggested that the I-band region of the titin consists mainly of the Ig and PEVK segments, and the magnitude of the elongation in each segment depends on the sarcomere length during the stretching (Trombitás et al. 1998). For example, the Ig segment is highly elongated at short sarcomere lengths, whereas the PEVK segment is substantially elongated at moderate to long sarcomere lengths (Trombitás et al. 1998). In Chapters 2 to 4, the shear moduli of the biarticular hamstring muscles were measured at a long muscle length (80% of maximal joint ROM). In this case, the magnitude of the elongation would be larger in the PEVK segment than in the Ig segment. Thus, the change in mechanical property of the PEVK segment, rather than that of the Ig segment, if any, greatly influences the changes in the passive muscle stiffness. In Chapters 2 and 3, the muscle lengths during SDL were relatively longer in LL (50 to 100% of exercise ROM) than in EW (0 to 100% of exercise ROM). Thus, the PEVK segment would be consistently elongated during SDL with LL, but not during SDL with EW. Such a continuous elongation of PEVK segment during SDL with LL may decrease the stiffness of PEVK segment, inducing a greater decrease in passive muscle stiffness in LL than in EW. Meanwhile, it was reported that the viscosity of titin gradually decreases under loading conditions, whereas the viscosity returns to the original state after unloading, referred to as thixotropic behavior (Kellermayer et al. 1997, 2001; Mártonfalvi et al. 2014). This phenomenon may be supported by the results in Chapters 2 and 3 that the shear modulus of SM decreased immediately after SDL with EW and LL, and then gradually returned to baseline. In Chapters 2 and 3, the exercise duration in a session of SDL was substantially longer in LL (150 s) than in EW (60 s). Therefore, the longer exercise duration may decrease the passive tension of titin, thereby leading to the greater decrease in SM stiffness in LL than in EW.

A few studies have investigated the chronic effects of resistance training on titin isoform expression (McGuigan et al. 2003; Kyröläinen et al. 2005). For example, the titin isoform expression of the vastus lateralis did not change after 8 weeks of jump squat training (McGuigan et al. 2003). Similarly, the titin isoform expression of the lateral gastrocnemius was not changed after 15 weeks of lower limb-resistance training (e.g., squat, deadlift, and calf raise [Kyröläinen et al. 2005]). These findings indicate that the titin isoform expression is unlikely to change after resistance training. The lack of change in the titin isoform expression may be a reason for the insignificant change in passive stiffness of the biarticular hamstring muscles after 10 weeks of SDL with LL in Chapter 4.

Muscle connective tissue

Muscle connective tissue is also a determinant of passive muscle stiffness. In Chapters 2 to 4, the biarticular hamstring muscles were manually outlined as large as possible, taking care to exclude epimysium, aponeurosis, artifacts (saturated area), and missing areas (unfilled region within the elasticity map) within each elastographic image. Thus, the muscle shear modulus measured in Chapters 2 to 4 reflects the mechanical properties of the perimysium and endomysium as well as those of the muscle fascicles. It was reported that the proportion of dry material to total dry muscle was greater in the perimysium than in the endomysium (Light et al. 1985). Additionally, the collagen fibers were suggested to generate the passive tension, especially during the late phase of muscle elongation (Woo et al. 1994). In Chapters 2 and 3, muscle lengths of the biarticular hamstrings during SDL were relatively longer in LL than in EW. Thus, the collagen fibers within the perimysium may be consistently elongated during SDL with LL than during SDL with EW. Such a continuous elongation of the collagen fibers within the perimysium may decrease the stiffness of perimysium, and thus result in the greater immediate decrease in SM stiffness in LL than in EW. Meanwhile, it was suggested that the muscle connective tissue exhibits thixotropic behavior (Chytil et al. 2010; Stecco et al. 2021). In Chapters 2 and 3, the exercise duration was longer in LL than in EW. The longer exercise duration may induce the greater immediate decrease in SM stiffness in LL than in EW through a decrease in the passive tension of muscle connective tissue.

To the best of my knowledge, no studies have directly examined the chronic effects of resistance training on the muscle connective tissue. Meanwhile, it was reported that the proportion of the connective tissue to the sampled muscle fibers did not differ between untrained participants and bodybuilders who had been performing resistance training for 6 to 10 years (Macdougall et al. 1984; Sale et al. 1987). These findings raise the possibility that the resistance training did not change the amount of the muscle connective tissue. Thus, 10 weeks of eccentric-only SDL training in Chapter 4 may also not change the amount of the muscle connective tissue, which may partially explain the insignificant changes in passive stiffness of the biarticular hamstring muscles.

In summary, the greater immediate decrease in SM stiffness after SDL with LL than after SDL with EW in Chapters 2 and 3 could be interpreted as follows: 1) the exercise-induced decreases in the number of sarcomeres in series within a muscle fascicle, if any, had no significant effect on the stable cross-bridges and titin of passive muscle stiffness, and 2) the PEVK segment of titin and the perimysium of the muscle connective tissue were significantly elongated for a long duration, which could induce a decrease in the passive tension of the determinants. Meanwhile, the lack of chronic changes in passive stiffness of the biarticular hamstring muscles in Chapter 4 could be interpreted as follows: 1) the training-induced increases in sarcomere and fascicle lengths, if any, did not significantly influence the determinants of passive muscle stiffness, and 2) resistance training did not change the Ca^{2+} ATPase activity, Ca^{2+} ATPase content, titin isoform expression, or proportion of the muscle connective tissue.

5.3. Intermuscular differences

In Chapters 2 and 3, the shear modulus of SM decreased immediately after resistance exercise of SDL with EW and LL, whereas the shear moduli of BFlh or ST did not. The muscle-specific immediate decrease in passive stiffness may be related to the architectural differences among the biarticular hamstring muscles. A previous anatomical study reported that the fascicle length of SM was the shortest among the hamstring muscles (Woodley and Mercer, 2005). Moreover, the change in a sarcomere length was larger in SM (1.865 μm) than in BFlh (0.886 μm) and ST (0.683 μm) between the anatomical position and the maximal hip flexed position with the knee extended (Cutts et al. 1988). These findings allow me to speculate that the change in a sarcomere length for a given amount of muscle-tendon unit elongation is greater in SM than in BFlh and ST. Thus, the shorter fascicle length in SM could explain the muscle-specific immediate decrease in passive stiffness after SDL with EW and LL. Meanwhile, the previous animal studies also reported the intermuscular differences in the architecture of titin and the amount of connective tissue (Light et al. 1985; Freiburg et al. 2000). For example, the length of the PEVK segment within titin was longer in the soleus than in the gastrocnemius muscles in rabbits (Freiburg et al. 2000). Additionally, the proportion of dry perimysium to total dry muscle was especially larger in ST and the gastrocnemius than in the psoas major, longissimus dorsi, pectoralis profundis, and sternomandibularis in cattle (Light et al. 1985). To the best of my knowledge, there are no human studies that investigated the differences in the architecture of titin and the amount of connective tissue among the human hamstring muscles. Such intermuscular differences in the hamstrings, if any, may partially explain the muscle-specific immediate decrease in passive stiffness.

5.4. Limitations and future directions

Measurement region of muscle shear modulus

In Chapters 2 to 4, the shear modulus was assessed only from the middle region (corresponding to the muscle belly) within each biarticular hamstring muscle to minimize the total measurement time. Such a measurement region within each muscle was determined based on the results of the reliability tests for the shear modulus measurements and the measurement procedures in a previous study (Miyamoto et al. 2017). Before starting the experiment in Chapter 2, I tested the intra-day reliability of the shear modulus measurements in the proximal, middle, and distal regions within BFlh, ST, and SM. The results indicate that there were no large regional differences in CVs and ICCs within each muscle (Table 5-1). Meanwhile, Miyamoto et al. (2020) reported proximo-distal differences in the shear modulus within the biarticular hamstring muscles. In addition, the absolute change in the shear modulus after static stretching was nonuniform between the proximal and distal regions within SM in the previous study (Miyamoto et al. 2020). Unfortunately, Miyamoto et al. (2020) did not measure the shear modulus in the middle region of SM. Therefore, it is unclear whether our results are extensible to other regions within each of the biarticular hamstrings muscles.

Table 5-1 Intra-day reliability of shear modulus measurement

Muscle	CV (%)			ICC		
	Proximal	Middle	Distal	Proximal	Middle	Distal
BFllh	4.3	5.2	7.9	0.99	0.95	0.85
ST	4.8	4.8	4.8	0.98	0.81	0.97
SM	3.3	6.0	3.6	0.96	0.94	0.96

CV, coefficient of variation; ICC, intraclass correlation coefficient; BFlh, biceps femoris long head; ST, semitendinosus; SM, semimembranosus

Participants

In Chapters 2 to 4, the participants were young males without neuromuscular diseases, musculoskeletal injuries, or sport specialization at the times of the experiments. It has been demonstrated that passive muscle stiffness differs depending on sex, age, and sporting events. For example, young males had higher passive stiffness of the medial and lateral gastrocnemius than young females (Miyamoto et al. 2018). Additionally, young males had higher passive stiffness of the medial gastrocnemius than older males (Hirata et al. 2020). Moreover, participants (males and females) without sport specialization had higher passive stiffness of SM than figure skaters and taekwondo players (Avrillon et al. 2019). Thus, it is unclear if the results of Chapters 2 to 4 are applicable to other populations (females, elderly, and athletes).

Types of exercises

The SDL was adopted to acutely and chronically decrease the passive stiffness of the biarticular hamstring muscles in Chapters 2 to 4. This is because SDL includes hip flexion with the knee extended, and thus may induce higher muscle elongation of the biarticular hamstring muscles compared to other hamstring exercises (e.g., Nordic hamstring curl and single-leg Roman chair [Hooren et al. 2022]). Meanwhile, a previous study (Miyamoto et al. 2017) found that passive hip flexion stretching with the knee extended decreased only the shear moduli of ST and SM, whereas passive knee extension stretching with the hip flexed decreased the shear moduli of BFlh, ST, and SM. These results suggest that the immediate changes in the passive stiffness of BFlh depend on the joint in which the passive stretching is performed. Thus, resistance exercise consisting of eccentric knee flexion with the hip flexed (e.g., seated leg curl) may induce different changes in BFlh stiffness from the results in Chapters 2 to 4. Meanwhile, I included SDL with eccentric-only contraction in Chapters 2 to 4. In the actual sports and clinical settings, SDL is generally performed with a combination of concentric and eccentric contractions. Hence, future study is warranted to investigate whether the findings in Chapters 2 to 4 is also applicable to SDL with a combination of concentric and eccentric contractions.

Underlying mechanism(s) of changes in passive muscle stiffness

In Chapters 2 and 3, the resistance exercise of SDL with EW and LL immediately decreased the shear modulus of SM, but not of BFlh or ST. It has been suggested that passive muscle stiffness is mainly determined by the number of stable cross-bridges (Hill, 1968), the amounts of titin (Freiburg et al. 2000) and muscle connective tissue (Gajdosik et al. 2001). Additionally, the determinants of the passive muscle stiffness may be affected by the number of sarcomeres in series within a muscle fascicle, sarcomere lengths, and fascicle lengths. To the best of my knowledge, there is no clear evidence of how the aforementioned factors are related to the immediate changes in the muscle shear modulus after resistance exercise. Thus, further research is warranted to elucidate the underlying mechanism(s) of exercise-induced changes in passive muscle stiffness.

5.5. Conclusion

The purpose of this thesis was to clarify the acute and chronic effects of resistance training on the passive stiffness of the hamstring muscles. The main findings of this thesis are the following three points. Firstly, a session of resistance exercise of SDL with EW immediately decreased the shear modulus of SM, whereas that with EN or CW did not immediately change the shear moduli of any of the biarticular hamstring muscles. Secondly, a session of resistance exercise of SDL with LL immediately decreased SM shear modulus, whereas that with SS or LS did not immediately change the shear moduli of any of the biarticular hamstring muscles. The magnitude of the immediate decrease in SM shear modulus was greater after SDL with LL than after SDL with EW. Thirdly, a 10-week training intervention using eccentric-only SDL with LL did not change the shear moduli of any of the biarticular hamstring muscles. These findings suggest that 1) the combination of long muscle lengths and a long exercise duration during resistance exercise is important to immediately decrease the passive stiffness of a specific muscle, and 2) even eccentric-only resistance training with long muscle lengths and a long exercise duration does not chronically change passive stiffness of the biarticular hamstring muscles.

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