博士論文（要約）

Changes in neuromuscular properties of plantar-flexor muscles after spinal cord injury

（脊髄損傷後の足関節底屈筋群における神経筋特性の変化）

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PREFACE

Spinal cord injury (SCI) is a pathological condition in which spinal cord is damaged by external force. An epidemiological survey revealed the number of SCI individuals in Japan is more than 100 thousand and has been on the increase by around 5 thousand each year (The Ministry of Health, Labour and Welfare of Japan, 2010).

After SCI, many individuals have impaired leg functions and are bound to a wheel-chair caused primarily by post-SCI motor paralysis. Motor paralysis following SCI inevitably prevents daily activities such as upright standing and bipedal walking; consequently, overall physical activity declines in affected patients. Previous studies have indicated that SCI individuals experience extreme muscle atrophy (Castro et al., 1999a, 1999b; Lotta et al., 1991), fiber transformation towards a fast-fatigable type (Grimby et al., 1976; Martin et al., 1992; Olsson et al., 2006), and decreased bone mineral density (Frey-Rindova et al., 2000; Szollar et al., 1998). This musculoskeletal degeneration is attributed largely to the dramatically decreased muscle activity and mechanical stress in the paralyzed limbs. Additionally, the reorganization of central nervous system after SCI also accompanies an elevation of spinal reflex (Little et al., 1999), which often leads to the involuntary intermitted muscle contraction such as clonus. Further, afferent signals from muscle contraction influence the basic excitability of motor neuron (plateau potential) (Pierrot-Deseilligny & Burke, 2012). In this regard, “neural” and “muscular” factors have interaction.

As a neuromuscular profile after SCI, to date, a previous research has shown that a spinal reflex excitability in individuals experiencing both complete and incomplete SCI was significantly higher than the excitability in healthy (Nakazawa et al., 2006). However, the relationship between the neuromuscular properties and the injury duration is not entirely clarified. These information, i.e., how the changes of the neuromuscular properties in SCI individuals progress with the injury duration, could guide clinicians in determining the appropriate period to start intervention.

The purpose of this thesis is to gain further insight into the changes in neuromuscular properties of muscles after SCI, especially, from the relevance to injury duration. To achieve this end, two experiments were conducted. In the first experiment (*experiment-1*), I investigated the relationship between the injury duration and neuromuscular properties of the plantar-flexor muscles in the SCI participants. In the second experiment (*experiment-2*), I further investigated the neuromuscular mechanism underlying the relation observed in the *experiment-1* by using the ultrasound measurement.

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CONTENTS OF THE THESIS

ABBREVIATIONS

Main abbreviations used in this thesis are as follows.

ADL: Activity of daily living

BWST: Body-weight support training

CNS: Central nervous system

DF: Dorsiflexion

EMG: Electromyogram

EMS: Electrical muscle stimulation

FES: Functional electrical stimulation

LG: Lateral head of gastrocnemius

MAS: Modified ashworth scale

MFL: Muscle fascicle length

MG: Medial head of the gastrocnemius

MRI: Magnetic resonance imaging

MTJ: Muscle tendon junction

MTU: Muscle tendon unit

PF: Plantarflexion

SCI: Spinal cord injury

Sol: Soleus

TA: Tibialis anterior

TERMINOLOGIES

Technical terms used in this thesis are defined as follows.

Partial correlation coefficient: correlation between two random variables (X, Y), with the effect of a set of controlling random variables removed. When the correlation coefficient between X and Y, Y and Z, Z and X were set as $R_{xy,}$ R_{yz}, R_{xz}, respectively, $R_{xy,z}$, i.e., the correlation coefficient between X and Y, controlled Z is calculated as follows,

$$R_{xy,z} = \frac{R_{xy} - R_{xz}R_{yz}}{\sqrt{1-R_{xz}^2}\sqrt{1-R_{yz}^2}}$$

Passive motion: joint motion conducted by a dynamometer

Passive torque: joint torque in which a muscle is at a rest condition

Time constant: time of changes to reach the two thirds (63.2%) of total, which typically used in engineering and research on motor learning

CHAPTER 1 BRIEF HISTORY OF THE STUDY

1.1. STUDY ON THE SPINAL CORD INJURY

Spinal cord injury (SCI) is a devastating events that spinal cord is traumatically damaged. In spite of the current progress of regenerative medicine, SCI is still a harmful condition for which there is yet no cure (Silva et al., 2014). In this section, I explain the basic knowledge about SCI to help readers to understand this thesis.

1.1.1. *Epidemiology: overview of the spinal cord injury*

There are approximately 40 per million SCI individuals in Japan (Singh et al., 2014). To say the least, 40 per million is quite a few, and high level in the world (Singh et al., 2014; Figure 1-1). The cause of SCI is mainly due to the traffic accident or fall (Lee et al., 2014; Figure 1-2), and a nationwide epidemiological survey conducted from 1990 to 1992 revealed that the ratio of male:female, complete:incomplete, and cervical:caudal-cervical injury were about 4:1, 3:1, and 1:2, respectively (Shingu et al., 1994, 1995). The bimodal peaks in age distribution (approximately 15~25 and 50~60 years) were also presented (Shingu et al., 1995; Figure 1-3).

1.1.2. *Injury severity of the spinal cord injury*

The injury severity of SCI is largely foreseeable by the injury localization. The details of the relationship between injury localization and functional outcomes is in Table 1-1. In addition, the completeness of injury also influences on the severity and prognosis. Complete or incomplete is determined by if sacral motor function is retained

or not. ASIA (American Spinal Cord Injury Association Impairment Scale: see Kirshblum et al., 2011; Figure 1-4) is the most widely used scale in the neurological classification of spinal cord injury. In ASIA, both motor and sensory function are assessed and eventually evaluated on the base of five grades (A, B, C, D, and E).

1.1.3. *Spasticity*

Spasticity is a major symptom observed in the upper motor neuron syndrome such as cerebral palsy, sclerosis, stroke, and spinal cord injury. Classically, The definition of spasticity "velocity-dependent increase in the stretch reflex" (Lance, 1980) is widely accepted especially in the research field, though velocity dependent increasing of articular resistance is often used in the clinical field.

1.1.4. *Modified ashworth scale*

Modified ashworth scale (Table 1-2) is a major clinical index to evaluate the degree of spasticity. In this scale, examiners passively rotated the joint in subjects at a constant angular velocity by hand, and the amount of resistance during the rotation was scored on five grades (0, 1, 1+, 2, and 3).

1.1.5. *Contracture*

A "contracture" is a term used for the limited range of motion that can occur in various disorders, such as local trauma and upper motor neuron syndrome. Note that involuntary contraction with spasticity could be the cause of contracture.

1.2. STUDY ON THE NEUROMUSCULAR ADAPTATION IN PARALYSIS MUSCLES

To date, numerous researchers have investigated the adaptation of skeletal muscle. Lieber (2010) argued these researches from the view point of "increased use" (such as electrical stimulation, stretch, and exercise) and "decreased use" (immobilization, bed rest, denervation). Generally, increased use causes the hypertrophy (Alway et al., 1990; Roy et al., 1982), Type II to I muscle fiber transformation (Eisenberg & Salmons, 1981; Lieber, 1988; Salmons & Vrbová, 1969), while decreased use oppositely results in the muscle atrophy (Lieber et al., 1988, 1989) and Type I to II fiber transformation (Grimby et al., 1976). Then, is spinal cord injury (SCI) in increased use or in decreased use? Since SCI individuals have more or less paralysis, we perhaps tempted to conclude that SCI is in the decreased use, but individuals of SCI often present spasticity, which accompany intermitted involuntary contraction, so-called "clonus", this fact may complicate the story. From the investigations on other neurological disorders, it has been revealed that spastic muscle properties were considerably different to normal healthy muscles. For example, spastic muscles have greater fiber size variabilities, increased numbers of "rounded" fibers, and, in some cases, increased extracellular space (Booth et al., 2001; Dietz et al., 1986; Ito et al., 1996; Romanini et al., 1989; Rose et al., 1994). In addition, while one study showed no difference in titin isoform (Olsson et al., 2006), increased elasticity of single muscle cell (Fridén & Lieber, 2003) and decreased that of muscle bundle (Lieber et al., 2003), which suggested compliant extracellular matrix, have been presented. Moreover, seemingly contradictory alterations of "longer sarcomere length in shorter muscle tendon unit length" were observed in spastic muscle of multiple sclerosis (MS) with contracture (Lieber & Fridén,

2002). Thereafter sarcomere length was "negatively" correlated to the range of motion (Pontén et al., 2007). From these findings, Lieber (2010) speculated that the decreasing of the number of sarcomeres occurred in spastic muscle with contracture.

Although these direct investigations are meaningful, these approaches entail the ethical constraints since the experiments need invasive operation. In contrast, the noninvasive angle-torque measurements using a dynamometer were classically conducted to quantify the limb stiffness (Katz & Rymer, 1989; Mirbagheri et al., 2001; Sinkjær et al., 1988). Because the passive torque contains both the neural and mechanical components (Lieber, Steinman, Barash, & Chambers, 2004; Figure 1-5), Sinkjær & Magnussen (1994) has separately evaluated them by subtracting the nonreflex torque increment assessed before the stretch reflex thresholds from total torque during sudden ankle perturbation (Figure 1-6A), and showed that intrinsic stiffness in plantar-flexor muscles were higher in spastic hemiparetic individuals (Figure 1-6B). On the other hand, Mirbagheri et al. (2001) applied the system identification technique (parallel cascade method) to the angle, electromyogram (EMG), and torque data during the random ankle perturbations (Figure 1-7A), and demonstrated the intrinsic stiffness as well as the reflex stiffness in plantar-flexor muscles were elevated in spastic SCI individuals (Figure 1-7B).

In addition to these angle-torque measurements, utilizing ultrasound imaging, recent studies have demonstrated the musculo-tendinous alterations in the individuals with neurological disorders such as stroke, cerebral palsy (CP), MS, amyotrophic lateral sclerosis (ALS), and SCI individuals. As the muscular changes in stroke impaired limb, smaller pennation angle due to atrophy and shorter fascicle length in gastrocnemius (Gas), which suggested the alteration of muscle force-length characteristics and the reduced number of sarcomeres, were presented (Gao & Zhang, 2008; Gao et al., 2009; Kwah et al., 2012). In contrast, as the tendinous changes in stroke, increased Achilles

tendon length, decreased cross-sectional area, stiffness, and elasticity were reported (Zhao et al., 2015). On the other hand, some reports indicated that there is no statistical differences in the fascicle lengths and pennation angle of Gas, soleus (Sol) (Ramsay et al., 2014a), and tibialis anterior (TA) (Ramsay et al., 2014b) as well as the Achilles tendon length (Kwah et al., 2012). Therefore, it is difficult and premature to conclude the changes in spastic muscle after stroke. Similarly, as the musculo-tendinous changes in spastic CP individuals, shorter Gas muscle bellies (Fry et al., 2004) and fascicle length (Cobley et al., 2013; Matthiasdottir et al., 2014; Mohagheghi et al., 2007), strain (Barber et al., 2011a), and longer tendon slack length (Barber et al., 2012) as well as no statistically significant changes of those (Barber et al., 2011b; Malaiya et al., 2007; Shortland et al., 2001) were presented. For MS individuals, no difference in slack length was reported. In addition, higher echo intensities, which suggested the altered tissue composition and/or fasciculation, were also demonstrated in CP (Pitcher et al., 2015) and ALS (Arts et al., 2008, 2011) individuals while no changes of that was also presented (Lee et al., 2010). For SCI individuals, although few studies have utilized the ultrasound imaging, some researches revealed that patellar tendon (Maganaris et al., 2006) and hamstrings tendon (Diong et al., 2012a) resulted in more compliant after SCI, while no significant difference was observed in Gas (Diong et al., 2013).

On the other hand, as time-course changes in neuromuscular adaptation after SCI, spinal shock and reorganization of neural system (Little et al., 1999; Figure 1-8; Rowland et al., 2008; Table 1-3), the elevation of spinal reflex excitabilities (Nakazawa et al., 2006; Figure 1-9) and muscle atrophy (Castro et al., 1999a; Figure 1-10) have been investigated. Additionally, one study has shown that the tendon elasticity profoundly decreased (Maganaris et al., 2006; Figure 1-11), while another report demonstrated that no elasticity change in Gas occurred (Diong et al., 2012a; Figure 1-12).Therefore, I would have to say, little is known about the time-course changes after

SCI in the mechanical properties of muscle tendon unit. Only one research longitudinally (= 1 years) assessed the gastrocnemius elasticity, and no change was reported (Diong et al., 2013; Figure 1-13). However, their research focused solely on the contracture, and with and without spasticity was not mentioned.

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CHAPTER 2 PURPOSE OF THE THESIS

2.1. Purpose and outline of the thesis

As mentioned in the *PREFACE* and *CHAPTER 1*, a comprehensive review on the previous researches on the neuromuscular changes after spinal cord injury (SCI) suggested that spasticity and contracture have considerable influences on the neuromuscular alterations after SCI. Additionally, In the clinical field, it is known that these symptoms altered with the injury duration. However, it should be pointed out that in previous studies, (1) no regard was given to whether the SCI participants with and without spasticity (Diong et al., 2012a, 2012b, 2013; Maganaris et al. 2006) and contracture (Maganaris et al. 2006), (2) little attention has been paid on the relevance to the injury duration (Diong et al., 2012a, 2012b; Maganaris et al. 2006). Hence, the relationships between the changes in neuromuscular properties of muscles and the durations after SCI have not entirely been understood.

The general purpose of this thesis is to provide further insight into the changes in neuromuscular properties of plantar-flexor muscles, after SCI, especially the relevance to injury duration from cross-sectional approach. To achieve this end, following two experiments were conducted.

2.2. Outline of the two experiments

Experiment 1: Articular mechanical and neural changes in plantar-flexor muscles after spinal cord injury (CHAPTER 3)

I divided the SCI participants on the basis of with and without spasticity, and investigated the mechanical changes in the plantar-flexor muscles based on angle-torque relationship obtained from passive flexion of ankle joint from 10° plantarflexion (PF) to 20° dorsiflexion (DF) at 5 deg/s using custom-made dynamometer (Kawashima et al., 2009; Negishi, 2013; Figure 2-1). Additionally, I also examined the neural alterations from stretch reflex measurement: gastrocnemius (Gas) and soleus (Sol) electromyogram (EMG) recording during passive flexion of ankle joint at 60, 90, 120, and 150 deg/s.

Experiment 2: Musculo-tendon mechanical changes in plantar flexor muscles after spinal cord injury (CHAPTER 4)

I further recorded the torque and ultrasound images of Gas MTJ (muscle tendon junction) and fascicles during passive flexion of ankle joint (10° PF to 20° DF at 5 deg/s). From these images, excursions of the MTJ and changes in muscle fascicle length were obtained. Additionally, total MTU (muscle tendon unit) length changes of the plantar-flexor muscles were estimated from angle changes based on the cadaver model (Grieve et al., 1978), thereafter, tendon length changes were estimated by subtracting the MTJ excursion from the MTU length changes. Consequently, stiffness indices of distal tendon, proximal portion of the Gas MTJ, and muscle fascicles were calculated to examine the mechanical changes after SCI with spasticity.

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CHAPTER 3 ARTICULAR MECHANICAL AND NEURAL CHANGES IN PLANTAR-FLEXOR MUSCLES AFTER SPINAL CORD INJURY (EXPERIMENT-1)

3.1. Introduction

As described in the *PREFACE* and *CHAPTER 1*, spinal cord injury (SCI) participants are subjected to several neuromuscular changes, thus the quantitative evaluations of neuromuscular properties are important in preventing secondary disorders such as contracture after SCI. In the past, the Modified Ashworth Scale (MAS; see Table 1-2 for detail) has been generally used to evaluate the neuromuscular properties after SCI (Biering-Sørensen et al., 2006). However, in MAS, joint resistance is subjectively assessed by an examiner; thus, this score lacks adequate sensitivity to distinguish the contributions from the mechanical and neural components (Grippo et al., 2011). Muscle viscoelasticity is a significant contributor to muscle tone, and it is therefore necessary to understand not only the neural but also the mechanical properties of the paralyzed region in SCI individuals.

The objective of the present study is to determine the effect of injury duration on plantar-flexor neuromuscular properties in individuals with chronic SCI and spasticity.

3.2. Methods

3.2.1. *Participants*

16 participants diagnosed with SCI (age, 33 ± 9 years; height, 172 ± 7 cm; weight, 62 ± 10 kg; injury localization, C6–T12; injury duration, 11–371 months) and spasticity (10 complete and six incomplete), 5 participants with complete SCI without spasticity (age: 30 ± 7 years; height: 174 ± 4 cm; weight: 67 ± 14 kg; injury localization: T9–L3; injury duration: 12–213 months), and 13 control participants without injury (age, 27 ± 5 years; height, 169 ± 10 cm; weight, 62 ± 10 kg; 10 male and three female) participated in this study. The population demographics are summarized in Table 3-1. There was no significant age difference among the three groups (SCI participants with spasticity, SCI participants without spasticity, and healthy participants; $F = 2.075$, $P = 0.1422$). Each participant provided written informed consent about the experimental procedure, and the experiment was approved by the National Rehabilitation Center for Persons with Disabilities (NRCD).

3.2.2. *Measurements*

The participants were seated on a custom dynamometer (Kawashima et al., 2009) with the hip and knee joints angled at 90 deg. The ankle joint was fixed onto the foot plate. The calf circumference and muscle thickness of the plantar-flexor muscles were measured at 30% of the calf length using a tape measure and ultrasonography (Prosound 2, Aloka, Japan) with a linear array probe (7.5 MHz). The calf circumference, MG, and LG thickness of participants #5, 6, 8 (Table 3-1), and four healthy control participants, as well as the Sol thickness of participants #1, 5, 6, 8, and five healthy control

participants, were not obtained, mainly due to the sudden crash of recording medium, seizing up in the apparatus, and electronic power outage. This lack of data might lead to Type I error.

The right ankle joint was rotated from 10° of plantar-flexion to 20° dorsiflexion at 5 deg/s. During the measurement, the ankle joint angle and plantar-flexion torque were recorded at a 1 kHz sampling frequency using a 16-bit analog-to-digital converter (Powerlab, AD Instruments, New South Wales, Australia). The muscle activities of the gastrocnemius (Gas), soleus (Sol), and tibialis anterior (TA) were also measured using surface electromyogram (EMG) with a bipolar electrode (DE-2.3, Delsys Inc., Boston, MA, USA) spaced 1 cm apart. The EMG signal was amplified (The Bagnoli-8 EMG System, Delsys) thereafter with band-pass filtered between 20 and 450 Hz. Some participants (#1, 2, 3, 4, and 5 in Table 3-1) had clonus (involuntary phasic contraction with spasticity).

In these participants, therefore, I took care not to miss the emergence of symptoms by visually monitoring the joint motion and the surface electromyography displayed on an oscilloscope, and if clonus occurred, the experiment was temporary stopped. The trial was repeated 6–10 times, and five trials without muscle activity of the lower limb were selected and analyzed further.

A represented raw waveform of ankle angle, Gas, Sol, and TA EMG during measurement were presented in Figure 3-1A. This profiles clearly showed the EMG of each muscle kept silent and passive motion was conducted properly. On the other hand, one participant (Table 3-1, participant 3) showed a remarkable muscle activity

exceeding ± 3 SD of baseline (Figure 3-1B), therefore the data from this participant was excluded.

The passive flexions of ankle joints at 5 deg/s were then followed by a stretch reflex test in the SCI participants with spasticity and healthy control participants only. Stretch reflexes were elicited by quick 10° toe-up rotations of the footplate from 0° with participants at rest. Four different angular velocities (60, 90, 120, and 150 deg/s) were applied five times in each participant in random order. The inter-stimulus intervals were not constant but were randomized within 4–9 s to avoid anticipation of forthcoming perturbations.

After all trials, the MAS of the right plantar-flexor was assessed. MAS is a clinical scale for evaluating the degree of spasticity (Bohannon et al., 1987), in which the joint was manually rotated by a therapist and resistance is assessed in five-level (0, 1, 1+, 2, 3, 4) (The detail was shown in Table 1-2).

3.2.3. *Analysis*

The Angles and torques data in five trials were averaged and digitally low-pass filtered using a fourth-order zero-lag Butterworth filter (Winter, 1990) with a 10-Hz cut-off frequency (Foure et al., 2012; Fouré et al., 2013; Nordez et al., 2006). Three different mathematical models were fitted to the angle (θ)–torque (τ) data as previously described (Diong et al., 2012a, 2012b, 2013, Hoang et al., 2005, 2007; Kawakami et al., 2008; Kwah et al., 2012; Magnusson et al., 1996; Nordez et al., 2010, 2006, 2009; Sten-Knudsen, 1953):

(i) Second-order polynomial (SOP) model (Eq. 2.1)

$$\tau(\theta) = A\theta^2 \tag{2.1}$$

where A is experimental constant;

(ii) Fourth-order polynomial (FOP) model (Eq. 2.2)

$$\tau(\theta) = m\theta^4 + n\theta^3 + o\theta^2 + p\theta + q \tag{2.2}$$

where m, n, o, p, and q are experimental constants;

(iii) Exponential model similar to the Sten-Knudsen (SK) model (Eq. (2.3))

$$\tau(\theta) = \frac{1}{\alpha}(e^{\alpha(\theta-\theta_{slack})} - 1) \tag{2.3}$$

where α and θ_{slack} are experimental constants.

Prior to fitting eq. 1, the angle (θ) and torque (τ) data reference points (initial values of passive motion, i.e., where the angle and torque values at 10° plantarflexion) were set to zero. The plantar-flexor, peak torque (torque waveform peak), and energy (the area under the angle-torque curve) were calculated from the normalized angle-torque data. In eqs. 2.2 and 2.3, the raw angle-torque data were used. Parameters in eq. 2.3 were determined by the non-linear least squares method [Levenberg-Marquardt algorithm (Marquardt, 1963)] using the optimization toolbox from MATLAB (The Mathworks, Natick, MA, USA). A total of 1200 (Nordez et al., 2006) data points were used in each fitting. The stiffness indices (SI_{SOP}, SI_{FOP}, SI_{SK}) and $Angle_{SLACK}$ were determined from equations described as follows:

$$SI_{SOP} = 2A \tag{2.4}$$

$$SI_{FOP} = \underset{-10 \leq \theta \leq 20}{\text{mean}} (4m\theta^3 + 3n\theta^2 + 2o\theta + p) \tag{2.5}$$

$$SI_{SK} = \alpha \tag{2.6}$$

$$Angle_{SLACK} = \theta_{slack} \tag{2.7}$$

SI_{FOP} is the average of the derivative of the fourth-order polynomial regression of angle-torque curve across all angle ranges (10° plantar-flexion to 20° dorsiflexion). The determination coefficient (R^2) of the angle–torque curve fitting using SOP, FOP, and SK models was also calculated. Additionally, the numerical simulations for eqs. 2.1, 2.2, and 2.3 were conducted to examine the validity of data fitting. I have paid attention to the effect of one parameter would not extremely exceed the range of maximum and minimum values of raw torque data, so that, the experimental constants in eqs. 2.1, 2.2, 2.3 were simulated as follows; from 0 to 1 at 0.001 intervals for *A*, from -0.00003 to 0.00003 at 0.000015 intervals for *m*, from -30 to 30 at intervals 15 for *n*, -0.05 to 0.05 at 0.025 intervals for *o*, from-2 to 10 at 0.1 intervals for *p*, from -1 to 1 at 0.1 intervals for α, from -30 to 30 at 1 intervals for θ_{SLACK}. Further, on the assumption of the differences between the fitting and raw angle-torque data follow normal distribution, akaike's information criteria (AIC) of three models were calculated from the maximally likelihood estimation. The reflex responses were analyzed as the peak-to-peak amplitude for 35 ms after perturbation. The SR gain and offset were calculated as the slope and interception, respectively, of the regression line in the plotted stretch response to angular velocity (see Figure 3-2 for detail). Additionally, in accordance with the

previous motor learning researches (Lang & Bastian, 1999; Savin et al., 2014), time constant was elucidated by non-linear data fitting with an exponential decay function of the following equation:

$$y = a - b * e^{-t/c} \tag{2.8}$$

where *a* is the final value that the exponential decay function approaches, *b* is the difference between the initial value and final value, *t* is the injury duration after SCI, and c (time constant) is the rate at which the changes reach the $(1 - e^{-1})$, i.e., ~63.2%, approximately two thirds of the final value. Data were fitted in the same manner as parameters in eq. 2.3.

3.2.4. *Statistics*

One-way analysis of variance (ANOVA) was used to compare the determination coefficients (R^2) between the three models, and *post-hoc* analysis was performed using Tukey HSD test. Group differences of all morphological, neural, mechanical variables were assessed one-way ANOVA, thereafter, Scheffé´post hoc comparison were applied. Before and after all variables were logarithmically transformed (Erni & Dietz, 2001) (except the Gas and Sol offsets, which have negative magnitudes), the Spearman's rank-correlation coefficient for the MAS and the Pearson's product-moment correlation coefficient for the other variables were calculated to determine the relationship between the injury duration and mechanical parameters. Before logarithmic transformation,

MAS values were corrected by designating the minimum data value as one. The relationships between all variables and the subject's age, the injury localization were also determined using the same procedure. The partial correlation coefficients, controlled the morphological factors, were also calculated for the mechanical parameters. The statistical significances of the correlation coefficients were assessed for SCI participants with spasticity. Statistical significance was set at $P < 0.05$.

3.3. Results

Representative angle–torque data (raw and normalized) and fitting data using the three models are illustrated in Figure 3-3. In both examples (Figure 3-3A: participant 12 in Table 3-1; Figure 3-3B: participant 13 in Table 3-1), the FOP model fits the angle-torque data well. However, data fitting using the SOP and SK models was not sufficient in some participants (Figure 3-3B: participant 13 in Table 1). Similarly, the collective determination coefficient in data fitting using the FOP model was very good ($R^2 = 0.9m99 \pm 0.001$) and significantly higher than in both the SOP ($R^2 = 0.869 \pm 0.135$) and SK (0.904 ± 0.130) models in all participants (n = 33). The same tendency was observed and was significant for SCI participants with spasticity (FOP model: $R^2 = 0.999 \pm 0.001$; SOP model: $R^2 = 0.850 \pm 0.120$; SK model: $R^2 = 0.883 \pm 0.114$, n = 15). The results of numerical simulations for SOP, FOP, and SK model were overlaid on the angle-torque relation in SCI participants (Figure 3-4). It was visually confirmed that the orientation of lines in SOP and SK model had particular direction, but that in FOP not.

Further, Figure 3-5 clearly showed that the AIC in FOP model indicated the lower values for almost all subjects than those in other 2 models.

Representative Angle-torque curves of all participants (except participant 3) were shown in Figure 3-6A, and SCI participants with spasticity was solely presented in Figure 3-6C. Figure 3-6B and D were generated by designating the angle and torque reference points (initial values of passive motion, i.e., where the angle and torque values at 10° PF) in Figure 3-6A and C as zero. Using the data in Figure 3-6B and D, I calculated the peak torque and energy. In addition, after polynomial and exponential regressions, the $SI_{SOP,}$ SI_{FOP}, SI_{SK}, and $Angle_{SLACK}$ were calculated.

MAS value was approximately 1+ (Figure 3-7A), and injury localization (which mainly explain the severity; see Table 1-1 for detail) was ranged from C6 to L12 (Table 3-1). These suggested that the severity of SCI participants with spasticity was not extremely high. As a group comparison, the lower values for the calf circumference, MG thickness, and LG Thickness were presented in Figure 3-7 B. While gastrocnemius stretch reflex gain was significantly greater in the Normal than that in the SCI with spasticity (Figure 3-7B), Soleus stretch reflex offset was higher in SCI participants than that in the Normal (Figure 3-7B). No statistical differences were observed in all mechanical parameters.

MAS was negatively correlated with the injury duration in the SCI participants with spasticity (Figure 3-8). Additionally, the calf circumference and MG thickness were negatively correlated to the injury duration (n = 13 excluding the missing data described previously) (Figure 3-8). However, there were no statistically significant

relationships between the neural parameters (Gas gain and offset, Sol gain and offset) and injury duration in the group. I found that peak torque and SI_{FOP} (stiffness index calculated from the fourth-order polynomial regression) were negatively correlated with the injury duration (n = 15). Muscle morphology theoretically affects passive tension in the muscles and tendons. The effects of these morphological variables (the calf circumference and MG, LG, and Sol thicknesses) were removed in calculating the partial correlation coefficients between the mechanical properties (peak torque, energy, SI_{SOP}, SI_{FOP}, SI_{SK}, and $Angle_{SLACK}$) and the injury duration (Table 3-2). The peak torque and SI_{FOP} were negatively correlated, even after removing the effects of the calf circumference and MG thickness, which significantly decreased with the injury duration. Additionally, I observed that subject's age and injury localization were not correlated with these results (Figure 3-9). Further, from the relationship between SI_{FOP} and the injury duration, I elucidate the time constant as 120.5447 months (approximately 10 years).

3.4. Discussion

I investigated the effect of injury duration on plantar-flexor elasticity in individuals with chronic SCI and found negative correlations between the clinical index of spasticity (MAS), morphologic parameters (calf circumference and MG thickness), mechanical parameters (peak torque and stiffness index), and injury duration in SCI participants with spasticity. Additionally, there were significant partial correlation

coefficients between the mechanical parameters and the injury duration except the morphological factor.

3.4.1. *Alteration of the ankle joint stiffness after spinal cord injury*

The calf circumference, MG thickness, and LG thickness were significantly smaller in SCI participants without spasticity (NS) than that of the Normal healthy control (N) (Figure 3-7B), and the percent changes of these indices were -18, -76, and -68% respectively. On the other hand, Akima et al. (1997) showed that approximately 10% declines of the physiological cross-sectional areas (PCSA) in MG, LG, and Sol occurred after 20 days bed rest, which changes is relatively slight. The difference in time-period exposed to muscle's inactivity (1~10 years vs. 20 days) and limb position (on wheel-chair vs. on bed) could partially explain the disparity in these study. Further, As clearly shown in Figure 3-8, the decline in calf circumference and MG thickness with injury duration indicates that muscle atrophy continues in SCI even during the chronic stage. Castro et al. (1999a) measured the cross-sectional area of the lower musculature (Gas, Sol, TA) during the 6 months following spinal injury and showed that the CSA of Gas decreased markedly. The present result showing a selectively decreased MG thickness is consistent with this previous finding (Castro et al., 1999a) and suggesting that atrophy progress not only acute (<6 months) but also chronic stage. Notably, involuntary muscle activity resulting from clonus and spasm with spasticity might contribute to attenuate atrophy (Gorgey & Dudley, 2008), therefore the decreasing

spasticity observed in this study, coupled with the period on a wheel-chair, likely accelerated the muscle atrophy.

The Ashworth scale (Ashworth, 1964) remains a major clinical scale for evaluating spasticity (Bohannon et al., 1987; Ghotbi et al., 2009; Rekand, 2010), but its validity and reliability are questioned by some reports (Malhotra et al., 2009; Sunnerhagen, 2010). A positive correlation has been reported between the Ashworth scale and indices of clonus and spasm (Benz et al., 2005), therefore the negative correlation between MAS and the injury duration observed in this study suggest that the frequency of involuntary muscle contraction due to spasm gradually decreases over time after SCI. In evaluating the extent of spasticity over time, I found no statistically significant correlation between the stretch reflex gain and offset in the lower muscles (MG, Sol) and high value of injury high value in Sol Stretch reflex offset. This result is consistent with the previous study (Nakazawa et al., 2006) that found that the stretch reflex peak-to-peak amplitude (SRPP) and SRPP/Mmax in complete and incomplete SCI participants were not correlated to the injury duration. In addition, as the comparison of difference among groups, they also indicated that higher spinal reflex excitability in SCI than that of the Normal, and corresponds to the increasing of Sol stretch reflex offset obtained in present study. I could not figure out why Gas stretch reflex was smaller than that of the Normal, insufficient velocity condition (60~150 deg/s), and EMG saturation in SCI participants might be considered. Their range of injury duration was 12~475 months, which is equivalent to that of present study (11~371 months). On the other hand, Little (1999) showed the augmentation of spinal

reflex excitability drastically occur during acute (<6 month) stage in SCI. Taken together, as a neural time-course after SCI, it is assumed that the most part of changes occur within approximately 1 year and spinal circuit excitability in SCI individuals would not markedly change during the chronic stage, at least, after 1year injury duration.

several reports showed that Mmax was smaller in chronic SCI participants than in age-matched healthy control participants (Boorman et al., 1996; Calancie et al., 1993; Huang et al., 2006; Murillo et al., 2011; Pelletier & Hicks, 2010; Stein et al., 1992; Thomas, 1997), presumably because of changes in proprioceptor function or muscle atrophy. In these studies, the time-dependent decline in Mmax may be caused by muscle atrophy, but because I did not measure Mmax in this study, I cannot conclude that the spinal reflex excitability in chronic SCI is maintained. Further research is needed to clarify this.

3.4.2. *Characteristics of the mechanical properties of the paralyzed ankle joint in SCI*

Previous research (Nordez et al., 2006) indicates that the mathematical models used to calculate the mechanical parameters greatly affect the experimental result. Essentially, the change in a mechanical parameter (e.g., stiffness) varied depending on the selected mathematical model applied to the angle-torque relation; thus, I evaluated several mathematical models (SOP, FOP, and SK) to calculate each mechanical parameter. In this study, the peak torque and stiffness index (SI_{FOP}) calculated from the

FOP model were inversely correlated with the injury duration. In addition, the determination coefficients for the angle-torque data fitted with the FOP model were significantly higher than those with alternative models (SOP, SK). Of course, this does not directly mean that the fitting with FOP model was most appropriate. However, numerical simulation clearly showed that the orientations these models can express were considerably different, i.e., while the fitting lines with FOP model covered every direction in the raw data, fitting lines with SOP and SK model only covered particular directions (Figure 3-4). These differences likely reflect inappropriate data fitting of SOP and SK model observed in some participants, and infer the FOP model was more plausible to depict the characteristics of the raw data. Additionally, the lowest values of AIC in FOP model support that this model selection seems to be reliable. Notably, SI_{SOP}, SI_{SK}, and $Angle_{SLACK}$ calculated using the SOP and SK models were not significantly correlated with the injury duration. This observation likely reflects the inappropriate data fitting in some participants, and the insufficient sample size; however, the underlying mechanism is unclear.

Additionally, the mechanical work was not significantly correlated with injury duration. This result is consistent with a prior study showing that work is not a spasticity indicator (Akman et al., 1999). Furthermore, the decreased peak torque and SI_{FOP} over time suggest that plantar-flexor elasticity decreases with injury duration. However, several studies have found that the plantar-flexor passive-elasticity in chronic SCI individuals was higher than in healthy age-matched participants (Diong et al., 2012b; Kwah et al., 2012; Mirbagheri et al., 2001). I speculate that plantar-flexor

elasticity in SCI participants increases with immobilization during the acute stage of injury (<1 years), thereafter, gradually decreases over time due in part to the muscle atrophy as observed in this study. In theory, muscle morphology influences passive tension. Thus, I calculated the partial correlation coefficient between mechanical parameters and the injury duration, where morphological parameters were controlled (Table 3-2); significant relationships persisted, therefore I concluded that tissue elasticity, i.e., Young's modulus, in the whole plantar-flexor was involved in the mechanical parameter changes.

I cannot determine whether the muscle or tendon plays an important role in changes in the plantar-flexor elasticity. While Olsson et al. (2006) found that the muscle changes occurred at the cell level and reflect muscle fiber transformation from Type I to Type II, Diong et al. (2012a) observed that the gastrocnemius muscle stiffness was comparable in SCI participants with contracture and the Normal. Additionally, Maganaris et al. (2006) showed that patellar tendon CSA, stiffness, and Young's modulus decreased profoundly in chronic (18–288 months after injury) complete SCI participants. Further, Kawakami et al. (2008) presented that contributing rate of achiness tendon stiffness to passive torque was greater than that of muscle belly (62% vs. 32%). Overall, these previous studies suggest that the tendon rather than the muscle plays a primary role in the decreasing plantar-flexor elasticity. However, further research is needed to clarify the different changes in muscle and tendon function with injury duration after SCI. One of the main findings of present experiment was to

elucidate the time constant quantitatively, this information could be the guide for clinicians to decide when to start rehabilitation program.

3.4.3. *Limitations*

In the present experiment, I did not measure the range of motion of plantar-dorsiflexion in the SCI participants. Therefore, it is unclear whether contracture occurred in SCI participants. However, I visually confirmed that all participants achieved a 20° dorsiflexion while keeping the heel in contact with the dynamometer footplate during the passive flexion of ankle joints. Additionally, I confirmed that muscle activity in the calf muscles (MG, Sol) and antagonist (TA) were low (within the baseline mean ± 3SD) during the passive flexion of ankle joints (representative participant in Figure 3-3A). Thus, the influence of articular alternation and muscle activity on the plantar-flexor torque was minimal, and the passive flexion of ankle joints was conducted appropriately.

3.5. Conclusion

In *experiment-1*, I found that the degree of spasticity (MAS), morphologic (circumference and MG muscle thickness), and plantar-flexor elasticity of spastic SCI individuals in chronic stage decreased with the injury duration. In addition, the profound changes of plantar-flexor elasticity occur for 10 years, while the spinal reflex excitabilities did not exhibit the remarkable changes in chronic stage.

Table 3-1. Population demographics.

Subject	Sex	Age (years)	Height (cm)	Weight (kg)	Lesion level	Injury type	With spasticity	MAS	Duration of injury (months)	Medication
1	M	20	181	57	C6	incomplete	Yes	2	11	Gabalon
2	M	30	170	71	T7	incomplete	Yes	2	14	-
3	M	22	178	64	C7	incomplete	Yes	2	18	ITB
4	M	45	171	64	T1	incomplete	Yes	2	19	-
5	M	49	173	70	T3, 4, 5	incomplete	Yes	1+	52	Dantrium, Lendormin
6	M	25	170	54	T5	incomplete	Yes	1+	63	-
7	M	23	175	61	T10	complete	Yes	1+	18	-
8	M	27	178	55	T11, 12	complete	Yes	2	30	-
9	F	27	172	50	T8, 9, 10	complete	Yes	0	36	-
10	M	27	171	62	T10, 11	complete	Yes	1	114	-
11	M	37	180	75	T12	complete	Yes	1	115	Ternelin
12	M	35	173	62	T9, 10	complete	Yes	1	144	-
13	M	33	174	77	T12	complete	Yes	1+	144	-
14	M	39	174	77	T8	complete	Yes	1+	155	-
15	F	40	163	55	T9	complete	Yes	1+	300	-
16	F	48	153	40	T5	complete	Yes	1	371	-
17	M	27	173	55	L3	complete	No	-	12	-
18	M	30	175	58	Th12,L1,L2	complete	No	-	15	-
19	M	21	178	60	Th9	complete	No	-	23	-
20	M	33	175	87	Th11,12	complete	No	-	198	-
21	M	41	168	75	Th10	complete	No	-	213	-

Abbreviations: MAS, modified ashworth scale, ITB, intrathecal baclofen therapy

Healthy control subjects (n=13): Age (27±6), Height (169±10), Weight (62±10)

Table 3-2. A, Partial correlation coefficient between the injury duration (months) and mechanical properties (peak torque, energy, SI_{SOP}, SI_{FOP}, SI_{SK}, $Angle_{SLACK}$), except muscle architecture (calf circumference, MG, LG, and Sol thickness) and level of injury. **B**, Corresponding P values to A. Abbreviations: MG, medial gastrocnemius; LG, lateral gastrocnemius; Sol, Soleus.

A

	calf circumference (mm)	MG (mm)	LG (mm)	Sol (mm)	Level of Injury
peak torque (Nm)	-0.70	-0.63	-0.80	-0.72	-0.79
energy (Nm・deg)	-0.70	-0.33	-0.72	-0.58	-0.67
SI_{SOP} (Nm/deg)	-0.69	-0.57	-0.78	-0.69	-0.77
SI_{FOP} (Nm/deg)	-0.70	-0.63	-0.80	-0.72	-0.79
SI_{SK} (Nm/deg)	-0.45	-0.25	-0.62	-0.57	-0.65
$Angle_{SLACK}$ (Nm/deg)	0.58	0.42	0.71	0.63	0.72

B

	calf circumference (mm)	MG (mm)	LG (mm)	Sol (mm)	Level of Injury
peak torque (Nm)	0.017	0.039	0.003	0.019	0.004
energy (Nm・deg)	0.017	0.319	0.012	0.077	0.024
SI_{SOP} (Nm/deg)	0.019	0.070	0.005	0.027	0.006
SI_{FOP} (Nm/deg)	0.016	0.038	0.003	0.018	0.004
SI_{SK} (Nm/deg)	0.163	0.461	0.042	0.087	0.032
$Angle_{SLACK}$ (Nm/deg)	0.061	0.201	0.014	0.052	0.013

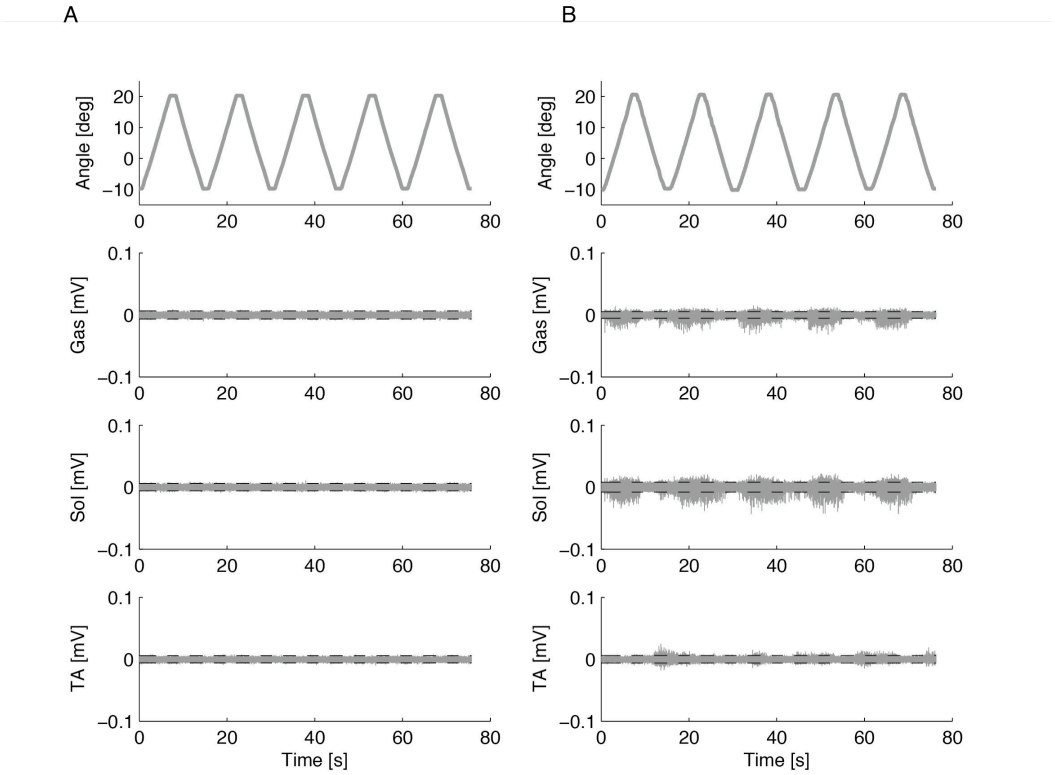

Figure 3-1. A, Representative angle, electromyogram of gastrocnemius (Gas), soleus (Sol), and tibialis anterior (TA) muscles waveform during the passive flexion of ankle joints. Broken lines indicate ± 3 SD of baseline. **B**, Angle and EMG waveform of an excluded participant (participant #3 in Table 3-1).

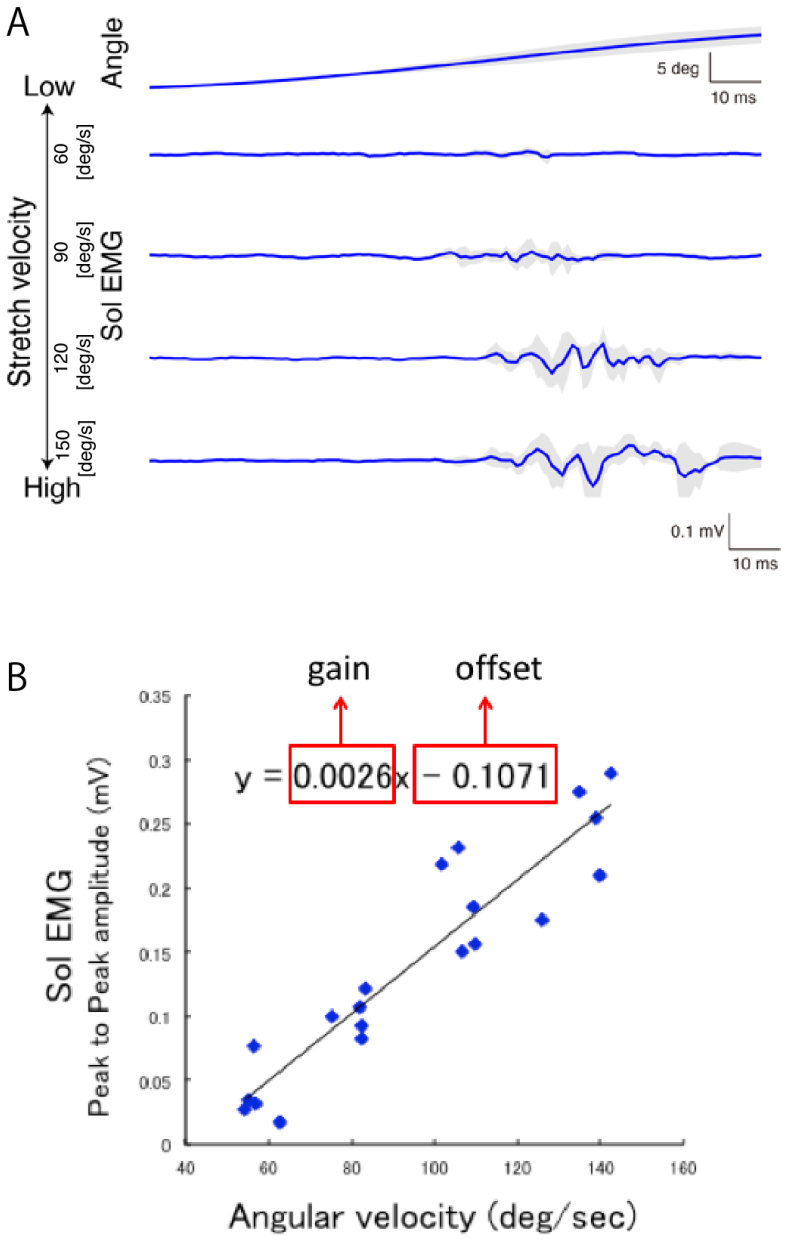

Figure 3-2 A, Typical Example of stretch reflex wave profile from spinal cord injury (SCI) subjects with spasticity under four velocity conditions (five trials ensemble average; gray areas mean SD). **B**, schematic diagram in calculating the stretch reflex gain and offset. Abbreviations: Sol, Soleus, EMG, electromyogram.

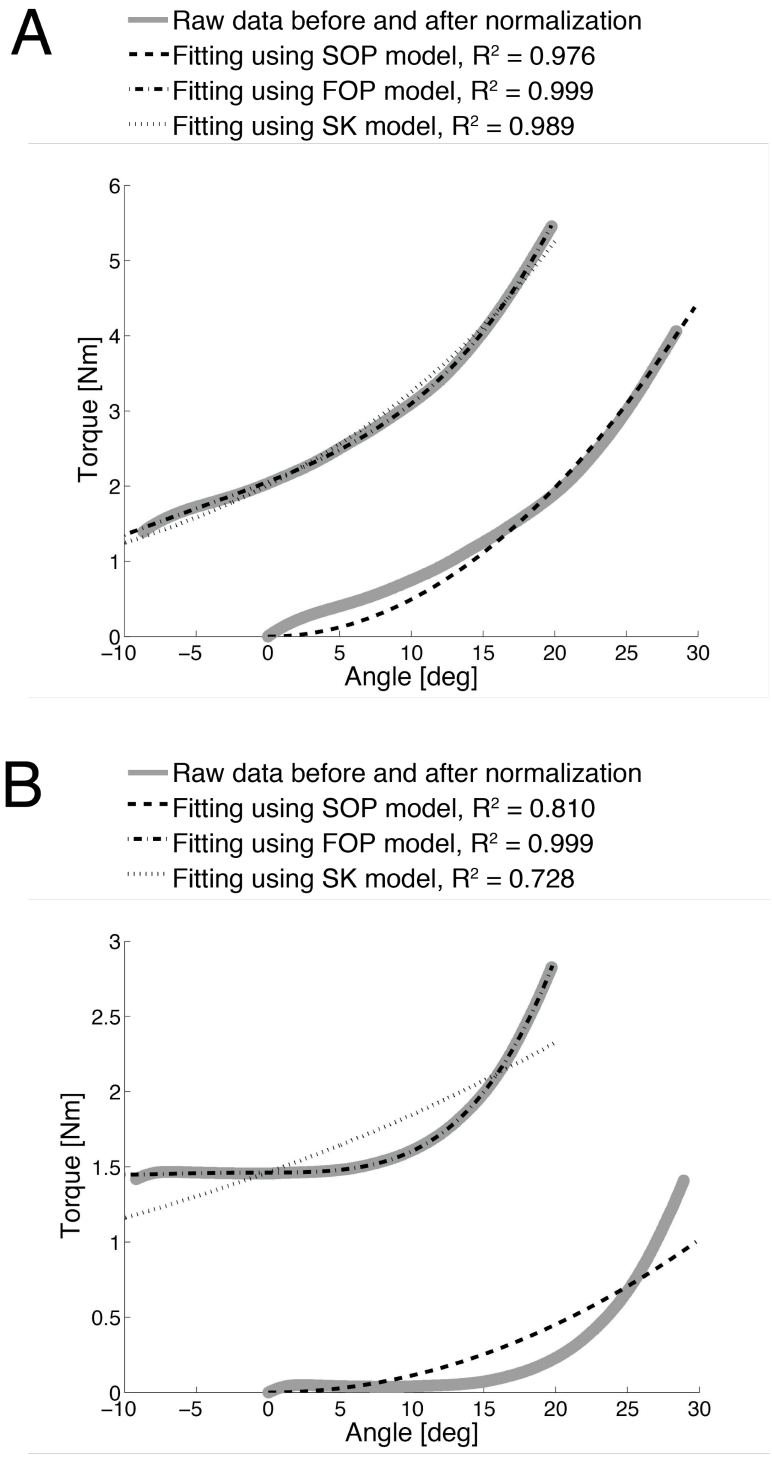

Figure 3-3. Representative angle-torque data fitting using three mathematical models (SOP, FOP, and SK). The fitting was good in all models for angle-torque data from participant 12 in Table 3-1 **(A)**. B, The SOP and SK model fittings were inferior compared with the FOP model, for angle-torque data from participant #13 in Table 3-1 **(B)**.

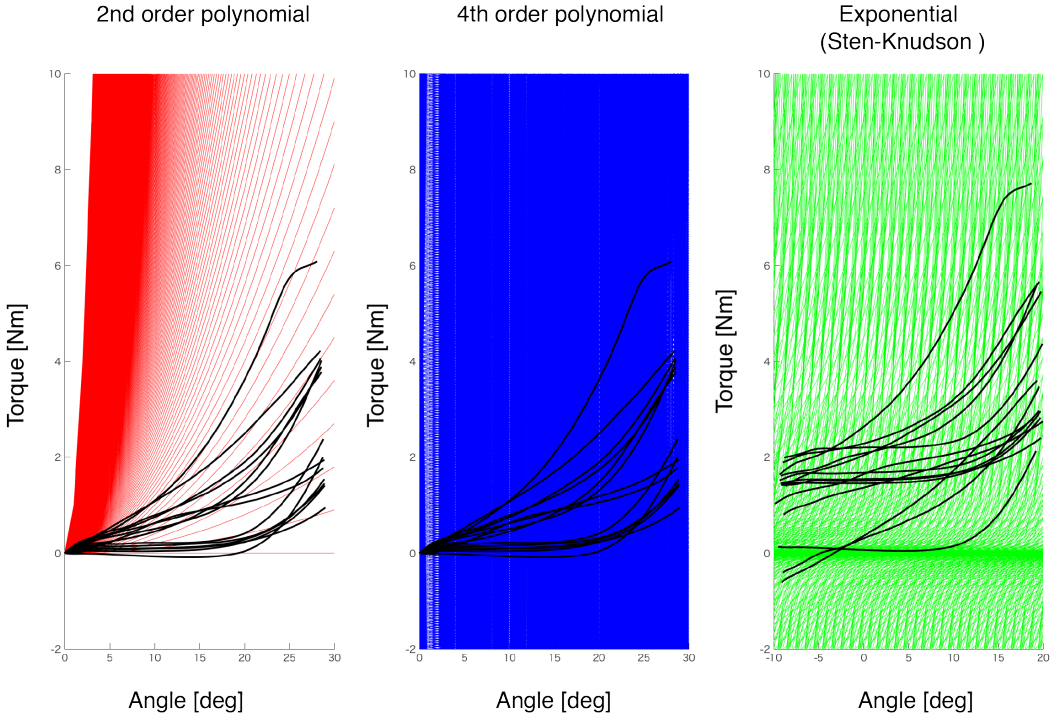

Figure 3-4 Black solid lines indicate angle-torque data in each spinal cord injury (SCI) participant with spasticity. Red, blue, green lines represent the simulated regression based on the 2nd order polynomial (SOP), 4th order polynomial (FOP), and exponential (Sten-Knudson; SK) models, respectively.

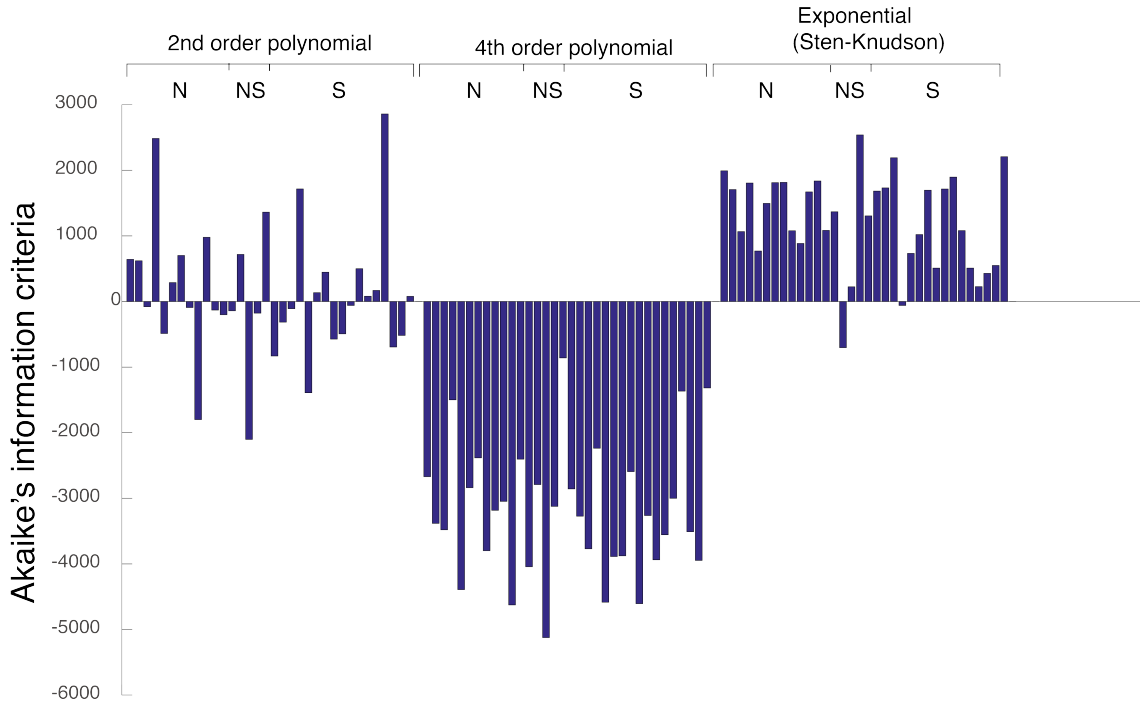

Figure 3-5 All individual data of Akaike's information criteria (AIC) calculated from data fitting of angle-torque data based on the 2nd order polynomial (SOP), 4th order polynomial (FOP), and exponential (Sten-Knudson; SK) models. Abbreviations: N, Normal healthy control participants; NS, spinal cord injury participants without spasticity; and S, spinal cord injury participants with spasticity, respectively.

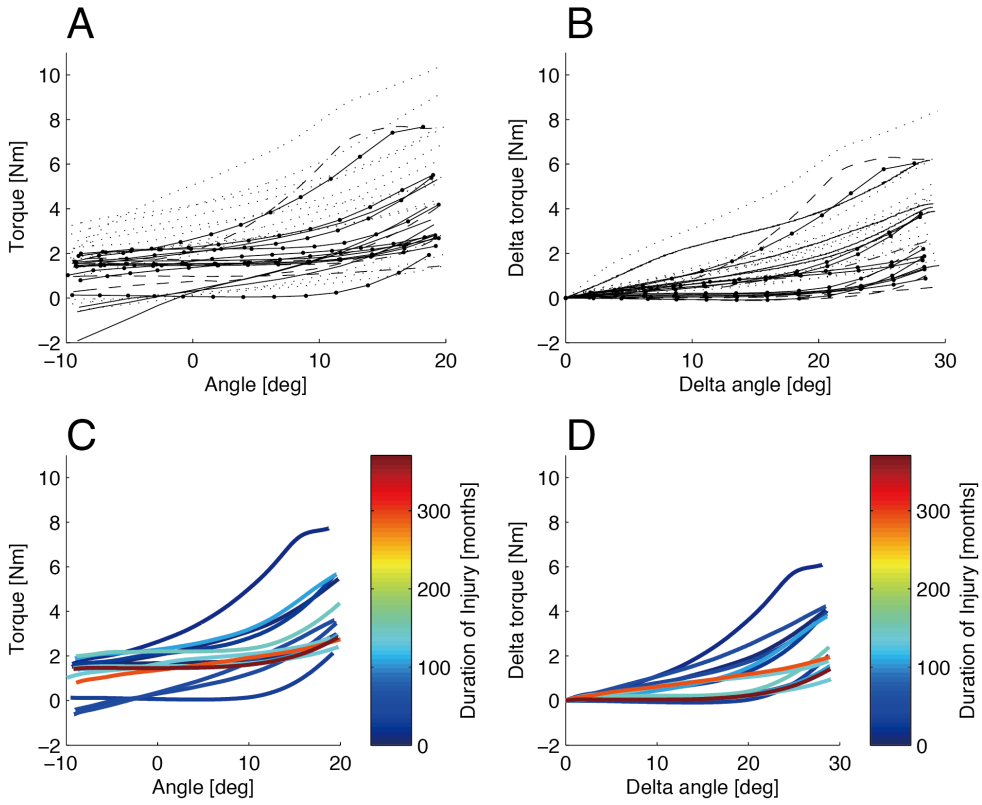

Figure 3-6 A, Angle-torque curves of plantar-flexor muscles for all complete spinal cord injury (SCI) participants with spasticity (chain), incomplete SCI participants with spasticity (solid), complete SCI participants without spasticity (dashed), and healthy control participants (dotted) except participant #3 in Table 3-1. **B**, the angle and torque reference points (where subject's angles were initial values at -10 °) in Figure 3-6A were set at zero. **C**, Angle-torque curve for SCI participants with spasticity (both complete and incomplete) from Figure 3-6A, colors of lines indicating the injury duration (months). **D**, the angle and torque reference points in Figure 3-6C were set at zero.

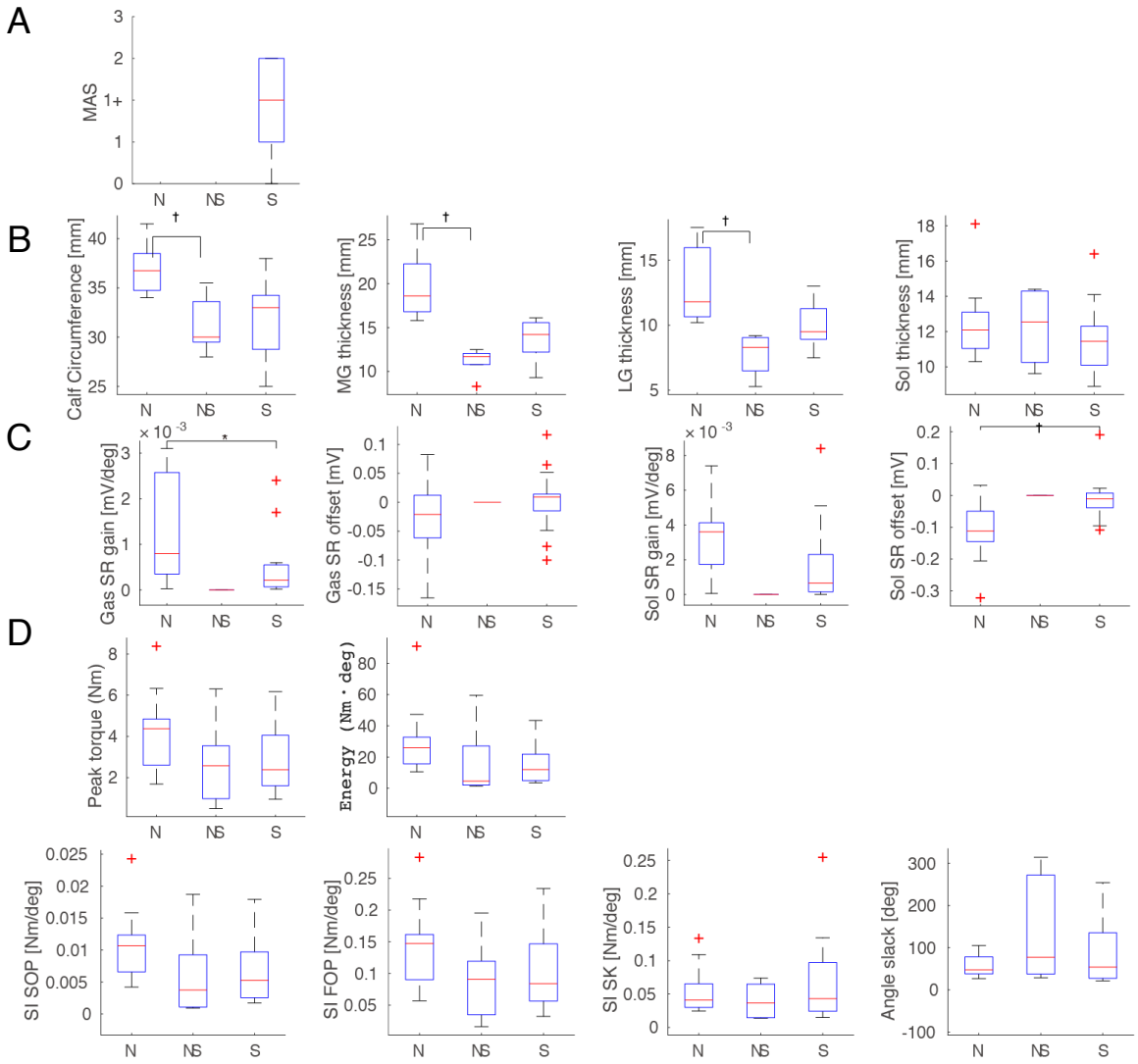

Figure 3-7 Box-and-whisker plots of clinical (**A**), morphological (**B**), neural (**C**), and mechanical (**D**) parameters. N, NS, and S means the Normal (normal healthy control) participants, spinal cord injury (SCI) participants without spasticity, and SCI participants with spasticity, respectively. To test group differences, One-way analysis of variance and Scheffé post hoc comparison were applied. Note. * $P < 0.05$, †, $P < -0.01$. Abbreviations: MAS, modified ashworth scale; MG, medial gastrocnemius; LG, lateral gastrocnemius; Sol, Soleus; gastrocnemius, Gas; SR, stretch reflex; SI, stiffness index; SOP second order polynomial; FOP, fourth order polynomial; SK, Sten-Knudson.

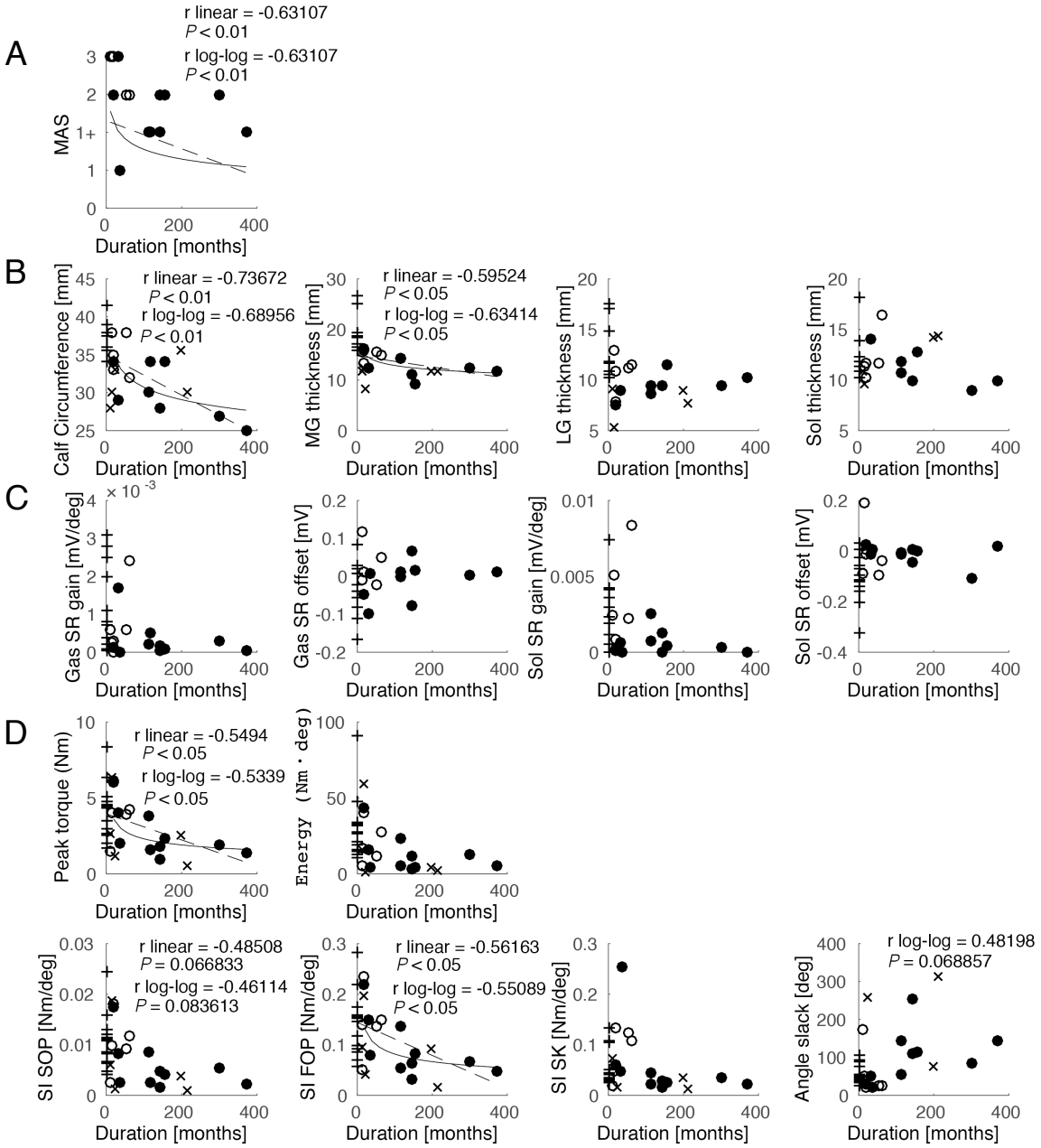

Figure 3-8. Relationship between clinical (**A**), morphological (**B**), neural (**C**), mechanical (**D**) parameters and injury duration (months). Complete SCI (spinal cord injury) participants with spasticity (filled circles), incomplete SCI participants with spasticity (open circles), complete SCI participants without spasticity (x), Normal healthy control (plus sign). Broken and Solid lines indicate the significance in the linear and logarithmically transformed correlation, respectively. Abbreviations: MAS, modified ashworth scale; MG, medial gastrocnemius; LG, lateral gastrocnemius; Sol, Soleus; gastrocnemius, Gas; SR, stretch reflex; SI, stiffness index; SOP second order polynomial; FOP, fourth order polynomial; SK, Sten-Knudson.

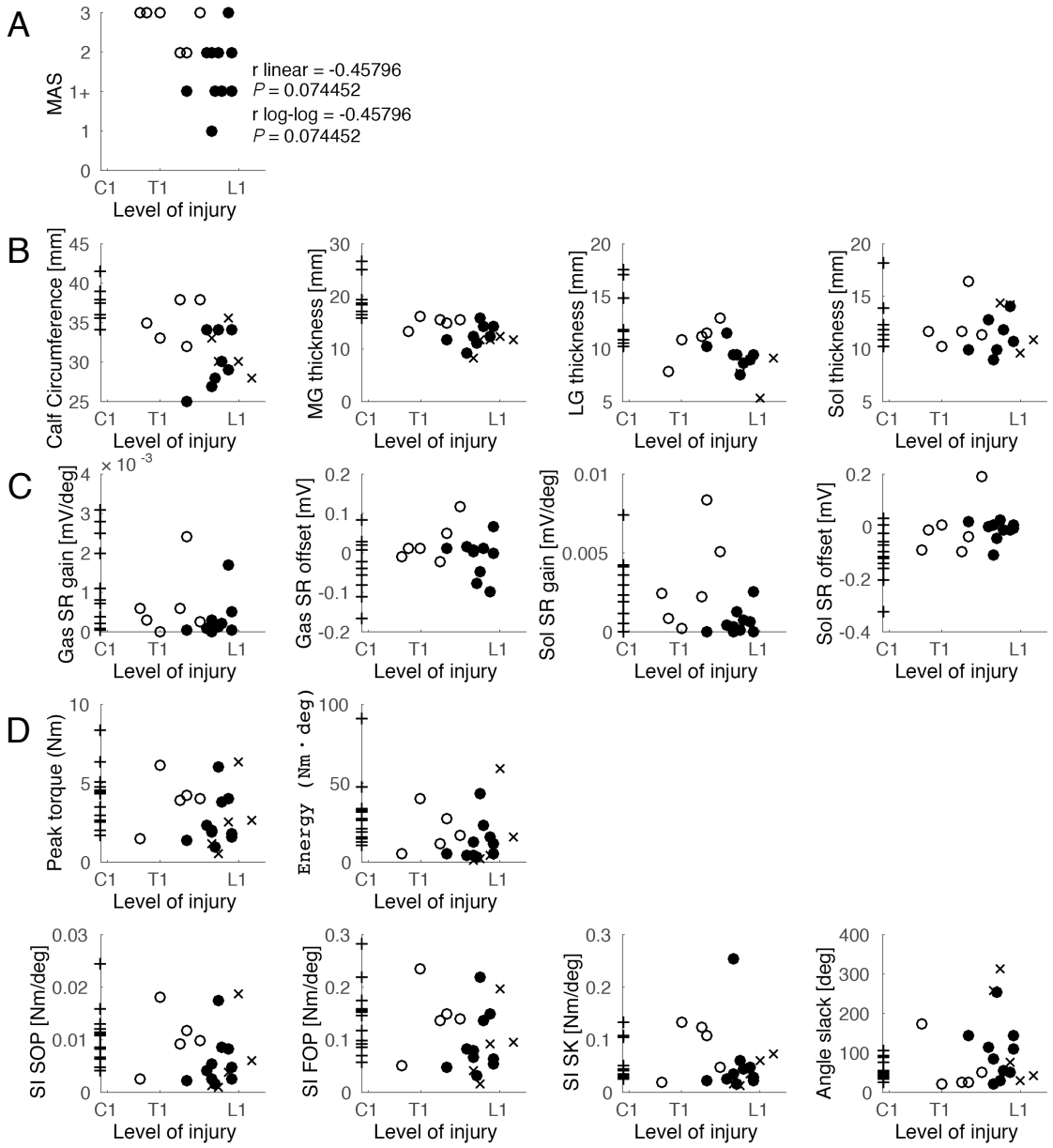

Figure 3-9. Relationships between clinical (**A**), morphological (**B**) neural (**C**), mechanical (**D**) parameters and injury localization (Level of injury). Complete SCI (spinal cord injury) participants with spasticity (filled circles), incomplete SCI participants with spasticity (open circles), complete SCI participants without spasticity (x), Normal healthy control (plus sign). Abbreviations: MAS, modified ashworth scale; MG, medial gastrocnemius; LG, lateral gastrocnemius; Sol, Soleus; gastrocnemius (Gas); SR, stretch reflex; SI, stiffness index; SOP second order polynomial; FOP, fourth order polynomial; SK, Sten-Knudson.

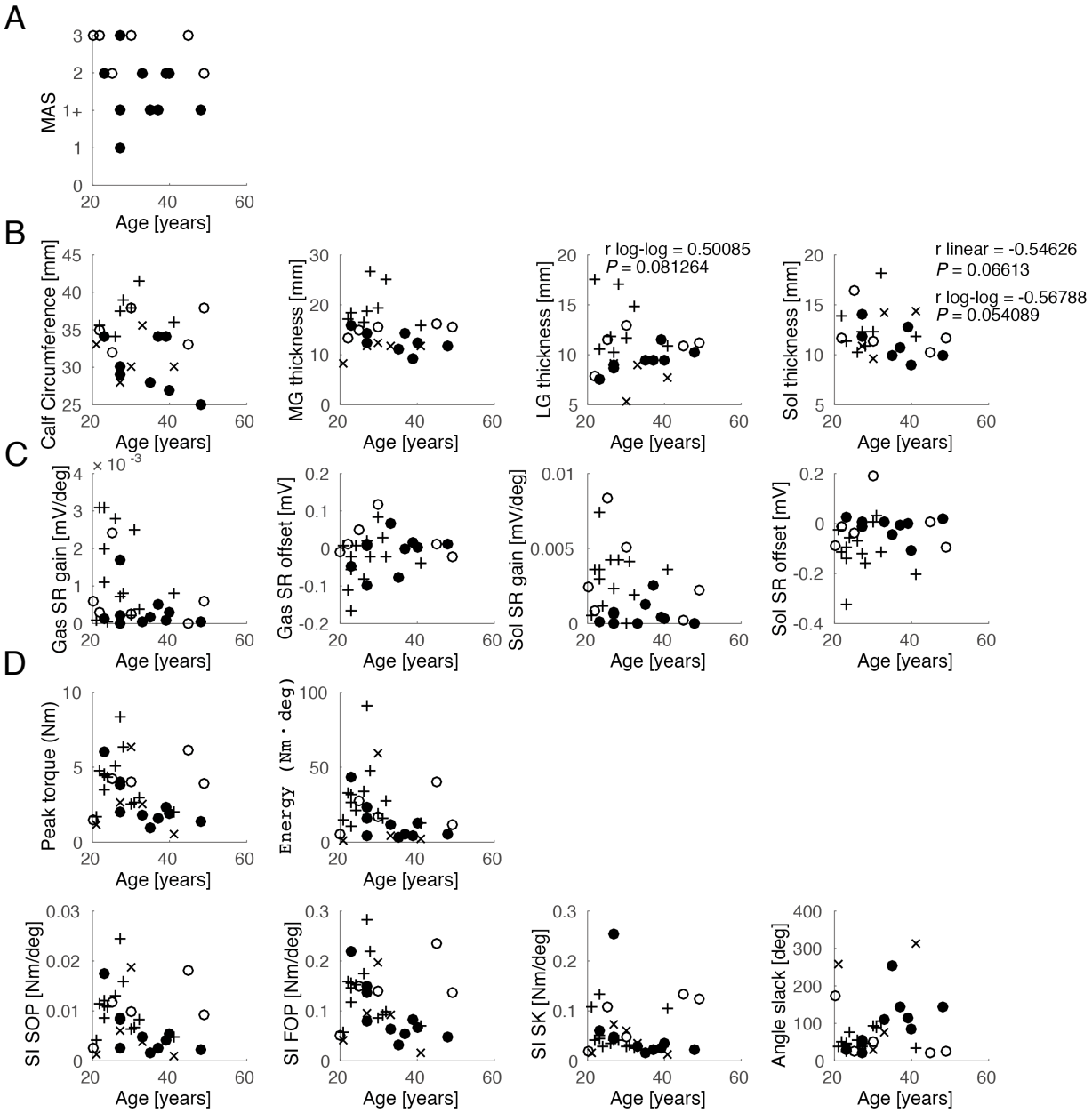

Figure 3-10 Relationships between clinical (**A**), morphological (**B**) neural (**C**), mechanical (**D**) parameters and participants age. Complete SCI (spinal cord injury) participants with spasticity (filled circles), incomplete SCI participants with spasticity (open circles), complete SCI participants without spasticity (x), Normal healthy control (plus sign). Abbreviations: MAS, modified ashworth scale; MG, medial gastrocnemius; LG, lateral gastrocnemius; Sol, Soleus; gastrocnemius (Gas); SR, stretch reflex; SI, stiffness index; SOP second order polynomial; FOP, fourth order polynomial; SK, Sten-Knudson.

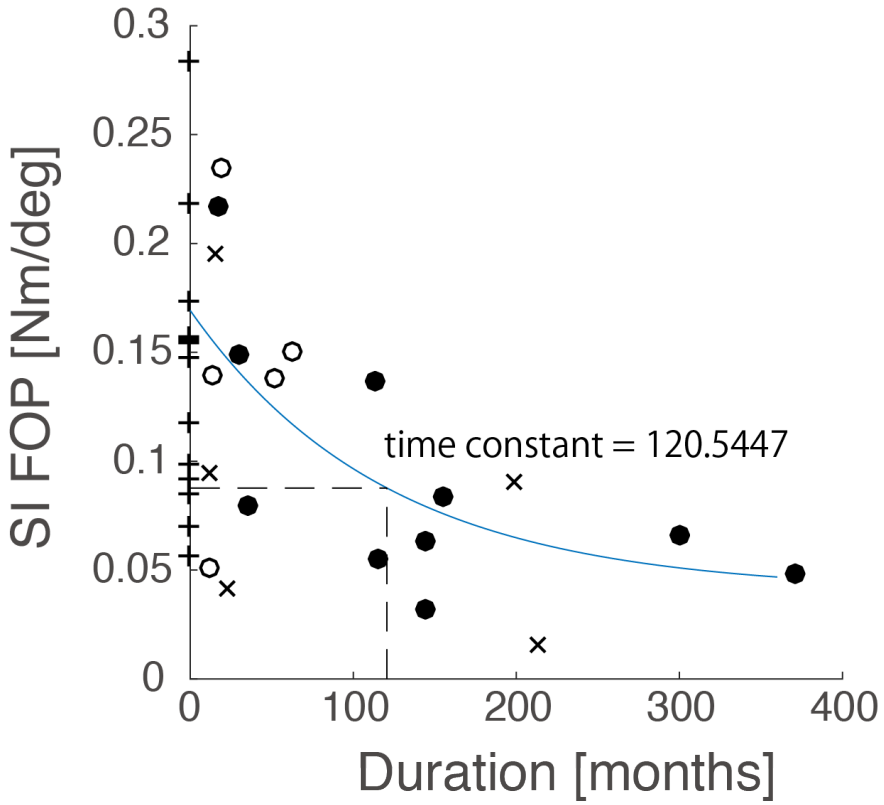

Figure 3-11 Relationship between Stiffness index based on fourth-order polynomial model (SI FOP) and injury duration. Complete spinal cord injury (SCI) participants with spasticity (filled circles), incomplete SCI participants with spasticity (open circles), complete SCI participants without spasticity (x). Blue solid line indicates non-linear regression for the SCI participants with spasticity, and chain line indicates analytically-derived time constant. Abbreviations: SI FOP, stiffness index; FOP, fourth order polynomial.

CHAPTER 4 MUSCULO-TENDON MECHANICAL CHANGES IN PLANTAR FLEXOR MUSCLES AFTER SPINAL CORD INJURY (EXPERIMENT-2)

This chapter is unpublicized because it will be submitted on a scholarly journal. The full version of the thesis shall be opened to the public within 5 years.

CHAPTER 5 GENERAL DISCUSSION

5.1. Summary of Findings

In this thesis, two experiments were executed to provide further insight into changes in neuromuscular properties of plantar-flexor muscles after spinal cord injury (SCI). The following findings and suggestions were obtained from these experiments.

Experiment-1 (CHAPTER 3):

In order to clarify the relationship between changes in neuromuscular properties of plantar-flexor muscles of SCI participants with spasticity and injury duration, I conducted the passive flexion of ankle joints from 10° plantarflexion to 20° dorsiflexion at 5 deg/s using a dynamometer. After the regression analysis for angle–passive torque relation by using polynomial and exponential mathematical models, mechanical indices were calculated. Furthermore, as a stretch reflex test, quick ankle joint rotations from anatomical position to 10° dorsiflexion were conducted at multiple speeds (60, 90, 120, and 150 deg/s), thereafter the stretch reflex gain and offset were calculated. As a result, I found the significant negative correlation between the mechanical parameters and the injury duration. Furthermore, the partial correlation coefficient except muscle morphological factors has still significant in one mechanical parameter (SI_{p4}). This result clearly showed the elasticity (Young's modulus) of the whole plantar-flexor

muscles was reduced with the injury duration after SCI. Time constant (120.5 months) of this changes also indicated that the elasticity continues to decline for approximately 10 years. Additionally, it was revealed that the spinal reflex excitability estimated by stretch reflex amplitude was elevated in the SCI participants with spasticity as group comparison, but not statistically related to the injury duration.

Experiment-2 (CHAPTER 4):

To identify the mechanism of results obtained in the *experiment-1*, i.e., whether tissue's changes were related to the decreasing of elasticity in the whole plantar-flexor muscles, the ultrasound imaging during passive flexion of ankle joint were applied under the same condition in the *experiment-1*. From the obtained tissue elongations–torques data, the stiffness indices of outer tendon (SI Tendon), proximal portion of muscle tendon junction (SI MTJ), and muscle fascicle (SI MFL) were calculated. As a result, SI MTJ and SI MFL have negative correlation with the injury duration, and the time constants of the changes in SI MTJ and SI MFL were 36.4 and 85.4 months (approximately 3 and 7 years), respectively. However, as for the partial correlation coefficients except the morphological factors, only one parameter (SI MTJ) significantly correlated with the injury duration. These results suggested that elasticity of tissues in proximal portion of gastrocnemius muscle tendon junction (MTJ) except fascicle (i.e., aponeurosis and/or connective tissues such as muscle fascia, perimysium, etc.) was decreased with the injury duration.

In the following section, I make some arguments for the results obtained in two experiments from the standpoint of basic and clinical significance.

5.2. A Unified-View of the research and Clinical Implications

5.2.1. *Neuromuscular changes after spinal cord injury*

In the *experiment-1*, I found the significant negative correlation between elasticity index of plantar-flexor muscles and injury duration. Considering the pretreatment of logarithmic transformation, this result indicated that profound changes in elasticity of the whole plantar-flexor muscles occur in an early stage of prognosis. The same was equally true for the observed changes in proximal portion of MTJ in the *experiment-2*. These trends were quantified by elucidating the time constants, and profound stiffness reductions in connective tissues, muscle fascicles, and the whole plantar-flexor muscles could occur within 3, 7, and 10 years, respectively. I cannot precisely identify the causes of different time-courses in these tissues; however, the changes in the amount of collagen contents and extracellular matrix observed in the spastic paralyzed muscle (Booth et al., 2001) could be the key to explain the difference; increasing of inferior materials led the tissue to more compliant. As for the muscle fiber itself, previous researches have shown that elevated elasticity (Young's modulus) was presented in spastic muscle (Fridén & Lieber, 2003; Lieber et al., 2003). Hence, it could be interpreted that those "qualitative" changes in muscle fiber were compromised by the morphological changes (i.e., muscle atrophy) and the alterations in fiber's surrounding

materials (i.e., extracellular matrix); thus, muscle fascicle resulted in more compliant. The fact that the partial correlation of coefficient of SI MFL was not statistically significant with the injury duration suggested that the influence of morphological changes would be more prominent. Further, the present study also indicated that the spinal reflex excitability remained at a high level in SCI individuals with spasticity in chronic stage. This result was inconsistent with the previous research (Nakazawa et al., 2006). Considering the subject's duration (11-371 months) of the present study, it was suggested that the profound elevation in spinal excitability occurs within approximately 1 years, and the change afterwards seems to be the moderate.

5.2.2. *Clinical implications*

The exercise is also recommended in SCI individuals as well as healthy individuals to prevent complication (Jacobs & Nash, 2004). In the simulation study, Lichtwark and Wilson (2008) estimated the optimal gastrocnemius length and tendon stiffness in walking and running based on Hill type model, and speculated that the "lower tendon stiffness" and "shorter muscle length" have the advantage in walking. However, this research assumed the locomotion in healthy individual. Therefore, the finding cannot be easily applicable to that in SCI individuals. An experimental study has demonstrated that the reduction in musculo-tendinous stiffness would lead to the decrease in the intramuscular compartment pressure stabilizing the force exertion, resulting in the decline of force output (Garfin et al., 1981). Additionally, the decline of

tendon elasticity was related to the decreased rate of force development (Bojsen-Møller et al., 2005). Furthermore, the elevated contribution of passive stiffness in paralyzed limb to the plantarflexion torque during walking was also presented (Lamontagne et al., 2000). Considering these findings, the declined time course of SI MTJ and MFL might have negative effects on the activities of daily living (ADL) and could worsen the quality of rehabilitation such as locomotive training. A previous simulation study also has shown that an excessive decrease in stiffness could lead to the reduction in walking efficacy, and this change would be more prominent when the stiffness reached the about 50% of an optimal value (Lichtwark & Wilson, 2007). Additionally, another research has suggested that the inevitable gait pattern changes happened when the stiffness alteration exceeded the approximately 50% of an initial value (Huang & Wang, 2012). Therefore, preventing the deteriorations in the plantar-flexor muscles would be important. One of the significance of this study was elucidating the time constants quantitatively (Figure 5-1). These quantifications clearly showed that the profound changes in the stiffness indices of proximal portion of gastrocnemius MTJ, muscle fascicles, and the whole plantar-flexor muscles occurred within 36.4, 85.4, and 120.5 months (approximately 3 and 7 years) after SCI, respectively. This information about duration of tissue degradation could provide a guide for SCI individuals and clinicians to consider when to start the rehabilitation and/or exercise program in accordance with the patient's pathological conditions.

Many literatures revealed that the positive effect of electrical muscle stimulation (EMS) training on the muscle atrophy after SCI, i.e., could prevent muscle atrophy

(Baldi et al., 1998; Gorgey et al., 2013; Ragnarsson, 2007; Shields & Dudley-Javoroski, 2006, 2007). It is still controversial what kind of intervention would be most appropriate for preventing the changes in "mechanical" properties of muscles after SCI. However, in healthy individual, previous reports indicated that long-term (>3 weeks) training increased the tendon (include aponeurosis) stiffness (Kubo et al., 2001). Thereafter, these augmentations were remarkable in the static (isometric) contraction, especially with long duration condition (Kubo et al., 2001, 2007, 2009). Considering these findings, it is suggested that electrical muscle stimulation with static (isometric) condition can be influential to prevent the reduction of elasticity in the aponeurosis and/or other connective tissues.

5.2.3. *Uniqueness of spinal cord injury*

To date, numerous studies were conducted to identify the changes in immobilized muscle, and bed rest paradigms were mainly adopted especially in vivo. However, as mentioned in the *CHAPTER 3* and *4*, a paralyzed muscle in SCI individual is substantially different from that in bed rest because of following factors: (1) limb position (flexed knee on a wheel-chair), (2) abnormality in nerve connection (reorganization of central nervous system), and (3) occurrence of involuntary contraction. Hence, the findings obtained in bed rest cannot be easily applicable to the SCI. Such uniqueness of SCI was also related to the profound gastrocnemius muscle atrophy observed in *experiment-1* and *-2*.

5.2.4. *Generalization: comparison with longitudinal studies*

The design of the present study was a “cross-sectional” approach for 21 SCI individuals in chronic stage (11-371 months after SCI) (Table 3-1). On the other hand, although some researchers have “longitudinally” investigated the neuromuscular properties after SCI, almost all attempts were ranged in acute (Little et al., 1999) or intermediate phases (<6 months; Castro et al., 1999a, 1999b). As neuromuscular changes in chronic phase, Diong et al. (2013) reported that there were no stitistically significant changes in the gastrocnemius elasticity. However, they focused on the contracture, and their observation period was relatively short (<12 months). One longitudinal study has focused on the chronic changes and shown the hormonal balances were altered from 1 to 3 years after SCI (Karapolat et al., 2015). This finding does not contradict the results in the present study, and explains a part of the background of neuromuscular changes after SCI in chronic stage. However, I also have to admit the weakness of the cross-sectional approach in the present study, e.g., the relatively small number of subjects coupled with the missing values. Further longitudinal assessment for several years might be needed to confirm the findings in this thesis.

5.3. Limitations

As mentioned in the *CHAPTER 4*, I estimated the total muscle tendon unit (MTU) length changes using cadaver regression model (Grieve et al., 1978) and tendon

excursions were calculated as the subtraction of the displacements of MTJ from total MTU length changes. Thus, the possibility for over/under estimation in tendon excursion cannot be ruled out although this approach was used in several studies (Kato et al., 2010; Mizuno et al., 2013; Morse et al., 2008; Nakamura et al., 2011). Development of new technologies to evaluate the tissue viscoelastic properties such as magnetic resonance (Mariappan et al., 2010) or ultrasound (Brandenburg et al., 2014) elastography, shear wave imaging, and high frequency ultrasonography (Mamou et al., 2012; Saegusa-Beecroft et al., 2013) could be the solution of these limitations in the future.

5.4. CONCLUSION OF THE THESIS

In order to provide further insight into the changes in neuromuscular properties of plantar-flexor muscles after SCI, in the thesis, I conducted two experiments focusing attention on the injury duration and with and without spasticity. As a result, it was concluded that, from a neural perspective, (1) the profound elevation of spinal reflex excitabilities occur within 1 year, and afterwards, the changes become moderate (*experiment-1*), and from a muscular perspective, stiffness of (2) proximal portion of gastrocnemius MTJ (such as aponeurosis and/or other connective tissues such as fascia, perimysium, etc.) (*experiment-2*), (3) muscle fascicles (*experiment-2*), and (4) the whole plantar-flexor muscles (*experiment-1*) was reduced with the injury duration after SCI with spasticity. The prominent changes of those occur within approximately 3, 7, and 10

years. The alterations in (2) and (4) mean qualitative changes, i.e., reductions in Young's modulus, while the changes in (3) is due to the muscle atrophy. This information would be helpful and could provide a guide for SCI individuals and clinicians to consider when to start the rehabilitation and/or exercise program in accordance with the patient's pathological conditions.

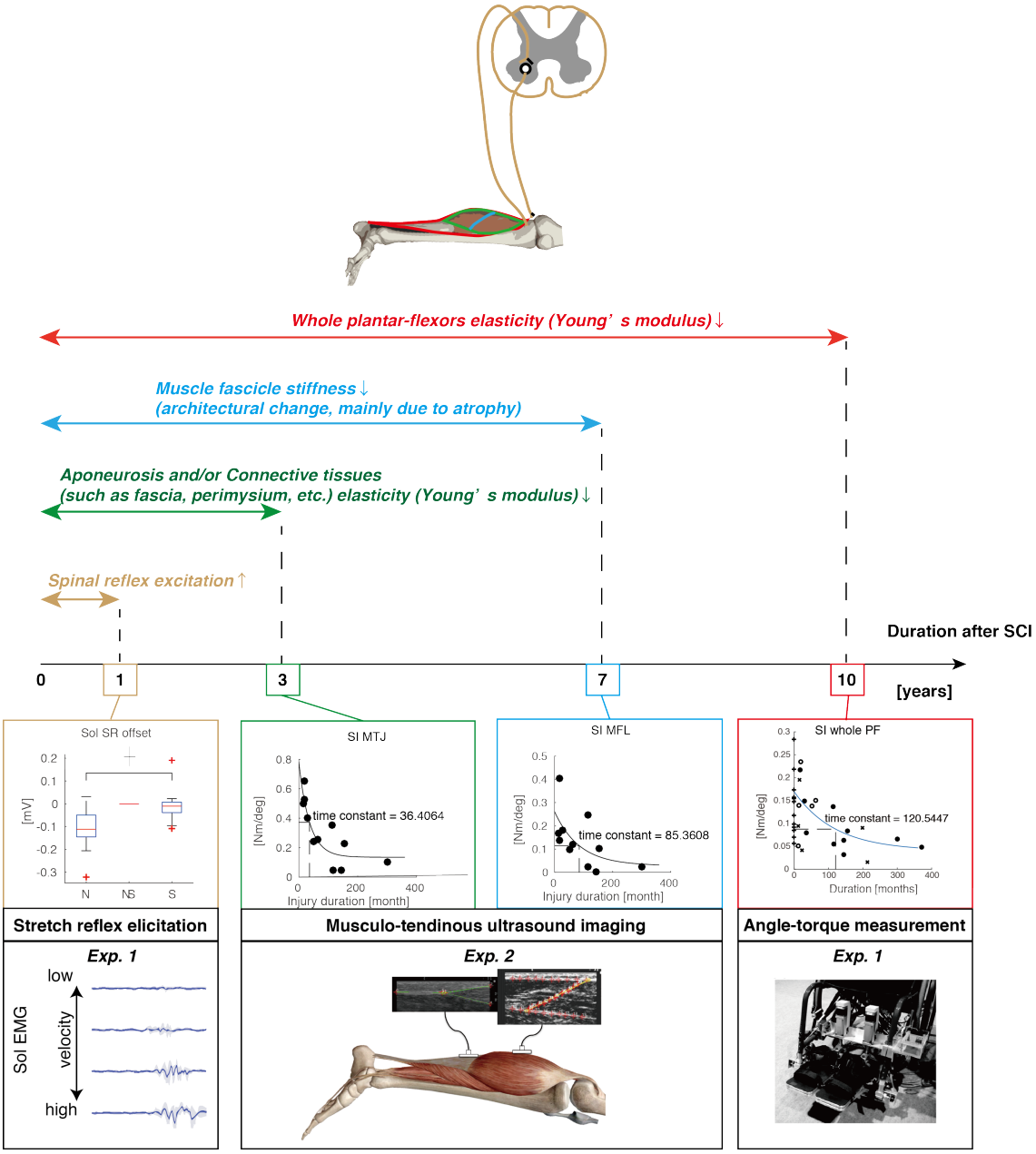

Figure 5-1 Schematic diagram of time-course changes in spastic plantar-flexor neuromuscular properties after spinal cord injury. Abbreviations: SCI, spinal cord injury; Sol, soleus; SI MTJ, stiffness index of the proximal portion of gastrocnemius muscle tendon junction; SI MFL, stiffness index of muscle fascicle; SI whole PF; stiffness index of whole plantar-flexor muscles.

APPENDIX

In the *experiment-2*, muscle tendon excursion was measured by optical flow approach (Lucas-Kanade algorithm). The Lucas-Kanade algorithm (Lucas & Kanade, 1981) is a widely used computer vision method for estimation of optical flow. In this chapter, I explain the summary of this method based on the description by Bradski and Kaehler (2008). Subsequently, I introduce recent studies on skeletal muscle using this method.

Overview of Lucas-Kanade algorithm

Tracking unidentified objects is important when we wish to determine what is interesting based on its motion or when an object's motion is precisely what makes it interesting. There are major two methods for achieving this: the Lucas-Kanade (Lucas & Kanade, 1981) and Horn-Schunck (Horn & Schunck, 1981) techniques, which represent what are often referred to as "sparse" and "dense" optical flow, respectively.

The most popular "sparse" tracking technique, Lucas-Kanade algorithm; this method also has an implementation that works with "image pyramids", allowing us to track faster motions.

The Lucas-Kanade algorithm, as originally proposed in 1981, was an attempt to produce dense results. Yet because the method is easily applied to a subset of the points in the input image, it has become an important sparse technique. The Lucas-Kanade

algorithm can be applied in a “sparse” context because it relies only on “local” information that is derived from some small window surrounding each of the points of interest. This is in contrast to the intrinsically global nature of the Horn and Schunck algorithm (more on this shortly). The disadvantage of using small local windows in Lucas-Kanade is that large motions can move points outside of the local window and thus become impossible for the algorithm to find. This problem led to development of the “pyramidal” Lucas-Kanade algorithm, which tracks starting from highest level of an image pyramid (lowest detail) and working down to lower levels (finer detail). Tracking over image pyramids allows large motions to be caught by local windows.

Three assumptions

The basic idea of the Lucas-Kanade algorithm rests on three assumptions as follows.

- Brightness Constancy
- Temporal Persistence or “small movements”
- Spatial Coherence

Brightness Constancy

The first assumption, “Brightness Constancy” means a pixel from the image of an object in the scene does not change in appearance as it (possibly) moves from frame to frame. In other words, this is just the requirement that “pixels in one tracked patch look

the same over time". This means we assume that the brightness of a pixel does not change as it is tracked from frame to frame.

Temporal Persistence

The second assumption, "Temporal Persistence", essentially means that "motions are small from frame to frame". In other words, we can view this change as approximating a derivative of the intensity with respect to time (i.e., we assert that the change between one frame and the next in a sequence is differentially small). In practice, the image motion of a surface patch changes slowly in time. This means the temporal increments are fast enough relative to the scale of motion in the image that the object does not move much from frame to frame.

Spatial Coherence

Neighboring points in a scene belong to the same surface, have similar motion, and project to nearby points on the image plane.

We now look at how these assumptions, which are illustrated in Figure A-1, lead us to an effective tracking algorithm. To understand the implications of these assumption, now, I explain the mathematical expression of that. The first assumptions, "Brightness Constancy" is simply rewritten as "pixel intensity exhibits no change over time". Thus, if we express the intensity of the pixel which we want to track as $I(x, y, t)$, first assumption is expressed as follows:

$$I(x, y, t) = I(x + \Delta x, y + \Delta y, t + \Delta t) \quad (1)$$

linearizing eq. (1) by applying a first-order Taylor expansion to the right-hand side yields the approximation:

$$I(x, y, t) = I(x, y, t) + \frac{\partial I}{\partial x}\Delta x + \frac{\partial I}{\partial y}\Delta y + \frac{\partial I}{\partial t}\Delta t \quad (2)$$

then, dividing eq. (2) by Δt, we obtain following equation:

$$\frac{\partial I}{\partial x}\frac{\Delta x}{\Delta t} + \frac{\partial I}{\partial y}\frac{\Delta y}{\Delta t} + \frac{\partial I}{\partial t} = 0 \quad (3)$$

in eq. (3), then, let $\frac{\partial I}{\partial x}$, $\frac{\partial I}{\partial y}$, and $\frac{\partial I}{\partial t}$ be I_x I_y, and I_t, which mean spatial derivatives of intensity along x-axis and y-axis direction, and temporal derivative of intensity, respectively. Based on the assumption of Temporal Persistence or "small movements", we can calculate I_x, I_y, and I_t. Thereafter, let $\frac{\Delta x}{\Delta t}$ and $\frac{\Delta y}{\Delta t}$ be *u* and *v,* these mean "optical flow" and need to estimate the object's next location. So that, we obtain "optical flow constraint equation" as follows:

$$I_x u + I_y v + I_t = 0 \quad (4)$$

further, eq. (4) can be simplified as follows:

$$[I_x \quad I_y]\begin{bmatrix} u \\ v \end{bmatrix} = -I_t \tag{5}$$

however, in eq. (4), the number of unknown variables are two: *u and v*, which means that measurements at the single-pixel level are under constrained and cannot be used to obtain a unique solution. Then, on the basis of third assumption "Spatial Coherence", which presume a local patch of pixels moves coherently, we can easily solve for the motion of central pixel by using the surrounding pixels to set up a system of equations. So that, if we use a 3-by-3 window (Figure A-2) of brightness values, we can set up nine equations as follows:

$$\begin{bmatrix} I_x(p_1) & I_y(p_1) \\ I_x(p_2) & I_y(p_2) \\ \vdots & \vdots \\ I_x(p_9) & I_y(p_9) \end{bmatrix}\begin{bmatrix} u \\ v \end{bmatrix} = -\begin{bmatrix} I_t(p_1) \\ I_t(p_2) \\ \vdots \\ I_t(p_9) \end{bmatrix} \tag{6}$$

to solve this equation for $\begin{bmatrix} u \\ v \end{bmatrix}$ first, we convert $\begin{bmatrix} I_x(p_1) & I_y(p_1) \\ I_x(p_2) & I_y(p_2) \\ \vdots & \vdots \\ I_x(p_9) & I_y(p_9) \end{bmatrix}$ in the form of square matrix by multiplying transposed matrix of that:

$$\begin{bmatrix} I_x(p_1) & I_x(p_2) & \dots & I_x(p_9) \\ I_y(p_1) & I_y(p_2) & \dots & I_y(p_9) \end{bmatrix} \begin{bmatrix} I_x(p_1) & I_y(p_1) \\ I_x(p_2) & I_y(p_2) \\ \vdots & \vdots \\ I_x(p_9) & I_y(p_9) \end{bmatrix} \begin{bmatrix} u \\ v \end{bmatrix}$$

$$= \begin{bmatrix} I_x(p_1) & I_x(p_2) & \dots & I_x(p_9) \\ I_y(p_1) & I_y(p_2) & \dots & I_y(p_9) \end{bmatrix} \begin{bmatrix} -I_t(p_1) \\ -I_t(p_2) \\ \vdots \\ -I_t(p_9) \end{bmatrix}$$

$$\begin{bmatrix} \sum I_x I_x & \sum I_x I_y \\ \sum I_x I_y & \sum I_y I_y \end{bmatrix} \begin{bmatrix} u \\ v \end{bmatrix} = - \begin{bmatrix} \sum I_x I_t \\ \sum I_y I_t \end{bmatrix} \tag{7}$$

thereafter, if the first term of left-hand side in eq. (7) is invertible, optical flow $\begin{bmatrix} u \\ v \end{bmatrix}$ can be calculated as follows:

$$\begin{bmatrix} u \\ v \end{bmatrix} = - \begin{bmatrix} \sum I_x I_x & \sum I_x I_y \\ \sum I_x I_y & \sum I_y I_y \end{bmatrix}^{-1} \begin{bmatrix} \sum I_x I_t \\ \sum I_y I_t \end{bmatrix}$$

then, when the first term of left-hand side in eq. (7) is invertible? That term is invertible when it has full rank 2, which occurs when it has "two large eigenvectors". This will happen in "image regions that include texture running in at least two directions". In this case, that term will have the best properties then when the tracking window is centered over a corner region in an image.

The problems in actual image

For most video cameras running at 30 Hz, large and non-coherent motions often take place, this creates dilemma, that is, we want a large window to catch large motions, but a large window too often breaks the coherent motion assumption.

Pyramidal iteration of tracking

To overcome this problem, pyramidal tracking was frequently used. That is, we can track first over larger spatial scales using an “image pyramid” (Figure A-3), and then, refine the initial motion velocity assumptions by working our way down the levels of the image pyramid until we arrive at the raw image pixels. Hence, the recommended technique is first to solve for optical flow at the top layer and then to use the resulting motion estimates as the starting point for the next layer down. We continue going down the pyramid in this manner until we reach the lowest level. So that, we minimize the violations of our motion assumptions, and can track faster and longer motions.

Applications of Lucas-Kanade algorithm to musculo-tendinous ultrasound image

This section is unpublicized online because it will be submitted on a scholarly journal. The full version of the thesis shall be opened to the public within 5 years.

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