Corticospinal drive does not contribute to increased torque production in the presence of residual force enhancement

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Abstract

Following active muscle stretch, a muscle's force capacity is increased, which is known as residual force enhancement (rFE). As earlier studies found modulations of cortical excitability in the presence of rFE, this study aimed to test whether corticospinal drive contributes to rFE. Fourteen participants performed submaximal plantar flexion stretch-hold and fixed-end contractions at 30% of their maximal voluntary soleus muscle activity in a dynamometer. During the steady state of the contractions, participants either received subthreshold or suprathreshold transcranial magnetic stimulation (TMS) of their motor cortex while triceps surae muscle responses to stimulation were obtained by electromyography (EMG) and net plantar flexion torque was recorded. B-mode ultrasound imaging was used to confirm muscle stretch during stretch-hold contractions in a subset of participants. Following stretch of the plantar flexors, an average rFE of 7% and 11% was observed for contractions with subthreshold and suprathreshold TMS, respectively. 41-46 milliseconds following subthreshold TMS, triceps surae muscle activity was suppressed by 19-25%, but no difference in suppression was found between contraction conditions. Similarly, the reduction in plantar flexion torque following subthreshold TMS was not different between contraction conditions. Motor evoked potentials, silent periods and superimposed twitches following suprathreshold stimulations were also not different between contraction conditions. As stimulations of the motor cortex by TMS did not result in any differences between stretch-hold and fixed-end contractions, we conclude that corticospinal drive does not contribute to the increased torque production in the presence of rFE following active muscle stretch.

New & Noteworthy

This study tested whether corticospinal drive contributes to the increased torque capacity in the presence of rFE. Through subthreshold and suprathreshold TMS of the motor cortex,

triceps surae muscle activity was respectively supressed or increased in the presence of rFE and during a reference contraction without rFE. As similar responses were observed between contraction contractions, we conclude that corticospinal drive likely does not contribute to the increased torque capacity in the presence of rFE.

Keywords: eccentric contraction, active muscle stretch, neural control, cortical excitability, torque enhancement

Introduction

It is well known that stretch of an active muscle results in increased force production during the isometric steady state following stretch compared with the steady-state force produced at the same muscle length and activation level during a fixed-end contraction. This is referred to as residual force enhancement (rFE), which was initially investigated *in situ* (Abbott and Aubert, 1952) and in isolated muscle fibres (Edman et al., 1982). Edman et al. (1982) suggested that rFE after active muscle stretch results from non-uniformities in sarcomere lengths. Since then, a variety of studies have investigated the development of rFE and suggested additional potential underlying mechanisms. These suggestions include stretch-induced increases in the number of attached cross-bridges, an increase in the average cross-bridge force and/or the attachment of the second myosin head (for topical reviews, see Edman, 2012; Herzog, 2014; Rassier, 2012). Additionally, titin could potentially explain rFE by increasing its stiffness in the presence of calcium and by potentially interacting with actin (Freundt and Linke, 2019; Herzog, 2018; Nishikawa, 2016).

Complementary to *in vitro* research, several *in vivo* studies have investigated rFE during electrically-stimulated and voluntary contractions (for reviews, see Chapman et al., 2018; Seiberl et al., 2015). rFE during voluntary contractions has been observed for small muscles of the thumb (Lee and Herzog, 2002), for large muscles of the lower limb (Pinniger and Cresswell, 2007; Seiberl et al., 2010) and during multi-joint multi-muscle contractions of the lower limbs (Hahn et al., 2010). The potential relevance of rFE during human movement was demonstrated in studies that investigated rFE during submaximal voluntary contractions and at joint angle configurations that mimic those of human locomotion (Paternoster et al., 2016; Seiberl et al., 2013).

Besides rFE, several *in vivo* studies have documented an activation reduction (AR) during the steady state after active muscle stretch. In contrast to rFE, which is observed when muscle

activity is matched between stretch-hold (STR) and fixed-end reference (REF) contractions, AR occurs when force or torque is matched between contraction conditions. AR refers to a reduced muscle activity level needed to maintain a given force/torque following active muscle stretch compared with fixed-end contractions at the same final muscle length. Several studies have reported AR and concluded that neuromuscular efficiency after active muscle stretch is increased, which might in part be explained by enhanced passive forces due to increased titin stiffness (Altenburg et al., 2008; Jones et al., 2016, 2016; Mazara et al., 2018; Oskouei and Herzog, 2005; Paquin and Power, 2018; Seiberl et al., 2012). However, Paquin and Power (2018) also found a rightward shift in the EMG-torque relationship following active stretch compared with fixed-end reference conditions, which indicates that rFE might alter the neuromuscular activation strategy.

Despite the presence of rFE after active muscle stretch during submaximal and maximal voluntary contractions, stretch contractions are often associated with neural inhibition (Duchateau and Enoka, 2016). However, whether neural inhibition occurs during active stretch under voluntary activation remains controversial (Hahn, 2018). The potentially unique control of stretch contractions and the observation of AR following active stretch has motivated a number of studies to investigate the neural control and/or neural modulations that occur during the isometric steady state following active muscle stretch (Altenburg et al., 2008; Contento et al., 2019; Hahn et al., 2012; Sypkes et al., 2018). For example, Altenburg et al. (2008) examined single motor unit behaviour of the vastus lateralis muscle during AR after active muscle stretch. These authors found similar discharge rates of VL motor units between STR and REF conditions, which led them to conclude that a derecruitment of motor units might have occurred during AR (Altenburg et al. 2008). Two further studies have used motor evoked potentials (MEPs) and cervicomedullary motor evoked potentials (CMEPs) to investigate modulations to the excitability of cortical and spinal sites in the presence of rFE (Hahn et al., 2012; Sypkes et al., 2018). During submaximal dorsiflexion contractions, Sypkes

et al. (2018) found unchanged TA MEPs, but reduced TA CMEPs, following active muscle stretch compared with fixed-end reference contractions. This is in line with the idea of reduced motor unit recruitment (Altenburg et al. 2008) and might be caused by tension-related afferent feedback as assessed by tendon-evoked inhibitory reflexes (Contento et al. 2019). Contrary to these findings under submaximal contraction intensities, Hahn et al. (2012) found larger MEPs, but unchanged CMEPs, following stretch of the plantar flexors during maximal voluntary contractions. Both the results of Hahn et al. (2012) and Sypkes et al. (2018) could be interpreted as an increase in cortical excitability in the presence of rFE. Further, enlarged V-waves during rFE in the Hahn and colleagues (2012) study suggest a greater motoneuronal output and/or an increase in stretch reflex excitability after active stretch (Hahn et al. 2012).

The above findings suggest that the underlying mechanisms of rFE during voluntary contractions are not purely mechanical in nature. However, it remains unclear whether the observed increase in cortical excitability contributes to the increased torque production in the presence of rFE. Therefore, this study was designed to investigate whether corticospinal drive contributes to increased torque production following active stretch of the plantar flexor muscles during submaximal voluntary contractions. This was examined by inhibiting and facilitating the motor cortex via transcranial magnetic stimulation (TMS) in the presence (stretch-hold condition) and absence (fixed-end reference condition) of rFE.

Based on previous research (Hahn et al., 2012; Sypkes et al., 2018), we predicted that inhibiting motor cortical neurons by subthreshold TMS (Davey et al., 1994; Petersen et al., 2001; Zuur et al., 2010) would lead to a larger suppression in muscle activity (EMG) and plantar flexion torque in the presence of rFE compared with fixed-end reference contractions with a matched background muscle activity. Conversely, facilitation of cortical interneurons and pyramidal neurons by suprathreshold TMS (Rossini et al., 2015; Rothwell, 1997) was predicted to result in greater superimposed twitches in the presence of rFE compared with the

fixed-end reference contractions with a matched background muscle activity. If both predictions hold, this would indicate that corticospinal drive contributes to the increased torque production in the presence of rFE.

Materials and Methods

Participants

Fourteen recreationally-active participants (six women, 26.7 ± 5.3 yrs., 1.77 ± 0.11 m, and 74.0 ± 16.8 kg) voluntarily participated in this study after providing free written informed consent. Participants were free of any neuromuscular disorders and injuries to their right lower limb. The experimental protocol was approved by the local Ethics Committee of the Faculty of Sport Science at Ruhr University Bochum, Germany.

Experimental set-up

During the experiment, the participants laid prone on a dynamometer bench with their upper body supported by their forearms and their right foot tightly strapped onto the footplate attachment of a dynamometer (IsoMed 2000, D&R Ferstl GmbH, GER). The axes of rotation of the ankle joint and the dynamometer were aligned prior to testing and the foot was firmly secured to the footplate using three straps (forefoot, ankle, heel) to minimize heel lift during the plantar flexion contractions. Additionally, the participants' hips were secured to the dynamometer bench with a belt. A computer monitor positioned in front of the participants was used to provide visual feedback of their soleus muscle activity (moving 0.25-s root-mean-square amplitude calculation) during the plantar flexion contractions. Net ankle joint torques and ankle joint angles of the right leg were sampled at 1 kHz using a 16-bit Power-3 1401 and Spike2 data collection system (v.8.01, Cambridge Electronics Design, Cambridge, UK)

Surface electromyography

Muscle activity and responses to TMS from soleus (SOL), medial gastrocnemius (MG) and lateral gastrocnemius (LG) muscles were recorded via bipolar surface electromyography (EMG). After skin preparation, self-adhesive surface electrodes (Ag/ AgCl, Kendall, ECG Electrodes, 8 mm recording diameter) were positioned with an inter-electrode distance of 20 mm over the triceps surae muscle bellies following the recommendations of SENIAM (Hermens et al., 1999). A reference electrode was placed over the right fibular head. Cables were fixed to the skin with tape to prevent motion artefacts. EMG signals were amplified 1000 times by a multichannel analogue amplifier (AnEMG12, Bioelectronica, Turin, IT) and band-pass filtered between 10 Hz and 4.4 kHz, prior to being sampled at 5 kHz. EMG and torque/angle data were synchronised and sampled using the same analogue-to-digital converter and software described above.

Transcranial Magnetic Stimulation (TMS)

TMS (MagPro Compact, MagVenture, Farum, Denmark) was used to either inhibit or facilitate the motor cortical area of the right plantar flexor muscles in the left hemisphere, slightly left of the vertex, via A double-cone coil (D-B80 Butterfly Coil, MagVenture, Farum, Denmark). The coil had monophasic current running through its centre in an anterior posterior direction. The vertex was marked on the scalp and defined as halfway between the left and right processus zygomaticus ossis temporalis and halfway between the os nasale and the external occipital protuberance. The vertex location helped to find the optimal location for TMS of the motor cortical area innervating the right plantar flexor muscles (TMS hotspot), which is generally defined as the position in which a single pulse stimulation evokes the largest peak-to-peak MEP amplitude in the target muscle (Siebner and Ziemann, 2007). In order to find the TMS hotspot, several stimuli were delivered while the TMS coil was slightly left, in front or behind the vertex, while participants performed fixed-end plantar flexion (PF) contractions at 30% of their maximal voluntary activity (MVA). Once the TMS hotspot was

located, the wings of the coil were marked on the scalp with a semi-permanent marker. Subthreshold and suprathreshold intensities were then determined by either decreasing or increasing the stimulator output. To achieve a suppression of soleus muscle activity, stimulator output was reduced in small increments until the threshold for facilitation was reached. This threshold was defined as the point when stimulation during 30% MVA resulted in visible MEP responses in only 50% of ten consecutive trials (Petersen et al., 2001). For facilitation, stimulator output was increased until MEPs were clearly visible in comparison to the immediately preceding background EMG in at least five consecutive trials.

Ultrasound imaging

Muscle fascicle behaviour of the MG from three participants was examined during familiarisation sessions using B-mode ultrasound imaging (LS128 CEXT-1Z, Telemed, Vilnius, Lithuania) to ensure muscle fascicle stretch occurred during ankle rotation in the stretch-hold condition. Ultrasound images were recorded using a flat, linear, 128-element transducer (LV7.5/60/128Z-2, 6 MHz, 60 x 50 mm (width x depth)) operating at ~60 fps in EchoWave II software (Telemed, Vilnius, Lithuania). The transducer was placed on the skin over the MG mid-muscle belly in the longitudinal plane and rotated to obtain a clear image with continuous aponeuroses and muscle fascicles. The position of the probe was maintained and marked on the skin in order to ensure consistent placement during the stretch-hold contractions.

Contraction conditions

The experiment involved participants performing submaximal plantar flexion contractions at 30% MVA. The background activity level was controlled throughout the contractions by having participants visually match their SOL EMG amplitude (moving 0.25-s root-mean-square (RMS) amplitude calculation) to a horizontal cursor on a screen. The conditions involved fixed-end reference contractions (REF) at 20° dorsiflexion (DF) and active stretch-

hold contractions (STR) from 0°-20° DF (0° refers to the sole of the foot perpendicular to the shank).

All contractions started with a 2-s linear ramp from 0-30% MVA. Following the ramp, the isometric steady state during the fixed-end reference contraction was maintained for 13-s. During the stretch-hold contractions, the ramps were followed by a 1-s isometric steady state at the initial ankle joint angle (0° DF), before the plantar flexor muscles were actively stretched to an ankle joint angle of 20° DF at an angular velocity of $30°s^{-1}$. Following active stretch, participants maintained another isometric steady state for ~11-s, resulting in an overall contraction duration of 15-s (Fig. 1).

Experimental protocol

Participants attended two sessions on two different days. In the first session, participants were familiarised with the test protocol and trained to perform maximal voluntary fixed-end contractions (MVC) and the submaximal contractions described above. Additionally, participants were familiarised with TMS.

The second session consisted of the test protocol (Fig. 2). After a short warm-up (eight submaximal PF contractions with increasing torque), at least two MVCs were performed to determine 100% MVA. Participants were instructed to push their forefoot into the footplate as hard as possible and to maintain the contractions, so that there was a clearly visible torque plateau. MVC torque was calculated as the difference between peak torque during the contraction and the mean baseline over 500 ms prior to the beginning of contraction. In order to ensure that the MVCs were repeatable, peak-to-peak torques were required to be within a 5% range. 100% MVA was determined as the smoothed (moving 0.25-s RMS amplitude calculation) SOL EMG amplitude at peak MVC torque from the best MVC. Three minutes of rest was provided between MVCs to minimise fatigue.

Following the MVCs, the TMS hotspot and the subthreshold and suprathreshold TMS intensities were determined during sustained fixed-end contractions at 30% MVA. Once hotspot and stimulation intensities were determined, 100 subthreshold stimulations were delivered for each contraction condition to study motor cortex inhibition. For this purpose, contractions from each condition were separated into ten sets with ten TMS (1 Hz) during the isometric steady state phase of the contractions at 20° DF starting 5-s after contraction onset (Fig. 1). Contractions were randomised and a minimum of three minutes rest was provided between each set. Facilitation of the motor cortex was investigated by providing 20 suprathreshold stimulations per contraction condition. Suprathreshold stimulations were delivered at the same time points as the subthreshold stimulations, but participants performed only two contractions per condition. The contractions with suprathreshold TMS were always performed after all sets of the subthreshold stimulations were finished. During all contractions, trials were excluded and repeated if participants could not maintain their soleus EMG activity within 25-35% MVA.

Data analysis

Residual Force Enhancement (rFE)

rFE for the subthreshold and suprathreshold conditions was determined by calculating the difference between the isometric steady-state torque during the STR and REF conditions. Net PF torque was averaged across contractions for each contraction condition and mean rFE was calculated during the isometric steady state at 20° DF, 500 ms after each TMS, for a time window of 490 ms. Stimulations delivered before the isometric steady state of SOL muscle activity were excluded.

TMS inhibition

Suppression of muscle activity (SOL, MG and LG) after subthreshold TMS was determined via averaged rectified raw EMG signals from each muscle and contraction condition.

Waveform averages were calculated over a time window of 100 ms after stimulation and again, stimulations delivered before the isometric steady state of SOL muscle activity were excluded. The latency and duration of EMG amplitude suppression were determined using the evaluation methods by Petersen et al. (2001) and Zuur et al. (2010). The onset of EMG amplitude suppression was marked when the EMG amplitude first dropped under the background EMG amplitude (mean value calculated 15–25 ms after stimulation) for at least 4-ms. The offset of EMG amplitude suppression was calculated when the EMG amplitude rose above the background EMG amplitude for 1 ms (Zuur et al., 2010) (Fig. 3). The mean EMG amplitude between the onset and offset of suppression was calculated and compared with the background EMG amplitude right before suppression.

Torque production following TMS-induced EMG amplitude suppression was analysed via moving correlations (50 ms time intervals) between the torque of the REF and STR conditions. A reduction in torque due to TMS-induced EMG amplitude suppression was identified when torque data of REF and STR contractions was correlated ($r \geq 0.7$). This data analysis was based on the random fluctuations in torque steadiness that should result in non-correlated torque signals. However, when torque was affected by the TMS-induced EMG amplitude suppression in a similar manner, positive correlations were expected. Once the time window of torque reduction was identified, the torque offset between the REF and STR conditions was removed by subtracting the mean torque difference between REF and STR over the first 10 ms after the onset of torque reduction. Finally, the mean torque during the period of TMS-induced torque reduction (i.e. during the times where $r \geq 0.7$) was calculated for REF and STR and compared between contraction conditions.

TMS facilitation

MEPs of SOL, MG and LG following suprathreshold TMS were calculated as peak-to-peak amplitudes from the raw EMG signals and averaged across contractions for each muscle and

contraction condition (Lewis et al., 2014) (Fig. 4). Additionally, the silent period (SP) duration was analysed for both contraction conditions as the time from stimulation to the end of the SP. The end of the SP was defined as the time when the EMG signal crossed the threefold standard deviation (SD) of the SP raw EMG following the MEP for the first time (Fig. 4). SD during the SP was determined as the smallest SD of the raw EMG over a moving 30ms window. Superimposed torque twitches following suprathreshold TMS were calculated separately as peak-to-peak amplitudes and averaged for each contraction condition.

Statistical analysis

All data were tested for normality using the Shapiro-Wilk test. Paired t-tests were used to test for significant differences (alpha level of 0.05) in triceps surae muscle activity, net plantar flexion torque, EMG amplitude suppression, torque reduction, MEP, SP and superimposed twitch amplitude between REF and STR conditions. Statistical analysis was performed using SPSS software (v22, IBM, New York, USA). Values are presented as mean ± SD in the text.

Results

Five and four participants were excluded from further analysis as they did not show rFE following the subthreshold (n=9) and suprathreshold (n=10) stimulations, respectively.

Contraction conditions

During both contraction conditions, participants managed to maintain a constant level of muscle activity (~30 % MVA). During the isometric steady state, EMG of SOL, MG and LG did not differ between STR and REF conditions during contractions with subthreshold TMS (SOL: $P = 0.308$, MG: $P = 0.091$, LG: $P = 0.144$; Fig. 5) or suprathreshold TMS (SOL: $P = 0.528$, MG: $P = 0.490$, LG: $P = 0.349$). Ultrasound imaging visually confirmed that the

muscle-tendon unit stretch during the 20° dynamometer rotation of the stretch-hold contraction resulted in fascicle stretch of the MG muscle at 30% MVA (n=3).

Contractions with subthreshold TMS

The isometric steady-state net plantar flexion torque in the STR condition exceeded the time-matched steady-state torque in the REF condition (STR: 105.9 ± 34.5 Nm; REF: 99.0 ± 33.4 Nm, $P = 0.002$), which resulted in mean rFE of 7.3 ± 4.2% (Fig. 6A).

Subthreshold stimulation of the motor cortex led to significant ($P < 0.001$) EMG amplitude suppression for SOL, MG and LG without any difference between STR and REF conditions (SOL: $P = 0.483$; MG: $P = 0.378$; LG: $P = 0.399$). The mean EMG amplitude suppression ranged between 19-25% of the background EMG amplitude, it occurred 41-46 ms after stimulation, and it lasted 10-15 ms in total (Table 1). The reduction in net plantar flexor torque following EMG amplitude suppression did not differ ($P = 0.228$) between STR and REF conditions.

Contractions with suprathreshold TMS

The isometric steady-state net plantar flexion torque in the STR condition exceeded the time-matched steady-state torque in the REF condition (STR: 89.5 ± 26.0 Nm; REF: 82.2 ± 27.2 Nm, $P = 0.002$), which resulted in mean rFE of 10.8 ± 10.0 % (Fig. 6B). For all muscles analysed (SOL, MG, LG), MEP and SP after suprathreshold TMS did not differ between STR and REF conditions (MEP: SOL: $P = 0.304$, MG: $P = 0.594$, LG: $P = 0.453$; SP: SOL: $P = 0.320$, MG: $P = 0.283$, LG: $P = 0.265$; Table 2). TMS-evoked superimposed twitches following suprathreshold stimulations were 9.1 ± 3.6 Nm (STR) and 8.6 ± 3.5 Nm (REF), and did not differ between STR and REF conditions ($P = 0.259$).

Discussion

326 The aim of this study was to determine whether corticospinal drive contributes to the
327 increased torque production in the presence of rFE following active stretch of the plantar
328 flexor muscles. To achieve this, reductions in EMG activity and torque production following
329 inhibition of motor cortical neurons, and increases in MEP amplitudes, SP durations and
330 superimposed twitch torque amplitudes following facilitation of motor cortical neurons were
331 compared between submaximal stretch-hold and fixed-end contractions.

332 Participants managed to keep muscle activity constant (30% MVA) throughout the fixed-end
333 reference contractions and during the isometric steady state of the stretch-hold contractions.
334 At this matched background EMG, mean rFE magnitudes of 7% and 11% were observed
335 during the steady state in STR compared with REF, which is in line with former studies
336 (Oskouei and Herzog, 2005; Paternoster et al., 2016; Pinniger and Cresswell, 2007; Seiberl et
337 al., 2013). Inhibition of motor cortical neurons by subthreshold TMS caused reductions in
338 EMG activity and net plantar flexion torque, however the reductions did not differ
339 significantly between contraction conditions. Similarly, MEP amplitudes, SP durations and
340 superimposed twitch torque amplitudes evoked by suprathreshold TMS were not significantly
341 different between STR and REF conditions.

342 Following subthreshold TMS, we found a suppression of triceps surae muscle activity by 19-
343 24% relative to the background activity. This is similar to the ~15% EMG amplitude
344 suppression in SOL induced by subthreshold TMS during walking and jumping (Petersen et
345 al., 2001; Zuur et al., 2010), but smaller compared with the 50% suppression in hand and arm
346 muscles during voluntary fixed-end contractions (Davey et al., 1994). Also, the latency and
347 the duration of the observed EMG amplitude suppression was similar to values reported
348 previously (Petersen et al., 2001; Zuur et al., 2010). Importantly, the TMS-induced EMG
349 amplitude suppression showed no difference between STR and REF conditions, which also
350 resulted in the same magnitudes of torque reduction for both contraction conditions. Contrary

351 to our first prediction, this indicates that corticospinal drive does not contribute to the
352 increased torque production following active muscle stretch.

353 Our data also does not support our second prediction that suprathreshold TMS elicits larger
354 superimposed twitch torque amplitudes in the presence of rFE compared with fixed-end
355 reference contractions (twitch amplitude of ~9 Nm for both contraction types). This further
356 supports the interpretation of the subthreshold TMS-induced EMG amplitude suppression that
357 corticospinal drive does not contribute to the increased torque production following active
358 muscle stretch. As the facilitation of the motor cortex with a given suprathreshold stimulation
359 intensity elicited comparable twitch torques for both contraction conditions, despite steady-
360 state torques after active stretch being enhanced because of rFE, our findings indicate that rFE
361 is a mechanical property of muscle (Freundt and Linke, 2019; Herzog, 2018; Nishikawa,
362 2016).

363 A limitation of the current study was that we only performed TMS of the motor cortex to
364 assess corticospinal excitability, which does not allow us to distinguish between cortical and
365 spinal aspects of corticospinal modulation. The unchanged MEP amplitudes and SP durations
366 that we found in the presence of rFE compared with the reference contractions is in line with
367 the findings of Sypkes et al. (2018) and indicate that the overall corticospinal excitability was
368 unaffected. However, unchanged MEPs do not exclude potential spinal modulations and the
369 smaller CMEP amplitudes in the presence of rFE observed by Sypkes et al. (2018) could be
370 interpreted as reduced spinal motoneuron excitability, which might be due to tension-related
371 afferent feedback from Golgi tendon organs (Contento et al. 2019). Accordingly, we assume
372 that the unchanged MEP amplitudes in our study reflect an increased cortical excitability,
373 which did not contribute to the increased torque production following active muscle stretch.

374 In conclusion, we found that subthreshold and suprathreshold TMS of motor cortical neurons
375 affected muscle activity and torque production, but the changes did not differ between stretch-

hold and fixed-end reference contractions. Contrary to our predictions, a contribution of corticospinal drive to the increased torque production following active muscle stretch could not be confirmed. However, it remains unknown whether and how the spinal modulations observed by others (Altenburg et al. 2008, Sypkes et al. 2018, Contento et al. 2019) affect torque production in the presence of rFE.

Disclosures

The authors report no conflicts of interest.

Endnote

At the request of the authors, readers are herein alerted to the fact that additional materials related to this manuscript may be found at https://figshare.com/articles/Frischholz_et_al_data_xlsx/11919828. These materials are not a part of this manuscript and have not undergone peer review by the American Physiological Society (APS). APS and the journal editors take no responsibility for these materials, for the website address, or for any links to or from it. [Data currently stored, but not publicly available. Data will be formally published, including details to accepted manuscript, once paper accepted.]

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484 Figure legends

485 Figure 1. Example data from the active stretch-hold (STR, dark blue) and fixed-end reference
486 (REF, light blue) contraction conditions. A: The traces show soleus (SOL) EMG (moving
487 0.25-s RMS amplitudes). Transcranial magnetic stimulation (TMS, vertical black lines) was
488 delivered at 1 Hz from 5-s after contraction onset (marked as time zero). In case the first
489 stimulation was delivered before SOL EMG reached the target level, the stimulation was
490 excluded from analysis. B: Traces show the corresponding crank arm angles.

491

492 Figure 2. A: Schematic diagram of the experimental protocol. The protocol started and ended
493 with maximal voluntary contractions (MVC, dark grey). Transcranial magnetic stimulation
494 (TMS) hotspot and sub-/suprathreshold intensities (light grey) were then determined before
495 data collection. Twenty contractions with subthreshold TMS (dark blue) and 4 contractions
496 with suprathreshold TMS (light blue) were separated into three sets, with 5 min rest between
497 each set. B: Schematic description of contractions within sets (blue bars) and delivery of TMS
498 (same procedure for sub- and suprathreshold TMS). Vertical black lines indicate the timing of
499 TMS during the submaximal contractions. Each set during subthreshold TMS consisted of 10
500 contractions of 15-s duration, followed by a 3 min rest.

501

502 Figure 3. Example data of rectified and averaged soleus (SOL) EMG from contractions with
503 subthreshold transcranial magnetic stimulation (TMS). The horizontal dotted line represents
504 the averaged background EMG amplitude. Vertical dashed lines indicate onset and offset of
505 EMG amplitude suppression following TMS. Time zero indicates the time of stimulation.

506

Figure 4. Representative example of motor evoked potentials (MEPs) and silent periods (SPs) from raw soleus (SOL) EMG signals elicited by suprathreshold transcranial magnetic stimulation (TMS). The light blue traces show the single MEPs (n = 12) and the dark blue trace shows the average of all SOL MEPs from one participant. The horizontal dashed black line indicates the duration of the silent period (SP) and the vertical black line indicates the peak-to-peak amplitude. Time zero indicates the time of stimulation.

Figure 5. Mean EMG amplitudes from the triceps surae muscles following subthreshold transcranial magnetic stimulation for individual participants (blue symbols and grey lines) and the group average (black horizontal lines) during active stretch-hold (STR) and fixed-end reference (REF) contractions.

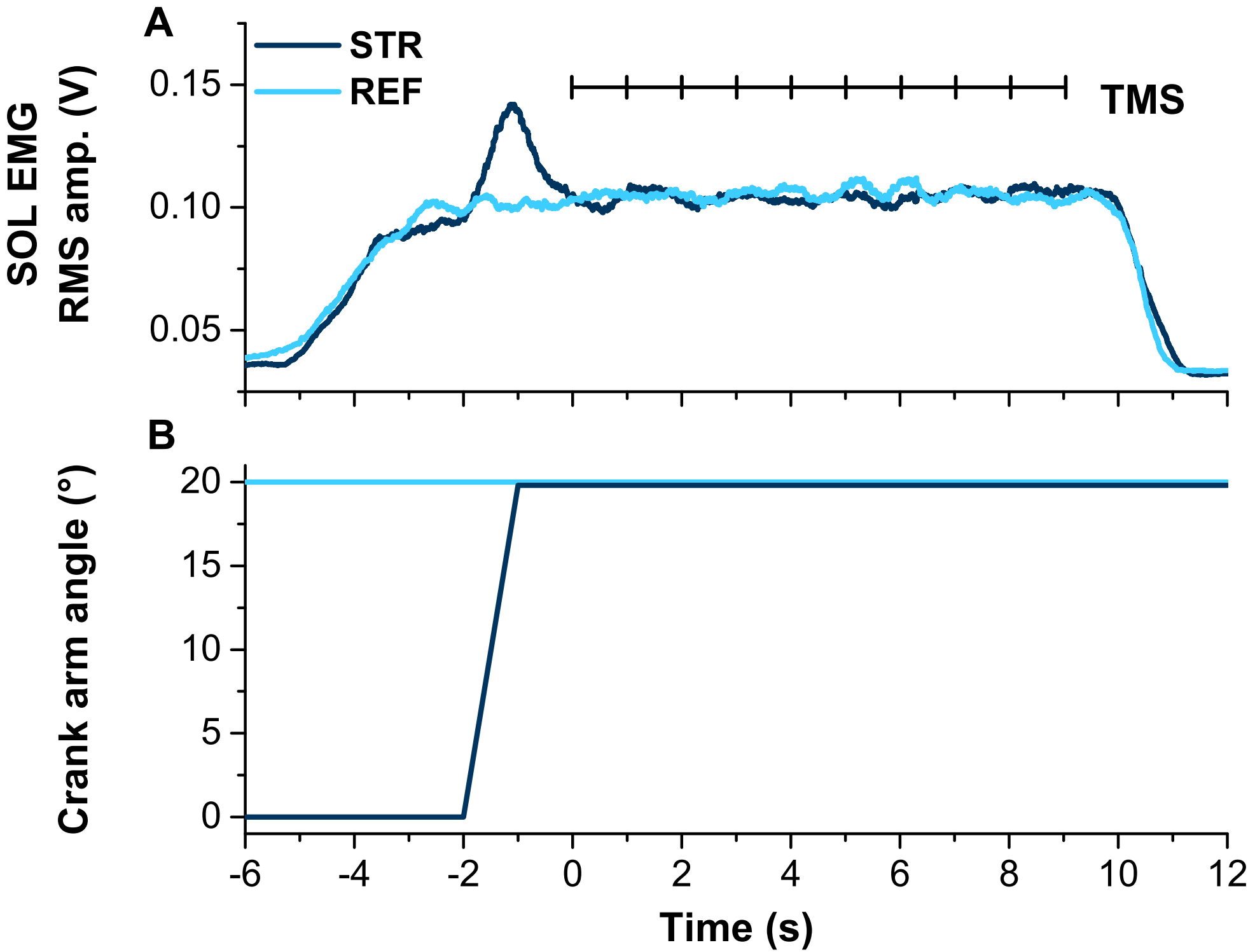
Figure 6. A: Individual mean net plantar flexion torque values 500-990 ms following transcranial magnetic stimulation (TMS) with subthreshold intensity for active stretch-hold (STR) and fixed-end reference (REF) contractions (blue symbols and grey lines). Black horizontal lines represent the group means. B: Individual mean net plantar flexion torque values 500-990-ms following TMS with suprathreshold intensity for active stretch-hold (STR) and fixed-end reference (REF) contractions (blue symbols and grey lines). Black horizontal lines represent the group means. *indicates that STR and REF were significantly different.

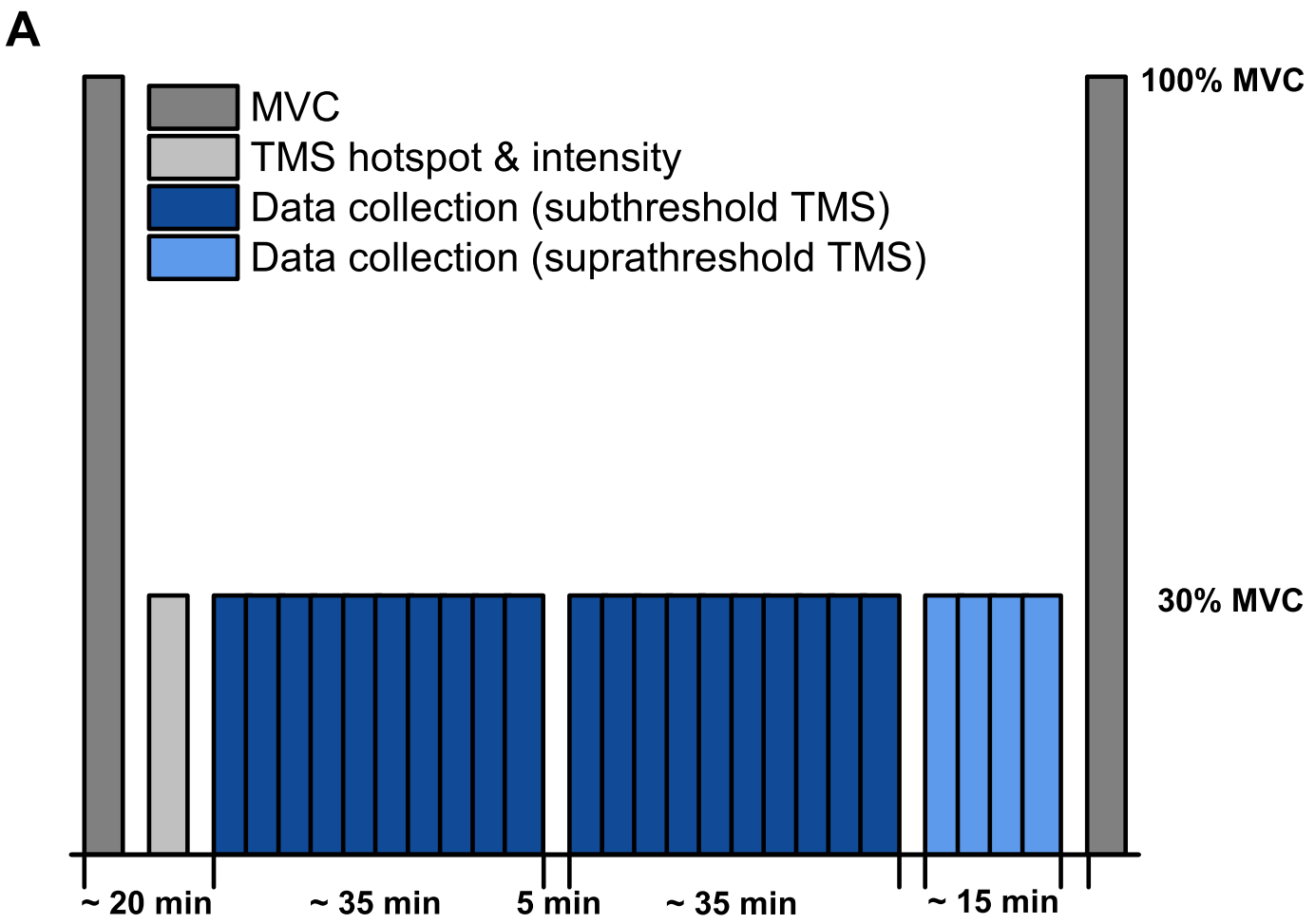
Table 1. Mean values ± SD of EMG amplitude suppression following subthreshold TMS from soleus (SOL), medial gastrocnemius (MG) and lateral gastrocnemius (LG) muscles during active stretch-hold (STR) and fixed-end reference (REF) contractions.

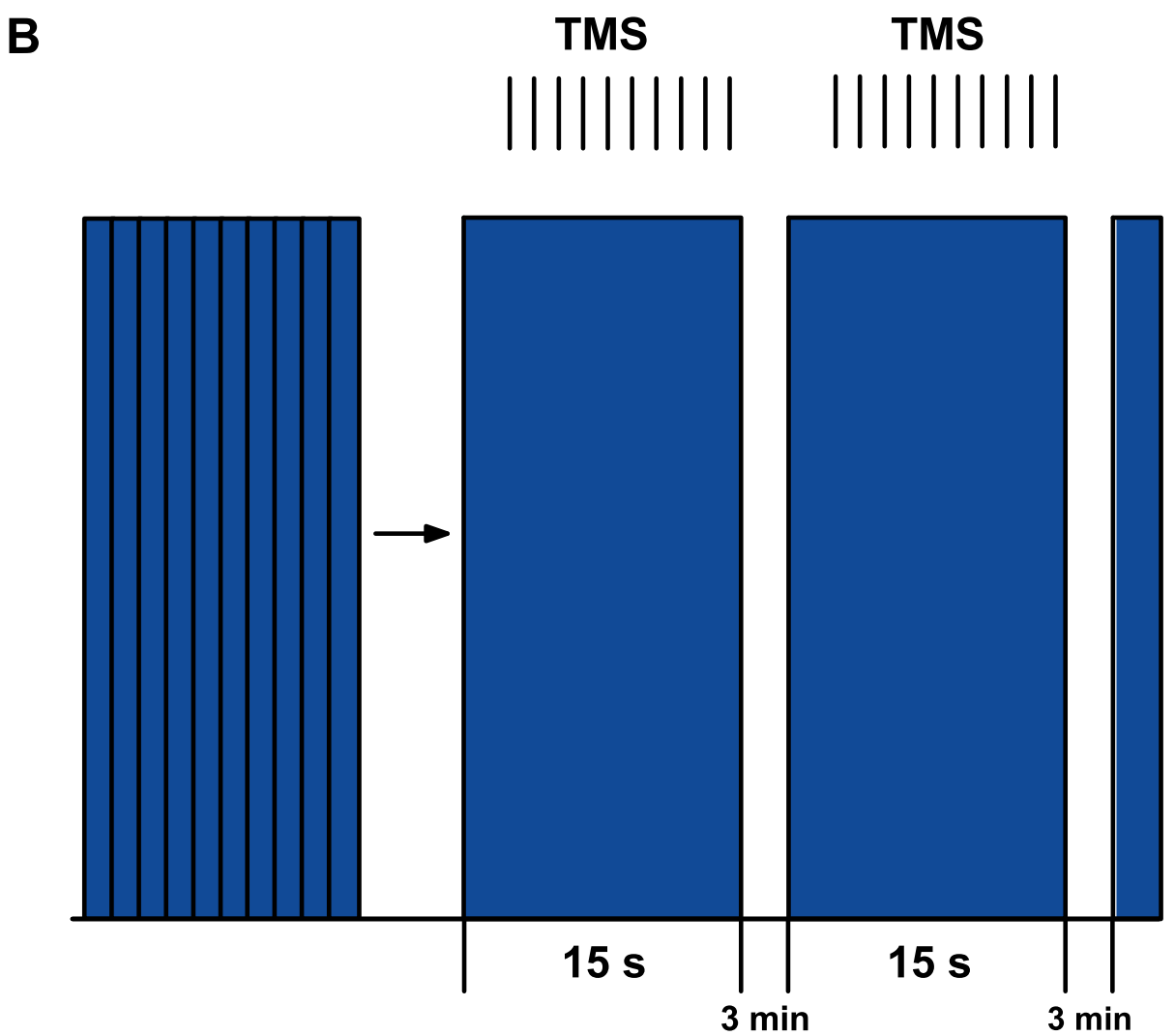
	SOL	**MG**	**LG**
EMG suppression REF [%]	23.5 ± 6.5	22.9 ± 8.4	19.4 ± 2.3
EMG suppression STR [%]	24.1 ± 5.1	23.7 ± 6.9	20.9 ± 6.4
Latency REF [ms]	44 ± 4	42 ±5	44 ± 5
Latency STR [ms]	44 ± 3	43 ± 6	46 ± 5
Duration REF [ms]	13 ± 4	13 ± 5	12 ± 5
Duration STR [ms]	13 ± 5	12 ± 4	10 ± 4

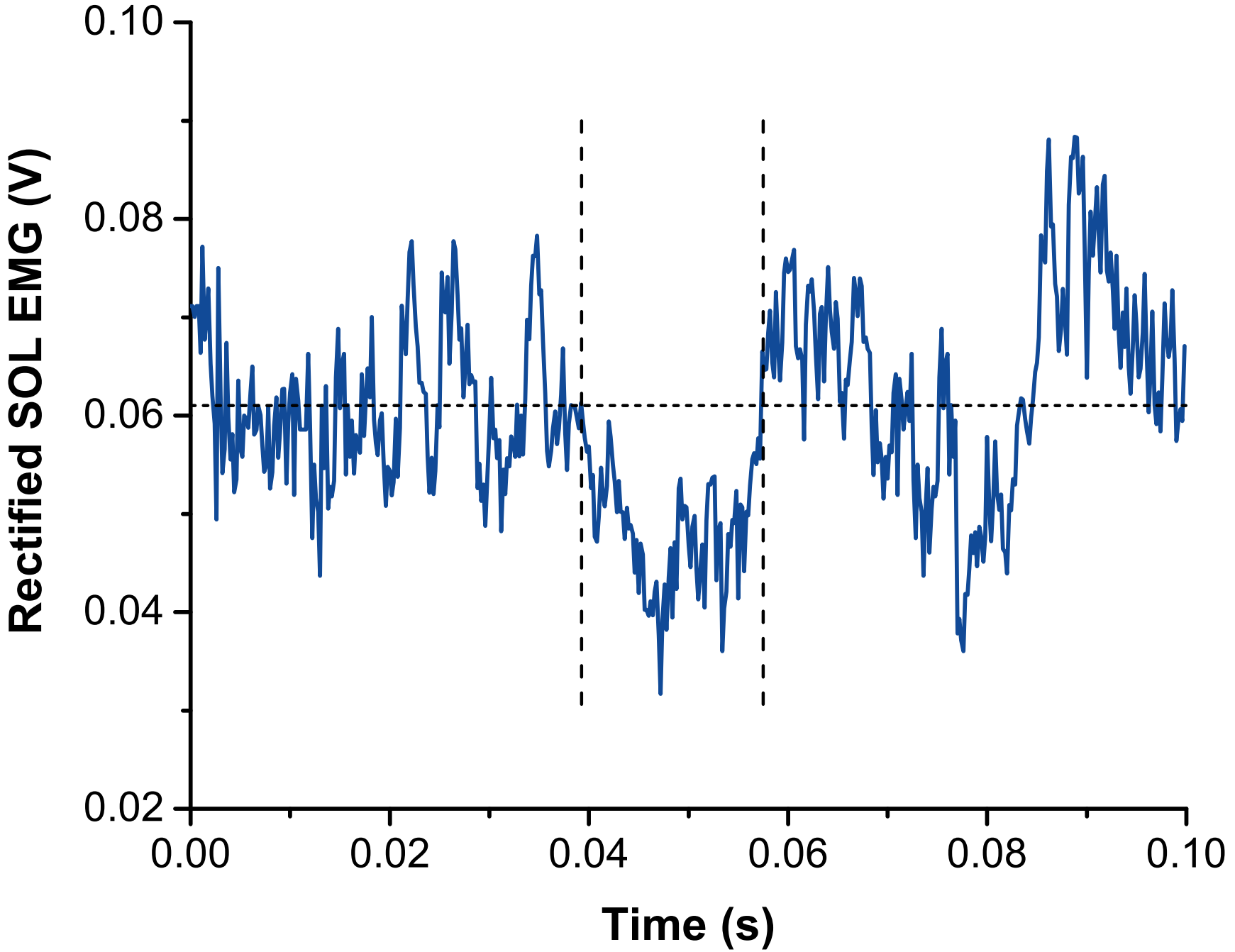
Table 2. Mean values ± SD of motor evoked potentials (MEP) and silent periods (SP) after suprathreshold TMS from soleus (SOL), medial gastrocnemius (MG) and lateral gastrocnemius (LG) muscles during active stretch-hold (STR) and fixed-end reference (REF) contractions.

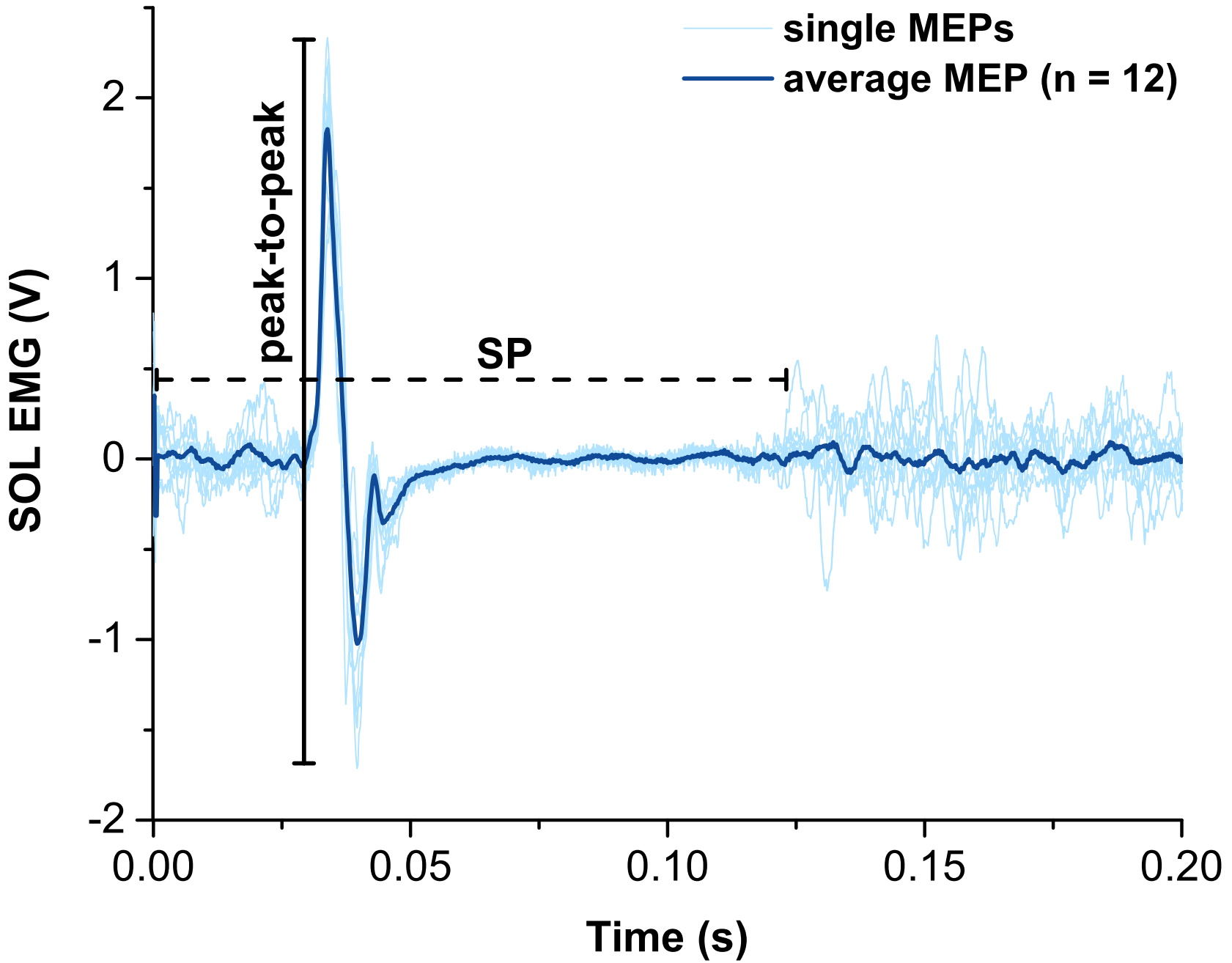
	SOL	**MG**	**LG**
MEP REF [V]	1.30 ± 0.78	2.54 ± 2.67	1.19 ± 0.46
MEP STR [V]	1.37 ± 0.72	2.70 ± 2.97	1.14 ± 0.50
SP REF [ms]	115 ± 31	112 ± 22	118 ± 29
SP STR [ms]	116 ± 31	115 ± 23	123 ± 34

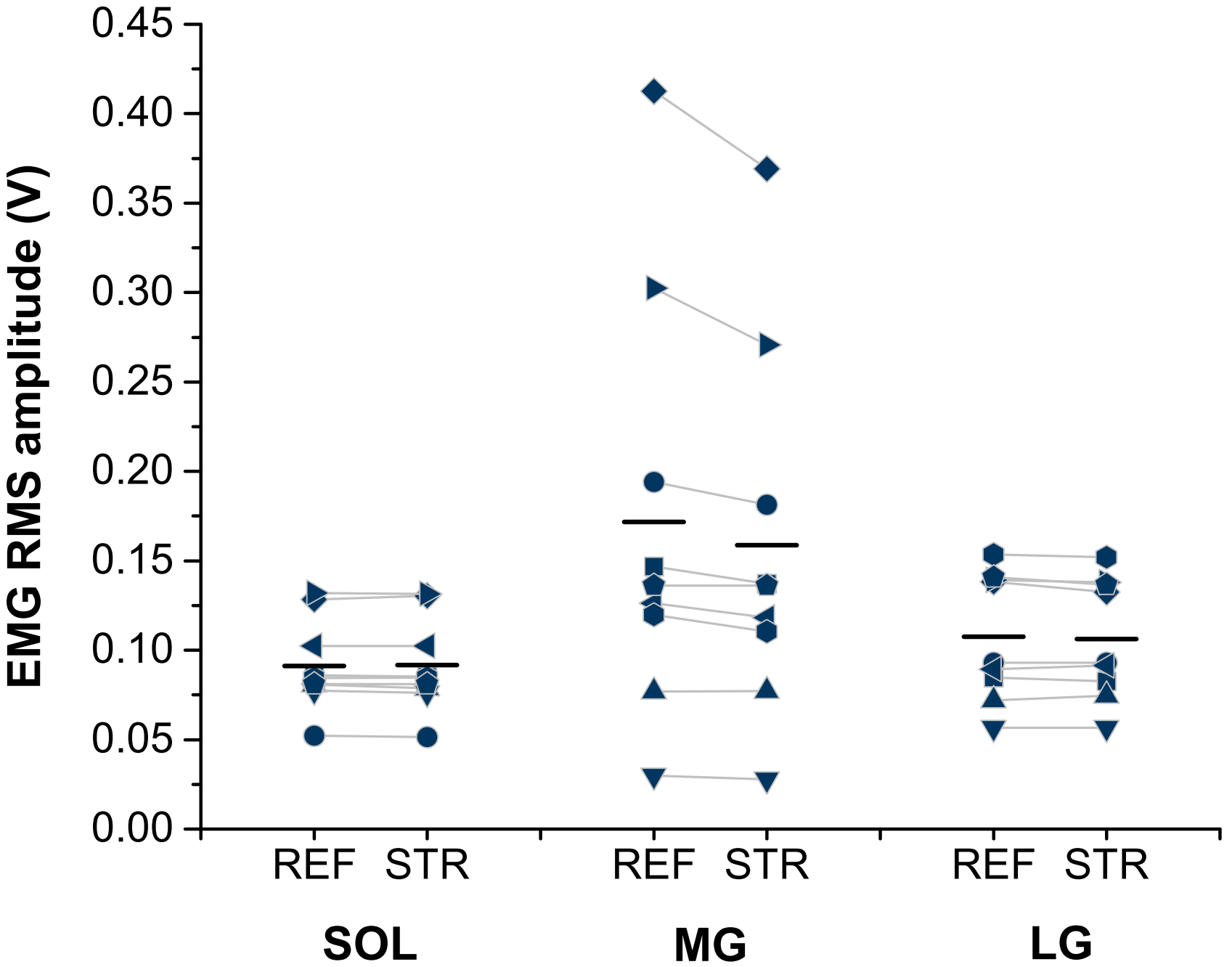

A
STR
REF
TMS
SOL EMG
RMS amp. (V)
0.15
0.10
0.05
B
Crank arm angle (°)
20
15
10
5
0
-6
-4
-2
0
2
4
6
8
10
12
Time (s)

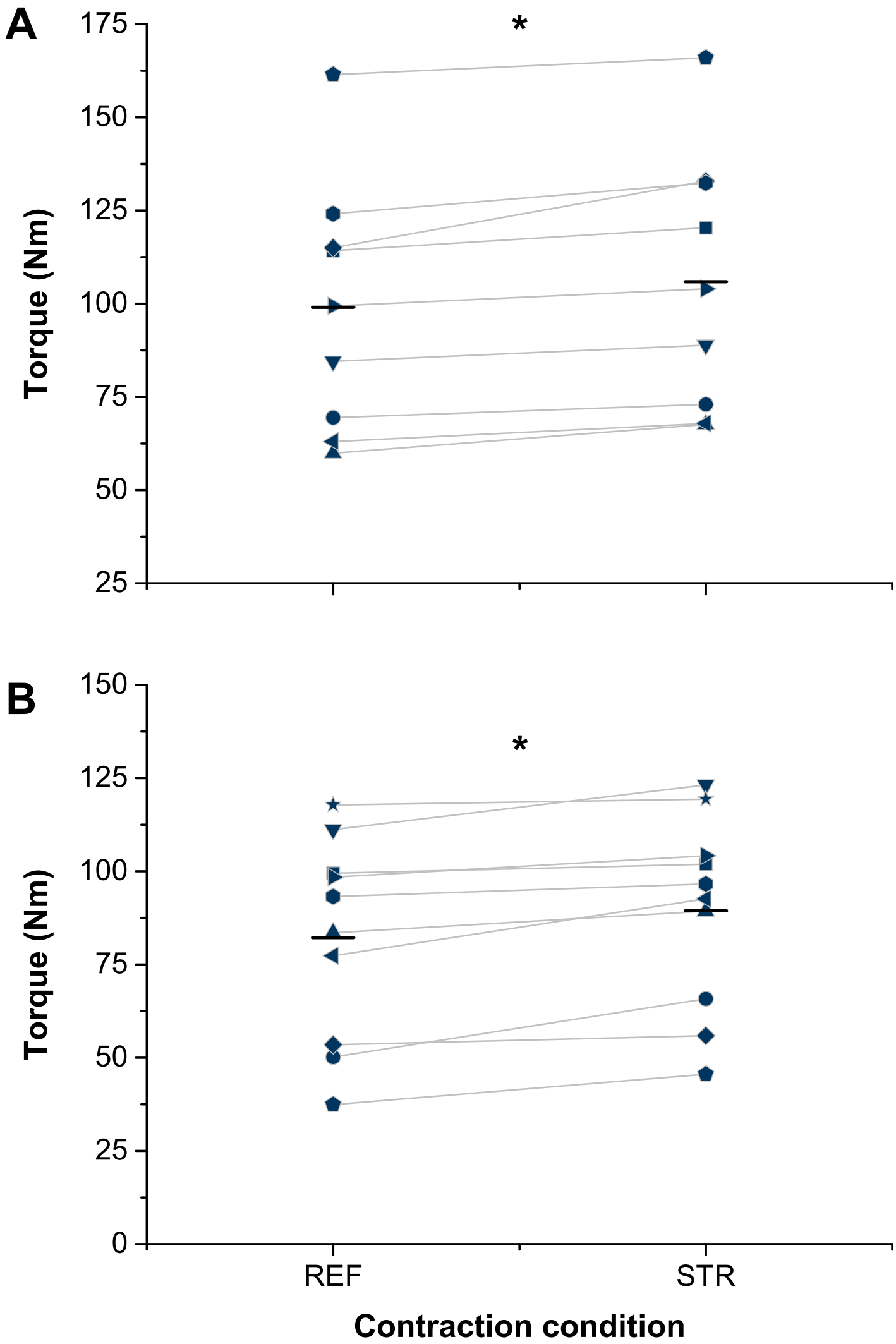

A
MVC
TMS hotspot & intensity
Data collection (subthreshold TMS)
Data collection (suprathreshold TMS)
100% MVC
30% MVC
~ 20 min
~ 35 min
5 min
~ 35 min
~ 15 min


B
TMS
TMS
15 s
15 s
3 min
3 min


0.10
0.08
0.06
0.04
0.02
Rectified SOL EMG (V)
0.00
0.02
0.04
0.06
0.08
0.10
Time (s)


single MEPs
average MEP (n = 12)
peak-to-peak
SP
SOL EMG (V)
2
1
0
-1
-2
0.00
0.05
0.10
0.15
0.20
Time (s)


EMG RMS amplitude (V)
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
REF
STR
REF
STR
REF
STR
SOL
MG
LG


A
175
150
125
100
75
50
25
Torque (Nm)
*
B
150
125
100
75
50
25
0
Torque (Nm)
*
REF
STR
Contraction condition