scientific reports

Check for updates

OPEN

Force overestimation during vascular occlusion is triggered by motor system inhibition

Yudai Takarada[1]✉ & Daichi Nozaki[2]

Low-intensity resistance exercise with vascular occlusion enhances human muscular strength by elevating neural drive to muscles, which is accompanied by the additional activation of fast-twitch fibers because of muscle fatigue. However, few previous studies have investigated the underlying neuromotor mechanisms from a perspective other than muscle fatigue. Notably, participants require more voluntary effort to exert muscular force to lift a weight immediately after vascular occlusion, indicating its acute effect on the force perception system without muscle fatigue. However, the major cause of force overestimation under these conditions remains unclear. We sought to elucidate the neural mechanism of force exertion combined with tourniquet-induced vascular occlusion, with special reference to exerted force perception, using motor evoked potentials in response to transcranial magnetic stimulation applied over the contralateral primary motor cortex as well as upper extremity H-reflex measurements. Rapid force overestimation was accompanied by the instantaneous inhibition of spinal motoneuron and corticospinal tract excitability. Thus, force overestimation may be caused by motor-related cortical areas functioning as the source of excitatory input to the corticospinal tract; participants would be unable to exert the same handgrip force as with normal blood flow without a compensatory input to the corticospinal tract from motor-related cortical areas.

Skeletal muscles can adapt to exercise stimuli via changes in their mechanical and metabolic properties. These changes are specific to the type of exercise stimulus; intense resistance exercise generally causes increases in muscular size and strength[1], whereas exercise with much smaller loads (i.e., endurance exercise) results in increased muscle oxidative capacity without a considerable increase in muscular size[2]. However, Takarada et al.[3–5] have previously shown that vascular occlusion induces marked hypertrophy and a concomitant increase in strength even when the exercise load for endurance exercise is much lower than that expected to induce muscular hypertrophy. These enhancing effects of ischemic resistance exercise on muscular strength have been supported by many other studies in the last 20 years. Nevertheless, little is known about the neural mechanisms underlying this enhancing effect of ischemic muscle contractions on human muscular strength, except via enhancement of the hypothalamic–pituitary system state (e.g., increased plasma concentrations of human growth hormone)[6] and enhancement of the spinal motoneuron state (e.g., the additional recruitment of fast-twitch fibers caused by muscle fatigue, with the intramuscular accumulation of metabolic subproducts such as lactate and protons)[3,4,6,7].

From a practical point of view, given its small mechanical stress and large effects on inducing muscular strength, the deliberate combination of low-intensity resistance exercise and moderate vascular occlusion is potentially useful not only for improving performance in athletes[4,5], but also for accelerating muscular strength recovery in aged people (including bedridden older adults) and for improving muscular function in patients undergoing postoperative rehabilitation[8,9]. We have noted that participants report the need for greater force to lift a weight when the resistance exercise begins with vascular occlusion but not repetitive muscle contractions. Indeed, participants require more voluntary effort to exert the muscular force to lift a weight when they are undergoing resistance exercise following vascular occlusion[10]. In this situation, the primary responsible factor for the overestimation of perceived force exertion during vascular occlusion is assumed to be the centrally generated motor command, as previously hypothesized by McCloskey[11–13]. However, the major cause of force overestimation remains unclear.

In the current study, we sought to elucidate the neural mechanism of force exertion when combined with vascular occlusion, with special reference to the perception of exerted force. To do this, we used motor evoked potentials (MEPs) in response to transcranial magnetic stimulation (TMS) applied over the contralateral primary motor cortex (M1) as well as upper extremity H-reflex measurements. First, we investigated the effects of vascular occlusion (with an applied tourniquet around the upper arm at approximately 200 mmHg) within 60 s

[1]Faculty of Sports Sciences, Waseda University, Saitama 359-1192, Japan. [2]Graduate School of Education, The University of Tokyo, Tokyo 113-0033, Japan. ✉email: takarada@waseda.jp

of handgrip force perception using a contralateral force-matching task, which was used to quantify the sensation of effort[11]. In this task, force is applied to one hand (the reference) and the participants attempt to exert the same amount of force with the other hand (the indicator) without visual feedback. The relationship between the level of force applied to the reference hand and that exerted by the indicator hand provides an objective indication of the sensation of effort in the reference hand. In the present study, the force-matching task was performed at given target forces (15%, 30%, or 45% of the maximal voluntary contraction [MVC]) with or without vascular occlusion. Second, we investigated the effects of vascular occlusion on the motor system state by examining MEPs in response to TMS applied over the contralateral M1 in the resting state and during handgrip force exertion at the three predetermined target force levels. Third, we investigated the effects of vascular occlusion on spinal motoneuron excitability by examining contraction-induced H-reflexes in response to median nerve stimulation (H-responses).

Of particular importance, we observed that rapid force overestimation occurred within 1 min of starting the occlusion; this was accompanied by the instantaneous suppression of both corticospinal tract and spinal motoneuron excitability. These results suggest that force overestimation during vascular occlusion may be caused by motor-related cortical areas functioning as the source of excitatory input to the M1 and/or the corticospinal tract to recruit more motoneuron drive to the muscles. This concept is supported by the finding that MVCs were unchanged between conditions with and without vascular occlusion. Our results provide the first objective evidence to suggest that rapid force overestimation during vascular occlusion is triggered by the instantaneous inhibition of both corticospinal tract and spinal motoneuron excitability.

Methods

Participants and general procedures

The present study was conducted in accordance with the Declaration of Helsinki of 1964, revised in 2013. All experimental procedures complied with relevant laws and institutional guidelines and were approved by the Human Research Ethics Committee of the Faculty of Sport Sciences of Waseda University (Approval Number: 2020 – 411). Three experiments examined the influence of transient occlusion on the perception of force (the bilateral force perception experiment), on MEPs in the flexor carpi radialis (FCR) muscle in response to TMS (the unilateral TMS experiment), and on contraction-induced H-responses in the FCR muscle (the H-response experiment) (Table 1). Thirty-four healthy Japanese right-handed males (evaluated using the Edinburgh Handedness Inventory)[14] were enrolled; 19 participated in the force perception and TMS experiments, completing one session in each experiment, and the other 15 participated in the H-response experiment, completing two sessions that were separated by at least 7 days. In each of the three experiments, all participants completed both the control and occlusion conditions. The force perception experiment was performed first, the TMS experiment was conducted within the following 7 days, and the H-response experiment was performed last. None of the participants reported neurological, psychiatric, or other contraindications to TMS[15]. Their mean age was 20.5 ± 1.0 years old (mean ± standard deviation, range 18–22 years). All participants provided both written and verbal informed consent. The mean height of the 15 participants in the H-response experiment was 169.8 ± 7.6 cm (mean ± standard deviation, range 160–190 cm), and that in the force perception and TMS experiments was 172.6 ± 6.9 cm (mean ± standard deviation, range 164–193 cm).

Bilateral force perception experiment

To first examine how vascular occlusion affects the perception of handgrip force, we used the contralateral force-matching method[11] because it allows quantification of the ongoing perception of exerted muscular force. In this method, participants are first required to generate a specified level of force by contracting the muscles of the reference limb in the presence of external feedback; they are then asked to match the subjective magnitude of this force using the muscles of the contralateral limb without the assistance of feedback.

Procedure and force measurement

Participants were placed in a seated position with their upper body upright. Their upper arm was inclined at about 45° in front of the body with the aid of an armrest. To measure handgrip force, the participants help handgrip devices (dimensions: approximately 154 (width) × 240 (depth) × 60 (height) mm; weight: approximately 0.65 kg; measuring range: 0–100 kg; resolution: 1/16,000 [amplifier], Takei Scientific Instruments Co., Ltd., Niigata, Japan) with a strain gauge (KFG-5–120-C1-16; Kyowa Electronic Instruments Co. Ltd., Tokyo, Japan) in their right and left hands. The measured force was amplified (AD240-A; TEAC Instruments Co., Kawasaki, Japan),

	Task	Intensity	Number of contractions (trials)	Rest period between control and occlusive conditions	Start time of occlusion	Duration of occlusion	Stimulation	Number of stimulation
Bilateral force matching experiment	squeezing handgrip devices with right for 6 s and the left hands for 3 s	15%, 30%, and 45%MVC	36 times: six times with 6-s rest periods at each given target force with or without occlusion	3 min	10 s before contraction	45 s	No stimulation	0
Unilaterla TMS experiment	squeezing handgrip devices by right hand for 3 - 4s	15%, 30%, and 45%MVC	36 times: six times with 3-s rest periods at each given target force with or without occlusion	3 min	10 s before contraction	48 s	TMS over left M1 during voluntary contration once every 4 - 5 s	36
H-response experiment	holding a 1kg weight plate and maintaining with right hand for 50 s	4% – 6% MVC	twise (with or without occlusion)	within 4 weeks	10 s before contraction	50 - 60 s	Median nerve stimulation during voluntary contration once every 5 s	20

Table 1. Specifics of task, contraction, intensity, rest, and stimulation in three experiments.

digitized (4 kHz), filtered using a Butterworth filter with a cutoff frequency of 10 Hz, and input into a visual feedback system (Panasonic CF-S9) with a display that showed the participants both the force exerted by their reference (right) hand and the predetermined target force level.

To begin, the maximum voluntary force of the right hand without vascular occlusion was measured. Because the force perception experiment involved participants with no experience in force exertion combined with vascular occlusion, participants performed three brief MVCs (1–2 s duration) on a cue given by an experimenter ("one, two, three, squeeze") with a 60-s inter-squeeze interval. The mean value was used as the maximal voluntary handgrip force, the value of which was subsequently used to calculate the three target force levels (15%, 30%, and 45% of the MVC). Under the experimental task, the participants were instructed to match the exerted force to the predetermined target force levels (the contralateral force-matching task). The participants were given at least 5 min of rest to eliminate the influence of postexercise facilitation after MVC, in accordance with a previous study[16]. The maximal voluntary handgrip force was re-measured both at the end of the experimental task (post-MVC) and in the occluded condition after 3 min (post-MVC with occlusion).

Each participant performed a force-matching task six times at each of the three target force levels without (control) or with (occluded) vascular occlusion, followed by a 3-min rest period. Six force-matching tasks (i.e., three target force levels with or without occlusion) were performed; thus, the numbers of participants in the 12 combinations of execution order were as follows: 15%/30%/45% MVC without occlusion: 2; 15%/45%/30% MVC without occlusion: 2; 30%/15%/45% MVC without occlusion: 2; 30%/45%/15% MVC without occlusion: 1; 45%/15%/30% MVC without occlusion: 1; 45%/30%/15% MVC without occlusion: 1; 15%/30%/45% MVC with occlusion: 1; 15%/45%/30% MVC with occlusion: 2; 30%/15%/45% MVC with occlusion: 2; 30%/45%/15% MVC with occlusion: 0; 45%/15%/30% MVC with occlusion: 2; 45%/30%/15% MVC with occlusion: 1.

Task

During the experimental task, only the force exerted in the reference hand was displayed on a personal computer (PC) monitor (Panasonic CF-S9) in the aforementioned visual system. Participants were seated in front of a table facing the monitor and were asked to align the force exerted by the reference hand with a predetermined target force indicated on the monitor using visual feedback. A start-of-trial cue ("one, two, three, right squeeze") was provided by an experimenter. After approximately 3 s, the experimenter provided another verbal signal ("one, two, three, left squeeze") and participants were required to squeeze the left handgrip device with their left (indicator) hand at a force level that matched the reference hand without visual feedback (the bilateral force-matching task) (Fig. 1a). When the participant was satisfied that they were applying a level of force with the indicator hand that matched that of the reference hand, they provided a verbal signal ("yes") to the experimenter. Visual feedback for the reference hand remained on the display throughout this period. An end-of-trial cue was provided by an experimenter approximately 7 s after the start of the reference-hand force exertion. The trial was performed six times with 6-s rest periods at each given target force with or without vascular occlusion, followed by a 3-min rest period.

Vascular occlusion was produced using a tourniquet, which was attached at the proximal end of the right upper arm. Once the participants confirmed that they felt no pain using the Verbal Rating Scale (VRS)[17] a pressure of approximately 200 mmHg began to be applied by pneumatic inflation approximately 10 s before the handgrip contractions started. This pressure was maintained throughout three handgrip contractions at each given target force with vascular occlusion and was released immediately after the end of the three handgrip contractions. The vascular occlusion in one force-matching task thus lasted for approximately 45 s. We confirmed that no participants experienced pins and needles in their right arm immediately after vascular occlusion. Before the force-matching task, all participants received the task instructions and practiced the handgrip force exertion until they were satisfied that they were able to apply a level of force with their indicator hand that matched that of the reference hand within approximately 1 s of beginning the handgrip force exertion of the indicator hand.

Analysis

Figure 1b, c show examples of the force data collected during the task. Because our preliminary work showed that it took approximately 1 s to perceive whether the forces exerted by both hands were the same after participants had fully practiced, the data used for the analysis were averaged over 500 ms, starting 1.5–2 s after the force was first applied to the handgrip by the indicator hand. Differences in exerted force between the reference and indicator hands were normalized as the matching value (MV [%]) as follows:

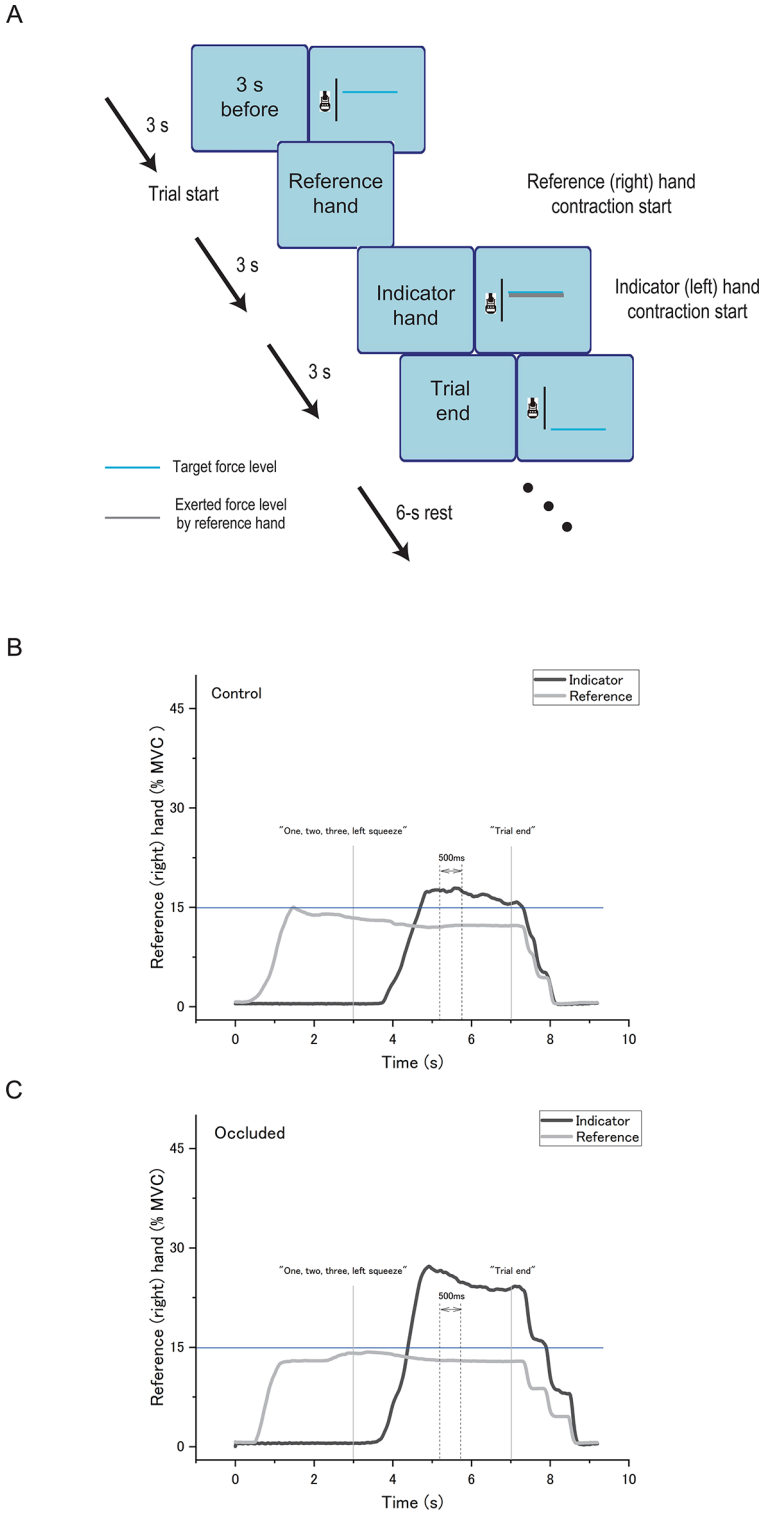
MV (%) = (handgrip force of the indicator hand – handgrip force of the reference hand) / handgrip force of the reference hand × 100.

Unilateral TMS experiment

To elucidate the possible neural mechanisms underlying the effects of vascular occlusion on perceived force exertion, we investigated the effects of transient vascular occlusion of the upper arm on the motor system state during both a resting state and force exertion.

Procedure and MEP measurement

Participants were placed in a seated position with their upper body upright. Their upper arm was inclined at about 45° in front of the body with the aid of an armrest and their forearm was supinated. Before the experimental task, 10 TMS stimuli were applied with an interstimulus interval of approximately 5 s in the presence or absence of vascular occlusion in the resting state, with more than 3 min of rest between the conditions without and with vascular occlusion (Fig. 2a). The experimental procedure was the same as that used in the following experimental task (Fig. 2b), with the exception of the handgrip muscular contractions. Force measurement was performed



using the same measurement system as that used in the force perception experiment. Six unilateral TMS tasks (i.e., three target force levels with or without occlusion) were performed in the same order as in the bilateral force perception experiment (see Bilateral force perception experiment).

Monophasic TMS pulses were administered to the left M1 (controlling the right hand) via a stimulator (M2002, Magstim, Whitland, UK) using a double-figure-eight-shaped coil (4150-00 Double 70-mm Alpha Coil, Magstim) with a maximum magnetic field strength of 1.55 T. Each participant sat upright with their elbows bent in front of them and their hands resting on their thighs. The M1 of each participant was mapped extensively using 5–10 stimuli, with the current direction of the coil placed perpendicular to the anatomically defined central sulcus, to find the area evoking the largest response from the FCR muscle (the hot spot). The TMS coil was then positioned over the hot spot of the left M1, which was determined as the area with the lowest resting motor threshold. This was defined as the lowest stimulus intensity that elicited MEPs with peak-to-peak amplitudes

◂**Fig. 1**. Experimental procedure. (**A**) Time course of a force perception experiment using the contralateral force-matching task. Approximately 3 s after starting the reference (right) hand contraction, participants were required to simultaneously squeeze the left handgrip device with their left (indicator) hand and to match force levels with the right hand without visual feedback. This trial was performed three times with a 6-s rest at each given target force (15%, 30%, 45% of the maximum voluntary contraction [MVC]) with or without vascular occlusion. One contralateral force-matching task lasted for approximately 45 s. (**B**, **C**) Typical recordings from a single participant of handgrip force during the contralateral force-matching task at a target force level of 15% of the MVC with and without vascular occlusion. Force data are expressed as the mean of three recordings. The recordings are aligned at the trial start (time 0). Blue line, target force level. Gray line, force exerted by the reference (right) hand. Black line, force exerted by the indicator (left) hand (i.e., perceived force). Force data were analyzed for the time period (bidirectional arrows) between the two vertically dashed lines. The two solid vertical lines indicate the time points of the two verbal signals provided by an experimenter: (1) to match the handgrip force applied by the reference (right) hand with the indicator hand, and (2) for the trial end (approximately 7 s after the start of the reference-hand force exertion).

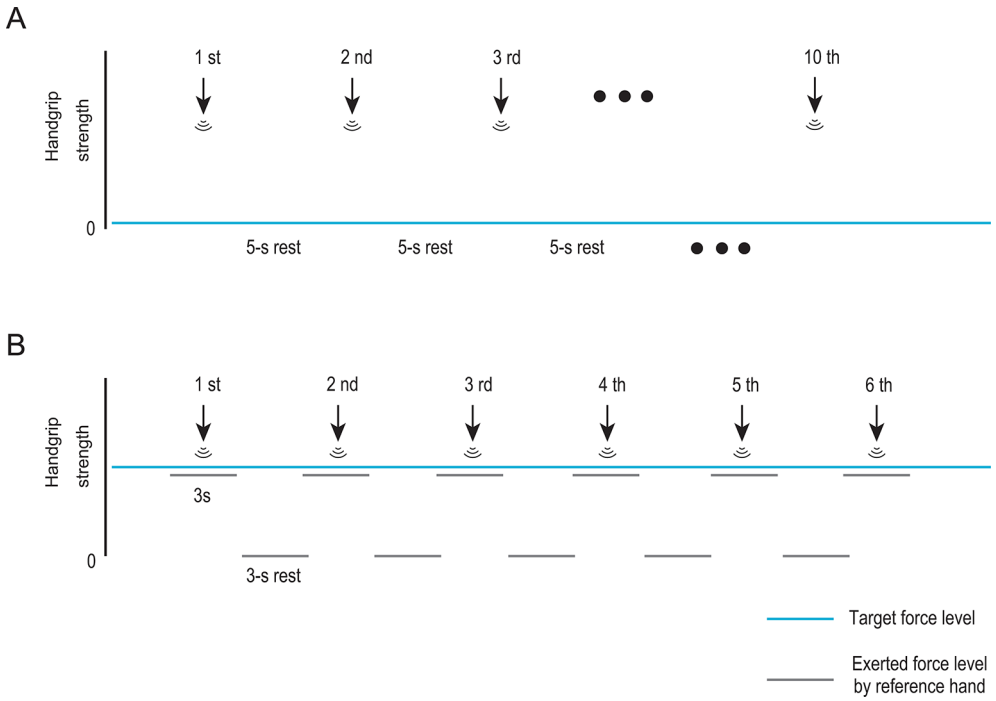


Fig. 2. Experimental procedure. First, 10 transcranial magnetic stimulation stimuli were applied at an interstimulus interval of approximately 5 s with or without vascular occlusion in the resting state, with more than a 3-min rest between the without and with vascular occlusion conditions (**A**). Second, the participant performed a unilateral force-matching task six times (approximately 3 s in duration), followed by an approximately 3-s rest period, at each of the three target force levels (15%, 30%, and 45% of the maximum voluntary contraction) with or without vascular occlusion (**B**). Force data are expressed as the mean of six recordings, which were spaced at least 5 min apart on the same day. One contralateral force-matching task lasted for approximately 48 s. The timing of each transcranial magnetic stimulation is indicated by an arrow. Stimulation was manually delivered over the target site 1–1.5 s after the participants began to exert hand-grip forces during each brief contraction.

greater than 50 μV in at least 5 of 10 trials[18]. The handle of the coil was pointed backward (approximately 45° laterally from the midsagittal line). During MEP recordings, participants were asked to remain in a resting state. The coil position was stabilized throughout the experiment using a coil stand constructed from multiple products (Manfrotto Distribution KK, Tokyo, Japan); however, we did not use a neuronavigation system to record the coil position. The optimal scalp position of M1 was marked directly onto the scalp with a black waterproof marker pen. The positioned coil was monitored continuously to maintain its consistent positioning throughout the experiment, and resting motor thresholds were 58.8% ± 8.7% (mean ± standard deviation, range 44–80%) of the maximum stimulator output.

Before the experimental tasks, the stimulus intensity was increased in 5–10% increments from 44 to 95% of the maximum stimulator output to determine the applied stimulus intensity for the unilateral force-matching task. The stimulus intensity needed to be sufficiently high for a single MEP waveform to be discriminated from the background electromyography (bEMG) activity[19]; two different muscular contraction intensities (approximately

75% and 100% of the MVC) were therefore adopted. The applied stimulus intensity was 70.1% ± 2.4% of the maximum stimulator output, equivalent to 115.2% ± 1.4% of the resting motor threshold. The applied stimulus intensity for each participant was constant in all conditions to allow the obtained measurements to be analyzed and compared within participants. Stimulation was manually delivered once over the target site at 1–1.5 s after the participants began to exert hand-grip forces during each brief contraction (3–4 s in duration), with a 3-s inter-squeeze interval in the unilateral force-matching task (Fig. 3a, b). Six measurement sessions (three target force levels with or without occlusion) were performed one time only within a single measurement session (Fig. 2b). Thus, the MEP was recorded six times for each measurement session and was recorded 36 times for each participant throughout the unilateral force-matching task.

Surface EMG was measured from the right FCR muscle via bipolar silver surface electrodes (10 mm in diameter, Nihon Kohden Co., Tokyo, Japan), with a constant interelectrode distance of 20 mm. The skin overlying the identified muscles was cleaned with alcohol pads prior to electrode placement. Signals (analysis time of 30 ms) were amplified using a bandpass filter (15 Hz–10 kHz) and digitized (MEG-6108; Nihon Kohden Co.) at a sampling rate of 4 kHz.

Task

During the experimental task, only the force exerted in the reference hand was displayed on a PC monitor (Panasonic CF-S9) in the same visual system as that used in the force perception experiment. Participants were seated in front of a table facing the monitor and were asked to align the force exerted by the reference hand with a predetermined target force indicated on the monitor using visual feedback (the unilateral force-matching task). The three predetermined target force levels were the same as those in the force perception experiment. A start-of-trial cue ("one, two, three, right squeeze") was provided by an experimenter. During the measurement session, the participant performed the unilateral force-matching task six times (approximately 3 s in duration), followed by an approximately 3-s rest period, at each of the three target force levels (15%, 30%, or 45% of the MVC) with or without vascular occlusion (Fig. 2b). A 3-min rest period was maintained between each measurement session. Under the occluded condition, the tourniquet was inflated to approximately 200 mmHg to restrict blood flow approximately 10 s before the handgrip contractions started. The vascular occlusion in one unilateral force-matching task thus lasted for approximately 48 s. The execution order was the same as that in the force perception experiment. Six measurement sessions (three target force levels with or without occlusion) were performed one time only within a single measurement session.

Analysis

To estimate the relative levels of responsiveness of the M1 to voluntary drive during voluntary handgrip contractions, the force produced by the superimposed twitch (superimposed twitch force) following TMS was expressed as a fraction of the pre-stimulus force at each TMS (Fig. 3a), in accordance with a previous study[20].

To measure bEMG, a rectified EMG signal with a period of 100 ms before TMS was integrated, with the force kept at the maximum force level (Fig. 3b). There were no trials with bEMG above 0.1 mV for the 100ms period. We calculated the averaged waveform of the MEP under the unilateral force-matching task (an average of six recordings) (see *Procedure and MEP measurement* for details). We then measured the latency from stimulus onset to the averaged MEP onset determined by visual inspection, as well as the peak-to-peak amplitude of each MEP from 10 ms to 40 ms after TMS, the size of which reflects corticospinal excitability[21,22] (Fig. 3c). These analyses were performed using analysis software (LabChart 7.3.8; ADInstruments, Tokyo, Japan). The silent period duration was taken as the time interval from the stimulus artifact to the return of continuous EMG[23,24] (Fig. 3c). Because it was difficult to determine the end of the silent period (because voluntary EMG activity recovers gradually rather than abruptly), the end of the silent period was determined as the moment at which the corresponding rectified EMG activity reached a value within two standard deviations of the rectified EMG signal in the period 100 ms before TMS[25,26], with careful visual inspection. However, two participants were excluded because the end of their silent period was relatively unclear— in one participant, a small burst of EMG occurred before the resumption of continuous activity, making it impossible to detect the silent period duration. These two exclusions meant that the data from just 17 participants were used for this experiment, and the trials of the 17 participants for the force perception experiment were adopted as the analysis targets.

H-response experiment

To examine how vascular occlusion affects spinal motoneurons during force exertion, we investigated the effects of transient vascular occlusion of the upper arm on the H-response during constant isometric contraction.

Procedure and H-response measurement

Participants were seated comfortably in a chair with their right forearm resting on a pillow. The elbow and shoulder were flexed at 100° and 15°, respectively. During recordings, the forearm was supinated and the wrist was flexed at approximately 15°. We tried to position the arms the same way across participants. The H-responses and motor responses were recorded with or without vascular occlusion at a pressure of 200 mmHg. Given that a previous report suggested that it is difficult to obtain H-responses from the FCR muscle in the absence of facilitation[27], each measurement was performed first at rest with or without vascular occlusion, and then with facilitation (a moderate voluntary contraction against resistance) with or without vascular occlusion within 4 weeks. In the facilitation condition, participants were asked to hold a 1-kg weight and maintain a constant background isometric contraction of the right FCR. Before these measurements, we estimated the effects of this weight on the neural drive to skeletal muscle, measured from EMG activity. In three healthy subjects (three males; 20–22 years old), the 1-kg weight produced an EMG signal that was approximately 4.1–5.8% of that recorded during the MVC of individual forearm muscles (i.e., the FCR).

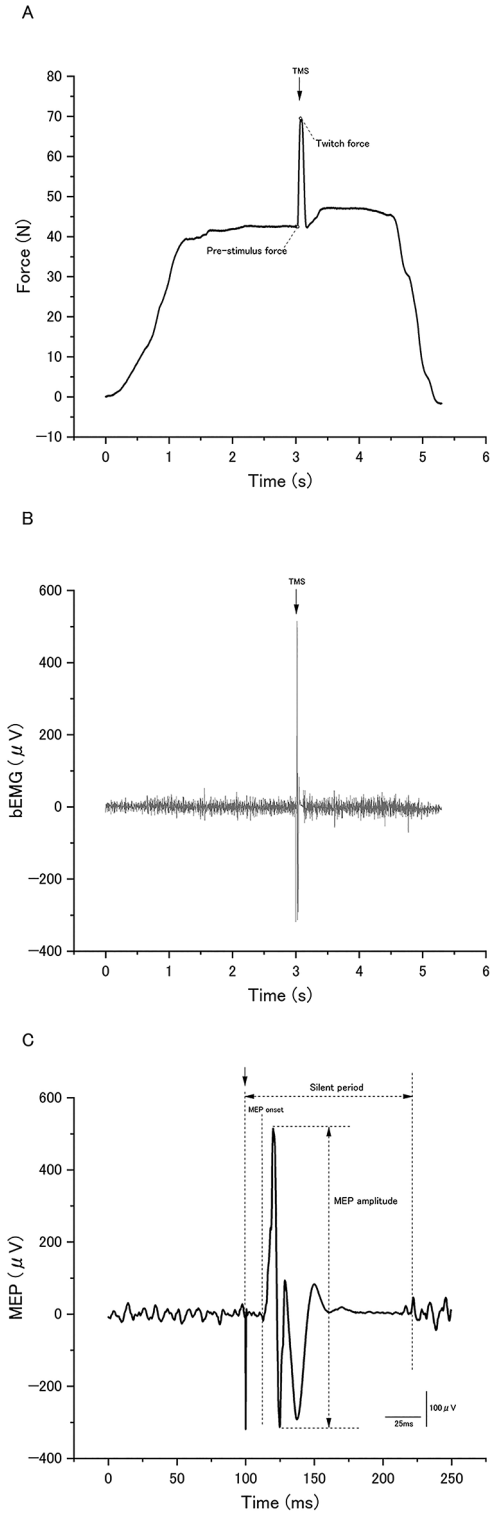


Fig. 3. Force, background electromyography (bEMG), and motor evoked potential (MEP) during isometric handgrip contractions. Typical recordings from a single participant of handgrip force (**A**), bEMG (**B**), and MEP waveforms (**C**) of the flexor carpi radialis during the unilateral force-matching task at the target force level (15% of the maximum voluntary contraction) without vascular occlusion. The recordings are expressed as an average of six recordings. The timing of transcranial magnetic stimulation (TMS) is indicated by an arrow. Dashed lines with gray dots indicate pre-stimulus and superimposed twitch forces. Bidirectional arrows indicate MEP amplitudes and the silent period duration.

Once the electrodes were applied (as described in the following paragraph), approximately 5 min of practice trials were used to familiarize the participants with the H-response stimulation and recording procedures. In all cases, the median nerve was stimulated once every 5 s, beginning at an intensity below the H-response threshold and increasing until the maximal motor (M)-response (M_{max}) was reached. To record the H-responses (10 traces), the stimulation intensity was set at an intensity that evoked reflexes of 5–10% of the M_{max} amplitude[28,29] on the ascending part of the recruitment curve[30]. The same stimulation intensity was repeated 10 times in each recording condition with or without vascular occlusion. The duration of each recording condition was approximately 50 s, followed by a 60-s rest. A pressure of 200 mmHg started to be applied by pneumatic inflation approximately 10 s before each recording condition; this pressure was maintained throughout each recording and was released immediately after recording ended. The vascular occlusion in one recording condition thus lasted for approximately 60 s.

Electrical stimuli were delivered by a stimulator (DC-940B, Nihon Kohden Co.) and an isolator (SM-940B, Nihon Kohden Co.) at each level of intensity at a rate of 0.2 Hz (duration, 1 ms)—which did not result in H-response depression when the H-responses were elicited during a background contraction of the FCR muscle[31]—to the right median nerve using flat-surfaced disk electrodes (12 mm wide, 46 mm long), with the cathode 24 mm proximal to the anode (9 mm in diameter). The electrodes were placed proximal to the antecubital fossa, approximately one-third of the distance from the lateral epicondyle to the bicep tendon[28,32]. After the appropriate stimulating and recording sites were determined, we marked the electrode locations with permanent marker to ensure that electrodes were placed in the same position across all stimulation intensities. The H-responses and motor responses in surface EMG were obtained from the right FCR muscle via bipolar silver surface electrodes (10 mm in diameter, Nihon Kohden Co.) attached to the skin with electroencephalography paste, with a constant interelectrode distance of 20 mm. FCR muscle bellies were identified by palpation during manually resisted wrist flexion. The skin overlying the identified muscles was cleaned with alcohol pads prior to electrode placement. A reference electrode was fixed on the skin overlying the lateral epicondyle near the elbow joint of the right arm. Signals (analysis time of 5 ms) were amplified using a bandpass filter (15 Hz–3 kHz) and digitized (MEG-6108; Nihon Kohden Co.) at a sampling rate of 10 kHz before being stored in the computer memory of a PC (LATITUDE D520, Dell Technologies, Round Rock, TX, USA). Wave data were inspected online and stored in the hard disk of the PC for the subsequent analysis of latencies and peak-to-peak amplitudes of H-responses and the M_{max} of the FCR. The M-response was elicited by the supramaximal stimulation of the median nerve at the antecubital fossa and was recorded with and without arterial occlusion.

To confirm the effects of transient vascular occlusion on the maximum voluntary handgrip force, we measured the maximal voluntary handgrip force in participants with experience of handgrip force exertion with vascular occlusion within 2 weeks after the H-response measurement. We asked these participants to perform three brief MVCs (1–2 s in duration) with the right hand with or without vascular occlusion on a cue given by an experimenter ("one, two, three, squeeze") with a 60-s inter-squeeze interval, as in the force perception experiment. Seven of the 15 participants first performed the brief MVCs without vascular occlusion, and then (after a 3-min rest period) performed them with vascular occlusion. The other eight participants performed the brief MVCs in reverse order. Vascular occlusion was produced using a tourniquet, which was attached at the proximal end of the right upper arm. A pressure of 200 mmHg started to be applied by pneumatic inflation approximately 10 s before each handgrip contraction started; this pressure was released immediately after the end of the handgrip contraction.

Analysis

The magnitudes of FCR H-responses and M-responses were evaluated by the peak-to-peak amplitudes of the EMG responses; these were measured in response to at least eight stimuli applied to the median nerve, and were averaged at each stimulation intensity for each participant[33,34]. The latencies of these averaged waves were measured from stimulus artifact to the start of each evoked action potential (Fig. 4a). The analyses were performed using analysis software (LabChart 8). The peak-to-peak amplitude values were also expressed as a proportion of the M_{max} values (Fig. 4b). All signals were visually inspected to ensure that the measurements obtained from the software were accurate. During the EMG measurements, force was exerted to hold a 1-kg weight and maintain a constant background isometric contraction of the right FCR.

The data used for the analysis were averaged over 500 ms after the peak was reached, and the mean value of three brief MVCs was used as the maximal voluntary handgrip force.

Statistics and power analysis

Significant differences in MEP amplitude and latency in a resting state, the magnitude and latency of the FCR H-response and M-response, and the maximum voluntary handgrip force with and without vascular occlusion were investigated using paired *t*-tests. Differences in maximal voluntary force among three MVCs (within-participant factors: MVC, post-MVC, and post-MVC with occlusion) were determined using one-way analysis of variance (ANOVA). Matching values, superimposed twitch forces, MEP amplitudes, silent period durations, and bEMG were analyzed using repeated-measures two-way ANOVA with within-participant factors of Intensity (15%, 30%, and 45% of the MVC), and Condition (with or without vascular occlusion). Greenhouse–Geisser corrections were applied when appropriate to adjust for non-sphericity, and degrees of freedom were changed using a correction coefficient. Post hoc multiple comparisons were performed using Holm's method. Data were analyzed using Jeffreys's Amazing Statistics Program (JASP ver. 0.17.2.1). A significance threshold of $p < 0.05$ was used for all tests. When the results of the main effect and interaction of the ANOVA are presented, Cohen's *d* and η^2 are also shown as an effect size index. The values of the effect size index (Cohen's *d)* were interpreted as 0.20, 0.50, and 0.80 for small, medium, and large effects, respectively[35]. η^2 is used to denote eta squared as an effect size index, the values of which were interpreted as 0.10, 0.25, and 0.40 for small, medium, and large effects,

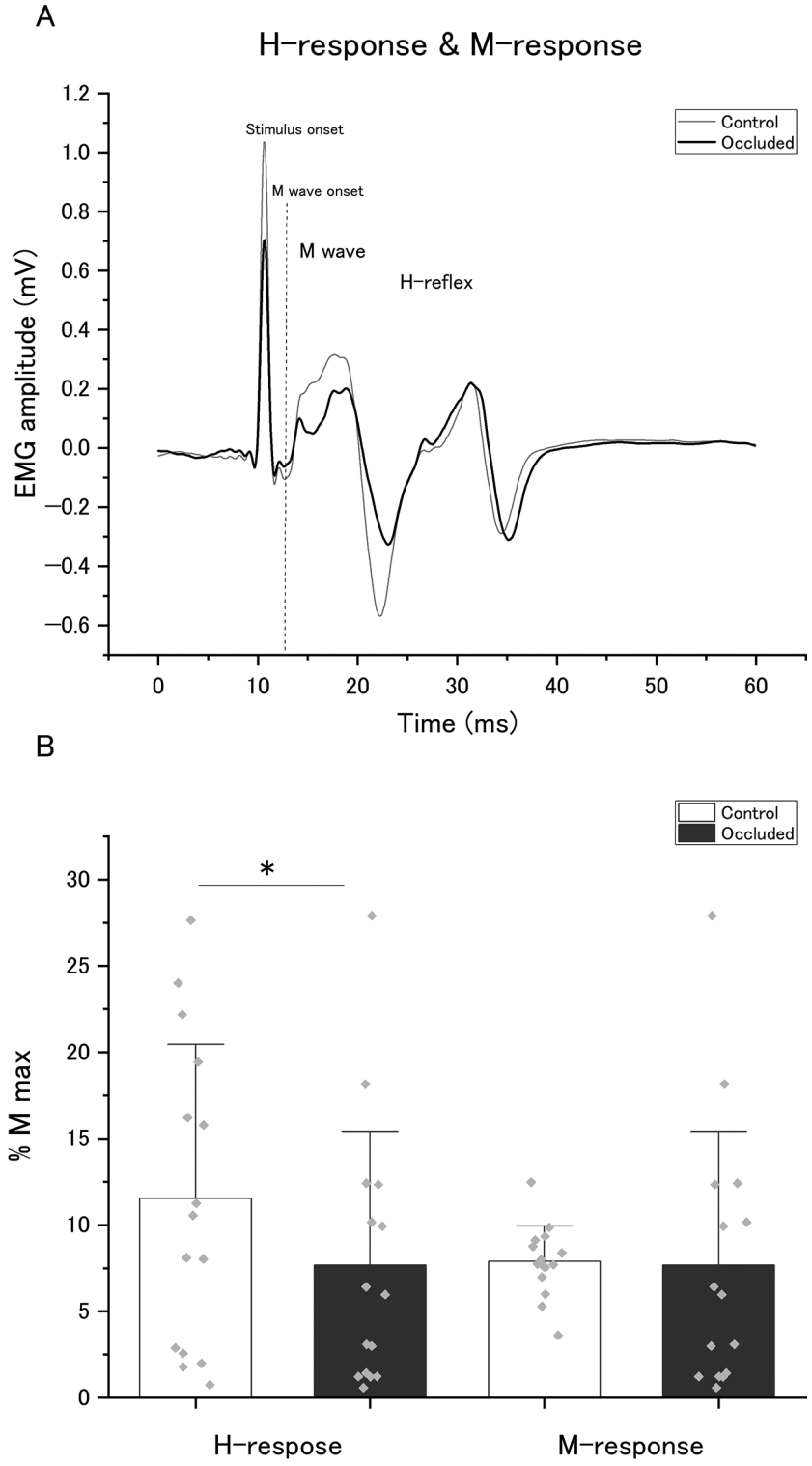


Fig. 4. Effects of transient vascular occlusion on contraction-induced H-reflexes. (**A**) Typical recordings from a single participant of the averaged flexor carpi radialis electromyography response (H-response) to median nerve stimulation (over eight responses) while holding a 1-kg weight with (black) or without (gray) vascular occlusion. The recording is aligned to the stimulation onset (time 0). (**B**) Mean contraction-induced H-response and M-response amplitudes with or without transient vascular occlusion. All values are normalized (% M_{max}) to the maximal M-responses while holding a 1-kg weight. Bar heights represent the mean across participants, with individual points plotted; error bars represent one standard deviation. $^{*}p < 0.05$, control versus occluded conditions. Vascular occlusion significantly attenuated contraction-induced H-reflex amplitudes.

respectively[35]. We therefore designed the experiment to have 80% power for detecting the effect size (0.25, η^2), using a significance level of 5%. We used G*Power 3.1 (Institut für Experimentelle Psychologie, Düsseldorf, Germany) to compute the required total sample size of the current study by conducting a repeated-measures ANOVA with within-participant factors, using 95% power (1 − β error probability). The computed required sample size was 14 participants for each experimental group. Unless otherwise noted as the standard deviation, data are expressed as the mean ± standard error of the mean.

Results

Bilateral force perception experiment

The participants were asked to determine the magnitude of the handgrip force exerted by the reference hand by producing a brief matching contraction with the indicator hand to numerically estimate the subjective effort required to exert the handgrip force of the reference hand. There were no significant differences in the maximal voluntary handgrip force before (321.4 ± 19.5 N) and after (327.4 ± 17.6 N) the contralateral force-matching task, or when combined with vascular occlusion (326.3 ± 14.4 N; $F[1.47, 23.52] = 0.58$; $p = 0.51$). Figure 4 shows the MVs in the control and occluded conditions at each level of target force; these were calculated as the difference in exerted handgrip force between the reference and indicator hands. The mean MVs in the control condition at the three different target levels were 54.6% ± 11.4%, 26.3% ± 9.1%, and 14.4% ± 8.3%, respectively, and those in the occluded condition were 83.2% ± 10.8%, 40.3% ± 8.6%, and 33.7% ± 8.4%, respectively. Two-way ANOVA revealed significant main effects of Intensity ($F[1.56, 25.01] = 24.2$; $p = 5.26 \times 10^{-6}$; effect size: $\eta^2 = 0.44$) and Condition ($F[1, 16] = 33.8$; $p = 2.61 \times 10^{-5}$), but no significant interaction between Intensity and Condition ($F[1.69, 27.15] = 2.81$; $p = 0.085$) (Fig. 5). These results indicate that, when combined with arterial occlusion, the handgrip force exerted by the indicator hand is significantly increased at all levels of target force during handgrip contractions of the reference hand. Together, these findings suggest that vascular occlusion leads to the overestimation of exerted force.

Unilateral TMS experiment

Resting-state MEP amplitudes were significantly lower in the occluded condition (179.7 ± 32.6 μV) than in the control condition (232.4 ± 40.0 μV; $t[17] = 3.97$, Cohen's $d = 0.96$; $p = 0.001$, paired t-test) (Fig. 6a, b). However, there were no significant differences in MEP amplitudes between the two conditions during force exertion, as shown in Table 2; Fig. 7a. Two-way ANOVA revealed no significant main effects of Intensity ($F[1.03, 16.57] = 2.43$; $p = 0.13$) or Condition ($F[1, 16] = 0.07$; $p = 0.79$), and no interaction between Intensity and Condition ($F[1.34, 21.48] = 0.082$; $p = 0.84$). There were no significant differences in MEP latencies between the two conditions during resting state (control: 14.4 ± 0.28 ms; occluded: 14.6 ± 0.27 ms; $t[17] = -1.52$, $p = 0.14$) or force exertion (main effects of Intensity [$F(1.52, 27.39) = 6.72$; $p = 0.007$], Condition [$F(1, 18) = 0.02$; $p = 0.88$], and interaction between Intensity and Condition [$F(1.91, 34.47) = 0.23$; $p = 0.78$]) (15% MVC, control: 12.1 ± 0.23 ms, occluded: 12.2 ± 0.28 ms; 30% MVC, control: 12.0 ± 0.53 ms, occluded: 12.1 ± 0.22 ms; 45% MVC, control: 11.8 ± 0.24 ms, occluded: 11.8 ± 0.23 ms).

The silent period duration was longer with vascular occlusion than without vascular occlusion. Two-way ANOVA revealed no significant main effect of Intensity ($F[1.37, 21.92] = 0.305$; $p = 0.661$) and no interaction

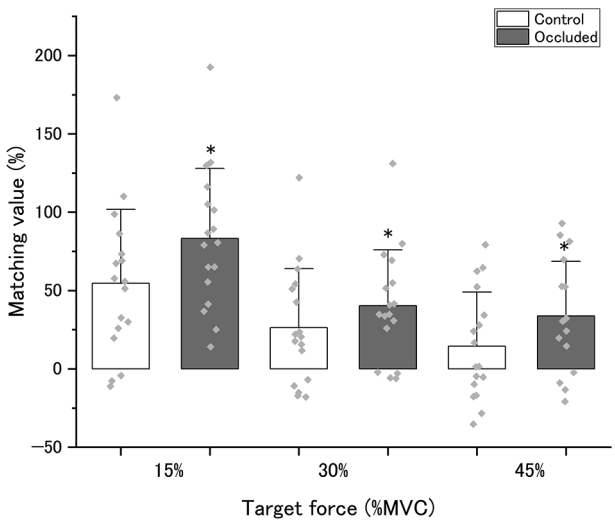


Fig. 5. Effects of transient vascular occlusion on sense of effort. Matching values with (filled) or without (unfilled) vascular occlusion at three different target forces (15%, 30%, and 45% of the maximum voluntary contraction [MVC]). Bar heights show the mean across participants, with individual points plotted; error bars represent one standard deviation. *$p < 0.05$, control versus occluded conditions. Note that at all three force levels (15%, 30%, and 45% of the MVC), the force exerted by the indicator (left) hand was significantly greater in the occluded condition than in the control condition.

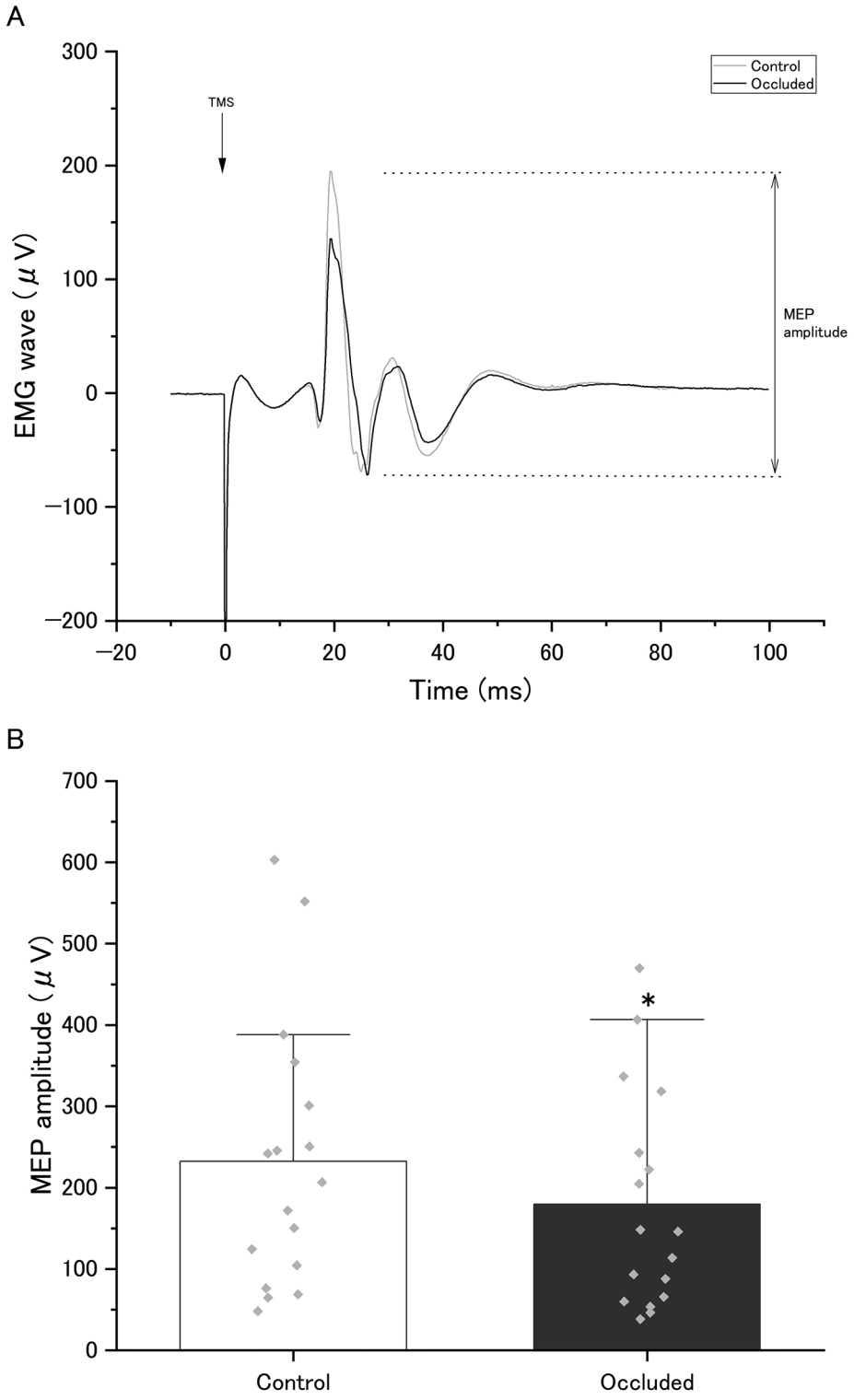


Fig. 6. Effects of transient vascular occlusion on motor evoked potential (MEP) amplitude during the resting state. (**A**) Typical recordings from a single participant of averaged MEP waveforms (averaged from 10 recordings) measured from the relaxed flexor carpi radialis muscle during the resting state with or without transient vascular occlusion. The timing of transcranial magnetic stimulation (TMS) is indicated by an arrow. (B) Mean MEP amplitudes during the resting state with or without transient vascular occlusion. Bar heights show the mean across participants, with individual points plotted; error bars represent one standard deviation. $^{*}p < 0.05$, control versus occluded conditions. Vascular occlusion significantly attenuated MEP amplitudes during the resting state.

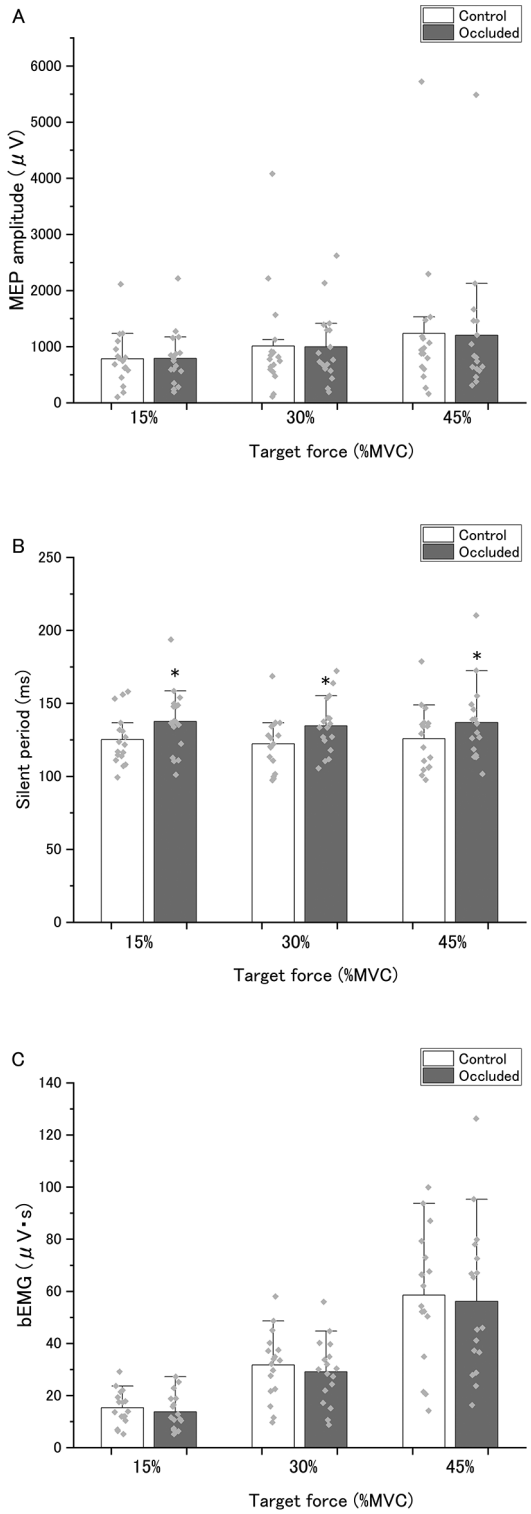


Fig. 7. Effects of transient vascular occlusion on motor evoked potential (MEP) amplitude, the silent period, and background electromyography (bEMG). (**A**) Amplitudes of MEPs of the flexor carpi radialis, (**B**) silent period durations, and (C) bEMG activity of the flexor carpi radialis during the unilateral force-matching task at the target force level (15% of the maximum voluntary contraction [MVC]) with or without transient vascular occlusion. Transient vascular occlusion significantly extended the duration of the cortical silent period. Bar heights show the mean across participants, with individual points plotted; error bars represent one standard deviation. $^{*}p < 0.05$, control versus occluded conditions.

	Superimposed twithc force (%)		Silent period (ms)		MEP amplitude (μV)		bEMG (μV·s)	
	Control	Occluded	Control	Occluded	Control	Occluded	Control	Occluded
15%MVC	53.5 ± 6.57	51.1 ± 8.0	125.1 ± 4.2	137.6 ± 5.4*	784.9 ± 114.7	791.2 ± 118.6	15.3 ± 1.59	13.7 ± 1.68
30%MVC	29.2 ± 4.6	29.5 ± 4.5	122.2 ± 4.4	134.6 ± 4.5*	1010.9 ± 225.8	997.0 ± 156.0	31.7 ± 3.13	29.1 ± 3.00
45%MVC	21.2 ± 3.0	19.2 ± 2.5	125.8 ± 5.1	136.9 ± 6.2*	1236.7 ± 306.1	1202.3 ± 293.9	58.5 ± 6.10	56.1 ± 7.03

Table 2. Twitch force, Silent period, MEP amplitude, and bEMG for conditions with and without occlusion.

	Control	Occluded
M-response latency (ms)	3.31 ± 0.12	3.2 ± 0.11
M-reponse amplitude (mV)	0.46 ± 0.05	0.41 ± 0.05
Mmax latency (ms)	2.81 ± 0.1	2.76 ± 0.11
Mmax amplitude (mV)	6.0 ± 0.67	6.1 ± 0.66
H-response latency (ms)	17.8 ± 0.64	17.7 ± 0.55
H-response amplitude (mV)	0.70 ± 0.18	0.51 ± 0.16*
H-response/Mmax (%)	11.5 ± 2.16	7.7 ± 1.87*
bEMG (mV · s)	0.031 ± 0.004	0.027 ± 0.004

Table 3. 3 H-response and M-response characteristics for conditions with and without occlusion.

between Intensity and Condition ($F[1.54, 24.06] = 0.073$; $p = 0.88$). However, there was a significant main effect of Condition ($F[1, 16] = 17.51$; $p = 7.00 \times 10^{-4}$; effect size: $\eta^2 = 0.18$) (Fig. 7b).

bEMG revealed no significant changes among the conditions during force exertion (main effects of Intensity [$F(1.07, 17.14) = 53.4$; $p = 8.31 \times 10^{-7}$], Condition [$F(1, 16) = 2.63$; $p = 0.12$], and interaction between Intensity and Condition [$F(1.10, 17.63) = 0.050$; $p = 0.84$]) (Fig. 7c).

Superimposed twitch force revealed no significant changes among the conditions during force exertion (main effects of Intensity [$F(1.49, 23.9) = 28.4$; $p = 2.28 \times 10^{-6}$], Condition [$F(1, 16) = 0.14$; $p = 0.71$], and interaction between Intensity and Condition [$F(1.38, 22.0) = 0.20$; $p = 0.73$]) (Table 2).

H-response experiment

The amplitudes of contraction-induced H-responses of the FCR were significantly lower in the occluded condition (0.51 ± 0.16 mV) than in the control condition (0.70 ± 0.18 μV; $t[14] = 3.31$, Cohen's $d = 0.53$; $p = 0.005$, paired t-test) (Table 3). However, there were no significant differences in M-response latencies ($t[14] = 1.67$, $p = 0.11$), M-response amplitudes ($t[14] = 1.13$, $p = 0.27$), M_{max} latencies ($t[14] = 1.52$, $p = 0.15$), M_{max} amplitudes ($t[14] = -0.43$, $p = 0.67$), H-response latencies ($t[14] = 1.03$, $p = 0.33$), or bEMG ($t[14] = 1.22$, $p = 0.24$) between the two conditions (Table 3; Fig. 4b). The H-response/M_{max} was significantly decreased ($t[14] = 3.88$, Cohen's $d = 0.46$; $p = 0.002$) with no changes in M-response amplitudes, expressed as a proportion of M_{max} ($t[14] = 1.76$, $p = 0.10$) (Fig. 4b). There were no significant differences in maximal voluntary handgrip force between the with (325.7 ± 19.3 N) and without (331.0 ± 18.0 N; $t[14] = -1.15$, $p = 0.26$) vascular occlusion conditions.

Together with the results of maximal voluntary handgrip force in the force perception experiment, our findings indicate that transient vascular occlusion has no effect on maximal voluntary handgrip force, regardless of prior experience of handgrip force exertion with vascular occlusion.

Discussion

In the present study, vascular occlusion by the application of a tourniquet to the proximal end of the reference (right) upper arm increased handgrip force by the indicator (left) hand in a contralateral force-matching task. This finding indicates that vascular occlusion causes the sensation of effort to increase, as evidenced by the overestimation of the exerted handgrip force. Furthermore, vascular occlusion significantly attenuated not only H-response amplitudes of the FCR in response to median nerve stimulation, but also MEP amplitudes in the

FCR muscle in response to TMS over the left M1 in the resting state. However, vascular occlusion combined with low-intensity handgrip contraction did not affect the bEMG or the MEP amplitude or latency in the FCR muscle. Together, these results suggest that vascular occlusion instantaneously inhibits both spinal motoneuron and corticospinal tract excitability, thus resulting in an overestimation of perceived force exertion. Moreover, the neural aspects of this force overestimation may be caused by motor-related cortical areas functioning as the source of excitatory input to the M1 and/or the corticospinal tract because the handgrip force level would be unable to be maintained without such a compensatory input to the spinal motoneurons via the M1 from motor-related cortical areas with increased activity in the neural centers.

It has been reported that, in the resting state, blood flow of the brachial artery is acutely restricted immediately after the initiation of occlusion (applied by an occlusion cuff attached to the proximal end of the upper arm at a pressure of 100 mmHg); during the occlusion, blood flow is maintained at approximately one-sixth of that before occlusion[3]. These observations indicate that, although it may not suppress the circulation completely (leading to vasodilatation), moderate vascular occlusion may compress the underlying arteries and veins[36], and might cause blood pooling in the capacitance vessels of the distal portion of the arm, with a concurrent decrease in blood flow through the arteries. Upon occlusion pressure release (i.e., reperfusion), blood flow immediately returns toward its resting level[3]. This post-occlusive hyperemia is enhanced only by low-intensity muscle contraction[3]. Similarly, a local distension of the vascular network with an enhancement of post-occlusive hyperemia is observed in low-intensity cycling exercise with vascular occlusion (applied by an occlusion cuff attached to the proximal end of both thighs), as a greater change in the diameter of the superficial femoral artery[37].

Vascular occlusion triggers the activity of group III and IV muscle afferent fibers even in the relaxing phase (without muscle contraction); this activity is directly proportional to the blood flow rate before occlusion[38]. The instantaneous activity of group III and IV muscle afferent fibers by arterial occlusion leads to more activated muscle (ischemic) contraction than that with natural blood flow (i.e., not ischemic)[38–42]. Furthermore, the acute reduction of arterial supply to the contracting muscle activates a new group of fibers, predominantly belonging to group IV, that is silent during normal contraction; these fibers are more activated by ischemic contractions than by those that are not ischemic[40,42]. It is thus reasonable to assume that the instantaneous activity of the group III and IV mechanosensitive units was increased in the ischemic handgrip contractions in the present study, although the vascular occlusion never caused pain (i.e., not as nociceptive stimuli), as indicated by unchanged pain/discomfort scale scores[17]. This may be because the vascular occlusion in the current study led to local distension of the vascular network. The vascular occlusion-induced instantaneous activity of group III and IV mechanosensitive units may have been partly responsible for the reduced H-response amplitudes (i.e., decreased reflex responses to the active motoneuron pool) in the condition with a 60-s vascular occlusion. Although few studies have investigated the impact of acute ischemia on the H-response and M-wave, one study reported that acute ischemia for 5 min via femoral artery occlusion reduces spinal excitability, as determined by the soleus H-reflex[43]. However, the precise mechanisms underlying such an instantaneous reduction in H-response amplitudes remain unclear.

In addition to mechanosensitive afferent fiber activity, the activity of metabosensitive afferent fibers is also increased by ischemic muscle contraction, and is additive to mechanosensitive afferent fiber activity. Vascular occlusion at approximately 100 mmHg for 5 min significantly increases plasma lactate concentrations even in the resting state; in combination with muscle contraction within 5 min, it markedly increases plasma lactate concentrations[3,6,37]. The resulting acidic intramuscular environment can stimulate sympathetic nerve activity through chemoreceptive reflexes, which are mediated by intramuscular metaboreceptors and group III and IV afferent fibers[44]. Moreover, the firing of these mechanosensitive and metabosensitive group III/IV muscle afferents not only decreases spinal motoneuron excitability[45] with a marked decline in the motor unit discharge rate[46], but also decreases the excitability of the contralateral M1[47].

In accordance with these neurophysiological alterations, vascular occlusion (applied by a tourniquet to the proximal end of the upper arm) in the present study significantly decreased not only the amplitudes of H-responses, but also the amplitudes of MEPs in the resting state, resulting in the corticospinal pathway generating less force for the same voluntary drive during occlusion. Nevertheless, no significant changes were observed in the amplitudes of MEP, bEMG, or superimposed twitch force during vascular occlusion combined with handgrip contraction. Together, these findings suggest that inhibition of the M1 and/or the corticospinal tract and spinal motoneurons may be compensated for by motor-related cortical areas functioning as the source of excitatory input to the spinal motoneurons to recruit more motoneurons to drive to muscles during the overestimation of exerted force. This is because handgrip force levels would be unable to be maintained without such a compensatory input to the M1 and/or the corticospinal tract from motor-related cortical areas. It is thus reasonable to assume that force overestimation during vascular occlusion is caused by motor-related cortical areas functioning as the source of excitatory input to spinal motoneurons via the corticospinal tract and/or the M1, with increased activity in the neural center (voluntary drive). This speculation is also supported by some previous studies[47,48] that suggest that the brain takes signals generated upstream of the M1 as an indicator of motor effort magnitude. Furthermore, the participants were estimating levels of handgrip force based on the perceived sense of effort[11], which may be linked to activity in neural centers upstream of the motor cortex[47], rather than a corollary discharge[49] of the motor command. A similar explanation likely applies to our results, indicating an increased silent period duration during handgrip contraction with vascular occlusion. When TMS is applied over the M1 during a voluntary contraction, the MEP is followed by a period of near silence in EMG, which lasts for more than 200 ms with a high-intensity stimulus[50,51]. Silent periods longer than 100 ms following TMS over the M1 are caused by inhibition within the cortex[23,50,52]; an increased silent period duration suggests increased cortical inhibition[53,54]. Thus, our observed lack of significant changes in the amplitudes of MEP, bEMG, and superimposed twitch force despite increased cortical inhibition may be because a compensatory function of motor-related cortical areas may act on the M1 and/or the corticospinal tract during the overestimation of

exerted force. This speculation is supported by the current result of unchanged handgrip force with and without occlusion; a compensatory input to the M1 and/or the corticospinal tract from motor-related cortical areas would enable participants to maintain the exerted force level, leading them to believe that both hands were using the same force in the force matching task. It should be noted, however, that the precondition for interpreting alterations in the occlusion condition without any detectable changes in MEP amplitude was that the peripheral properties of the neuromuscular system were very similar throughout the two conditions.

In addition to the proposed cortical compensatory mechanism for a reduction in spinal and corticospinal excitability with vascular occlusion, presumably caused by increased III/IV afferent inhibition, we cannot deny that force overestimation may also be triggered by events at the spinal level. Vascular occlusion reportedly attenuates H-response amplitudes of the FCR in response to median nerve stimulation, thus suggesting that the decreased afferent (e.g., somatosensory) input to motoneurons from the occluded limb is induced by the promotion of presynaptic inhibition[55]. This may be partly because MEP amplitude in the resting state was significantly decreased in the occluded condition. It is therefore possible that the overestimation of exerted force during occlusion may be caused by less feedback regarding afferent information for the same handgrip force level. These neurophysiological alterations at the spinal level may also be involved in increasing the silent period duration. This speculation is supported by the reported attenuation of P23 amplitudes generated in the contralateral somatosensory cortex (S1)[10]. However, we believe that participants might place an exclusive emphasis upon the sense of effort in situations in which there is a large mismatch between the sense of muscular force (mediated by large-diameter cutaneous, joint, or muscle afferents) and the sense of effort, as previously shown[12,48,56].

The present study provides evidence that rapid force overestimation during unfatigued contraction with vascular occlusion might be triggered by the instantaneous inhibition of both spinal motoneurons and corticospinal tract excitability, which may be caused by compensatory activity in motor-related cortical areas, with increased voluntary drive. That is, vascular occlusion was able to briefly enhance the motor system state to maintain the same level of output force. Given its small mechanical stress and instantaneous enhancing effect on motor system activity, a combination of low-intensity muscle contractions and moderate vascular occlusion is potentially useful for accelerating the recovery of muscular strength in aged people (including bedridden older adults) and for improving muscular function during postoperative rehabilitation[8,9]. This enhancing effect on motor system activity during ischemic contraction will also be useful for athletes and coaches because human voluntary force exertion in the motor system is relatively inhibited, and there is a latent ability to produce additional force that is unable to be produced during ordinary force exertion[19,57–60]. A combination of vascular occlusion and MVC will induce the enhancing effect on motor system activity, thus resulting in additional force production[3–5]. This is because maximal voluntary force is unchanged with and without vascular occlusion, despite vascular occlusion-induced motor system inhibition; in the present study, the motor system inhibition was complemented under restricted blood flow. If there is no excitatory neuronal input to the M1, which corresponds to the occlusion-induced inhibition of M1 and/or corticospinal tract, and spinal motoneurons, then muscular force production levels will decrease when combined with vascular occlusion.

In practice, repetitive muscular contractions combined with vascular occlusion in long-term exercise training can induce increased muscle mass and muscular strength, even when the levels of muscular force are much lower than those expected to induce muscular hypertrophy[3–5]. The neural mechanisms underlying the effects of an externally applied occlusive stimulus have been interpreted as the additional recruitment of fast-twitch fibers in an ischemic condition caused by muscle fatigue[3,4,6,7]. That is, fast-twitch muscle fibers would be preferentially and/or additionally activated even if the level of force were much lower than that expected to recruit them because of muscle fatigue (with increased metabolic products caused by muscular contraction)[3,6,7,61,62]. However, the preferential and/or additional recruitment of fast-twitch fibers would also be induced by increased compensatory neuronal inputs to spinal motoneurons immediately after vascular occlusion.

Several possible limitations of the present study should be considered. First, we cannot deny the possibility that ischemia of the forearm and/or the tourniquet itself causes the mechanical deformation of the nerves and interferes with conduction of the peripheral nerve. Indeed, several previous studies have demonstrated that tourniquet-induced vascular occlusion of the upper arm markedly attenuates the amplitudes of early-latency somatosensory evoked potentials and Erb's potentials to median nerve stimuli at the wrist[63,64]. However, we previously observed no significant changes in the peak latencies and amplitudes of nerve action potentials, early-latency somatosensory evoked potentials (i.e., N20) with median nerve stimuli during arterial occlusion (250 mmHg)[10]. This inconsistency in the effects of vascular occlusion on median nerve function may be mainly caused by differences in occlusion duration rather than degrees of occlusion. In previous studies, the vascular occlusion duration was relatively long (from 24 min[63] to 30 min[64]), whereas this duration did not exceed 150 s in our previous experiments or 60 s in the current study. It may therefore be that vascular occlusion with a short duration (≤ 150 s) does not induce any deterioration in median nerve function, at up to a tourniquet-induced inflation pressure of 250 mmHg. This conclusion is supported by the results of our M-response experiments, in which no significant differences between the control and occluded conditions were observed in the amplitudes or time integrals of M-waves, indicating that such arterial occlusion in the upper arm does not produce any substantial changes in the M-response.

Second, we must consider that the relatively small number of TMS trials (10) might have produced a large variability in MEP amplitudes, although the number of trials in the present study was decided based on measurements of MEP size in a previous study[25]. However, we believe that even if this effect occurred, it was unlikely to have canceled out the observed attenuation of MEP amplitudes caused by vascular occlusion during a resting state. This is because we were able to observe significant MEP amplitude attenuation in the occluded condition, despite relatively high variability in MEP amplitudes. Moreover, to prevent such confounding effects as much as possible, we investigated the effects of vascular occlusion on the motor system in a resting state before

the unilateral force-matching task in the TMS experiment. Under these conditions, there were no differences in the numbers of TMS trials between participants, suggesting that there were no effects of TMS trial numbers during handgrip contractions on MEPs in a resting state.

Third, methodological limitations should be noted because the present study used three different experiments: bilateral force perception, unilateral TMS, and H-response experiments. We used the contralateral force-matching method[11] in the bilateral force perception experiment to allow quantification of the ongoing perception of exerted muscular force. However, the application of TMS over the contralateral (left) M1 during the unilateral (right) muscular contraction in the force-matching task may have affected the formation of force perception more or less because of transient decreases in exerting force immediately after TMS. Thus, unlike in the bilateral force-matching task, the application of TMS over the left M1 during right muscular contraction had to be performed during handgrip force exertion by the right hand in the unilateral TMS experiment. Regarding H-response measurements, we used the H-reflex during voluntary muscle contraction for facilitation because of the relative difficulty and large variability when evoking the H-reflex in the FCR without facilitation[27]. The contraction-induced H-reflex method used in the present study, in which participants hold a light weight (e.g., 0.50 kg) to facilitate the H-reflexes of individual muscles (e.g., the FCR), has good reliability in terms of the amplitude and latency of the response[28,32,33]. We therefore believe that the contraction-induced H-response results reflect the activity in the spinal cord during ongoing voluntary muscle contraction with or without vascular occlusion. Nonetheless, we must note that, because of methodological differences in three different experiments, we cannot assume that the activities of the descending motor pathways were the same. We therefore recommend the careful interpretation of the present results: rapid force overestimation during vascular occlusion might be triggered by the instantaneous inhibition of both spinal motoneurons and corticospinal tract excitability. To address these limitations, a future study should investigate the effects of vascular occlusion on excitatory input from the motor cortex to the corticospinal tract and/or frontal and parietal cortex activity using functional magnetic resonance imaging.

Data availability

All data generated or analyzed during this study are included in this published article. Data will be made available from the corresponding author on reasonable request.

Received: 16 May 2024; Accepted: 5 March 2025
Published online: 13 March 2025

References

1. McDonagh, M. J. N. & Davies, C. T. M. Adaptive response of mammalian skeletal muscle to exercise with high loads. *Eur. J. Appl. Physiol. Occup. Physiol.* **52**, 139–155 (1984).
2. Holloszy, J. O. & Booth, F. W. Biochemical adaptations to endurance exercise in muscle. *Annu. Rev. Physiol.* **38**, 273–291 (1976).
3. Takarada, Y. et al. Effects of resistance exercise combined with moderate vascular occlusion on muscular function in humans. *J. Appl. Physiol.* **88**, 2097–2106 (2000).
4. Takarada, Y., Sato, Y. & N Ishii. Effects of resistance exercise combined with vascular occlusion on muscle function in athletes. *Eur. J. Appl. Physiol.* **86**, 308–314 (2002).
5. Takarada, Y., Tsuruta, T. & Ishii, N. Cooperative effects of exercise and occlusive stimuli on muscular function in low-intensity resistance exercise with moderate vascular occlusion. *Jpn J. Physiol.* **54**, 585–592 (2004).
6. Takarada, Y. et al. Rapid increase in plasma growth hormone after low-intensity resistance exercise with vascular occlusion. *J. Appl. Physiol.* **88**, 61–65 (2000).
7. Wernbom, M. & Aagaard, P. Muscle fibre activation and fatigue with low-load blood flow restricted resistance exercise—An integrative physiology review. *Acta Physiol.* **228**, 1–48 (2019).
8. Takarada, Y., Takazawa, H. & Ishii, N. Applications of vascular occlusion diminish disuse atrophy of knee extensor muscles. *Med. Sci. Sports Exerc.* **32**, 2035–2039 (2000).
9. Tanaka, Y. & Takarada, Y. The impact of aerobic exercise training with vascular occlusion in patients with chronic heart failure: aerobic exercise training with vascular occlusion in CHF. *ESC Heart Fail.* https://doi.org/10.1002/ehf2.12285 (2018).
10. Takarada, Y., Nozaki, D. & Taira, M. Force overestimation during tourniquet-induced transient occlusion of the brachial artery and possible underlying neuralmechanisms. *Neurosci. Res.* **54**, 38–42 (2006).
11. McCloskey, D. I., Ebeling, P. & Goodwin, G. M. Estimation of weights and tensions and apparent involvement of a "sense of effort". *Exp. Neurol.* **42**, 220–232 (1974).
12. McCloskey, D. I. Kinesthetic sensibility. *Physiol. Rev.* **58**, 763–820 (1978).
13. McCloskey, D. I. Corollary discharge and motor commands and perception. In: Handbook of Physiology (eds. Brookhart, J.M. & Mountcastle, V.B.) 1415–1447 (American Physiological Society,1981).
14. Oldfield, R. C. The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* **9**, 97–113 (1971).
15. Rossi, M., Hallett, P. M., Rossini, A. & Pascual-Leone Group SoTC. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin. Neurophysiol.* **120**, 2008–2039 (2009). .
16. Samii, A., Wassermann, E. M., Ikoma, K., Mercuri, B. & Hallett, M. Characterization of postexercise facilitation and depression of motor evoked potentials to transcranial magnetic stimulation. *Neurology* **46**, 1376–1382 (1996).
17. Berker, M. & Hughes, B. Using a tool for pain assessment. *Nurs. Times.* **86**, 50–52 (1990).
18. Rossini, P. M. et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalogr. Clin. Neurophysiol.* **91**, 79–92 (1994).
19. Takarada, Y. & Nozaki, D. Maximal voluntary force strengthened by the enhancement of motor system state through barely visible priming words with reward. *PLoS ON.* **9**, e109422 (2014).
20. Gandevia, S. C., Allen, G. M., Butler, J. E. & Taylor, J. L. Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. *J. Physiol.* **490**, 529–536 (1996).
21. Rothwell, J. C. Techniques and mechanisms of action of transcranial stimulation of the human motor cortex. *J. Neurosci. Methods.* **74**, 113–122 (1971).
22. Petersen, N. T., Pyndt, N. T. & Nielsen, J. B. Investigating human motor control by transcranial magnetic stimulation. *Exp. Brain Res.* **152**, 1–16 (2003).
23. Inghilleri, M., Berardelli, A., Cruccu, G. & Manfredi, M. Silent period evoked by transcranial stimulation of the human cortex and cervicomedullary junction. *J. Physiol.* **466**, 521–534 (1993).

24. Damron, L. A., Dearth, D. J., Hoffman, R. L. & Clark, B. C. Quantification of the corticospinal silent period evoked via transcranial magnetic stimulation. *J. Neurosci. Methods*. **173**, 121–128 (2008).
25. Groppa, S. et al. A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. *Clin. Neurophysiol.* **123**, 858–882 (2012).
26. Duclay, J., Pasquet, B., Martin, A. & Duchateau, J. Specific modulation of spinal and cortical excitabilities during lengthening and shortening submaximal and maximal contractions in plantar flexor muscles. *J. Appl. Physiol.* **117**, 1440–1450 (2014).
27. Miller, T. A., Newall, A. R. & Jackson, D. A. H-reflexes in the upper extremity and the effects of voluntary contraction. *Electromyogr. Clin. Neurophysiol.* **35**, 121–128 (1995).
28. Christie, A. D., Inglis, J. G., Boucher, J. P. & Gabriel, D. A. Reliability of the FCR H-reflex. *J. Clin. Neurophysiol.* **22**, 204–209 (2005).
29. Capozio, A., Chakrabarty, S. & Astill, S. Reliability of the TMS-conditioned monosynaptic reflex in the flexor carpi radialis muscle. *Neurosci. Lett.* **745**, 135622 (2021).
30. Pierrot-Deseilligny, E. & Mazevet, D. The monosynaptic reflex: a tool to investigate motor control in humans. Interest and limits. *Neurophysiol. Clin. Neurophysiol.* **30**, 67–80 (2000).
31. Burke, D., Adams, R. W. & Skuse, N. F. The effects of voluntary contraction on the H-reflex of human limb muscles. *Brain* 112, 417 – 33 (1989).
32. Jaberzadeh, S., Scutter, S., Warden-Flood, A. & Nazeran, H. Between-days reliability of H-reflexes in human flexor carpi radialis. *Arch. Phys. Med. Rehabil.* **85**, 1168–1173 (2004).
33. Stowe, A. M. et al. Between-day reliability of upper extremity H-reflexes. *J. Neurosci. Methods*. **170**, 317–323 (2008).
34. Burke, D. Clinical uses of H reflexes of upper and lower limb muscles. *Clin. Neurophysiol.* **1**, 9–17 (2016).
35. Cohen, J. *Statistical Power Analysis for the Behavioral Sciences* 2nd edn (Lawrence Erlbaum Associates, 1988).
36. Wolthuis, R. A., Bergman, S. A. & Nicogossian, A. E. Physiological effects of locally applied reduced pressure in man. *Physiol. Rev.* **54**, 566–595 (1974).
37. Takarada, Y. & Ito, M. Endocrine and hyperemic responses to low-intensity aerobic exercise with vascular occlusion. *Sport Sci. Res.* **9**, 350–365 (2012).
38. Haouzi, P., Chenuel, B. & Huszczuk, A. Sensing vascular distension in skeletal muscle by slow conducting afferent fibers: neurophysiological basis and implication for respiratory control. *J. Appl. Physiol.* **96**, 407–418 (2004).
39. Haouzi, P., Hill, J. M., Lewis, B. K. & Kaufman, M. P. Responses of group III and IV muscle afferents to distension of the peripheral vascular bed. *J. Appl. Physiol.* **87**, 545–553 (1999).
40. Mense, S. & Stahnke, M. Responses in muscle afferent fibres of slow conduction velocity to contractions and ischaemia in the Cat. *J. Physiol.* **342**, 383–397 (1983).
41. Mense, S. & Meyer, H. Different types of slowly conducting afferent units in Cat skeletal muscle and tendon. *J. Physiol.* **363**, 403–417 (1985).
42. Kaufman, M. P., Rotto, D. M. & Rybicki, K. J. Pressor reflex response to static muscular contraction: its afferent arm and possible neurotransmitters. *Am. J. Cardiol.* **62**, 58–62 (1988).
43. Zakutansky, D. W., Kitano, K., Wallace, J. P. & Koceja, D. M. H-reflex and motor responses to acute ischemia in apparently healthy individuals. *J. Clin. Neurophysiol.* **22**, 210–215 (2005).
44. Victor, R. G. & Seals, D. R. Reflex stimulation of sympathetic. *Outflow Dur. Rhythmic Exerc. Hum. Am. J. Physiol. Heart Circ. Physiol.* **257**, H2017–H2024 (1989).
45. Martin, P. G. et al. Fatigue sensitive afferents inhibit extensor but not flexor motoneurons in humans. *J. Neurosci.* **26**, 4796–4802 (2006).
46. Lowe, T. W., Tenan, M. S., Shah, K. & Grifn, L. Low–load blood flow restriction reduces time–to–minimum single motor unit discharge rate. *Exp. Brain Res.* **241**, 2795–2805 (2023).
47. Carson, R. G., Riek, S. & Shahbazpour, N. Central and peripheral mediation of human force sensation following eccentric or concentric contractions. *J. Physiol.* **539**, 913–925 (2002).
48. Takarada, Y., Mima, T., Abe, M., Nakatsuka, M. & Taira, M. Inhibition of the primary motor cortex can alter one's sense of effort: effects of low-frequency rTMS. *Neurosci. Res.* **89**, 54–60 (2014).
49. Sperry, R. W. Neural basis of the spontaneous optokinetic response produced by visual neural inversion. *J. Comp. Physiol. Psychol.* **43**, 482–489 (1950).
50. Fuhr, P., Agostino, R. & Hallett, M. Spinal motor neuron excitability during the silent period after cortical stimulation. *Electroencephalogr. Clin. Neurophysiol.* **81**, 257–262 (1991).
51. Holmgren, H., Larsson, L-E. & Pedersen, S. Late muscular responses to transcranial cortical stimulation in man. *Electroencephalogr. Clin. Neurophysiol.* **75**, 161–172 (1990).
52. Triggs, W. J. et al. Cortical and spinal motor excitability during the transcranial magnetic stimulation silent period in humans. *Brain Res.* **628**, 39–48 (1993).
53. Ziemann, U. & Hallett, M. Basic neurophysiological studies with TMS. In Transcranial Magnetic Stimulation in Neuropsychiatry (eds (eds George, M. S. & Belmaker, R. H.) 45–98 (American Psychiatry, (2000).
54. Säisänen, L. et al. Factors influencing cortical silent period: optimized stimulus location, intensity and muscle contraction. *J. Neurosci. Methods*. **169**, 231–238 (2008).
55. McNeil, C. J., Butler, J. E., Taylor, J. L. & Gandevia, S. C. Testing the excitability of human motoneurons. *Front. Hum. Neurosci.* **7**, 152 (2013).
56. Sidhu, S. K. et al. Fatigue-related group III/IV muscle afferent feedback facilitates intracortical Inhibition during locomotor exercise. *J. Physiol.* **596**, 4789–4801 (2018).
57. Moritani, T. et al. Oxygen availability and motor unit activity in humans. *Eur. J. Appl. Physiol.* **64**, 552–556 (1992).
58. Sundberg, C. J. Exercise and training during graded leg ischaemia in healthy man with special reference to effects on skeletal muscle. *Acta Physiol. Scand. Suppl.* **615**, 1–50 (1994).
59. Ikai, M. & Steinhaus, A. H. Some factors modifying the expression of human strength. *J. Appl. Physiol.* **16**, 157–163 (1961).
60. McComas, B. A. Y. Extent of motor unit activation during effort. *J. Appl. Physiol.* **51**, 1131–1135 (1981).
61. Takarada, Y. & Nozaki, D. Motivational goal-priming with or without awareness produces faster and stronger force exertion. *Sci. Rep.* **8**, 10135 (2018).
62. Takarada, Y. & Nozaki, D. The unconscious mental inhibiting process of human maximal voluntary contraction. *Psychol. Res.* **86**, 1458–1466 (2022).
63. Nishihira, Y. et al. Somatosensory evoked potentials following voluntary movement during upper arm compression. *Electromyogr. Clin. Neurophysiol.* **36**, 21–28 (1996).
64. Yamada, T., Muroga, T. & Kimura, J. Tourniquet-induced ischemia and somatosensory evoked potentials. *Neurology* **31**, 1524–1529 (1981).

Acknowledgements

The authors would like to thank Mr. Shinichi Shioya for technical assistance with the H-reflex stimulation and recording procedures.

Author contributions

The complete list of author contributions to the paper according to the CRediT model is as follows: (1) Conceptualization Y.T.; (2) Data curation Y.T.; (3) Formal analysis Y.T.; (4) Grant acquisition Y.T.; (5) Investigation Y.T.; (6) Methodology Y.T.; (7) Project administration Y.T.; (8) Resources Y.T.; (9) Software Y.T.; (10) Supervision Y.T.; 11. Validation Y.T.; 12. Visualization Y.T.; 13. Writing original draft Y.T.; 14. Writing-review and editing Y.T. and D.N.; 15. Statistical Analysis Y.T.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to Y.T.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.