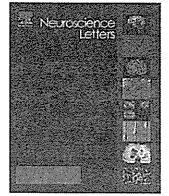


55. **Di Lazzaro V, Oliviero A, Profice P, Insola A, Mazzone P, et al.** (1999) Direct demonstration of interhemispheric inhibition of the human motor cortex produced transcranial magnetic stimulation. *Exp Brain Res* 124: 520–524.
56. **Li J-Y, Lai P-H, Chen R** (2013) Transcallosal inhibition in patients with callosal infarction. *J Neurophysiol* 109: 659–665.
57. **Gerloff C, Cohen LG, Floeter MK, Chen R, Corwell B, et al.** (1999) Inhibitory influence of the ipsilateral motor cortex on responses to stimulation of the human cortex and pyramidal tract. *J Physiol (Lond)* 510: 249–259.
58. **Nowak DA, Grefkes C, Ameli M, Fink GR** (2009) Interhemispheric competition after stroke: brain stimulation to enhance recovery of function of the affected hand. *Neurorehabil Neural Repair* 23: 641–656.
59. **Tazoe T, Perez MA** (2014) Selective activation of ipsilateral motor pathways in intact humans. *J Neurosci* 34: 13924–13934.
60. **Bradnam LV, Stinear CM, Byblow WD** (2011) Cathodal transcranial direct current stimulation suppresses ipsilateral projections to presumed propriospinal neurons of the proximal upper limb. *J Neurophysiol* 105: 2582–2589.
61. **Rehme AK, Fink GR, von Cramon DY, Grefkes C** (2011) The role of the contralesional motor cortex for motor recovery in the early days after stroke assessed with longitudinal fMRI. *Cereb Cortex* 21: 756–768.
62. **Bradnam LV, Stinear CM, Barber PA, Byblow WD** (2012) Contralesional hemisphere control of the proximal paretic upper limb following stroke. *Cereb Cortex* 22: 2662–2671.



Velocity-dependent suppression of the soleus H-reflex during robot-assisted passive stepping



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H I G H L I G H T S

- The effect of passive stepping velocity on the soleus H-reflex was studied.
- The H-reflexes were smaller during passive stepping than standing.
- The H-reflexes significantly decreased at faster stepping velocities.

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The amplitude of the Hoffmann (H)-reflex in the soleus (Sol) muscle is known to be suppressed during passive stepping compared with during passive standing. The reduction of the H-reflex is not due to load-related afferent inputs, but rather to movement-related afferent inputs from the lower limbs. To elucidate the underlying neural mechanisms of this inhibition, we investigated the effects of the stepping velocity on the Sol H-reflex during robot-assisted passive stepping in 11 healthy subjects. The Sol H-reflexes were recorded during passive standing and stepping at five stepping velocities (stride frequencies: 14, 21, 28, 35, and 42 min⁻¹) in the air. The Sol H-reflexes were significantly inhibited during passive stepping as compared with during passive standing, and reduced in size as the stepping velocity increased. These results indicate that the extent of H-reflex suppression increases with increasing movement-related afferent inputs from the lower limbs during passive stepping. The velocity dependence suggests that the Ia afferent inputs from lower-limb muscles around the hip and knee joints are most probably related to this inhibition.

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1. Introduction

It is well known that the amplitude of the soleus (Sol) Hoffmann (H)-reflex is strongly modulated in a task-dependent manner [2,3]. For example, the Sol H-reflex is significantly reduced during

walking compared with during standing [2], and is further reduced during running compared with during walking [3]. Although presynaptic inhibition of Ia terminals is conceived to be a key mechanism in task-dependent H-reflex modulation [2,3], it is not yet fully understood to what extent, and how afferent inputs during walking is involved in this reduction. In previous studies, passive pedaling or manually assisted stepping was used to investigate the effect of afferent input, as it has been technically difficult to eliminate descending inputs from the higher nervous centers during “natural” walking [1,12]. By using these paradigms, Brooke and co-workers found in their series of experiments that the extent of inhibition was dependent on the pedaling velocity [6,12]. Needless to say, however, pedaling and walking are kinetically and kinematically

Abbreviations: AFO, ankle foot orthosis; BF, biceps femoris; BGA, background electromyographic activity; DGO, driven-gait orthosis; EMG, electromyography; H-reflex, hoffmann reflex; M_{max} , maximum direct motor response; MT, motor threshold; RF, rectus femoris; RMS, root mean square; SCI, spinal cord injury; Sol, soleus; TA, tibialis anterior.

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different. It would be better that the effect of afferent input on the ol H-reflex during walking is studied in passive movements more similar to “natural” walking.

Recently, a passive stepping paradigm with a driven-gait orthosis (DGO; Lokomat[®], Hocoma AG, Switzerland) has been used to investigate the effects of afferent input on H-reflex excitability during walking [9,14]. As DGO can support the upright posture of the subjects and impose passive stepping movements that are much similar to “walking,” it is useful to elucidate the effects of afferent input during walking without any influence from descending inputs from the higher nervous centers. By using this technique, Kamibayashi et al. found that the Sol H-reflex was reduced during passive stepping with the DGO as compared with during passive standing. As this reduction occurred irrespective of body-weight condition, it was considered that the reduction of the H-reflex was not due to load-related afferent inputs but rather to movement-related afferent inputs from the lower limbs [9]. Taking this result and the series of Brooke et al. into consideration, we hypothesized that the Sol H-reflex during passive stepping would be more reduced as stepping velocity is increased because the amount of movement-related afferent inputs should increase with increasing stepping velocity. The present study was designed to test this hypothesis. Therefore, the purpose of this study is to elucidate the effect of stepping velocity on the Sol H-reflex during robot-assisted stepping.

2. Methods

Eleven healthy male subjects [age, 28 ± 4.9 (SD) years] participated in this study, which was conducted with the approval of the local ethics committee of the national rehabilitation center for persons with disabilities (Saitama, Japan). All subjects participated in the main experiment, and six of them participated in an additional experiment. None of the subjects had any known history of neurological disorders. Each subject provided informed consent to participate in the experimental procedures, which were done in accordance with the Declaration of Helsinki.

A DGO system (Lokomat, Hocoma AG) was used in this study. A detailed description of the device can be found elsewhere [7]. The subjects were fixed into the DGO with a harness around the hip, and cuffs around the legs, and connected to a body-weight support system. To eliminate the effects of load-related afferent inputs, the body weight was fully removed by using an upper body harness that was connected to the DGO system. The feet of the subject were not in contact with the ground during passive stepping (called “air stepping”). The knee and hip joints were moved passively from 0° to 60° flexion and from 30° flexion to 15° extension, respectively. As the DGO provided no ankle movements, ankle joints were fixed at neutral position by using ankle foot orthoses (AFOs). The subjects were instructed to relax, and not to intervene with the movements imposed by the DGO during the passive stepping, and practiced the passive stepping task for a few minutes before recording. To examine the effect of velocity, passive stepping was performed at five different stride frequencies (14, 21, 28, 35, and 42 min^{-1}). Stride frequency (min^{-1}) was defined as the number of strides per minute. Because the ranges of motions in the lower-limb joints were constant, the stepping velocity was directly related to stride frequency. Electrical stimuli to elicit the H-reflex were delivered at the mid-stance phase of the right leg. In addition to passive stepping, H-reflexes were also recorded during passive standing “in the air.” In our preliminary study, we investigated the effect of stepping velocity on the H-reflex at six different phases. The data showed that there were no phase-dependent differences in the modulation of H-reflex with increases in stepping velocity (unpublished data). Therefore, we investigated only a single phase

in the current study. There were two reasons why we selected the mid-stance phase of the right leg: (i) as the knee joint is extended at the mid-stance phase, the stimulus efficacy to the nerve is better at this phase than at the other phases; (ii) the position of the right leg at the mid-stance phase is similar to that at a passive standing condition, which we thought was important when H-reflex is compared across different motor tasks or conditions. Each experiment was performed for 2.0–2.5 h.

The electromyographs (EMGs) were recorded from the Sol, tibialis anterior (TA), rectus femoris (RF), and biceps femoris (BF) muscles of the right leg by using bipolar Ag/AgCl surface electrodes (F-150S, $18 \times 36 \text{ mm}$; Nihon Kohden, Japan) with an interelectrode distance of 10 mm. The raw EMG signals were amplified ($\times 1000$), and band-pass filtered between 15 Hz and 3 kHz by using a bioamplifier system (MEG-6108, Nihon Kohden, Japan). Actual hip and knee joint angular displacements of both orthotic legs were recorded with the DGO potentiometers. All EMG and joint angular signals were digitized at a sampling rate of 5 kHz by using a data acquisition system [Micro1401, Cambridge Electronic Design (CED), UK] and were stored in a computer with Spike2 software (CED).

The Sol H-reflexes were elicited by stimulating the right tibial nerve (rectangular pulse, 1-ms duration) with an electrical stimulator (SEN-7203, Nihon Kohden, Japan), with a cathode ($18 \times 36 \text{ mm}$) on the popliteal fossa and an anode ($50 \times 50 \text{ mm}$) placed over the patella. The electrodes were secured with adhesive tape and elastic bands to prevent their movement during the experiment. During passive stepping, an output signal from the DGO system was used as a trigger signal for electrical stimulation. The minimum interstimulus interval was 5 s. The H-reflexes were recorded at the stimulus intensity with an M-wave size of 10% maximal direct motor response (M_{max}) in each condition. H-reflex ($n = 12$ sweeps) amplitudes were measured during passive standing and stepping at each of the five different velocities. As there was a possibility that the M_{max} would vary across different conditions and over the time course of an experiment [8], the amplitude of M_{max} ($n = 3$ sweeps) was measured in each condition.

As an additional experiment, the recruitment curves of the M-wave and H-reflex were constructed in 6 of the 11 subjects. The recruitment curves were recorded during passive standing and passive stepping at two different velocity conditions (slow: 14 min^{-1} and fast: 28, 35, or 42 min^{-1}). The stimulus intensity was gradually increased from below the threshold of the H-reflex to above the maximum M-wave, with three stimuli being delivered at each level.

The peak-to-peak amplitudes of the H-reflex, M-wave, and M_{max} evoked at each condition were measured offline. To minimize individual variability, the H-reflex and M-wave amplitude were each expressed as a percentage of M_{max} (H/M_{max} and M/M_{max} , respectively). The background electromyographic activity (BGA) levels in the right Sol TA, RF, and BF muscles were determined as the root mean square (RMS) values of the EMG signals for 50 ms before stimulation.

For the analysis of the recruitment curve, the motor threshold (MT) was defined as the lowest stimulus intensity needed to produce an M-wave of $100 \mu\text{V}$ [11]. The stimulus intensities were expressed as a ratio of MT. The amplitudes of the H-reflex and M-wave were normalized to the corresponding M_{max} . The ascending limb of each individual recruitment curve of the H-reflex was fitted by using the following sigmoid function [10]:

$$H(S) = \frac{H_{\text{max}}}{1 + e^{m(S_{50}-S)}} \quad (1)$$

where S is the stimulus intensity, H_{max} is the maximum H-reflex, m is the slope of the function, S_{50} is the stimulus at 50% of the H_{max} , and $H(S)$ is the H-reflex amplitude at a given stimulus value (S).

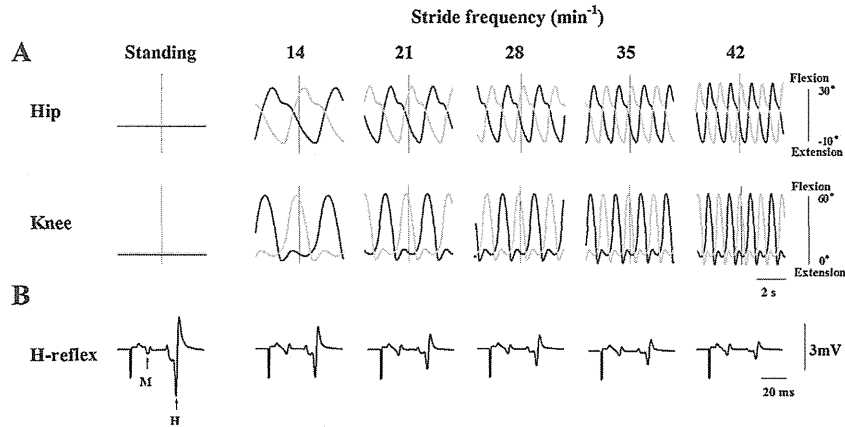


Fig. 1. (A) Typical averaged data ($n = 12$ sweeps) of right (black lines) and left (gray lines) hip and knee joint angular displacements ($^{\circ}$), obtained from a single subject during passive standing and passive stepping at five different velocity conditions ($14, 21, 28, 35,$ and 42 min^{-1}). Each vertical line represents timing of stimulation. (B) Typical averaged data ($n = 12$ sweeps) of Sol H-reflex waveforms during passive standing and passive stepping at five different velocity conditions.

Hslp was defined as the slope of the ascending limb of the recruitment curve at $50\% H_{\text{max}}$. This slope was determined by using the following equation:

$$\text{Hslp} = \frac{m(H_{\text{max}})}{4} \quad (2)$$

The threshold (Hth) of the H-reflex was calculated as the x -intercept of the tangent of the function at S_{50} .

Differences in the H/M_{max} and M/M_{max} were tested with a one-way repeated measures analysis of variance (rANOVA) [passive standing and stepping (five different velocity conditions)]. Differences in the H_{max} , Hslp and Hth were also tested with a one-way rANOVA [passive standing and stepping (slow and fast conditions)]. Post hoc Bonferroni tests were used to identify the significant differences among these conditions. Descriptive statistics included mean + standard error of the mean (SEM). For all tests, the level of significance was set at $P < 0.05$.

3. Results

Fig. 1A shows representative hip and knee joint angular displacements ($^{\circ}$) during passive standing and during passive stepping at five different velocity conditions, obtained from a single subject. As the hip and knee joint trajectories of the DGO were controlled by a computer, the joint movements were highly repeatable. The stimulus phase was identical across the five different velocity conditions. It was observed that the H-reflexes were inhibited during passive stepping as compared with during passive standing and

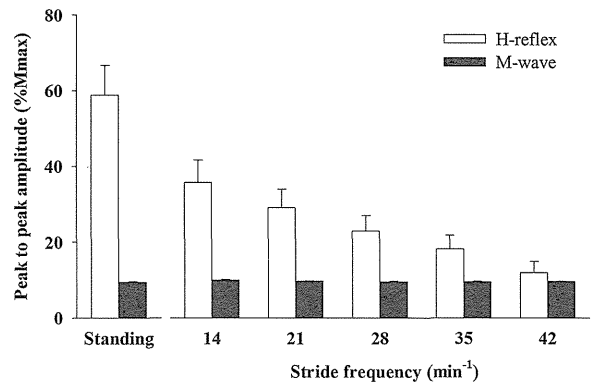


Fig. 2. Peak- to-peak amplitudes of the H-reflex and M-wave in the Sol muscle during passive standing and passive stepping at five different velocity conditions. Filled and open bars represent the average value, and the error bar represents the standard error of the mean (+SEM) for the 11 subjects.

were decreased with increasing velocity; however, the sizes of the M-wave were the same across different velocity conditions (Fig. 1B).

Fig. 2 shows the mean values (+SEM) of the amplitude of the H-reflex and M-wave for 11 subjects. A one-way rANOVA showed that there was a significant main effect [$F(1.83, 18.33) = 47.37, P < 0.001$]. The H-reflexes in each stepping condition were reduced compared with the value at the standing condition (all $P < 0.001$). In the stepping conditions, the H-reflexes were significantly decreased as the

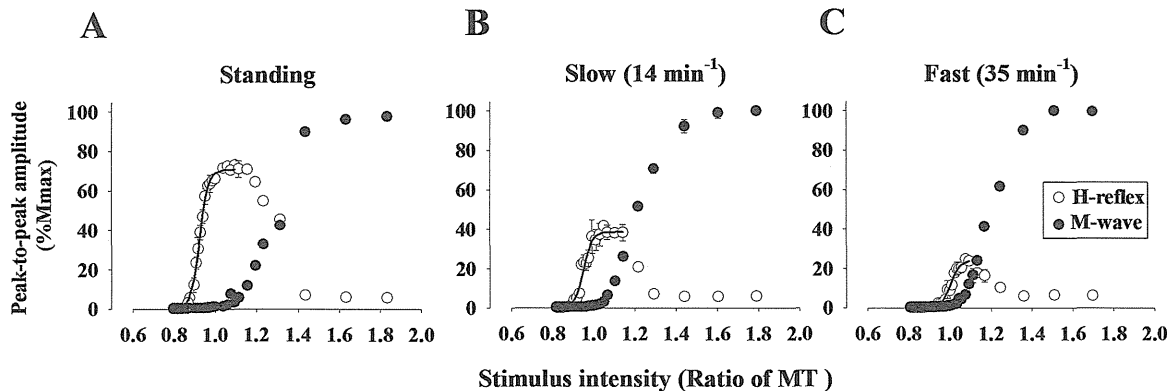


Fig. 3. Examples of recruitment curves of the M-wave and H-reflex during passive standing (A), and stepping at 14 min^{-1} (B), and 35 min^{-1} (C), obtained from a single subject. Each plot shows the mean value of three responses at each stimulus intensity. The sigmoid fitting curves are shown as black lines. MT: motor threshold.

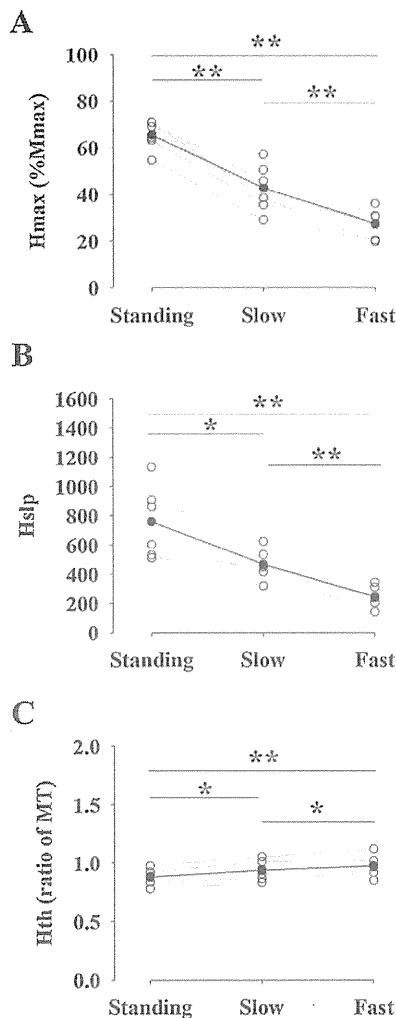


Fig. 4. Three parameters (H_{max} , H_{slp} , and H_{th}) calculated from the sigmoid function. Filled circles represent the averaged value. Open circles represent the magnitude of the H-reflex in each subject. Significant differences among the three different conditions, * $P < 0.05$, ** $P < 0.01$.

stepping velocity increased ($P = 0.001–0.045$), except in the case of 28 vs. 35 min^{-1} ($P = 0.289$) and 35 vs. 42 min^{-1} ($P = 1.02$). As for the M-wave amplitudes, the main effect was not significant [$F(5, 50) = 1.97$, $P = 0.819$]. The BGA levels in the right leg muscles were negligibly small.

Fig. 3 shows the H-reflex recruitment curves during passive standing (A) and passive stepping at slow (B) and fast (C) conditions obtained from a single subject. The maximal H-reflex size was decreased as the velocity increased.

Fig. 4 shows the results for three parameters of the recruitment curves. A one-way rANOVA showed that there was a significant main effect in each parameter [H_{max} : $F(2, 10) = 67.78$, $P < 0.001$, H_{slp} : $F(2, 10) = 26.19$, $P < 0.001$; H_{th} : $F(2, 10) = 40.97$, $P < 0.001$]. The H_{max} (A) and H_{slp} (B) were significantly smaller in the stepping conditions than in the standing condition ($P < 0.05$). The H_{th} (C) was significantly higher in the stepping conditions than in the standing condition ($P < 0.05$). Furthermore, H_{max} (A) and H_{slp} (B) were significantly smaller in the fast stepping condition than in the slow stepping condition ($P < 0.01$). The H_{th} (C) was significantly higher in the fast stepping condition than in the slow stepping condition ($P < 0.05$).

4. Discussion

The aim of this study was to clarify the effect of stepping velocity on the Sol H-reflex during robot-assisted passive stepping. In this study, passive stepping at five different velocity conditions was provided by the use of a DGO. Our results provide the first demonstration of a velocity-dependent suppression of the Sol H-reflex during passive stepping. The neuronal mechanisms underlying the present results will be discussed below.

In this study, the amplitude of the M-wave elicited in the Sol was used as an index of the consistency of the stimulus intensity, which is an important parameter in human H-reflex experiments involving movements [16]. As no significant main effect was found in M-wave amplitude (M/M_{max}), the afferent volley evoked at each condition was considered to be maintained at a constant level. In addition, as the ankle joint was fixed at the same joint position by using an AFO, there was only little change in Sol muscle geometry with respect to the surface electrodes. To eliminate any influence from the higher nervous centers, the subjects were instructed to relax and not to intervene with the movements imposed by the DGO for passive stepping. As a result, the average BGAs at each velocity were at the noise level, which demonstrated that all subjects were able to achieve the passive stepping. Therefore, the observed changes in the excitability of the Sol H-reflex were most probably due to somatosensory inputs from the lower limbs during passive stepping in the DGO.

In the present study, the H-reflexes were inhibited during passive stepping as compared with during passive standing, and were reduced in size as the stepping velocity increased. During stepping with the DGO, the hip and knee joints were moved passively, whereas the ankle joints were immobilized by using AFOs. As passive stepping was conducted in the air, load-related afferents would not be activated. Therefore, the H-reflex inhibition observed in the present study could be attributed to movement-related afferent inputs from the lower limbs. Furthermore, it was considered that the velocity dependent suppression of the H-reflex was caused by increasing movement-related afferent inputs from the lower limbs, including muscle, joint, cutaneous afferents, etc. In the main experiments, only one stimulus intensity (M-wave size = 10% M_{max}) was used for H-reflex recording to minimize experiment time, although it is better to apply stimulation at various intensities to know the H-reflex excitability in more detail. In additional experiments ($n = 6$), to reveal the input/output relation of the spinal neural circuit mediating H-reflex, we recorded the H-reflex at various stimulus intensities during passive standing and stepping (fast and slow conditions) and constructed the recruitment curves. The characteristics of the curves can be described with three parameters: H_{th} , H_{slp} , and H_{max} [10]. The changes in the parameters also indicated that the H-reflex excitabilities were more inhibited as the stepping velocity increased (Fig. 4) and provided a more strong evidence of velocity-dependent suppression of the H-reflex during passive stepping.

In previous studies, a passive pedaling or manually assisted stepping has been used to investigate the effect of afferent inputs on the H-reflex during “walking” [1,12]. However, there are differences both in kinematics and kinetics between pedaling and walking. First, the seated posture during pedaling differs from the upright posture during walking. Second, the movement patterns of pedaling differ from that of walking. The DGO used in this study can support the upright posture of the subjects and impose a passive stepping movement that is similar to “natural” walking. In a previous study, Brooke’s team reported that H-reflexes were inhibited during passive pedaling as compared with during sitting, and were reduced in size as the pedaling velocity increased [12]. Despite these differences between stepping and pedaling, the present results were consistent with the

results with the pedaling paradigm. Thus, it was considered that the velocity-dependent suppression during passive movements was not related to the posture and movements patterns. Rather, the movement-related somatosensory inputs, which are not context dependent, most probably play the major role in the H-reflex suppression observed during both passive pedaling and stepping. In the series of experiments by Brooke and co-worker they demonstrated that the suppression of the H-reflex during passive pedaling was mainly due to Ia afferent inputs in the knee extensor muscles [4,5,13]. With the present results, we cannot directly identify the source of velocity-dependent suppression of the H-reflex during passive stepping. Although the discharge of different types of sensory receptors in the legs should be increased with increasing velocity, it is considered that, on the basis of the work of Brooke's team, the velocity-dependent suppression during passive stepping might have been due to the increasing discharge rate of Ia afferents.

Concerning the clinical relevance of this study, symptoms of spasticity are often experienced by persons with spinal cord injury (SCI) and are thought to be related to hyper-excitability of the spinal reflex. Furthermore, velocity-dependent modulation is impaired in these patients during walking [15]. In our previous study, we showed that Sol H-reflexes were inhibited during passive stepping as compared with during passive standing, not only in healthy subjects but also in those with clinically motor-complete (SCI) [9]. These results suggest that spinal neural circuits may mediate the Sol H-reflex inhibition during passive stepping, and that there may be a potential therapeutic use for passive stepping (e.g., body-weight supported treadmill training) for the management of spasticity in ankle extensor muscles after SCI and for the improvement of spinal reflex modulations.

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References

- [1] J.D. Brooke, J. Cheng, J.E. Misiaszek, K. Lafferty, Amplitude modulation of the soleus H reflex in the human during active and passive stepping movements, *J. Neurophysiol.* 73 (1995) 102–111.
- [2] C. Capaday, R.B. Stein, Amplitude modulation of the soleus H-reflex in the human during walking and standing, *J. Neurosci.: Off. J. Soc. Neurosci.* 6 (1986) 1308–1313.
- [3] C. Capaday, R.B. Stein, Difference in the amplitude of the human soleus H reflex during walking and running, *J. Physiol.* 392 (1987) 513–522.
- [4] J. Cheng, J.D. Brooke, J.E. Misiaszek, W.R. Staines, The relationship between the kinematics of passive movement, the stretch of extensor muscles of the leg and the change induced in the gain of the soleus H reflex in humans, *Brain Res.* 672 (1995) 89–96.
- [5] J. Cheng, J.D. Brooke, W.R. Staines, J.E. Misiaszek, J. Hoare, Long-lasting conditioning of the human soleus H reflex following quadriceps tendon tap, *Brain Res.* 681 (1995) 197–200.
- [6] D.F. Collins, W.E. McIlroy, J.D. Brooke, Contralateral inhibition of soleus H reflexes with different velocities of passive movement of the opposite leg, *Brain Res.* 603 (1993) 96–101.
- [7] G. Colombo, M. Joerg, R. Schreier, V. Dietz, Treadmill training of paraplegic patients using a robotic orthosis, *J. Rehabil. Res. Dev.* 37 (2000) 693–700.
- [8] C. Crone, L.L. Johnsen, H. Hultborn, G.B. Orsnes, Amplitude of the maximum motor response (M_{max}) in human muscles typically decreases during the course of an experiment, *Exp. Brain Res. Exp. Hirnforschung. Exp. Cerebrale.* 124 (1999) 265–270.
- [9] K. Kamibayashi, T. Nakajima, M. Fujita, M. Takahashi, T. Ogawa, M. Akai, K. Nakazawa, Effect of sensory inputs on the soleus H-reflex amplitude during robotic passive stepping in humans, *Exp. Brain Res. Exp. Hirnforschung. Exp. Cerebrale.* 202 (2010) 385–395.
- [10] M. Klimstra, E.P. Zehr, A sigmoid function is the best fit for the ascending limb of the Hoffmann reflex recruitment curve, *Exp. Brain Res. Exp. Hirnforschung. Exp. Cerebrale* 186 (2008) 93–105.
- [11] J.C. Lamy, C. Ho, A. Badel, R.T. Arrigo, M. Boakye, Modulation of soleus H reflex by spinal DC stimulation in humans, *J. Neurophysiol.* 108 (2012) 906–914.
- [12] W.E. McIlroy, D.F. Collins, J.D. Brooke, Movement features and H-reflex modulation. II. Passive rotation, movement velocity and single leg movement, *Brain Res.* 582 (1992) 85–93.
- [13] J.E. Misiaszek, J.K. Barclay, J.D. Brooke, Inhibition of canine H reflexes during locomotor-like rotation about the knee arises from muscle mechanoreceptors in quadriceps, *J. Neurophysiol.* 73 (1995) 2499–2506.
- [14] T. Nakajima, T. Kitamura, K. Kamibayashi, T. Komiyama, E.P. Zehr, S.R. Hundza, K. Nakazawa, Robotic-assisted stepping modulates monosynaptic reflexes in forearm muscles in the human, *J. Neurophysiol.* 106 (2011) 1679–1687.
- [15] C.P. Phadke, F.J. Thompson, C.G. Kukulka, P.M. Nair, M.G. Bowden, S. Madhavan, M.H. Trimble, A.L. Behrman, Soleus H-reflex modulation after motor incomplete spinal cord injury: effects of body position and walking speed, *J. Spinal Cord Med.* 33 (2010) 371–378.
- [16] E.P. Zehr, Considerations for use of the Hoffmann reflex in exercise studies, *Eur. J. Appl. Physiol.* 86 (2002) 455–468.

Potential Role of pNF-H, a Biomarker of Axonal Damage in the Central Nervous System, as a Predictive Marker of Chemotherapy-Induced Cognitive Impairment

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Abstract

Purpose: Chemotherapy-induced cognitive impairment (CICI) is a clinically significant problem. Previous studies using magnetic resonance imaging indicated structural changes in the cerebral white matter of patients with CICI. Phosphorylated neurofilament heavy subunit (pNF-H), a major structural protein in axons, was recently reported to be elevated in the serum of patients with some central nervous system disorders. We performed a cross-sectional analysis of neuropsychological test results and serum pNF-H levels in patients undergoing adjuvant chemotherapy for breast cancer. Our hypothesis was that CICI is accompanied by axonal damage that can be detected by elevated serum pNF-H levels.

Experimental Design: Seventy-six patients with early breast cancer in various phases of treatment (naïve to chemotherapy; after one, three, or seven cycles of chemotherapy; or with a history of chemotherapy) were assessed by self-administered

neuropsychological tests and a single pNF-H measurement. The χ^2 and Mann-Whitney tests were used for statistical analysis.

Results: Increased pNF-H levels were observed in 28.8% of the patients who underwent chemotherapy, but in none of the chemotherapy-naïve patients or patients with a history of chemotherapy. The pNF-H-positive rate increased significantly in proportion to the number of chemotherapy cycles (one cycle, 5.0%; three cycles, 31.6%; seven cycles, 55.0%; $P < 0.05$). No significant differences in neuropsychological test results were observed among the groups.

Conclusions: The serum pNF-H level in patients undergoing chemotherapy for breast cancer increased in a cumulative dose-dependent manner, suggesting its potential application as a biomarker of neural damage after chemotherapy. *Clin Cancer Res*; 21(6); 1348–52. ©2015 AACR.

Introduction

Chemotherapy can offer long-term survival for patients with cancer, especially patients with breast cancer and blood cancer. However, several recent reports (1–3) have revealed that chemotherapy may induce cognitive dysfunction such as deficits in attention, concentration, executive function, verbal or visual learning, and processing speed (4, 5). For example, one study showed that patients' attention and memory task abilities were worse after than before chemotherapy. However, healthy subjects exhibited progressive improvement in these abilities with task repetition (6). Although the cognitive dysfunction following chemotherapy is usually less

severe, it sometimes impairs activities of daily living and quality of life to the point of debilitation. Recently, such chemotherapy-induced cognitive impairment (CICI) has been recognized as a clinically significant issue in patients treated with chemotherapy and cancer survivors with a history of chemotherapy (1–3, 7–9). Elucidation of the mechanisms of and diagnostic and therapeutic measures for CICI is urgently required.

One proposed mechanism of CICI is direct neurotoxicity by chemotherapy itself (7). Magnetic resonance imaging studies have demonstrated lower integrity of cerebral white matter (location of myelinated axons) rather than gray matter (location of neuronal cell bodies) in patients with CICI than in healthy subjects (10–12). However, these findings do not directly indicate whether the decreased integrity of the cerebral white matter is caused by damage to axons themselves, which are the main components of white matter, or by Wallerian axonal degeneration following neuronal damage.

Various tissues in the central nervous system (CNS), including neurons, axons, and glia, release several lines of proteins into the cerebral spinal fluid and/or peripheral blood flow when damaged (13–17). The use of some of these proteins in the cerebral spinal fluid and/or blood has been explored as objective biomarkers of the severity of neuronal damage. Increased levels of the circulating phosphorylated form of the high-molecular-weight neurofilament subunit (pNF-H), a major structural protein in central and

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Translational Relevance

Chemotherapy-induced cognitive impairment (CICI) has been recognized as a clinically significant issue in patients treated with chemotherapy and cancer survivors with a history of chemotherapy. Elucidation of the mechanisms of and diagnostic and therapeutic measures for CICI is urgently required. This study investigated CICI with a particular focus on the phosphorylated form of the high-molecular-weight neurofilament heavy subunit NF-H (pNF-H), a major structural protein in axons, as a predictive marker of CICI. We found that the serum pNF-H level in patients undergoing chemotherapy for breast cancer increased in a cumulative dose-dependent manner, suggesting the potential application of pNF-H as a biomarker of neural damage after chemotherapy. It might be useful to investigate the mechanisms and severity indexes of CICI and neuronal toxicity of chemotherapy on the CNS by using pNF-H as a surrogate marker, rather than subjective cognitive test batteries.

peripheral axons, were recently reported in a rodent model of spinal cord injury (17). The pNF-H level is associated with the severity of spinal cord injury (18) and may have adequate sensitivity to serve as a biomarker of treatment efficacy in patients with spinal cord injury (19). Increased pNF-H levels are also observed in patients with supraspinal CNS damage, such as that caused by multiple sclerosis (20), febrile seizures (21), hypoxic-ischemic encephalopathy (22), acute intracerebral hemorrhage, and other conditions. Thus, pNF-H has potential as an effective biomarker of CNS damage caused by either neuronal or axonal injury.

The purpose of the present study was to evaluate the potential role of pNF-H as a predictive marker of CICI. We performed a cross-sectional analysis of the results of neuropsychological tests, which can be conducted in the clinical setting and serum pNF-H levels in patients with breast cancer undergoing adjuvant chemotherapy.

Patients and Methods

Patients

Patients were eligible if they had histologic proof of early breast cancer. Patients were ineligible if they were <18 or >70 years of age, had a psychiatric disorder, or had a history of chemotherapy for another malignancy. The patients were assigned to various phases of treatment (before chemotherapy, after one cycle of chemotherapy, after three cycles of chemotherapy, after seven cycles of chemotherapy, and survivors who had undergone previous chemotherapy). Both neoadjuvant and adjuvant chemotherapy were eligible for inclusion. Various chemotherapy regimens were acceptable. The use of hormone agents was permitted in the group of patients who had undergone previous chemotherapy. These hormone agents including both tamoxifen and aromatase inhibitors were applied to the patients according to their menopausal status. Written informed consent was obtained from all participants. This study was approved by the institutional review board of our hospital and supported by Health Labour Sciences Research Grant from the Japanese Ministry of Health, Labour and Welfare (H24-Ganrinsho-ippan-011 and H26-Kakushinntekigan-ippan-060).

Methods

All patients underwent self-administered neuropsychological tests and pNF-H level measurements at once when they were assigned. The EuroQOL-5 Dimension questionnaire (EQ-5D), Hospital Anxiety and Depression Scale (HAD), State-Trait Anxiety Inventory (STAI), PainDETECT questionnaire, Epworth Sleepiness Scale (ESS), Raven's Colored Progressive Matrices (RCPM), Cognitive Failure Questionnaire (CFQ), Japanese version of the Brief Fatigue Inventory (BFI-J), Japanese version of the Newest Vital Sign (NVS-J), and Japanese Adult Reading Test (JART) were used. The EQ-5D is a scale of health-related quality of life (23), and the HAD is a self-assessment scale of anxiety and depression in the setting of an outpatient clinic of a medical hospital (24). The STAI is a 40-item instrument that measures transient and enduring levels of anxiety (25). PainDETECT is a screening questionnaire used to identify peripheral neuropathy and neuropathic pain (26). The ESS is a scale of subjective sleepiness (27). The RCPM is a standardized test designed to measure nonverbal intellectual capacity (28). The CFQ assesses self-reported cognitive functioning (29). The BFI-J is a nine-item questionnaire designed to assess fatigue in patients with cancer (30, 31). The NVS-J measures health literacy in Japanese adults (32). The JART evaluates the premorbid intelligence quotient (33). The serum pNF-H level was determined with a commercially available enzyme-linked immunosorbent assay kit (Human Phosphorylated Neurofilament H ELISA; BioVendor), following the manufacturer's protocol. The serum samples were diluted 3-fold before the analysis. pNF-H levels of >70.5 pg/mL were considered to be positive (18).

All statistical analyses were performed using SPSS software. The χ^2 and Mann-Whitney tests were used to compare data. Statistical significance was assessed at $P < 0.05$.

Results

A total of 76 patients participated in this study. Five patients were naïve to chemotherapy; 20 had completed 1 cycle of chemotherapy, 20 had completed 3 cycles of chemotherapy, 19 had completed 7 cycles of chemotherapy, and 12 had completed chemotherapy at least 24 months before this study. The patients' demographic data and neuropsychological test results are summarized in Tables 1 and 2. An increased pNF-H level was observed in 28.8% of the patients who underwent chemotherapy, but in none of the chemotherapy-naïve patients or patients who had undergone previous chemotherapy. The pNF-H-positive rate in each patient group treated with chemotherapy increased significantly in proportion to the number of chemotherapy cycles (one cycle, 5.0%; three cycles, 31.6%; seven cycles, 55.0%; $P < 0.05$; Fig. 1). The average pain intensity in each group according to the PainDETECT was 3.0 ± 2.3 , 1.8 ± 1.8 , 1.7 ± 2.0 , 1.6 ± 1.6 , and 1.6 ± 1.9 (naïve to chemotherapy, after one cycle of chemotherapy, after three cycles of chemotherapy, after seven cycles of chemotherapy, and previously treated by chemotherapy, respectively; $P = 0.65$). The PainDETECT numbness scores in each group were 2.2 ± 1.3 , 1.2 ± 0.4 , 2.0 ± 1.0 , 1.6 ± 1.1 , and 1.8 ± 0.8 , respectively ($P = 0.14$). Symptoms of peripheral neuropathy in the patients with increasing pNF-H levels were not salient and were comparable with those in pNF-H-negative patients. The average PainDETECT pain intensity and numbness scores in the pNF-H-positive versus pNF-H-negative patients were 1.6 ± 1.8 versus 1.8 ± 1.8 ($P = 0.62$) and $1.5 \pm$

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Table 1. Patient demographics

	Chemotherapy naïve (N = 5)	After 1 cycle of chemotherapy (N = 20)	After 3 cycles of chemotherapy (N = 20)	After 7 cycles of chemotherapy (N = 19)	Patients with a history of previous chemotherapy (N = 12)
Age (median), y	49	45.5	45	50	46
Postmenopausal, n (%)	3 (60)	3 (15)	9 (45)	7 (35)	6 (50)
Hormonal therapy, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	9 (75)
Chemotherapy regimen, n (%)					
FEC	N/A	1 (5)	4 (20)	N/A	N/A
AC	N/A	15 (75)	9 (45)	N/A	N/A
DOC	N/A	4 (20)	7 (35)	N/A	N/A
FEC-DOC	N/A	N/A	N/A	15 (79)	8 (67)
AC-DOC	N/A	N/A	N/A	3 (16)	0 (0)
Other	N/A	N/A	N/A	1 (5)	4 (33)
Days from first chemotherapy (median)	N/A	21	63	154	944

Abbreviations: AC, adriamycin and cyclophosphamide; DOC, docetaxel; FEC, 5-fluorouracil, epirubicin, and cyclophosphamide; N/A, not applicable.

1.1 versus 1.7 ± 0.9 ($P = 0.18$), respectively. No significant differences were observed in the neuropsychological test results among the patient groups (Table 3).

Discussion

In the present study, some of the patients treated with chemotherapy showed increased serum pNF-H levels, and the chemotherapy-associated pNF-H positivity rate increased in a cumulative dose-dependent manner. These findings indicate that the serum pNF-H level in patients treated with chemotherapy is probably derived from axon degeneration in the CNS and that neuronal toxicity by chemotherapy operates temporally because none of the patients who had completed chemotherapy at least 24 months before the study showed increasing pNF-H levels. These results suggest a potential application of pNF-H as a biomarker of neural damage in the CNS after chemotherapy. In patients with chemotherapy-induced peripheral neuropathy, it is already known that chemotherapy impairs function of the peripheral nervous system in a cumulative dose-dependent

manner (34, 35). However, among the present study participants, few patients showed peripheral neuropathy and neuropathic pain, and cumulative dose-dependent development of neuropathy was not observed. Furthermore, although pNF-H is certainly included in the peripheral nerve axon and oxaliplatin can lead to loss of pNF-H immunoreactivity in the dorsal root ganglion (36), symptoms of peripheral neuropathy with increasing pNF-H levels were not salient in the present patients, as in pNF-H-negative patients.

We found no differences in the neuropsychological test results between patients with and without increasing pNF-H levels. These tests failed to detect cognitive decline from the pretreatment to posttreatment period in almost all of the patients who still scored within the normal range in the posttreatment period, possibly because of high premorbid cognitive function. Previous studies (6, 10, 11) used more dedicated cognitive test batteries assessed by an experimenter, and the tests were applied much later than in the present study. The time points at which the patients were assessed by neuropsychological tests vary among previous studies, but many tests were conducted from 1 month to 1 year after

Table 2. Results of neuropsychological tests among groups sorted by treatment phase

	Chemotherapy naïve (N = 5)	After 1 cycle of chemotherapy (N = 20)	After 3 cycles of chemotherapy (N = 20)	After 7 cycles of chemotherapy (N = 19)	Patients with a history of previous chemotherapy (N = 12)
pNF-H-positive, n (%)	0 (0)	1 (5)	6 (30)	11 (57.8)	0 (0)
CFQ ^a (mean, SD)	21.5 ± 8.3	19.4 ± 11.0	17.5 ± 10.8	18.4 ± 11.4	25.6 ± 10.9
HADS ^b					
Anxiety subscale (mean, SD)	5.0 ± 2.9	5.5 ± 2.8	3.7 ± 2.8	5.0 ± 2.9	4.3 ± 2.9
Depression subscale (mean, SD)	3.4 ± 4.0	4.7 ± 3.4	3.9 ± 3.4	4.0 ± 3.6	2.8 ± 3.4
STAI ^b					
State anxiety (mean, SD)	41.6 ± 11.6	43.3 ± 11.1	38.3 ± 11.4	42.0 ± 11.9	36.9 ± 11.2
Trait anxiety (mean, SD)	45 ± 9.8	44.9 ± 11.4	35.0 ± 11.4	42.7 ± 11.9	38.1 ± 11.2
ESS ^b (mean, SD)	6.6 ± 4.0	10.5 ± 4.2	9.2 ± 4.1	8.0 ± 3.8	8.8 ± 3.8
BFI-J ^b (mean, SD)	34.5 ± 17.5	16 ± 14.8	16.3 ± 14.7	17.3 ± 15.8	21.9 ± 15.7
NVS ^a (mean, SD)	4.6 ± 0.7	4.2 ± 1.1	4.5 ± 1.1	4.6 ± 1.2	4.2 ± 1.1
JART ^a (mean, SD)					
Full-scale IQ	109.6 ± 7.5	106.7 ± 9.4	110.5 ± 9.2	110.2 ± 10.0	110.9 ± 9.6
Verbal IQ	111.4 ± 8.6	108.1 ± 10.6	112.4 ± 10.5	112.1 ± 11.3	112.8 ± 10.9
Performance IQ	106.3 ± 5.5	104.2 ± 6.9	107.0 ± 6.8	106.8 ± 7.3	107.2 ± 7.1
RCPM ^a (mean, SD)	36.0 ± 1.3	36.0 ± 0.7	35.8 ± 0.7	35.8 ± 0.8	35.5 ± 0.7
PainDETECT ^b (mean, SD)	4.1 ± 4.7	4.0 ± 4.8	3.7 ± 4.7	4.2 ± 5.0	4.2 ± 4.7
EQ5D ^b (mean, SD)	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1

Abbreviations: CFQ, cognitive failure questionnaire; EQ5D, EuroQOL-5 Dimension questionnaire; HADS, Hospital Anxiety and Depression Scale; IQ, intelligence quotient; pNF-H, phosphorylated form of the high-molecular-weight neurofilament subunit.

^aThe score would decrease when the function declines.^bThe score would increase when the function declines.

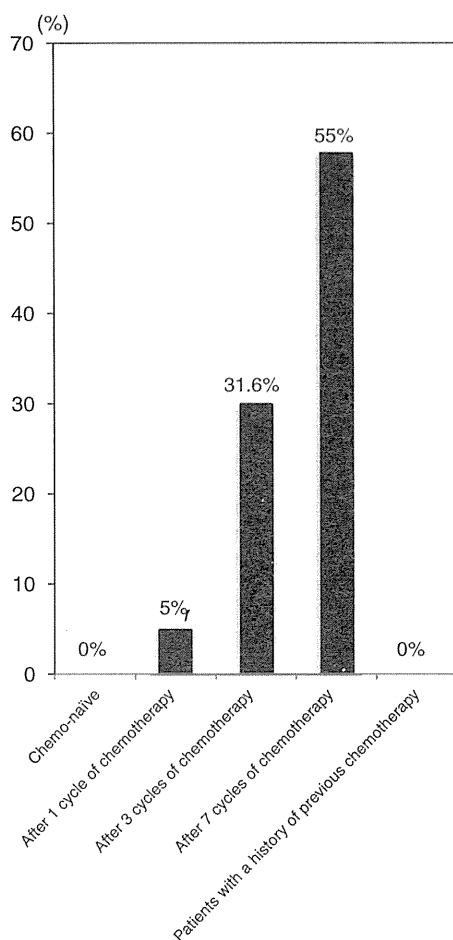


Figure 1. Rate of pNF-H positivity in each patient group treated with chemotherapy. pNF-H, phosphorylated form of the high-molecular-weight neurofilament subunit.

chemotherapy (3, 10–12, 37). We used self-administered neuropsychological tests to screen CICI in common clinical settings using simple cognitive test batteries. However, such tests might not be sensitive enough to detect subtle changes in CICI. Whether intimate relationships exist between the pNF-H level and dedicated cognitive test batteries requires further assessment. Subjective complaints regarding cognitive decline after chemotherapy and other cancer treatments might be associated with depression, anxiety, and fatigue (2, 3). Most of the participants in the present study showed no depression or anxiety. Moreover, the cognitive decline was not found among the patients who went into menopause or used some hormonal therapy, although menopausal status and hormone therapy might affect the cognitive function (38). These may have been related to the fact that the neuropsychological test results did not detect cognitive decline. It might be useful to investigate the mechanisms and severity indices of CICI and neural toxicity of chemotherapy on the CNS using pNF-H as a surrogate marker rather than subjective cognitive test batteries.

Table 3. Results of neuropsychological tests among groups sorted by pNF-H level

	pNF-H-positive (N = 18)	pNF-H-negative (N = 56)	P
CFQ ^a (mean, SD)	18.8 ± 11.2	21.1 ± 10.6	0.77
HADS ^b			
Anxiety subscale (mean, SD)	4.3 ± 2.8	4.9 ± 2.8	0.35
Depression subscale (mean, SD)	4.5 ± 3.5	3.9 ± 3.3	0.83
STAI ^b			
State anxiety (mean, SD)	40.1 ± 11.2	42.8 ± 10.9	0.79
Trait anxiety (mean, SD)	40.2 ± 11.3	43.1 ± 11.2	0.41
ESS ^b (mean, SD)	8.5 ± 4.2	9.5 ± 4.1	0.26
BFI-J ^b (mean, SD)	18.1 ± 14.4	19.1 ± 14.9	0.74
NVS ^a (mean, SD)	4.8 ± 1.2	4.5 ± 1.1	0.18
JART ^a			
Full-scale IQ (mean, SD)	110.4 ± 9.3	113.4 ± 9.3	0.79
Verbal IQ (mean, SD)	112.3 ± 10.6	115.2 ± 10.6	0.79
Performance IQ (mean, SD)	107.0 ± 6.8	110.1 ± 6.8	0.79
RCPM ^a (mean, SD)	35.9 ± 0.7	37.0 ± 0.7	0.37
PainDETECT ^b (mean, SD)	4.2 ± 4.9	4.1 ± 4.7	0.26
EQ5D ^b (mean, SD)	0.8 ± 0.1	0.8 ± 0.1	0.87

Abbreviations: CFQ, cognitive failure questionnaire; EQ5D, EuroQOL-5 Dimension questionnaire; HADS, Hospital Anxiety and Depression Scale; IQ, intelligence quotient; pNF-H, phosphorylated form of the high-molecular-weight neurofilament subunit.

^aThe score would decrease when the function declines.

^bThe score would increase when the function declines.

Conclusions

The serum pNF-H level in patients with breast cancer treated with chemotherapy increased in a cumulative dose-dependent manner. Axonal damage in the CNS can be cumulatively caused by chemotherapy, which might eventually lead to CICI. The present findings suggest a potential application of pNF-H as a biomarker of CNS damage after chemotherapy. However, the self-administered neuropsychological tests used in this study did not demonstrate significant cognitive impairment. A prospective cohort study is needed to validate the usefulness of pNF-H for assessment of CICI.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: A. Natori, M. Sumitani, T. Yamauchi, H. Yamauchi
Development of methodology: A. Natori, T. Ogata, T. Kogure, T. Yamauchi, H. Yamauchi

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Natori, T. Ogata, T. Yamauchi, H. Yamauchi

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Natori, M. Sumitani, T. Kogure, T. Yamauchi, H. Yamauchi

Writing, review, and/or revision of the manuscript: A. Natori, T. Kogure, T. Yamauchi, H. Yamauchi

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Natori, T. Yamauchi, H. Yamauchi
Study supervision: H. Yamauchi

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References

1. Jim HSL, Phillips KM, Chait S, Faul LA, Popa MA, Lee Y-H, et al. Meta-analysis of cognitive functioning in breast cancer survivors previously treated with standard-dose chemotherapy. *J Clin Oncol* 2012;30:3578–87.
2. O'Farrell E, MacKenzie J, Collins B. Clearing the air: a review of our current understanding of "chemo fog." *Curr Oncol Rep* 2013;15:260–9.
3. Collins B, MacKenzie J, Stewart A, Bielajew C, Verma S. Cognitive effects of chemotherapy in post-menopausal breast cancer patients 1 year after treatment. *Psycho Oncol* 2009;18:134–43.
4. Wefel JS, Witgert ME, Meyers CA. Neuropsychological sequelae of non-central nervous system cancer and cancer therapy. *Neuropsychol Rev* 2008;18:121–31.
5. Correa DD, Ahles TA. Neurocognitive changes in cancer survivors. *Cancer J* 2008;14:396–400.
6. Collins B, MacKenzie J, Tasca GA, Scherling C, Smith A. Cognitive effects of chemotherapy in breast cancer patients: a dose-response study. *Psychooncology* 2013;22:1517–27.
7. Ahles TA, Saykin AJ. Candidate mechanisms for chemotherapy-induced cognitive changes. *Nat Rev Cancer* 2007;7:192–201.
8. Wefel JS, Vardy J, Ahles T, Schagen SB. International Cognition and Cancer Task Force recommendations to harmonise studies of cognitive function in patients with cancer. *Lancet Oncol* 2011;12:703–8.
9. Silverman DH, Dy CJ, Castellon SA, Lai J, Pio BS, Abraham L, et al. Altered frontocortical, cerebellar, and basal ganglia activity in adjuvant-treated breast cancer survivors 5–10 years after chemotherapy. *Breast Cancer Res Treat* 2007;103:303–11.
10. Deprez S, Amant F, Yigit R, Porke K, Verhoeven J, Van den Stock J, et al. Chemotherapy-induced structural changes in cerebral white matter and its correlation with impaired cognitive functioning in breast cancer patients. *Hum Brain Mapp* 2011;32:480–93.
11. Deprez S, Amant F, Smeets A, Peeters R, Leemans A, Van Hecke W, et al. Longitudinal assessment of chemotherapy-induced structural changes in cerebral white matter and its correlation with impaired cognitive functioning. *J Clin Oncol* 2012;30:274–81.
12. Inagaki M, Yoshikawa E, Matsuoka Y, Sugawara Y, Nakano T, Akechi T, et al. Smaller regional volumes of brain gray and white matter demonstrated in breast cancer survivors exposed to adjuvant chemotherapy. *Cancer* 2007;109:146–56.
13. Loy DN, Sroufe AE, Pelt JL, Burke DA, Cao QL, Talbott JF, et al. Serum biomarkers for experimental acute spinal cord injury: rapid elevation of neuron-specific enolase and S-100beta. *Neurosurgery* 2005;56:391–7; discussion 7.
14. Cao F, Yang XF, Liu WG, Hu WW, Li G, Zheng XJ, et al. Elevation of neuron-specific enolase and S-100beta protein level in experimental acute spinal cord injury. *J Clin Neurosci* 2008;15:541–4.
15. Pouw MH, Hosman AJ, van Middendorp JJ, Verbeek MM, Vos PE, van de Meent H. Biomarkers in spinal cord injury. *Spinal Cord* 2009;47:519–25.
16. Kwon BK, Stammers AM, Belanger LM, Bernardo A, Chan D, Bishop CM, et al. Cerebrospinal fluid inflammatory cytokines and biomarkers of injury severity in acute human spinal cord injury. *J Neurotrauma* 2010;27:669–82.
17. Shaw G, Yang C, Ellis R, Anderson K, Parker Mickle J, Scheff S, et al. Hyperphosphorylated neurofilament NF-H is a serum biomarker of axonal injury. *Biochem Biophys Res Commun* 2005;336:1268–77.
18. Hayakawa K, Okazaki R, Ishii K, Ueno T, Izawa N, Tanaka Y, et al. Phosphorylated neurofilament subunit NF-H as a biomarker for evaluating the severity of spinal cord injury patients, a pilot study. *Spinal Cord* 2012;50:493–6.
19. Ueno T, Ohori Y, Ito J, Hoshikawa S, Yamamoto S, Nakamura K, et al. Hyperphosphorylated neurofilament NF-H as a biomarker of the efficacy of minocycline therapy for spinal cord injury. *Spinal Cord* 2011;49:333–6.
20. Gresle MM, Liu Y, Dagley LF, Haartsen J, Pearson F, Purcell AW, et al. Serum phosphorylated neurofilament-heavy chain levels in multiple sclerosis patients. *J Neurol Neurosurg Psychiatry* 2014;85:1209–13.
21. Matsushige T, Inoue H, Fukunaga S, Hasegawa S, Okuda M, Ichijima T. Serum neurofilament concentrations in children with prolonged febrile seizures. *J Neurol Sci* 2012;321:39–42.
22. Douglas-Escobar M, Weiss MD. Biomarkers of hypoxic-ischemic encephalopathy in newborns. *Front Neurol* 2012;3:144.
23. EuroQol Group. EuroQol—a new facility for the measurement of health-related quality of life. *Health Policy* 1990;16:199–208.
24. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand* 1983;67:361–70.
25. Spielberger CD, Gorsuch RL, Lushene R, Vagg PR, Jacobs GA. Manual for the state-trait anxiety inventory. Palo Alto, CA: Consulting Psychologists Press; 1983.
26. Freynhagen R, Baron R, Gockel U, Tolle TR. painDETECT: a new screening questionnaire to identify neuropathic components in patients with back pain. *Curr Med Res Opin* 2006;22:1911–20.
27. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep* 1991;14:540–5.
28. Raven JC. Colored progressive matrices sets A, Ab, B. Oxford: Oxford Psychologists Press Ltd.; 1947, 1995.
29. Broadbent DE, Cooper PF, FitzGerald P, Parkes KR. The cognitive failures questionnaire (CFQ) and its correlates. *Br J Clin Psychol* 1982;21:1–16.
30. Mendoza TR, Wang XS, Cleeland CS, Morrissey M, Johnson BA, Wendt JJK, et al. The rapid assessment of fatigue severity in cancer patients: use of the brief fatigue inventory. *Cancer* 1999;85:1186–96.
31. Okuyama T, Wang XS, Akechi T, Mendoza TR, Hosaka T, Cleeland CS, et al. Validation study of the Japanese version of the brief fatigue inventory. *J Pain Symptom Manage* 2003;25:106–17.
32. Powers BJ, Trinh JV, Bosworth HB. Can this patient read and understand written health information? *JAMA* 2010;304:76–84.
33. Matsuoka K, Uno M, Kasai K, Koyama K, Kim Y. Estimation of premorbid IQ in individuals with Alzheimer's disease using Japanese ideographic script (Kanji) compound words: Japanese version of national adult reading test. *Psychiatry Clin Neurosci* 2006;60:332–9.
34. Cavaletti G, Cornblath DR, Merkies ISJ, Postma TJ, Rossi E, Frigeni B, et al. The chemotherapy-induced peripheral neuropathy outcome measures standardization study: from consensus to the first validity and reliability findings. *Ann Oncol* 2013;24:454–62.
35. Stubblefield MD, Burstein HJ, Burton AW, Custodio CM, Deng GE, Ho M, et al. NCCN task force report: management of neuropathy in cancer. *J Natl Compr Canc Netw* 2009;7:S1–S26.
36. Jamieson SM, Subramaniam J, Liu JJ, Jong NN, Ip V, Connor B, et al. Oxaliplatin-induced loss of phosphorylated heavy neurofilament subunit neuronal immunoreactivity in rat DRG tissue. *Mol Pain* 2009;5:66.
37. McDonald BC, Conroy SK, Ahles TA, West JD, Saykin AJ. Gray matter reduction associated with systemic chemotherapy for breast cancer: a prospective MRI study. *Breast Cancer Res Treat* 2010;123:819–28.
38. Collins B, MacKenzie J, Stewart A, Bielajew C, Verma S. Cognitive effects of hormonal therapy in early stage breast cancer patients: a prospective study. *Psychooncology* 2009;18:811–21.

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Potential Role of pNF-H, a Biomarker of Axonal Damage in the Central Nervous System, as a Predictive Marker of Chemotherapy-Induced Cognitive Impairment

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ORIGINAL ARTICLE

Mechanical and neural changes in plantar-flexor muscles after spinal cord injury in humans

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Study design: Cross-sectional study.

Objectives: To determine the effect of injury duration on plantar-flexor elastic properties in individuals with chronic spinal cord injury (SCI) and spasticity.

Setting: National Rehabilitation Center for Persons with Disabilities, Japan.

Methods: A total of 16 chronic SCI patients (age, 33 ± 9.3 years; injury localization, C6-T12; injury duration, 11–371 months) participated. Spasticity of the ankle plantar-flexors was assessed using the Modified Ashworth Scale (MAS). The calf circumference and muscle thickness of the medial gastrocnemius (MG), lateral gastrocnemius and soleus were assessed using tape measure and ultrasonography. In addition, the ankle was rotated from 10° plantar-flexion to 20° dorsiflexion at 5 deg s^{-1} with a dynamometer, and the ankle angle and torque were recorded. After normalizing the data (the initial points of angle and torque were set to zero), we calculated the peak torque and energy. Furthermore, angle–torque data (before and after normalization) were fitted with a second- and fourth-order polynomial, and exponential (Sten–Knudsen) models, and stiffness indices (SI_{SOP} , SI_{FOP} , SI_{SK}) and $Angle_{SLACK}$ (the angle at which plantar-flexor passive torque equals zero) were calculated. The stretch reflex gain and offset were determined from 0 – 10° dorsiflexion at 50, 90, 120 and 150 deg s^{-1} . After logarithmic transformation, Pearson's correlation coefficients were calculated.

Results: MAS, calf circumference, MG thickness, peak torque and SI_{FOP} significantly decreased with injury duration (r log-log = -0.63 , -0.69 , -0.63 , -0.53 and -0.55 , respectively, $P < 0.05$). The peak torque and SI_{FOP} maintained significant relationships even after excluding impacts from muscle morphology.

Conclusion: Plantar-flexor elasticity in chronic SCI patients decreased with increased injury duration.

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INTRODUCTION

Motor paralysis following spinal cord injury (SCI) inevitably prevents daily activities such as upright standing and bipedal walking; consequently, physical activity declines in affected patients. Previous studies have indicated that SCI patients experience extreme muscle atrophy,^{1–3} fiber transformation towards a fast-fatigable type^{4–6} and decreased bone mineral density.^{7,8} This musculoskeletal degeneration is attributed largely to the dramatically decreased muscle activity and mechanical stress in the paralyzed limbs, caused primarily by post-SCI motor paralysis.

Quantitative evaluation of the muscle viscoelasticity and tone is important in preventing secondary disorders after SCI. The Modified Ashworth Scale (MAS) is generally used to measure muscle tone and spasticity,⁹ but this score lacks adequate sensitivity to distinguish the contributions from the mechanical and neural components.¹⁰ In a study of the neural profile after SCI, we reported that spinal reflex excitability in participants experiencing both complete and incomplete SCI are significantly higher than the excitability in healthy young participants, but the H_{max}/M_{max} did not change significantly in incomplete SCI participants.¹¹ In addition, the change in $H_{max}/$

M_{max} was not associated with the chronicity of injury in SCI participants. 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 patients. To date, the relationship between the mechanical properties and the duration of injury has not been reported. This information could guide clinicians in determining the appropriate intervention for the patient's pathologic condition.

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

PATIENTS AND METHODS

Participants

Sixteen 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 6 incomplete), five 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 healthy control participants (age, 27 ± 5 years; height, 169 ± 10 cm; weight, 62 ± 10 kg; 10 male and 3 female) participated in this study. The population demographics

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Table 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 2 A: partial correlation coefficient between mechanical properties and injury duration (months), excluding muscle architecture and injury level. B: associated P-values

	Calf circumference (mm)	MG (mm)	LG (mm)	Sol (mm)	Level of injury
A					
Peak torque (Nm)	-0.70	-0.63	-0.80	-0.72	-0.79
Energy (Nm per deg)	-0.70	-0.33	-0.72	-0.58	-0.67
SI _{SOP} (Nm per deg)	-0.69	-0.57	-0.78	-0.69	-0.77
SI _{FOP} (Nm per deg)	-0.70	-0.63	-0.80	-0.72	-0.79
SI _{SK} (Nm per deg)	-0.45	-0.25	-0.62	-0.57	-0.65
Angle _{SLACK} (Nm per deg)	0.58	0.42	0.71	0.63	0.72
B					
Peak torque (Nm)	0.017	0.039	0.003	0.019	0.004
Energy (Nm per deg)	0.017	0.319	0.012	0.077	0.024
SI _{SOP} (Nm per deg)	0.019	0.070	0.005	0.027	0.006
SI _{FOP} (Nm per deg)	0.016	0.038	0.003	0.018	0.004
SI _{SK} (Nm per deg)	0.163	0.461	0.042	0.087	0.032
Angle _{SLACK} (Nm per deg)	0.061	0.201	0.014	0.052	0.013

Abbreviations: LG, lateral gastrocnemius; MG, medial gastrocnemius; Sol, soleus.

are summarized in Table 1. There was no statistical age difference among the three groups (SCI participants with spasticity, SCI participants without spasticity and healthy participants; F -value = 2.075, $P = 0.1422$). Each participant provided written informed consent for the experimental procedure, which was approved by the National Rehabilitation Center for Persons with Disabilities (NRCDD).

Measurements

The participants were seated with their hip and knee joints angled at 90°. The ankle joint was fixed onto the foot plate of a custom dynamometer.¹² The calf circumference and muscle thickness of the plantar-flexors were measured at 30% of the calf length using a tape measure and ultrasonography (Prosound 2, Aloka, Tokyo, Japan) with a linear array probe (7.5 MHz). The calf circumference, medial gastrocnemius (Gas (MG)) and lateral Gas thicknesses of participants 5, 6 and 8, and four healthy control participants, as well as the

soleus (Sol) thickness of participants 1, 5, 6 and 8, and five healthy control participants, were not obtained. We have stated that 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 degs⁻¹. During the measurement, 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, Bella Vista, NSW, Australia). The muscle activity of the Gas, Sol and tibialis anterior were also measured using surface electromyograms with a bipolar electrode (DE-2.3, Delsys, Boston, MA, USA) spaced 1 cm apart. The electromyograms signal was amplified (The Bagnoli-8 EMG System, Delsys) with band-pass filtering between 20 Hz and 450 Hz. Some participants (#1, 2, 3, 4 and 5 in Table 2) had clonus. In those participants, therefore, we 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 temporarily stopped. The trial was repeated 6–10 times,

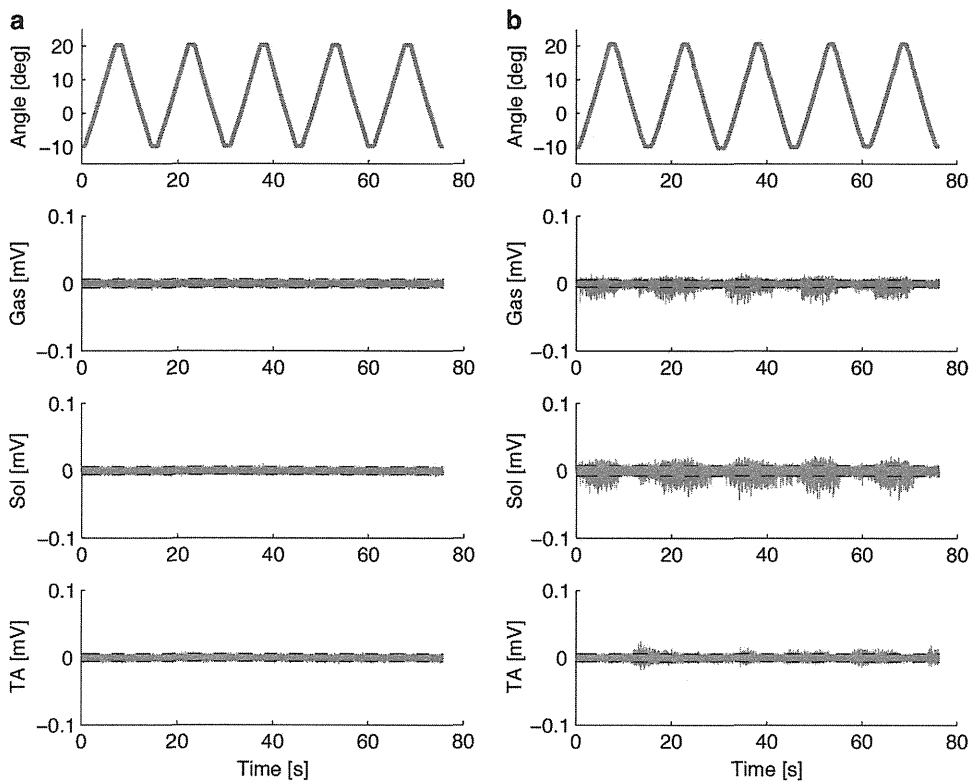


Figure 1 (a) Representative angle and electromyograms (EMG; Gas, Sol, TA) waveform during passive motion test. Broken lines indicate ± 3 s.d. of baseline. (b) Angle and EMG waveform of an excluded participant (participant 3 in Table 1).

and five trials without muscle activity of the lower limb were selected and analyzed further. One participant (Table 1, participant 3) showed remarkable muscle activity exceeding ± 3 s.d. of baseline (Figure 1b), therefore the data from this participant was excluded.

The passive motion test was 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^{-1}) were applied five times in each participant in a 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.

Analysis

Angle and torque data were averaged in five trials and digitally low-pass filtered using a fourth-order zero-lag Butterworth filter¹³ with a 10-Hz cut-off frequency.^{14–16} Three different mathematical models were fitted to the angle (θ)–torque (τ) data as previously described:^{16–27}

(i) Second-order polynomial (SOP) model (Equation 1)

$$\tau(\theta) = a\theta^2, \quad (1)$$

where a is experimental constant;

(ii) Fourth-order polynomial (FOP) model (Equation 2)

$$\tau(\theta) = m\theta^4 + n\theta^3 + o\theta^2 + p\theta + q, \quad (2)$$

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

(iii) Exponential model similar to the Sten–Knudsen (SK) model (Equation (3))

$$\tau(\theta) = \frac{1}{\alpha} e^{\alpha(\theta - \theta_{\text{slack}})}, \quad (3)$$

where α and θ_{slack} are experimental constants.

Prior to fitting Equation 1, the angle (θ) and torque (τ) data reference points were set to zero. The plantar-flexor, peak torque (torque waveform peak), and

energy (the area between the angle–torque curve and the line torque equaling zero) were calculated from the normalized angle–torque data. In Equations 2 and 3, the raw angle–torque data were used. Equation 3 fitting parameters were determined by the non-linear least square method (Levenberg-Marquardt algorithm²⁸) using the optimization toolbox from MATLAB (The Mathworks, Natick, MA, USA). A total of 1200¹⁶ data points were used in each fitting. The stiffness indices (SI_{SOP} , SI_{FOP} , SI_{SK}) and $Angle_{\text{SLACK}}$ were determined from these equations as follows:

$$SI_{\text{SOP}} = 2a \quad (4)$$

$$SI_{\text{FOP}} = \text{mean}_{-10 \leq \theta \leq 20} (4m\theta^3 + 3n\theta^2 + 2o\theta + p) \quad (5)$$

$$SI_{\text{SK}} = \alpha \quad (6)$$

$$Angle_{\text{SLACK}} = \theta_{\text{slack}} \quad (7)$$

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. 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 of the regression line in the plotted stretch response to angular velocity.

Statistics

One-way analysis of variance was used to compare the determination coefficients (R^2) between the three models, and *post hoc* analysis was performed using Tukey’s HSD test. All variables were logarithmically transformed²⁹ except the Gas and Sol offsets, which have negative magnitudes. Then, 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. In addition, the relationships between all variables and injury localization were

determined using the same procedure. Before logarithmic transformation, MAS values were corrected by designating the minimum data value as one. The partial correlation coefficient, excluding morphological parameters, was 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 $P < 0.05$.

RESULTS

Representative angle–torque data (raw and normalized) and fitting data using the three models are illustrated in Figure 2. In both

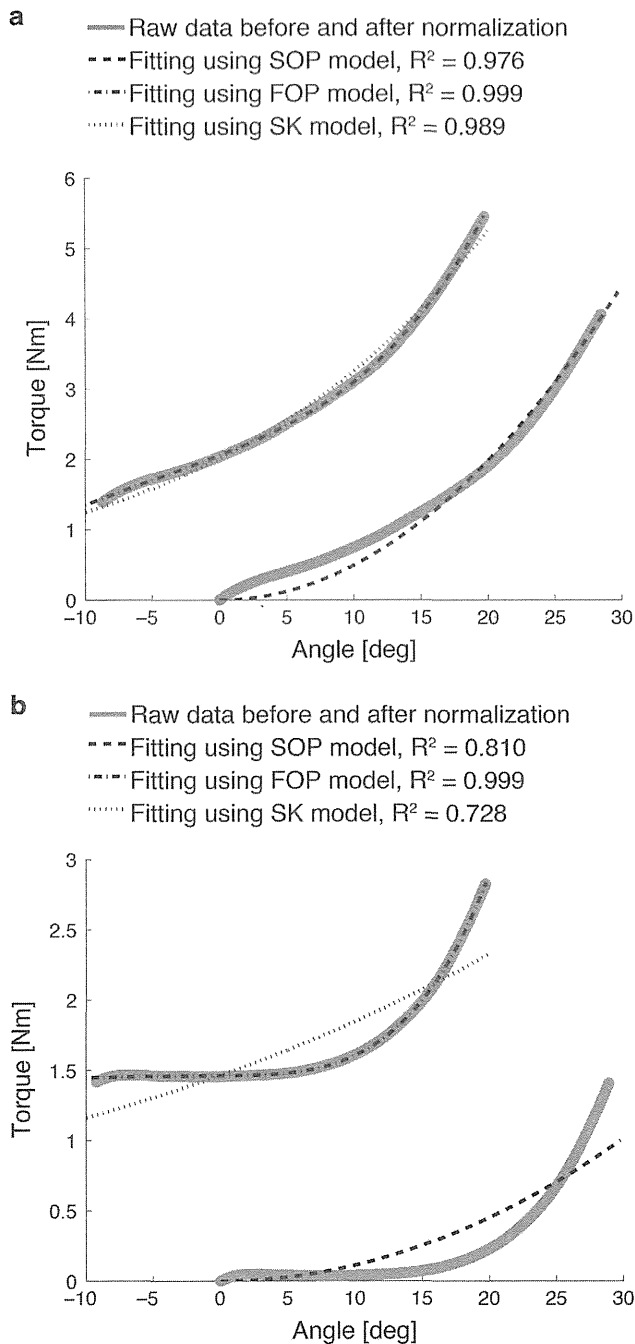


Figure 2 Representative angle–torque data fitting using three mathematical models (SOP, FOP and SK). (a) The fitting was good in all models, which were fitted to angle–torque data from participant 12 in Table 1. (b) The SOP and SK model fittings were inferior compared with the FOP model, based on angle–torque data from participant 13 in Table 1.

examples (Figure 2a: participant 12 in Table 1; Figure 2b: participant 13 in Table 1), the FOP model fits the angle–torque data well. However, data fitting using the SOP and SK models is poor in some participants (Figure 2b: participant 13 in Table 1). Similarly, the collective determination coefficient data fitting using the FOP model was very good ($R^2 = 0.999 \pm 0.001$) and significantly higher than in both the SOP ($R^2 = 0.869 \pm 0.135$) and SK (0.904 ± 0.130) models in all the participants ($n = 33$). This trend also occurred and was significant in SCI participants with spasticity (FOP model: $R^2 = 0.999 \pm 0.001$; SOP model: $R^2 = 0.850 \pm 0.120$; SK model: $R^2 = 0.883 \pm 0.114$, $n = 15$). MAS negatively correlated with the injury duration in the SCI participants with spasticity (Figure 3). In addition, the calf circumference and MG thickness were negatively correlated to the injury duration ($n = 13$ excluding the missing data described previously; Figure 3). 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.

Angle–torque representative curves of all participants (excluding participant 3) are shown in Figure 4a. After the polynomial and exponential regression, the SI_{FOP} , SI_{SK} and $Angle_{SLACK}$ were calculated, and Figure 4b was generated by designating the angle and torque reference points in Figure 4a as zero. Using the data in Figure 4a, we calculated the peak torque, energy and SI_{SOP} , and found that the peak torque and SI_{FOP} (stiffness index calculated from the fourth-order polynomial regression) were inversely correlated with the injury duration ($n = 15$).

Muscle morphology theoretically affects passive tension in the muscles and tendons. The effects of these morphologic variables (calf circumference and MG, lateral Gas, and Sol thicknesses) were excluded by 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 2). The peak torque and SI_{FOP} were negatively correlated, even after excluding the effects of the calf circumference and MG thickness, which significantly decreased with the injury duration. In addition, we confirmed that the injury severity was not correlated with these results (Figure 5).

DISCUSSION

We 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. In addition, there were significant partial correlation coefficients between the mechanical parameters and the injury duration excluding morphologic parameters.

Alteration of the ankle joint stiffness after SCI

As clearly shown in Figure 3, 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.*² measured the cross-sectional area of the lower musculature (Gas, Sol and tibialis anterior) during the 6 months following spinal injury and showed that the CSA of Gas decreased significantly. The present result showing a selectively decreased MG thickness is consistent with previous findings.² Notably, involuntary muscle activity resulting from clonus and spasm with spasticity might contribute to attenuate atrophy,³⁰ therefore the decreasing spasticity observed in this study, coupled with the immobilization, likely accelerated the muscle atrophy.

The Ashworth scale³¹ remains a major clinical scale for evaluating spasticity,^{32–34} but its validity and reliability are questioned by some

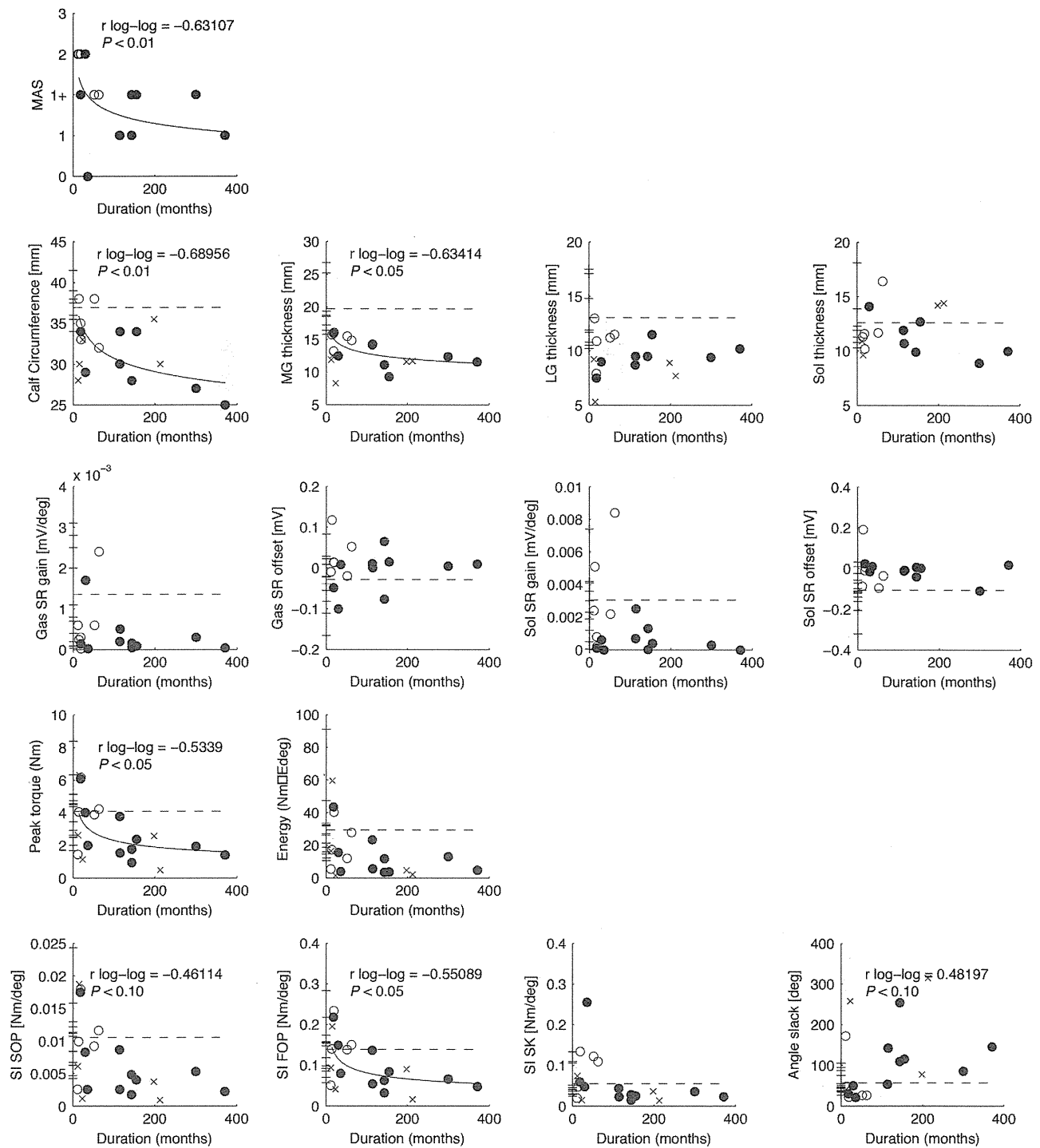


Figure 3 Relationship between mechanical variables and injury duration (months). Complete SCI participants with spasticity (filled circles), incomplete SCI participants with spasticity (open circles), complete SCI participants without spasticity (x), and healthy participants (horizontal bar) are shown. The horizontal dashed line indicates the mean value in healthy participants.

reports.^{35,36} A positive correlation has been reported between the Ashworth scale and indices of clonus and spasm,³⁷ therefore the negative correlation between MAS and the injury duration observed in this study suggest that the frequency of involuntary muscle contraction because of spasm gradually decreases over time after SCI. In evaluating the extent of spasticity over time, we found no statistically significant correlation between the stretch reflex gain and offset in the lower

muscles (MG, Sol) and injury duration. This result is consistent with our previous study¹¹ that found that the stretch reflex peak-to-peak amplitude and stretch reflex peak-to-peak amplitude/Mmax in complete and incomplete SCI participants were not correlated to the time post-injury, suggesting that spinal circuit excitability in SCI patients does not change during the chronic stage. However, several reports showed that Mmax was decreased more in the chronic SCI

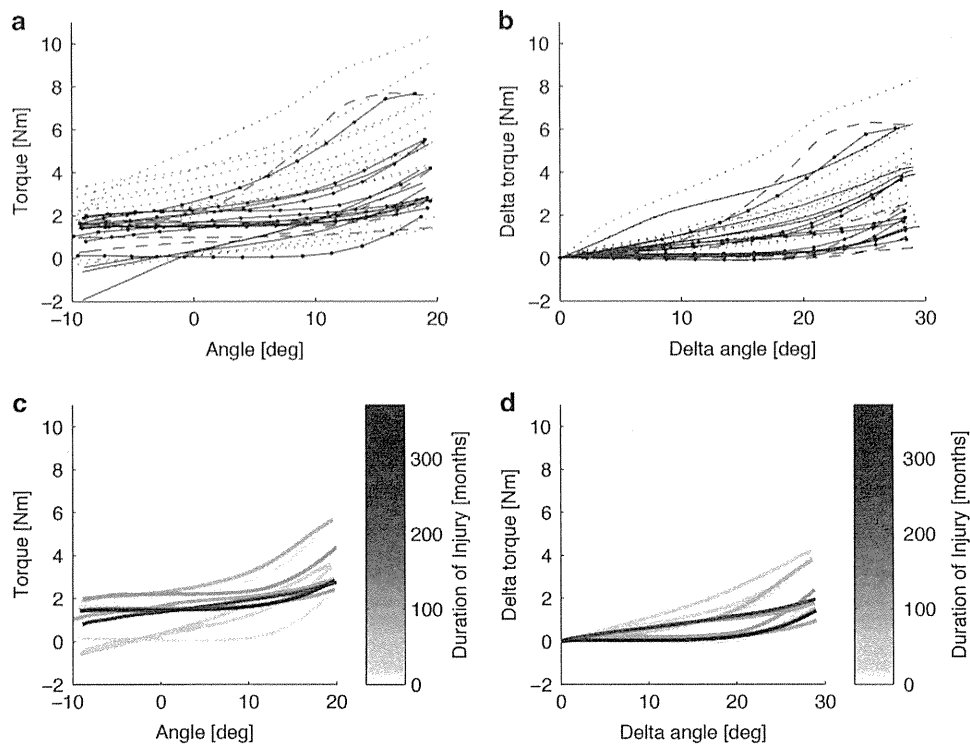


Figure 4 (a) Angle–torque curves of plantar-flexor muscles for all complete SCI participants with spasticity (chain), incomplete SCI participants with spasticity (solid), complete SCI participants without spasticity (dashed) and healthy control participants (dotted), excluding participant 3. (b) The angle and torque reference points in a were set at zero. (c) Angle–torque curve for SCI participants with spasticity (both complete and incomplete) from a, with grayscale intensities of lines indicating injury duration (months). (d) The angle and torque reference points in c were set at zero.

participants than in the age-matched healthy control participants,^{38–44} presumably because of the changes in proprioceptor function or muscle atrophy. In these studies, the time-dependent decline in Mmax may be caused by muscle atrophy, but because we did not measure Mmax in our study, we cannot conclude that the spinal reflex excitability in chronic SCI is maintained. Further research is needed to clarify this relationship.

Characteristics of the mechanical properties of the paralyzed ankle joint in SCI

Previous research¹⁶ indicates that the mathematical models used to calculate the mechanical parameters greatly impact the experimental result. Essentially, the change in a mechanical parameter (for example, stiffness) varied depending on the selected mathematical model applied to the angle–torque relation; thus, we 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. The determination coefficients for the angle–torque data fitted with the FOP model were significantly higher than the coefficients based on the alternative models (SOP, SK), therefore the result (SI_{FOP}) calculated using the FOP model was deemed to be the most 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 observed in some participants and the insufficient sample size; however, the underlying mechanism is unclear.

In addition, work was not significantly correlated with injury duration, which is consistent with a prior study asserting that work is not a spasticity indicator.⁴⁵ 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 the chronic SCI patients was higher than observed in the healthy age-matched participants.^{24,26,46} We speculate that plantar-flexor elasticity in SCI patients increases with immobilization during the acute stage of injury (<1 year), and thereafter, elasticity gradually decreases over time due in part to the muscle atrophy observed in this study. In theory, muscle morphology influences passive tension. Thus, we calculated the partial correlation coefficient between mechanical parameters and injury duration to exclude effects from morphological parameters (Table 2); significant relationships persisted, therefore we concluded that tissue elasticity in the plantar-flexor was involved in the mechanical parameter changes.

We cannot determine whether the muscle or tendon caused the plantar-flexor elasticity change. Theoretically, both muscle and tendon arranged in series can affect the total tissue stiffness,⁴⁷ a relationship that has been experimentally confirmed in the plantar-flexors of healthy participants.¹⁷ In contrast, Diong *et al.*²⁴ observed that the Gas muscle stiffness is increased in SCI participants, whereas Olson *et al.*⁶ found that the muscle changes occur at the cell level and reflect muscle fiber transformation from Type I to Type II. In addition, Maganaris⁴⁸ showed that patellar tendon CSA, stiffness, and Young's modulus decreased significantly in chronic (18–288 months after injury) complete SCI patients. Overall, these studies suggest that the tendon rather than the muscle has a primary role in the decreasing plantar-flexor elasticity. Further research is needed to clarify the different changes in muscle and tendon function over time following a SCI.

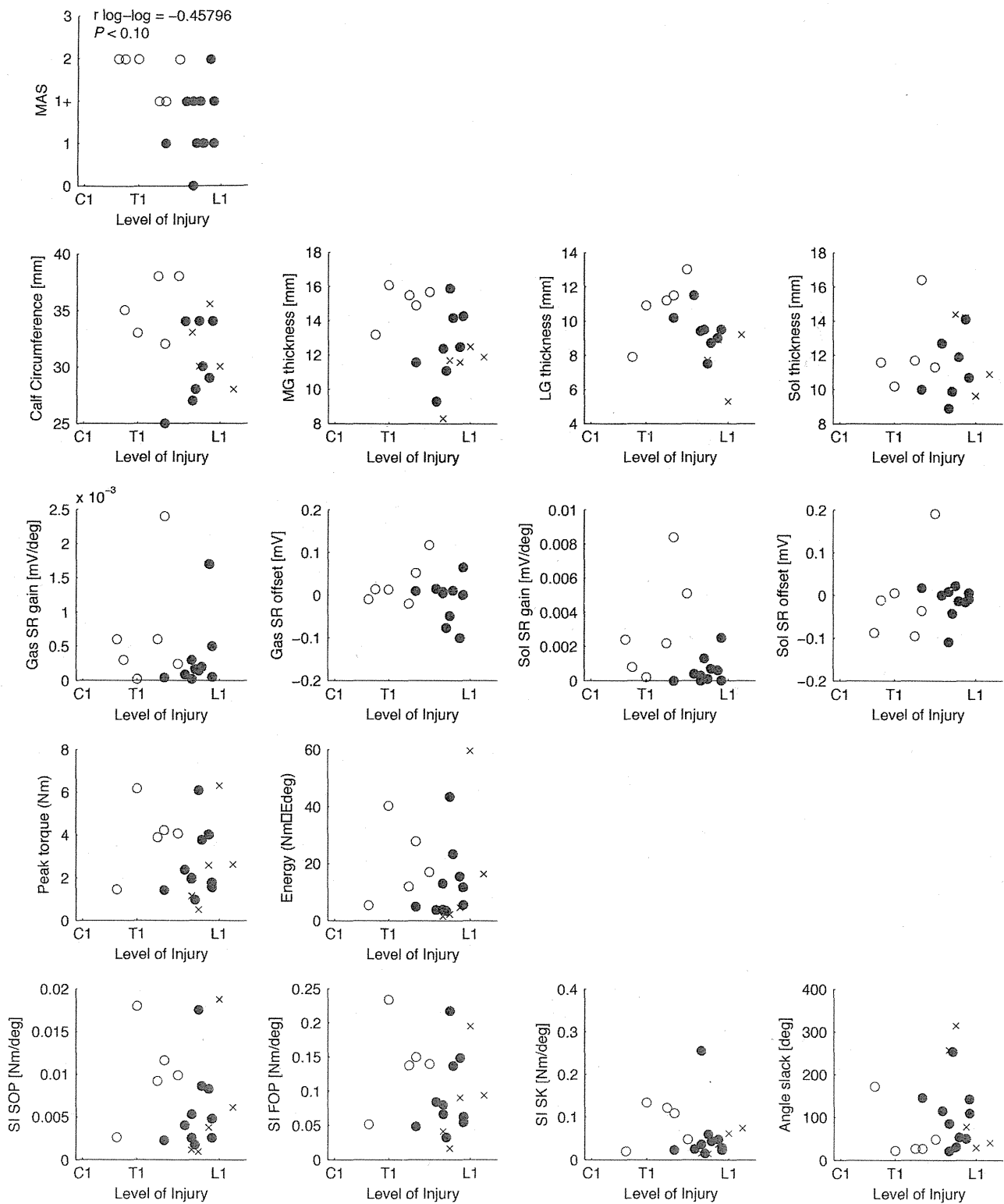


Figure 5 Relationships between mechanical parameters and injury location. Complete SCI participants with spasticity (filled circles), incomplete SCI participants with spasticity (open circles), complete SCI participants without spasticity (x).

Limitations

We 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, we visually confirmed that all

participants achieved a 20° dorsiflexion while keeping the heel in contact with the dynamometer footplate during the passive motion test. In addition, we confirmed that muscle activity in the calf muscles (MG, Sol) and antagonist (tibialis anterior) were low (within the

baseline mean \pm 3 s.d.) during the passive motion test (representative participant in Figure 2a). Thus, the influence of articular alternation and muscle activity on the plantar-flexor torque was minimal, and the passive motion test was conducted appropriately.

In conclusion, the degree of spasticity (MAS), morphologic (circumference and MG muscle thickness) and mechanical (muscle-tendon elasticity) properties of the plantar-flexors in chronic SCI patients with spasticity decreased with the injury duration. Therapeutic intervention, such as FES or BWST, may be needed to prevent these sequelae in chronic SCI patients.

DATA ARCHIVING

There were no data to deposit.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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- Lotta S, Scelsi R, Alfonsi E, Saitta A, Nicolotti D, Epifani P *et al*. Morphometric and neurophysiological analysis of skeletal muscle in paraplegic patients with traumatic cord lesion. *Paraplegia* 1991; **29**: 247–252.
- Castro MJ, Apple DF Jr, Hillegass EA, Dudley GA. Influence of complete spinal cord injury on skeletal muscle cross-sectional area within the first 6 months of injury. *Eur J Appl Physiol Occup Physiol* 1999; **80**: 373–378.
- Castro MJ, Apple DF, Staron RS, Campos GE, Dudley GA. Influence of complete spinal cord injury on skeletal muscle within 6 mo of injury. *J Appl Physiol* 1999; **86**: 350–358.
- Grimby G, Broberg C, Krotkiewska I, Krotkiewski M. Muscle fiber composition in patients with traumatic cord lesion. *Scand J Rehabil Med* 1976; **8**: 37–42.
- Martin TP, Stein RB, Hoepfner PH, Parthomore JD, Defos LJ. Influence of electrical stimulation on the morphological and metabolic properties of paralyzed muscle. *J Appl Physiol* 1992; **72**: 1401–1406.
- Olsson MC, Krüger M, Meyer LH, Ahnlund L, Gransberg L, Linke WA *et al*. Fibre type-specific increase in passive muscle tension in spinal cord-injured subjects with spasticity. *J Physiol* 2006; **577**: 339–352.
- Frey-Rindova P, de Bruin ED, Stüssi E, Dambacher MA, Dietz V. Bone mineral density in upper and lower extremities during 12 months after spinal cord injury measured by peripheral quantitative computed tomography. *Spinal Cord* 2000; **38**: 26–32.
- Szollar SM, Martin EM, Sartoris DJ, Parthomore JG, Defos LJ. Bone mineral density and indexes of bone metabolism in spinal cord injury. *Am J Phys Med Rehabil* 1998; **77**: 28–35.
- Biering-Sørensen F, Nielsen JB, Klinge K. Spasticity-assessment: a review. *Spinal Cord* 2006; **44**: 708–722.
- Grippo A, Carrai R, Hawamdeh Z, Falsini C, Aito S, Pinto F *et al*. Biomechanical and electromyographic assessment of spastic hypertonus in motor complete traumatic spinal cord-injured individuals. *Spinal Cord* 2011; **49**: 142–148.
- Nakazawa K, Kawashima N, Akai M. Enhanced stretch reflex excitability of the soleus muscle in persons with incomplete rather than complete chronic spinal cord injury. *Arch Phys Med Rehabil* 2006; **87**: 71–75.
- Kawashima N, Suzuki R, Nakazawa K, Ohta Y. Novel home-based rehabilitation device to prevent secondary diseases for patients with spinal cord injury. *2009 IEEE Int Conf Rehabil Robot* 2009; 349–353.
- Winter DA. *Biomechanics and Motor Control of Human Movement*. John Wiley & Sons, 1990.
- Fouré A, Nordez A, Cornu C. Effects of plyometric training on passive stiffness of gastrocnemii muscles and Achilles tendon. *Eur J Appl Physiol* 2012; **112**: 2849–2857.
- Fouré A, Nordez A, Cornu C. Effects of eccentric training on mechanical properties of the plantar flexor muscle-tendon complex. *J Appl Physiol* 2013; **114**: 523–537.
- Nordez A, Cornu C, McNair PJ. Acute effects of static stretching on passive stiffness of the hamstring muscles calculated using different mathematical models. *Clin Biomech* 2006; **21**: 755–760.
- Kawakami Y, Kanehisa H, Fukunaga T. The relationship between passive ankle plantar flexion joint torque and gastrocnemius muscle and achilles tendon stiffness: implications for flexibility. *J Orthop* 2008; **38**: 269–276.
- Sten-Knudsen O. Torsional elasticity of the isolated cross striated muscle fibre. *Acta Physiol Scand* 1953; **28**: 1–240.
- Nordez A, Fouré A, Dombroski EW, Mariot JP, Cornu C, McNair PJ *et al*. Improvements to Hoang *et al.*'s method for measuring passive length-tension properties of human gastrocnemius muscle *in vivo*. *J Biomech* 2010; **43**: 379–382.
- Nordez A, Casari P, McNair PJ, Cornu C. Characterization and modelling of the musculoarticular complex mechanical behavior in passive conditions. Effects of cyclic and static stretching. *IRBM* 2009; **30**: 20–29.
- Diong J, Harvey LA, Kwah LK, Clarke JL, Bilston LE, Gandevia SC *et al*. Gastrocnemius muscle contracture after spinal cord injury: a longitudinal study. *Am J Phys Med Rehabil* 2012; **92**: 1–10.
- Hoang PD, Gorman RB, Todd G, Gandevia SC, Herbert RD. A new method for measuring passive length-tension properties of human gastrocnemius muscle *in vivo*. *J Biomech* 2005; **38**: 1333–1341.
- Hoang PD, Herbert RD, Todd G, Gorman RB, Gandevia SC. Passive mechanical properties of human gastrocnemius muscle tendon units, muscle fascicles and tendons *in vivo*. *J Exp Biol* 2007; **210**: 4159–4168.
- Diong JH, Herbert RD, Harvey LA, Kwah LK, Clarke JL, Hoang PD *et al*. Passive mechanical properties of the gastrocnemius after spinal cord injury. *Muscle Nerve* 2012; **46**: 237–245.
- Magnusson SP, Simonsen EB, Aagaard P, Kjaer M. Biomechanical responses to repeated stretches in human hamstring muscle *in vivo*. *Am J Sports Med* 1996; **24**: 622–628.
- Kwah LK, Herbert RD, Harvey LA, Diong J, Clarke JL, Martin JH *et al*. Passive mechanical properties of gastrocnemius muscles of people with ankle contracture after stroke. *Arch Phys Med Rehabil* 2012; **93**: 1185–1190.
- Diong JHL, Herbert RD, Kwah LK, Clarke JL, Harvey LA. Mechanisms of increased passive compliance of hamstring muscle-tendon units after spinal cord injury. *Clin Biomech* 2012; **27**: 893–898.
- Marquardt D. An algorithm for least-squares estimation of nonlinear parameters. *J Soc Ind Appl* 1963; **151**: 859–860.
- Erni T, Dietz V. Obstacle avoidance during human walking: learning rate and cross-modal transfer. *J Physiol* 2001; **534**: 303–312.
- Gorgey AS, Dudley GA. Spasticity may defend skeletal muscle size and composition after incomplete spinal cord injury. *Spinal Cord* 2008; **46**: 96–102.
- Ashworth B. Preliminary trial of carisoprodol in multiple sclerosis. *Practitioner* 1964; **192**: 540–542.
- Bohannon R, Smith M. Interrater reliability of a modified Ashworth scale of muscle spasticity. *Phys Ther* 1987; **67**: 206–207.
- Ghotbi N, Ansari NN, Naghdi S, Hasson S, Jamshidpour B, Amiri S. Inter-rater reliability of the Modified Modified Ashworth Scale in assessing lower limb muscle spasticity. *Brain Inj* 2009; **23**: 815–819.
- Rekand T. Clinical assessment and management of spasticity: a review. *Acta Neurol Scand* 2010; **122**: 62–66.
- Malhotra S, Pandyan AD, Day CR, Jones PW, Hermens H. Spasticity, an impairment that is poorly defined and poorly measured. *Clin Rehabil* 2009; **23**: 651–658.
- Fleuren KS, Voerman GE, Erren-Wolters CV, Snoek GJ, Rietman JS, Hermens HJ *et al*. Stop using the Ashworth Scale for the assessment of spasticity. *J Neurol Neurosurg Psychiatry* 2010; **81**: 46–52.
- Benz EN, Hornby TG, Bode RK, Scheidt RA, Schmitz BD. A physiologically based clinical measure for spastic reflexes in spinal cord injury. *Arch Phys Med Rehabil* 2005; **86**: 52–59.
- Pelletier CA, Hicks AL. The length-tension relationship of human dorsiflexor and plantarflexor muscles after spinal cord injury. *Spinal Cord* 2010; **48**: 202–206.
- Stein RB, Gordon T, Jefferson J, Sharfenberger A, Yang JF, de Zepetnek JT *et al*. Optimal stimulation of paralyzed muscle after human spinal cord injury. *J Appl Physiol* 1992; **72**: 1393–1400.
- Thomas C. Contractile properties of human thenar muscles paralyzed by spinal cord injury. *Muscle Nerve* 1997; **20**: 788–799.
- Huang C-Y, Wang C-H, Hwang I-S. Characterization of the mechanical and neural components of spastic hypertonia with modified H reflex. *J Electromyogr Kinesiol* 2006; **16**: 384–391.
- Calancie B, Broton JG, Klose KJ, Traad M, Difini J, Ayyar DR. Evidence that alterations in presynaptic inhibition contribute to segmental hypo- and hyperexcitability after spinal cord injury in man. *Electroencephalogr Clin Neurophysiol* 1993; **89**: 177–186.
- Murillo N, Kumru H, Vidal-Samsó J, Benito J, Medina J, Navarro X *et al*. Decrease of spasticity with muscle vibration in patients with spinal cord injury. *Clin Neurophysiol* 2011; **122**: 1183–1189.
- Boorman GI, Lee RG, Becker WJ, Windhorst UR. Impaired 'natural reciprocal inhibition' in patients with spasticity due to incomplete spinal cord injury. *Electroencephalogr Clin Neurophysiol* 1996; **101**: 84–92.
- Akman MN, Bengi R, Karatas M, Kilinç S, Sözyay S, Ozker R. Assessment of spasticity using isokinetic dynamometry in patients with spinal cord injury. *Spinal Cord* 1999; **37**: 638–643.
- Mirbagheri MM, Barbeau H, Ladouceur M, Kearney RE. Intrinsic and reflex stiffness in normal and spastic, spinal cord injured subjects. *Exp Brain Res* 2001; **141**: 446–459.
- Cook CS, McDonagh MJ. Measurement of muscle and tendon stiffness in man. *Eur J Appl Physiol Occup Physiol* 1996; **72**: 380–382.
- Maganaris CN, Reeves ND, Rittweger J, Sargeant AJ, Jones DA, Gerrits K *et al*. Adaptive response of human tendon to paralysis. *Muscle Nerve* 2006; **33**: 85–92.