

1 Continuous monitoring of electromyography (EMG),
2 mechanomyography (MMG), sonomyography (SMG) and torque
3 output during ramp and step isometric contractions

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1 **Abstract**

2 In this study we simultaneously collected ultrasound images, EMG, MMG from the
3 rectus femoris (RF) muscle and torque signal from the leg extensor muscle group of nine
4 male subjects (mean±SD, age = 30.7±4.9 years; body weight = 67.0±8.4 kg; height =
5 170.4±6.9 cm) during step, ramp increasing, and decreasing at three different rates (50%,
6 25% and 17% MVC/s). The muscle architectural parameters extracted from ultrasound
7 imaging, which reflect muscle contractions, were defined as sonomyography (SMG) in
8 this study. The cross-sectional area (CSA) and aspect ratio between muscle width and
9 thickness (width/thickness) were extracted from ultrasound images. The results showed
10 that the CSA of RF muscles decreased by 7.25±4.07% when muscle torque
11 output changed from 0% to 90% MVC, and the aspect ratio decreased by
12 41.66±7.96%. The muscle contraction level and SMG data were strongly
13 correlated ($R^2=0.961$, $P=0.003$, for CSA and $R^2=0.999$, $P<0.001$, for
14 width/thickness ratio). The data indicated a significant differences ($P<0.05$) in
15 percentage changes for CSA and aspect ratio among step, ramp increasing, and
16 decreasing contractions. The normalized EMG RMS in ramp increasing was
17 8.25±4.00% higher than step ($P=0.002$). The normalized MMG RMS of step
18 contraction was significantly lower than ramp increasing and decreasing, with
19 averaged differences of 12.22±3.37% ($P=0.001$) and 12.06±3.37% ($P=0.001$),
20 respectively. The results of this study demonstrated that the CSA and aspect ratio,
21 i.e., SMG signals, can provide useful information about muscle contractions. They
22 may therefore complement EMG and MMG for studying muscle activation
23 strategies under different conditions.

1 Index Terms- **Muscle; Soft Tissue; Ultrasound; Sonomyography; SMG;**
2 **Mechanomyography; MMG; Electromyography; EMG.**

3

4 **1. Introduction**

5 Electromyography (EMG) is generated by a record of the electrical discharges of active
6 motor units (MU) during the muscle activation [1], and the root mean square (RMS)
7 magnitude of EMG is commonly used to describe the time-domain information of the
8 EMG signal [2]. As the “mechanical counterpart” of the motor unit electrical activity
9 measured by EMG, mechanomyography (MMG) is a recording of mechanical oscillation
10 that is detected from the body surface overlying the muscle [3, 4]. It has been suggested
11 that the lateral oscillations detected by MMG can be decomposed into three parts: (1) a
12 gross lateral movement at the beginning of a muscle contraction, (2) smaller subsequent
13 lateral oscillations produced at the resonant frequency of the muscle, and (3) dimensional
14 changes of the muscle fiber [4, 5].

15 As the index of torque during muscle contraction [5, 6], EMG and MMG signals can each
16 provide information on various aspects of muscle function. For example, EMG has now
17 been widely used to study muscle fatigue [7, 8], muscle pathology [9-12], prosthetic
18 device control [13, 14], etc. The features of the MMG signal have been used to reflect the
19 kinematic and physiological characteristics of postural control [15], concentric muscle
20 contractions [16], and cycle ergometry [17,18], as well as to detect various muscular
21 disorders, including cerebral palsy [19], myotonic dystrophy [20], low back pain [21],
22 and muscle fatigue [22]. Furthermore, studies have been conducted with EMG and MMG

1 simultaneously to examine skeletal muscle characteristics. For example, EMG and MMG
2 were used to compare agonist vs. antagonist muscles in old vs. young women [6] and to
3 estimate the influence of torque changes during relaxation from maximal voluntary
4 contraction (MVC) of elbow flexors at different joint angles [23]. Additionally,
5 complementary knowledge was provided by collecting EMG and MMG during
6 concentric, isometric and eccentric contractions at different MVC [24]. These modalities
7 were also used to investigate the effect of acute static stretching on the biceps brachii [23]
8 and to assess lower-back muscle fatigue [21].

9 Recently, many studies have been performed using EMG and MMG to identify the motor
10 control strategies involved in force/torque production during isometric ramp increasing or
11 step contraction, in which the force/torque is alternately linearly increased or steadily
12 maintained [25]. Investigating the differences between various muscle contraction
13 protocols may guide exercise testing and training [26]. The amplitude and frequency of
14 MMG and EMG were examined with torque during ramp increasing or step contraction
15 [4, 27-30]. For example, EMG has been used to illustrate the different characteristics of
16 ramp increasing vs. step contractions [31-34], and the relationship between MMG and
17 force/torque has also been compared [35].

18 On the other hand, ultrasonography has been effectively employed to evaluate the
19 morphological changes in muscle thickness or displacement [36-40], muscle fiber,
20 pennation angle [44, 45], and cross-sectional area [46, 47]. It has also been suggested that
21 ultrasound parameters may characterize muscular pain, injury and dysfunction [48-52].
22 Moreover, ultrasonography has been used along with EMG to provide more

1 comprehensive information about the activities and properties of skeletal muscles [53-57].
2 We have recently proposed using the real-time muscle morphological change detected by
3 ultrasound, namely sonomyography (SMG), for the prosthetic control [58,59] and for
4 assessment of isometric muscle contraction [60-62] and isotonic contraction [63,64].

5 According to the literature, EMG and MMG have been used as indicators to compare
6 different motor control strategies in ramp increasing vs. step contractions
7 [25,26,29,31,32,35]. Yet the utility of the architectural changes detected by ultrasound for
8 evaluation of the difference between these contractions has not been comprehensively
9 investigated. Since data suggest that the motor control strategy may differ between ramp
10 increasing and step contractions [25,26,29,31,32,35], it is possible that different motor
11 control strategies may be recruited during step, ramp increasing and decreasing, in which
12 torque is produced to a greater or lesser degree. Therefore, the purpose of this study was
13 to simultaneously collect and compare EMG, MMG and SMG vs. torque relationships
14 during ramp increasing, decreasing and step contractions and hopefully to investigate the
15 differences in the motor control strategy of ramp and step contractions with respect to
16 morphological characteristics, including muscle cross-sectional area (CSA) and aspect
17 ratio between width and thickness (width/thickness ratio).

18 **2. Methods**

19 *2.1. Subjects*

20 Nine healthy male adults (mean±SD, age = 30.7±4.9 years; body weight = 67.0±8.4 kg;
21 height = 170.4±6.9 cm) volunteered to participate in this study. No participant had a

1 history of neuromuscular disorders, and all were aware of experimental purposes and
2 procedures. Human subject ethical approval was obtained from the relevant committee in
3 the authors' institution, and informed consent was obtained from each subject prior to the
4 experiment.

5 *2.2. Experiment protocol*

6 The subject was seated with the right leg at a flexion angle of 90° below the horizontal
7 plane on a calibrated dynamometer (Humac/Norm Testing and Rehabilitation System,
8 Computer Sports Medicine, Inc., Massachusetts, USA). Straps on the subject's trunk
9 were used to stabilize his position (Fig. 1). The testing position of the subject was
10 in accordance with the Humac/Norm User's Guide (Humac/Norm Testing and
11 Rehabilitation System, Computer Sports Medicine, Inc., Massachusetts, USA. 2006).
12 The subject was required to put forth his maximal effort of isometric knee
13 extension for a period of 3 s with verbal encouragement provided. The maximal
14 voluntary contraction (MVC) was defined as the highest value of torque recorded during
15 the entire isometric contraction [65]. A rest of 5 min was allowed before the subject
16 performed a second MVC test. The MVC torque was then calculated by averaging the
17 two recorded highest torque values from the two tests.

18 The subject was instructed to perform step, ramp increasing, and decreasing contractions,
19 the order of which was chosen randomly. For the step contraction, the subject was
20 required to produce a stable sub-maximal MVC torque (15, 30, 45, 60, 75, and 90%
21 MVC) respectively for 4 s. During the ramp increasing and decreasing, each subject was
22 instructed to produce torques increasing from 0 to 90% of his MVC, decreasing back to

1 zero linearly within 4, 8, and 12 s, during which the corresponding contraction rates were
2 50% MVC/s, 25% MVC/s, and 17% MVC/s, respectively. The reason for choosing 90%
3 MVC as the highest value was to avoid muscle fatigue. During each contraction, a
4 template and the real-time torque values were shown on a computer screen. The former
5 served as a target, and these two lines helped subjects to adjust their torque productions in
6 real time. Each test was repeated twice with a rest of 5 min between two adjacent trials.
7 Before the actual test, subjects practiced producing a smooth ramp contraction for several
8 times. The ramp contractions with tracking error (TE) smaller than 3% MVC [28] were
9 selected for subsequent data processing. The tracking error was defined as follows:

$$10 \quad T = \sqrt{\frac{1}{N-1} \sum_{n=1}^N (Torque(n) - Template(n))^2} \quad (1)$$

11 where *Torque* (*n*) is the actual torque recorded during each test, *Template* (*n*) is the
12 pre-designed linear pattern shown on a computer screen to guide the ramp contraction,
13 and *N* is the number of torque signal data points recorded during each test.

14 2.3. Data Acquisition

15 The isometric torque generated by the right quadriceps femoris muscle was measured
16 using a Norm dynamometer (Humac/Norm Testing and Rehabilitation System, Computer
17 Sports Medicine, Inc., Massachusetts, USA) and sampled at a frequency of 25 Hz, which
18 was the frame rate of B-mode ultrasound imaging. A commercial ultrasound scanner
19 (Ultrasound Diagnostic Scanner, EUB-8500, Hitachi Medical Corporation, Tokyo, Japan)
20 with a 12 MHz linear array ultrasound probe (L53L, Hitachi Medical Corporation, Tokyo,
21 Japan) was used to collect the ultrasound images. The long axis of the ultrasound probe
22 was arranged perpendicularly to the long axis of the thigh on its superior aspect,

1 three-fifths of the distance from the anterior superior iliac spine to the superior patellar
2 border [66, 67]. The ultrasound probe was fixed by a multi-degree custom-designed
3 adjustable bracket. Ultrasound gel was applied between the skin and probe to serve as an
4 acoustic coupling medium (Fig. 1). The probe was adjusted to optimally visualize the
5 muscle fiber [56] and the position was marked to ensure that the probe was placed at the
6 same site every time. Before the test began, the ultrasound images were collected for the
7 subject under the relaxed state for normalization of the parameters collected during the
8 contraction tests. The B-mode ultrasound images were displayed in real time and
9 digitized by a video card (NI PCI-1411, National Instruments, Austin, USA) at 25 Hz for
10 later analysis.

11 Two surface EMG electrodes were placed on the rectus femoris (RF) muscle belly
12 parallel with the long axis of the muscle, and the ultrasound probe was positioned
13 between them. It has been earlier reported that the ultrasound gel used during the test
14 generates negligible effects to the EMG recording [68]. An MMG sensor was placed
15 close to one of the EMG electrodes, which was at a shorter distance from the knee than
16 the other electrode (Fig. 1). The surface EMG signal was captured by the EMG bipolar
17 Ag-AgCl electrodes (Axon System, Inc., NY, USA), and a reference EMG electrode was
18 placed near the kneecap. The MMG signal was detected using an accelerometer
19 (EGAS-FS-10-/V05, Measurement Specialties, Inc., France) fixed with two-sided tape.
20 The surface EMG and MMG signals were amplified by a custom-designed amplifier with
21 a gain of 2000, filtered separately by 10-400 Hz, 5-100Hz band-pass analog filters within
22 the amplifier, and digitized by a 12-bit data acquisition card (NI-DAQ 6024E, National
23 Instruments Corporation, Austin, TX, USA) with a sampling rate of 1 KHz. Ultrasound

1 images, surface EMG, MMG and torque signals were simultaneously collected and stored
2 by software for ultrasonic measurement of motion and elasticity (UMME,
3 <http://www.tups.org>) for further analysis. The time delay between the different data
4 collection systems was calibrated using a method similar to that described by Huang et al.
5 [68]. As the transducer moved cyclically up and down in a water tank, the two signals
6 representing the ultrasound image and the simulated EMG, respectively, were collected
7 and stored. The time delay between the data sets was calculated using a cross-correlation
8 algorithm. The details can be found in our earlier study [68].

9 *2.4. Data Analysis*

10 All signals were processed off-line using a program written in Matlab (Version 7.3,
11 MathWorks, Inc., Massachusetts, USA). During each sub-maximal step contraction, a 1-s
12 epoch of data was selected from the middle of the 4-s muscle contraction duration. The
13 torque value was represented by the mean of the data within the 1-s epoch (Fig. 2). For
14 ramp contraction, 256-ms epochs of data were selected from the centre of the torque to
15 match the value in step contraction (Fig. 3). The mean torque, and root mean square
16 (RMS) values of EMG and MMG were calculated for each epoch, and the EMG/MMG
17 RMSs were expressed as a percentage of their maximal values at 90% MVC. B-mode
18 ultrasound images were synchronized with the torque signal. In other words, a given
19 B-mode ultrasound image frame corresponded to a given torque value point. The B-mode
20 ultrasound images corresponding to torque values that were close to the averaged torque
21 value calculated during step contraction were selected for ramp increasing, decreasing
22 and step contractions. The width, thickness and cross-sectional area (CSA) of the RF
23 muscle were measured from the ultrasound images [69] using ImageJ software (ImageJ,

1 National Institutes of Health, USA) as shown in Fig. 4. In addition to CSA, the
2 width/thickness ratio of the RF muscle was used to describe the morphological change
3 during contractions (Fig. 5). The ultrasound parameters were then normalized as the
4 percentage change, with the reference value taken while the subject was relaxed in the
5 same position.

6 A total of 294 ($6 \times 6 \times 2$ (step) + $6 \times 6 \times 3 \times 2$ (ramp) + 6×1 (relaxed state)) ultrasound
7 images were measured twice by the same investigator. The intra-class correlation (ICC)
8 based on one-way random model and standard error of the measurement (SEM) were
9 performed to evaluate intra-observer repeatability. The relationships for CSA,
10 width/thickness ratio, EMG and MMG RMS vs. % MVC were investigated by a power
11 model for the averaged value across the nine subjects and three types of isometric
12 contractions (step, ramp increasing, and decreasing). Four separate three-way repeated
13 measure analyses of variance (ANOVAs) (movement patterns [step vs. ramp increasing
14 vs. decreasing] \times % MVC [15% vs. 30% vs. 45% vs. 60% vs. 75% vs. 90%] \times velocity
15 [50% MVC/s vs. 25% MVC/s vs. 17% MVC/s]) were used to analyze normalized
16 EMG/MMG RMS, CSA, and width/thickness ratio, respectively. If there were no
17 significant interaction effect among these three factors, two- and one-way repeated
18 measures ANOVAs were followed up with Bonferroni pairwise comparisons [35]. The
19 relationships between CSA, width/thickness ratio, MMG RMS, EMG RMS versus
20 %MVC were examined for each individual and the mean values for all the subjects.
21 Polynomial regression analysis (linear, quadratic, and cubic) were used to examine the
22 relationships. All the data were analyzed using SPSS (SPSS Inc. Chicago, IL, USA).

1 Statistical significance was set at the 5% probability level.

2 **3. Results**

3 *3.1 Composite (mean) of ultrasound measurements of the RF muscle during step* 4 *and ramp increasing, decreasing contractions*

5 The ICC for width, thickness and CSA measurements of RF muscle in this study
6 were 0.986, 0.987, and 0.978 respectively, and the SEM were 0.13 cm, 0.04 cm and
7 0.48 cm² (Table 1). The overall values of CSA and width/thickness ratio from the nine
8 subjects' RF muscles were 9.83±3.06 cm² (mean ± SD) and 2.99±0.57, respectively,
9 during the relaxed state. As shown in Fig. 6, the relationships between both the
10 percentage change of CSA and width/thickness ratio vs. % MVC could be well
11 represented by cubic functions ($R^2 = 0.961$, $P = 0.003$, for CSA and $R^2 = 0.999$,
12 $P < 0.001$, for width/thickness ratio).

13 There were no significant three- or two-way interactions among the three factors
14 (movement patterns×% MVC×velocity, all $P > 0.05$), but the effects of the movement
15 patterns ($P < 0.001$) and % MVC ($P = 0.012$) on the CSA were significant. The percentage
16 change at 15% MVC was found to be significantly lower than at 75% MVC (reduced by
17 2.94±0.93%, $P = 0.026$) or 90% MVC (reduced by 3.21±0.93%, $P = 0.010$). The percentage
18 change of CSA during step was significantly lower than during ramp decreasing, with
19 mean difference of 2.75±0.82% ($P = 0.003$). While the corresponding reduction compared
20 with ramp increasing was 0.64±0.82%, no significant difference was demonstrated
21 ($p > 0.999$). In addition, the percentage change of CSA during ramp increasing was
22 2.11±0.58% lower than during ramp decreasing with significant effect ($P = 0.001$) (Fig. 7).

1 For the width/thickness ratio, there were no significant three-way or two-way
2 interactions for movement patterns×velocity and movement patterns×% MVC, but
3 there was a significant effect for two-way interactions for movement patterns×%
4 MVC (P=0.002). As shown in Fig. 8, we noted that the change of width/thickness
5 ratio in ramp decreasing was $5.42\pm 1.65\%$ and $6.93\pm 1.17\%$ higher than in both
6 step and ramp increasing, respectively (P=0.003 and P<0.001), and the difference
7 between step and ramp increasing was not significant (P>0.999).

8

9 *3.2 Composite (mean) of the EMG and MMG RMS of RF muscle during step,*
10 *ramp increasing, and decreasing contractions*

11 As shown in Fig. 9, the overall relationships of both the normalized EMG RMS and
12 MMG RMS vs. % MVC were well represented by cubic models with high R² values
13 (R² = 0.999, P<0.001, for EMG RMS and R² = 0.999, P<0.001, for MMG RMS).
14 Movement patterns and % MVC were each found to significantly affect the EMG
15 RMS (both P<0.001). It was demonstrated that the normalized EMG RMS was
16 increased as the increase of torque (all P<0.05). The normalized EMG RMS in
17 ramp increasing was $8.25\pm 4.00\%$ higher than step (P=0.002) (P<0.001). Although
18 the average normalized EMG RMS of ramp decreasing was $4.25\pm 2.38\%$ higher
19 than, step contraction and $4.00\pm 1.68\%$ lower than ramp increasing, no significant
20 difference was demonstrated (P=0.225 and 0.054) (Fig. 10).

21 The normalized MMG RMS of step contraction was significantly lower than ramp
22 increasing and decreasing, with averaged differences of $12.22\pm 3.37\%$ (P=0.001)
23 and $12.06\pm 3.37\%$ (P=0.001), respectively. The difference between ramp

1 increasing and decreasing was small and not obvious ($0.16 \pm 2.38\%$, Fig. 11). Significant
2 effects of velocity and % MVC for normalized MMG RMS were found (both
3 $P < 0.01$). MMG RMS was only found to be significantly higher at a velocity of 50%
4 MVC/s than at 17% MVC/s ($P < 0.001$). Moreover, the averaged MMG RMS
5 became significantly higher as % MVC increased (all $P < 0.05$), with the exception
6 of the change from 15% to 30% MVC, and 30% MVC to 45% MVC ($P > 0.999$) (Fig.
7 9).

8 *3.3 Inter-individual variability of polynomial regression analysis of CSA, width/thickness* 9 *ratio, EMG RMS, MMG RMS*

10 Table 1 shows the polynomial regression analyses for the individual CSA,
11 width/thickness ratio, EMG RMS, MMG RMS versus isometric torque relationships. For
12 the individual patterns of CSA, five of the nine subjects exhibited cubic, three exhibited a
13 quadratic; for width/thickness ratio, five best fit with cubic, two with quadratic and one
14 with linear regression. For EMG and MMG RMS, there are still five subjects exhibit
15 cubic. However, three exhibit quadratic for EMG RMS whereas only one exhibit
16 quadratic and linear for MMG RMS.

17 **4. Discussion**

18 In this paper, ultrasound images, EMG, MMG and torque signals were continuously
19 collected from the RF muscles of nine male subjects during step, ramp increasing, and
20 decreasing contractions at rates of 50, 25 and 17% MVC/s. The CSA and width/thickness
21 ratio were extracted from the ultrasound images to describe the architectural changes of

1 the RF muscle during isometric contractions. The intra-operator reliability for
2 ultrasound measurements of RF muscle in this study was excellent, ranging from
3 0.978 to 0.987 (Table 1). This reliability in ultrasound measurement was achieved
4 by careful control of the transducer's position and orientation. Studies have shown
5 that muscle functions, such as the amplitude of force produced, joint angle change,
6 muscle contraction pattern (step or ramp) are closely related to architectural
7 characteristics such as CSA [65-71] and thickness [36,60, 63,64,72-74]. The CSA of RF
8 muscle of the younger male participants in this study ($9.83 \pm 3.06 \text{ cm}^2$) was similar to
9 those observed in [71] using magnetic resonance imaging. In another study using
10 ultrasonography, the reported CSA of RF muscle was $4.6 \pm 1.33 \text{ cm}^2$ for the older
11 male subjects [66]. The CSA of RF muscle reported was smaller mainly because
12 the subjects recruited were much older (around 60 years old) than that used in
13 current study. It has been suggested that reduction in muscle CSA develops
14 during the aging process due to a decrease in motor units [75].

15 During the isometric contractions, the CSA of the RF muscle was measured by
16 directly tracing the position of the aponeurosis, which separates the RF muscle
17 from the neighboring muscles of the quadriceps femoris (Fig. 4). The results
18 showed that the CSA decreased more as torque increased, with an average
19 percentage change from $6.61 \pm 9.03\%$ (15% MVC) to $10.27 \pm 8.77\%$ (90% MVC)
20 (Fig. 6A). On the other hand, the percentage change of width/thickness ratio
21 indicated that as torque increased, the width of the RF muscle diminished, while
22 the thickness increased (Fig. 6B). During the development of quadriceps femoris'
23 torque output, the RF muscle is often regarded as the bi-articular muscle that

1 transfers moments between joints [76] and directs force and movement [77], while
2 the other three vasti are synergistic, mono-articular muscles that generate force
3 [78-79]. Ploutz-Snyder et al. suggested that the RF muscle was the inactive
4 muscle, based on their findings of no change in CSA after squat exercises [71]. In
5 contrast, in the present study, although the CSA of the RF muscle decreased with
6 increasing torques, the results of the width/thickness ratio suggested that the RF
7 muscle was actively involved in isometric torque development, as indicated by its
8 increasing thickness and decreasing width. These observations strongly support
9 the concept that the RF muscle is likely to act as a mono-articular body [79].
10 However, to support this conclusion, future studies are warranted.

11 The morphological differences in the RF muscle in step, ramp increasing, and
12 decreasing may suggest different activation strategies in each of these three
13 isometric contractions. Movement patterns significantly affect the CSA and
14 width/thickness ratio ($P < 0.05$). Both the CSA and width/thickness ratio of RF
15 muscle were significantly higher in step compared with ramp decreasing, and the
16 width/thickness ratio of ramp increasing was significantly higher than of ramp
17 decreasing at all velocities ($P < 0.05$). Akima et al. suggested that not all muscle
18 fibers are recruited, even at the MVC, for subjects with low physical training [80];
19 furthermore, according to the muscle size principle, motor units are recruited in
20 the order of their recruitment thresholds [81]. As muscle torque developed, the
21 CSA and width/thickness ratio of RF muscle decreased. Thus, the smaller values
22 seen in ramp increasing compared with step may indicate that more muscle motor
23 units were recruited and that more fast-twitch muscle fibers may have been

1 activated for ramp increasing than for step. The motor units of fast-twitch were
2 controlled by the large size of motor neuron, which was recruited at higher thresholds
3 [82]. Therefore, the increased ratio between the recruited motor unit of fast-twitch and
4 slow-twitch in ramp increasing might allow subjects to gradually increase torque; this
5 mechanism may require extra effort to recruit high-threshold motor units. Ramp
6 decreasing occurs in a reverse process compared to ramp increasing. However, the CSA
7 and width/thickness ratio of RF muscle in ramp decreasing was deduced by a
8 comparison to ramp increasing (Fig. 7 and 8). As the torque was linearly reduced, the
9 deformation of the RF muscle in ramp decreasing could not restore muscle function to the
10 same level as in ramp increasing, possibly due to viscoelasticity of the skeletal muscle
11 and surrounding media. Another reason may be that the effort needed to produce the
12 same level of torque in the torque-increasing process was lower than for
13 torque-decreasing, which was used to recruit high-threshold motor units. Other reasons
14 too may affect muscle behavior during different movement patterns, such as the
15 actin-myosin cross-bridge activity, intramuscular pressure, muscle stiffness, the
16 mechanical properties of neighboring tissues [24], and the ratio of fast-twitch and
17 slow-twitch fibers [83]. Since the ratio of the fibre type could vary greatly in different
18 types of muscle [84], the current SMG parameters should be tested on other skeletal
19 muscles for a solid conclusion. The findings of a curvilinear increase in EMG and
20 MMG amplitude vs. torque were consistent with those reported in previous studies
21 [29, 85]. EMG and MMG RMS increased progressively with increasing torque at
22 all velocities, suggesting that more muscle motor units were recruited as torque
23 developed.

1 Both EMG and MMG amplitude in the RF muscle were found to be smaller in step
2 contraction than in ramp increasing, which was consistent with results from the
3 plantar flexors in studies performed by Bilodeau et al. [86] and Ryan et al. [28].
4 However, in a departure from previous studies examining the EMG and MMG
5 performance in step vs. ramp increasing contractions [25,32,35,87-88], the
6 present study also investigated the EMG and MMG amplitude in ramp decreasing
7 contractions in order to comprehensively compare all of the movement protocols
8 that may be used in muscle training or rehabilitation. We found that the EMG RMS
9 of ramp decreasing contractions was significantly smaller than that of ramp
10 increasing and bigger than that of the step test. However, the difference between
11 ramp increasing and decreasing was not significant in the MMG RMS. The
12 contraction rate did have a significant effect on the MMG RMS, perhaps because
13 MMG signals are easily contaminated by noise during muscle contractions [89]. At
14 higher rates, the low-frequency movement at the beginning or end of isometric
15 contractions would affect the MMG signals. This may also explain the large increase in
16 MMG RMS at 60% MVC of ramp decreasing contractions at a rate of 50% MVC/s (Fig.
17 11A). Our results are in line with findings from previous studies that have indicated that
18 the isometric contraction patterns ramp increasing, ramp decreasing and step may each
19 require motor control strategies that differ in, for example, the relative contributions of
20 motor unit recruitment and firing rate modulation [31,35,83].

21 Moreover, the differences among ramp increasing, decreasing and step
22 contractions were observed in the electrophysiological (EMG), mechanical (MMG),
23 morphological (SMG) responses. SMG parameters at most percentages of MVC

1 increased from ramp decreasing to ramp increasing to step, while EMG and MMG RMS
2 parameters increased from step to ramp decreasing to ramp increasing. We noticed a
3 discrepancy between the SMG parameters and EMG/MMG RMS during ramp increasing
4 and decreasing contraction; this may indicate that the morphological parameter would not
5 always yield the same conclusion as the electrophysiological or mechanical parameter.
6 The mechanisms responsible for this phenomenon should be further investigated, and if
7 this finding can be confirmed by testing more subjects, SMG parameters may provide
8 information complementary to EMG and MMG.

9 The results of inter-individual variability of polynomial regression analysis indicated that
10 the composite CSA, width/thickness ratio, EMG RMS and MMG RMS versus isometric
11 torque relationship were all best fit with a cubic model. However, only 55.5% (five of
12 nine) exhibited the same cubic patterns as the composite relationship (Table 1). The
13 quadratic relationship was take 33.3%, 22.2%,33.3%, 11.1% for CSA, width/thickness
14 ratio, MMG RMS and EMG RMS. There was only 11.1% exhibit linear for
15 width/thickness ratio and MMG RMS, whereas 11.1% of the individual patterns exhibited
16 no significant relationship for CSA, width/thickness ratio, EMG RMS versus torque and
17 22.2% for MMG RMS. The EMG, MMG result of the present study was consistent
18 with previous studies suggesting that the surface EMG and MMG amplitude versus
19 isometric torque relationship should be evaluated on a subject-by-subject basis [90, 91]
20 and that there were differences between subjects for the MMG amplitude versus
21 isometric torques [92, 93]. On the other hand, the SMG parameters also showed the
22 inter-individual variability. This may caused by the differences in motor control strategies
23 [94, 95] or muscle fiber type composition [96], which deserves further study.

1 Based on the sample size of 9 and alpha of 0.05, the calculated mean statistical power for
2 the parameters showed significances between different percentages of MVC is 0.61; that
3 for comparison between ramp increasing and decreasing parameters is 0.65; and that for
4 comparison between ramp and step parameters is 0.71. The overall mean statistical power
5 is 0.66 for all parameters. It is noted that statistical powers of the parameters were
6 relatively small, particularly for SMG and MMG parameters. This result is consistent
7 with the large standard deviations observed for the SMG and MMG parameters. In future
8 studies, more subjects are required and the reasons for the relatively larger variations of
9 SMG and MMG parameters among subjects should be investigated

10 To sum up, the combination of ultrasonography, EMG and MMG recordings during three
11 isometric contractions revealed the non-linear relationships between SMG (CSA,
12 width/thickness ratio) and torque, EMG and torque, and MMG and torque for the RF
13 muscle. As the utilization of different motor control strategies under various movement
14 patterns has not been comprehensively studied, the present study provides data regarding
15 morphological changes of the RF muscle in different movement patterns (ramp increasing,
16 decreasing, and step) using ultrasound. We found that the different movement patterns
17 significantly affect the CSA and width/thickness ratio; in other words, our ultrasound
18 measurement data of the RF muscle suggested that different movement patterns may use
19 different motor control strategies to control muscle morphology. The EMG/MMG data
20 from this study were consistent with findings from previous studies and support the
21 suggestion that different motor control strategies may be utilized during various
22 movement patterns. The discrepancy between SMG parameters and EMG/MMG RMS
23 regarding to ramp increasing and decreasing contraction should be further studied.

1 Furthermore, the reasons for the relatively larger variations of SMG parameters among
2 subjects should be investigated in future studies. The recording system described here can
3 be used to obtain more comprehensive information regarding muscle activities by
4 simultaneously recording electrophysiological, mechanical, and morphological responses
5 in human skeletal muscle activity.

6

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10 for his help in obtaining the bracket, as well as all of our subjects for their kind support of
11 this study.

12

1 **Figure Captions**

2 Fig. 1. Experimental setup for collecting ultrasound images, EMG, MMG and torque
3 signals from the subject's right rectus femoris (RF) muscle during isometric ramp
4 increasing, decreasing and step contractions. The ultrasound probe was aligned
5 perpendicularly to the RF muscle belly using a multi-degree adjustable bracket. The
6 two surface EMG electrodes were located at the two sides of the ultrasound
7 transducer and in parallel with the muscle fibers. The MMG sensor was placed near
8 the EMG electrode.

9 Fig. 2. A typical example of MMG and EMG signals recorded from RF muscle of subject
10 C during step contraction at the 45% MVC level. The signals (MMG, EMG, torque)
11 between the dashed lines were selected to represent the characteristics of a 45%
12 MVC step.

13 Fig. 3. A typical example of MMG and EMG signals recorded from the RF muscle of
14 subject C during ramp contractions at the rate of 25% MVC/s. The vertical dashed
15 boxes represent twelve segments of data points analyzed as MVC increased from
16 15% to 90% and again as MVC decreased from 90% to 15% at 15% intervals.

17 Fig. 4. (A) The aponeuroses appear as hyperechoic strips and boundaries differentiating
18 RF muscle from other neighboring quadriceps muscles (vastus lateralis (VL), vastus
19 intermedius (VI), and vastus medialis (VM)). The central aponeurosis of the RF
20 muscle appears as a comma-shaped hyperechoic band [69]. (B) The CSA, width and
21 thickness were measured by ImageJ software for further analysis.

22 Fig. 5. Typical ultrasound images at 0%, 30%, 60%, and 90% MVC contraction levels
23 during ramp contractions at the rate of 17% MVC/s from subject C.

1 Fig. 6. The relationship between (A) averaged percentage change of cross-sectional area
2 vs. % MVC, and (B) the averaged percentage change of width/thickness ratio vs. %
3 MVC across nine subjects in isometric contraction.

4 Fig. 7. The percentage change of cross-sectional area at different isometric torque (MVC)
5 during the step and ramp increasing, decreasing contractions with torque changing
6 rates of (A) 50% MVC/s, (B) 25% MVC/s, and (C) 17% MVC/s. Values are mean \pm
7 SD (N=9).

8 Fig. 8. The percentage change of width/thickness ratio at different isometric torque
9 (MVC) during step, ramp increasing, and decreasing contractions with torque at
10 rates of (A) 50% MVC/s, (B) 25% MVC/s, and (C) 17% MVC/s. Values are mean \pm
11 SD (N=9).

12 Fig. 9. Normalized (A) EMG RMS vs. % MVC and (B) MMG RMS vs. % MVC for nine
13 subjects in isometric contraction.

14 Fig. 10. Normalized EMG RMS during step, ramp increasing, and decreasing
15 contractions with torque at rates of (A) 50% MVC/s, (B) 25% MVC/s, and (C) 17%
16 MVC/s. Values are mean \pm SD (N=9).

17 Fig. 11. Normalized MMG RMS at different isometric torques (MVC) during step, ramp
18 increasing, and decreasing contractions with torque at rates of (A) 50% MVC/s, (B)
19 25% MVC/s, and (C) 17% MVC/s. Values are mean \pm SD (N=9).

20

1 Table 1. The intra-class correlation coefficients (ICC) and standard errors of
2 measurement (SEM) for the width, thickness, and cross-sectional area (CSA) of rectus
3 femoris (RF) muscle from two repeated ultrasound measurements made by one
4 operator.

Ultrasound parameter	ICC	95% CI lower	95% CI upper	SEM
Width	0.986	0.985	0.988	0.13 cm
Thickness	0.987	0.986	0.989	0.04 cm
CSA	0.978	0.975	0.981	0.48 cm ²

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1 Table 2. The polynomial regression models for the change of cross-sectional area
 2 (CSA), width/thickness ratio, mechanomyography and electromyography root mean
 3 square (MMG RMS and EMG RMS) vs. percent maximal voluntary contraction (%
 4 MVC) relationship.

Subject	CSA		Width/thickness ratio		EMG RMS		MMG RMS	
	Model	R ²	Model	R ²	Model	R ²	Model	R ²
1	Cubic	0.992	Linear	0.831	Cubic	0.997	Cubic	0.997
2	Cubic	0.999	Cubic	0.987	NS	NS	Cubic	0.986
3	Cubic	0.990	Quadratic	0.998	Quadratic	0.996	Cubic	0.999
4	Quadratic	0.926	Cubic	0.999	Quadratic	0.999	Cubic	0.999
5	Cubic	0.998	Cubic	0.997	Cubic	0.999	Cubic	0.998
6	Cubic	0.999	Cubic	0.994	Cubic	0.999	NS	NS
7	Quadratic	0.942	Quadratic	0.993	Cubic	0.999	Linear	0.723
8	NS	NS	Cubic	0.982	Cubic	0.981	NS	NS
9	Quadratic	0.934	NS	NS	Quadratic	0.986	Quadratic	0.986
Mean	Cubic	0.998	Cubic	0.999	Cubic	0.998	Cubic	0.999

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6 NS: no significant model (P>0.05)

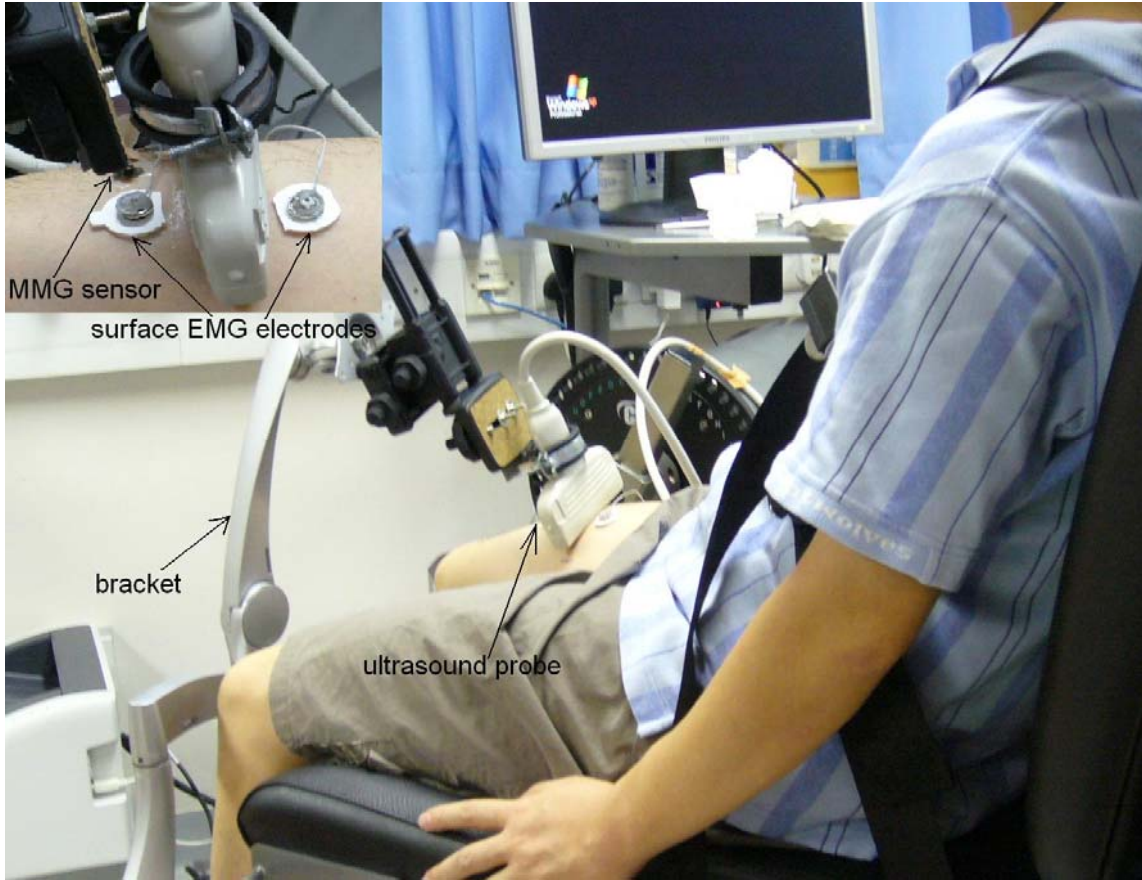
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1 **Figures**



2

3 **Fig. 1**

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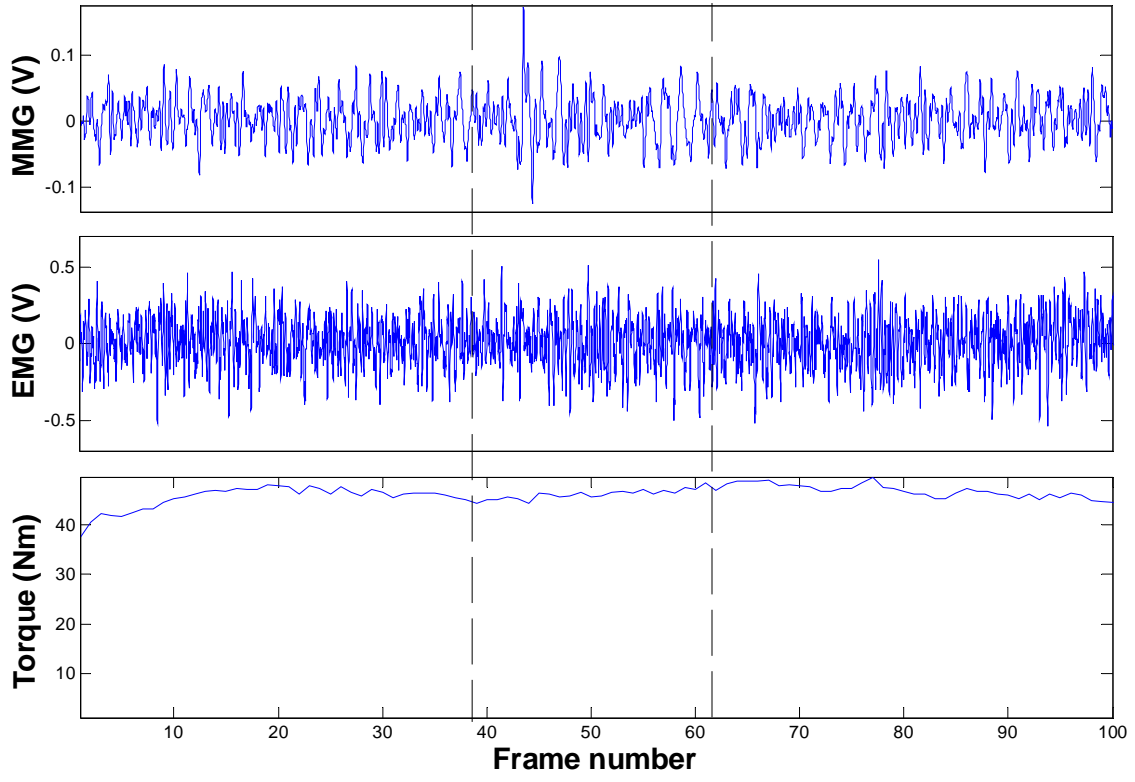
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2 **Fig. 2**

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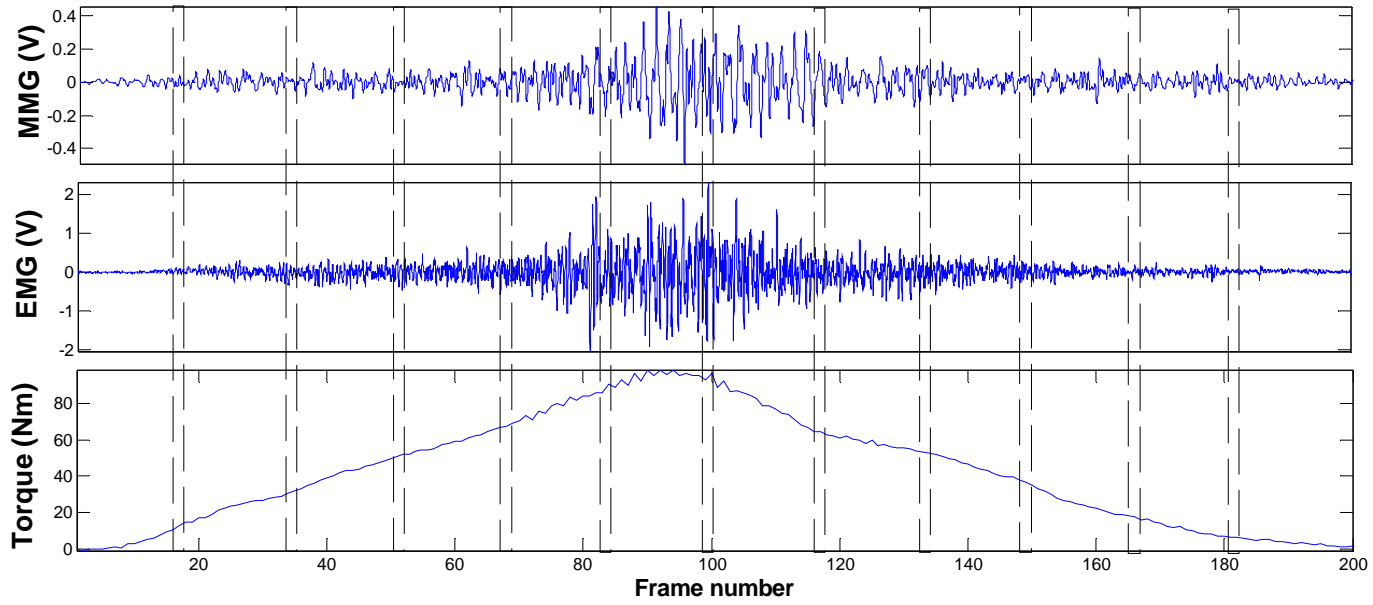
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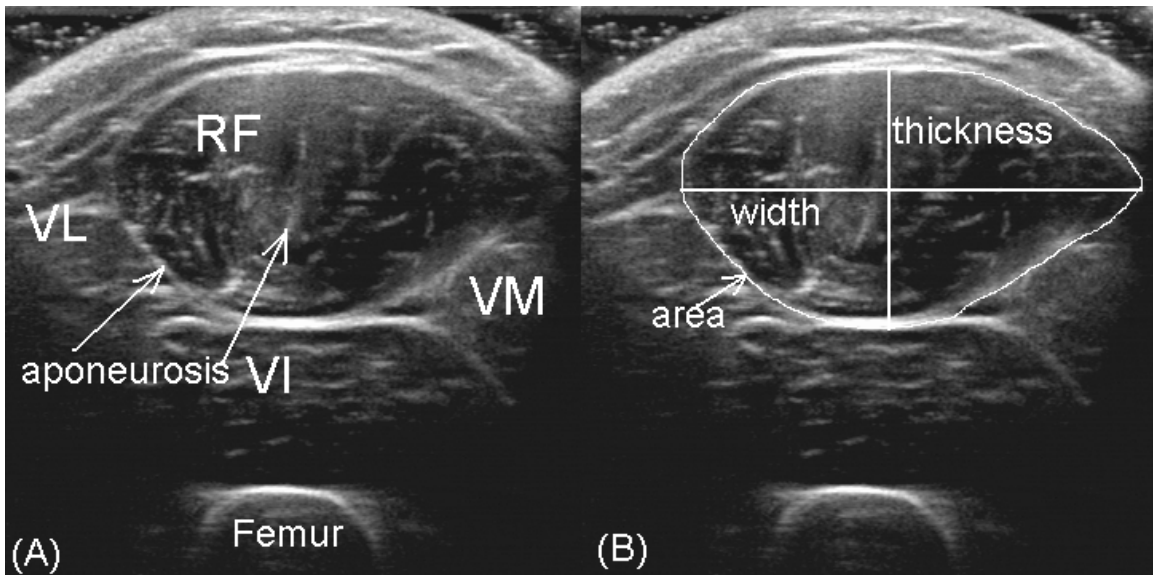


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2 **Fig. 3**

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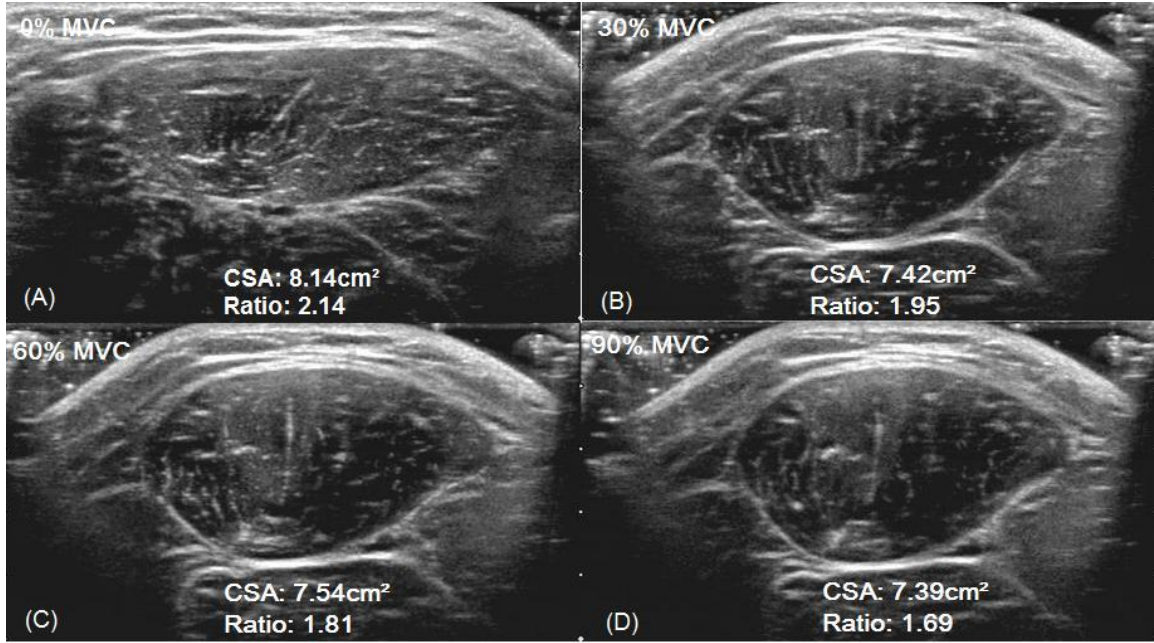
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6 **Fig. 4**

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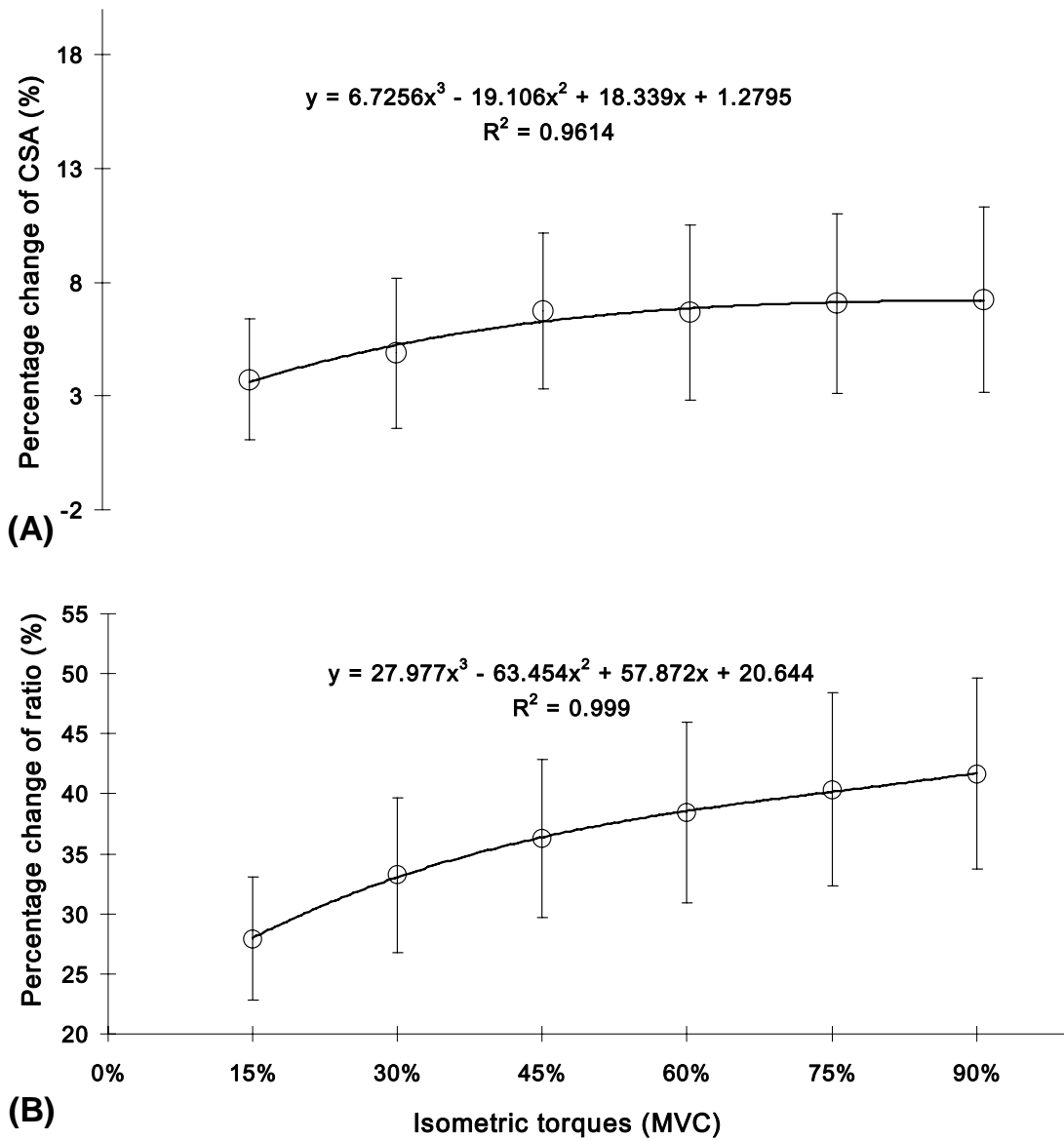


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3 **Fig. 5**

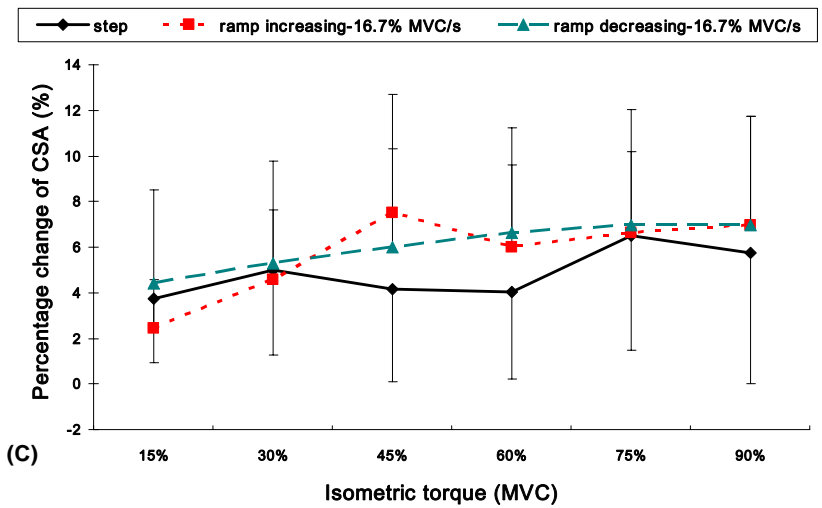
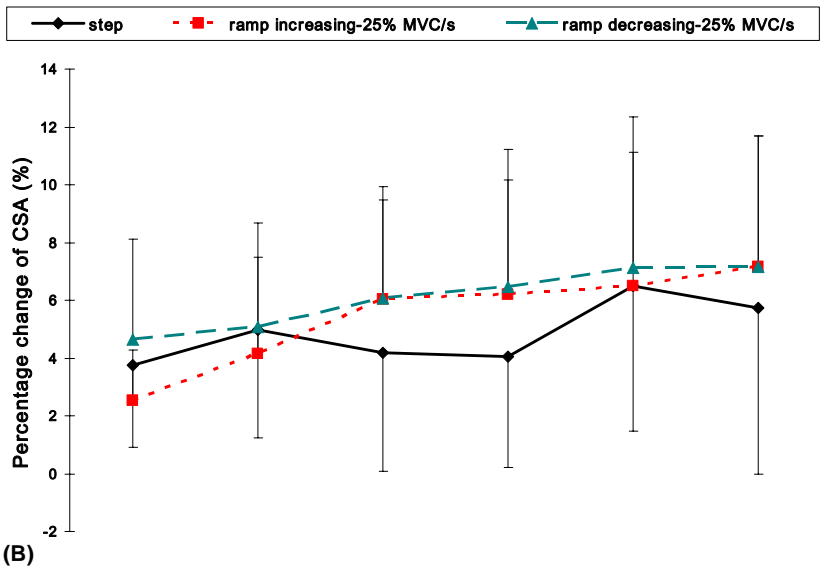
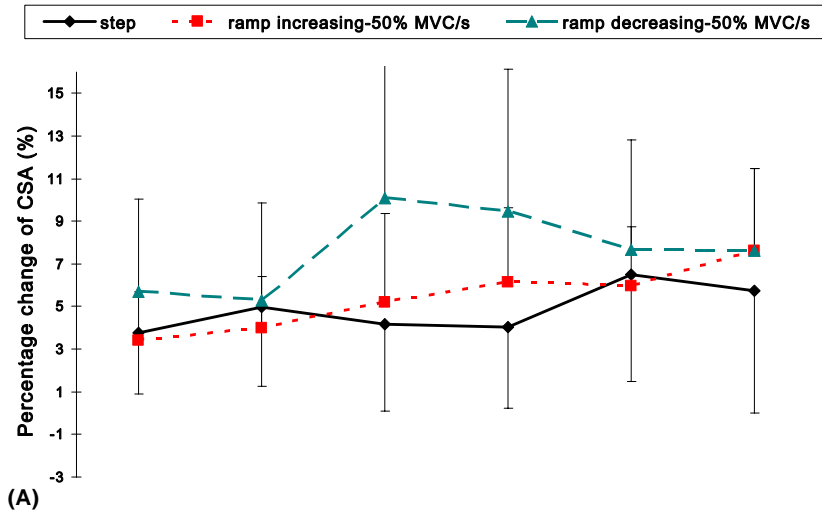
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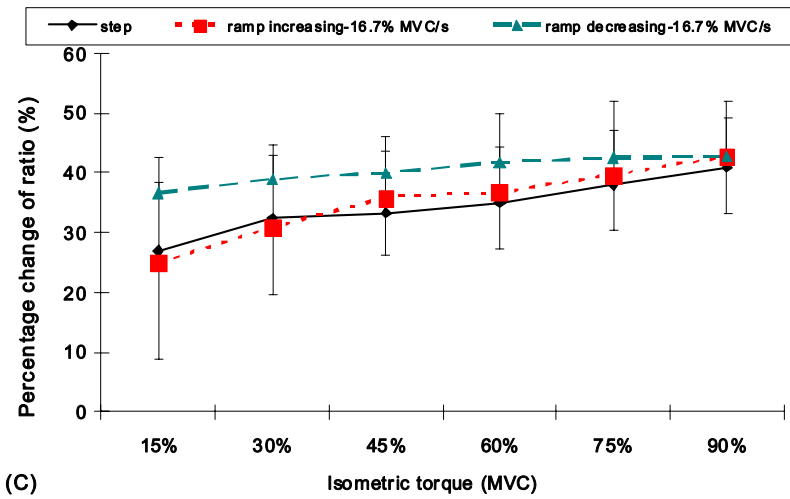
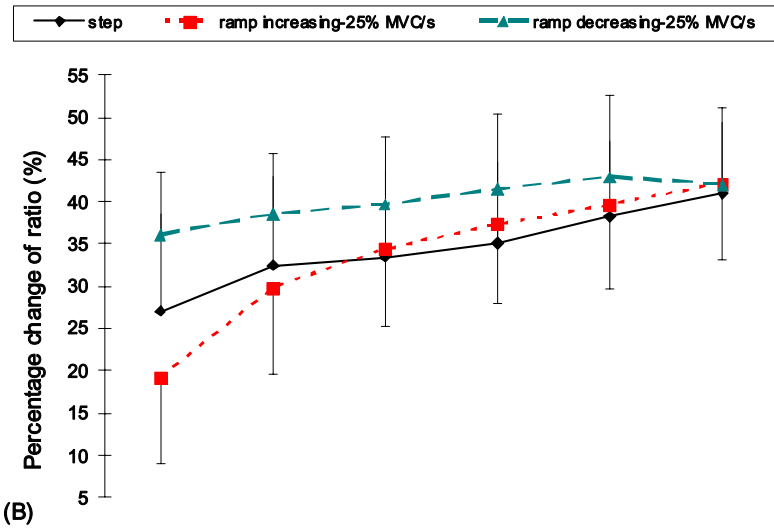
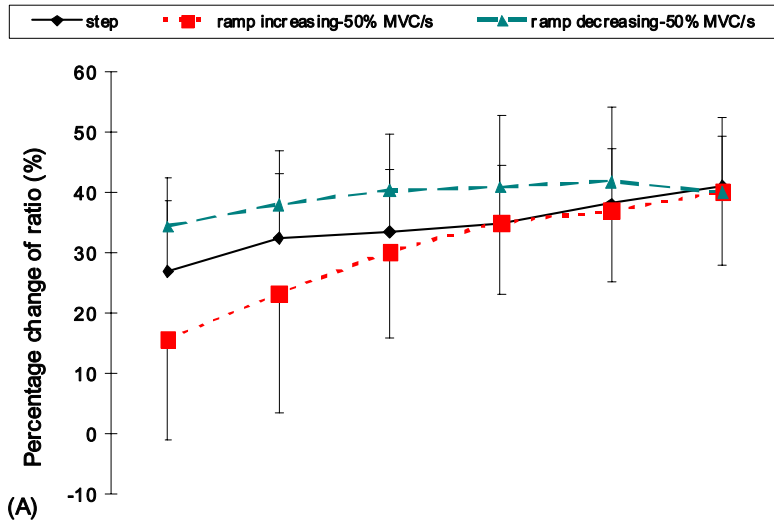


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Fig.6

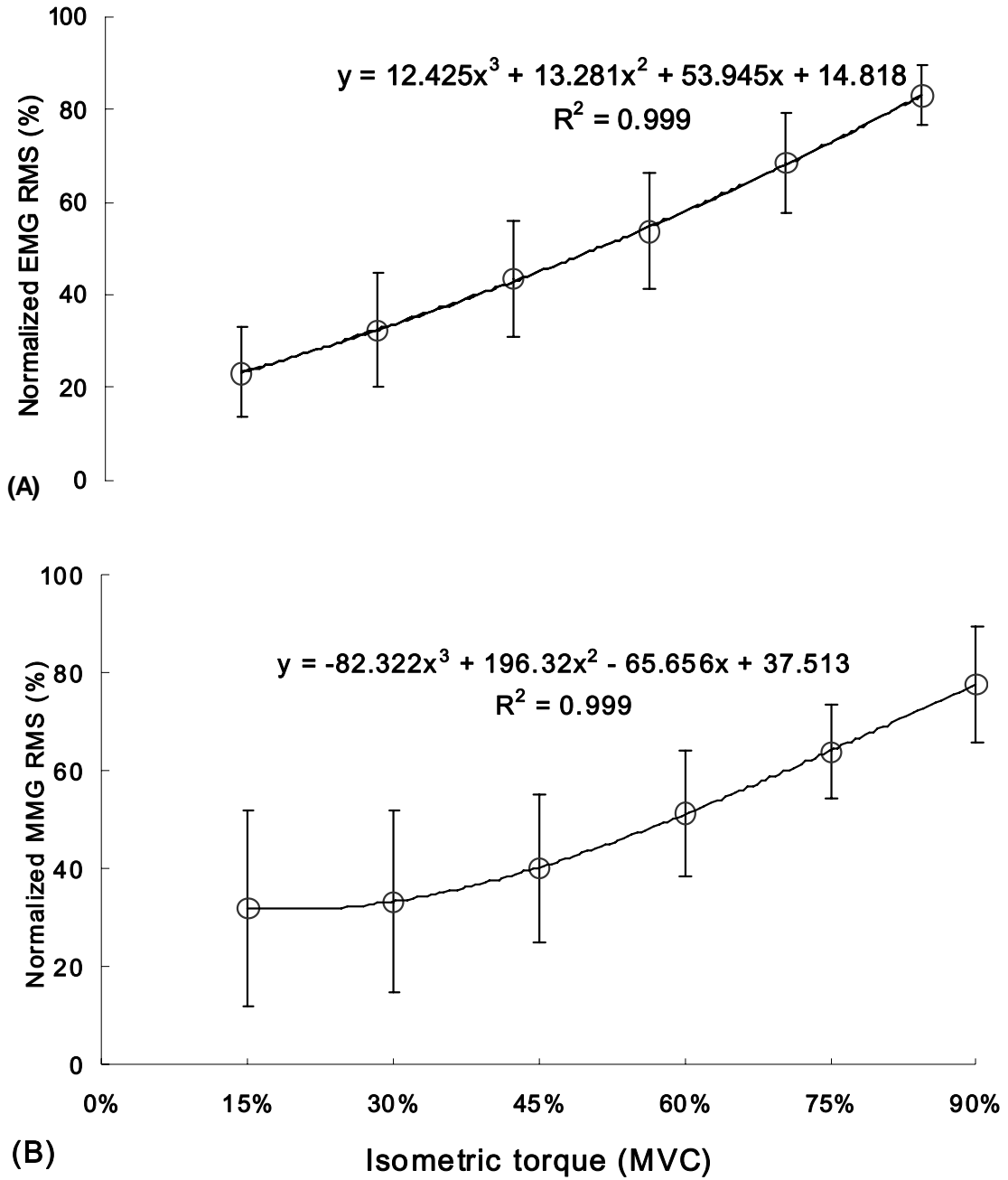


1 **Fig. 7**



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1 **Fig. 8**



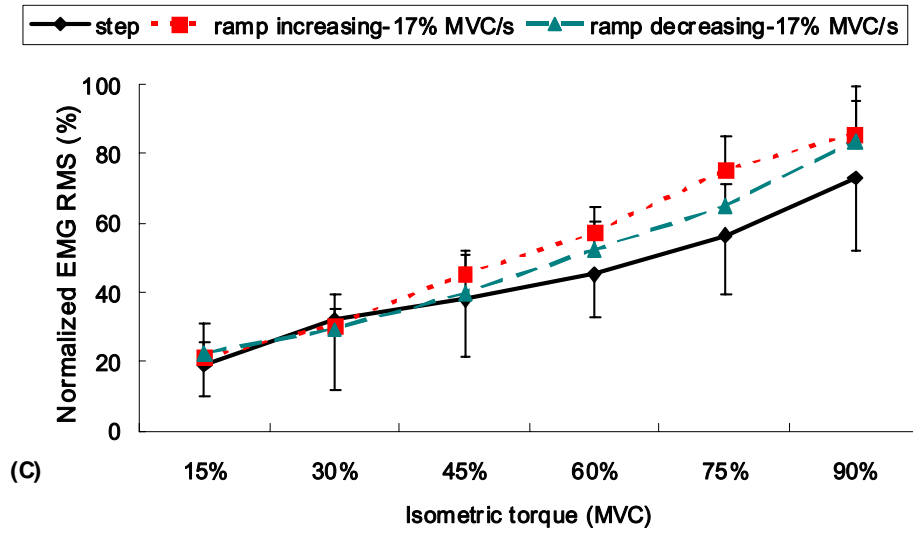
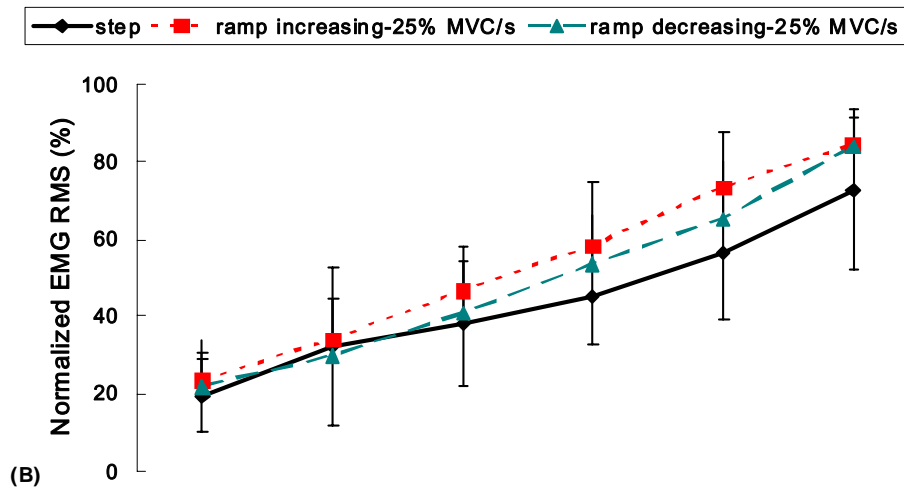
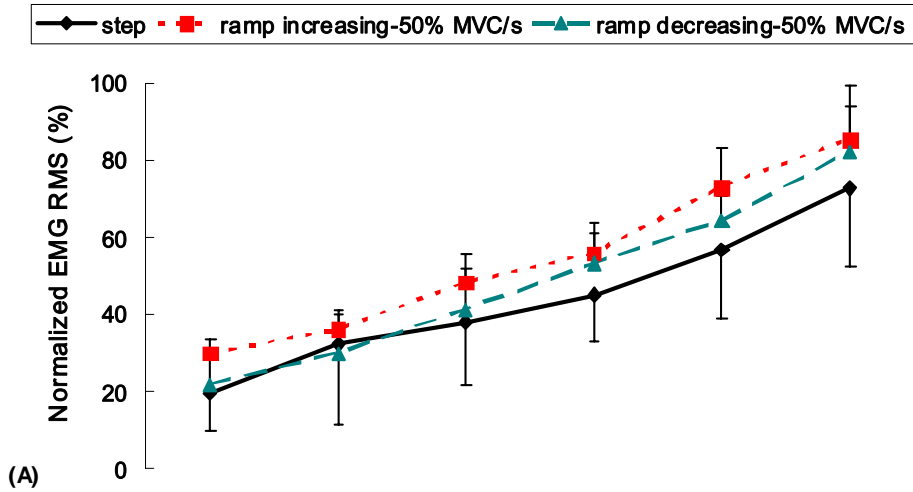
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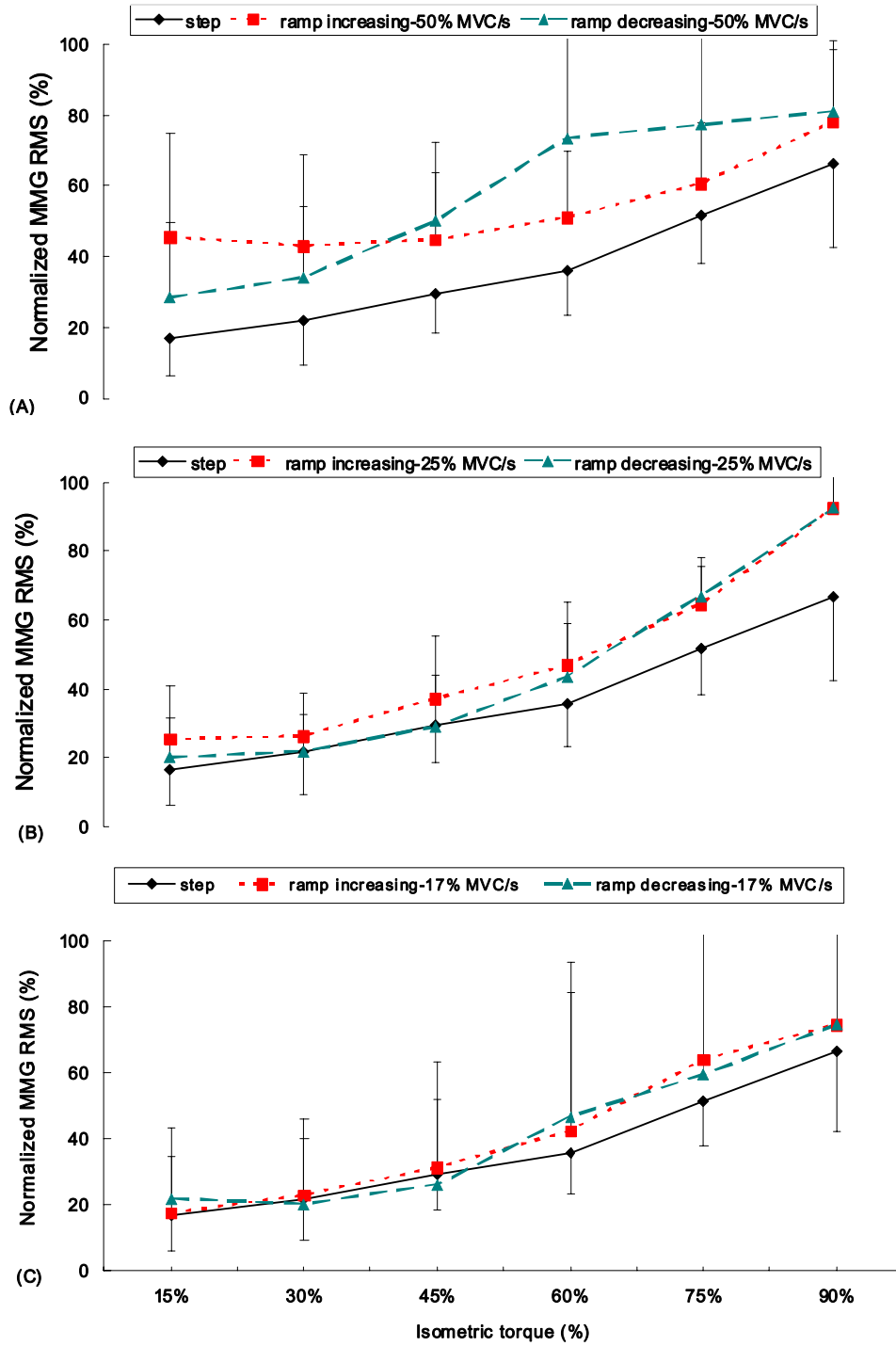
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6 **Fig. 9**



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2 Fig. 10



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2 Fig. 11

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