Repeatability and reliability of muscle relaxation properties induced by motor cortical stimulation


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Molenaar JP, Voermans NC, de Jong LA, Stegeman DF, Door- duin J, van Engelen BG. Repeatability and reliability of muscle relaxation properties induced by motor cortical stimulation. J Appl Physiol 124: 1597–1604, 2018. First published March 15, 2018; doi:10.1152/japplphysiol.00455.2017.—Impaired muscle relaxation is the main feature of many neuromuscular disorders. However, few tests are available to quantify muscle relaxation. Transcranial magnetic stimulation (TMS) of the motor cortex can induce muscle relaxation by abruptly inhibiting corticospinal drive. The aim of our study was to investigate whether repeatability and reliability of TMS-induced relaxation are greater than voluntary relaxation. Furthermore, effects of sex, cooling, and fatigue on muscle relaxation properties were studied. Muscle relaxation of deep finger flexors was assessed in 25 healthy subjects (14 men and 11 women, age 39.1±12.7 and 45.3±8.7 yr, respectively) with handgrip dynamometry. All outcome measures showed greater repeatability and reliability in TMS-induced relaxation compared with voluntary relaxation. The within-subject coefficient of variability of normalized peak relaxation rate was lower in TMS-induced relaxation than in voluntary relaxation (3.0% vs. 19.7% in men and 6.1% vs. 14.3% in women). The repeatability coefficient was lower (1.3 vs. 6.1 s⁻¹ in men and 2.3 vs. 3.1 s⁻¹ in women) and the intraclass correlation coefficient was higher (0.95 vs. 0.53 in men and 0.78 vs. 0.69 in women) for TMS-induced relaxation compared with voluntary relaxation. TMS enabled demonstration of slowing effects of sex, muscle cooling, and muscle fatigue on relaxation properties that voluntary relaxation could not. In conclusion, repeatability and reliability of TMS-induced muscle relaxation were greater compared with voluntary muscle relaxation. TMS-induced muscle relaxation has the potential to be used in clinical practice for diagnostic purposes and therapy effect monitoring in patients with impaired muscle relaxation.

NEW & NOTEWORTHY Transcranial magnetic stimulation (TMS)-induced muscle relaxation demonstrates greater repeatability and reliability compared with voluntary relaxation, represented by the ability to demonstrate typical effects of sex, cooling, and fatigue on muscle relaxation properties that were not seen in voluntary relaxation. In clinical practice, TMS-induced muscle relaxation could be used for diagnostic purposes and therapy effect monitoring. Furthermore, fewer subjects will be needed for future studies when using TMS to demonstrate differences in muscle relaxation properties.

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INTRODUCTION

Impaired muscle relaxation is the main feature of several neuromuscular disorders, e.g., myotonic dystrophy, nondystrophic myotonias, or Brody disease (33, 38). Muscle relaxation is an important part of the cross-bridge cycle and represents the sum of all processes that follow the halt of neural drive to the muscle fibers (12, 23). However, in clinical practice and the scientific literature there is an emphasis on muscle contraction (strength), while muscle relaxation is often overlooked (29). Consequently, there are multiple methods to quantify muscle strength, such as the Medical Research Council (MRC) scale or muscle dynamometry, whereas only limited in vivo tests are available to measure muscle relaxation properties. In patients with neuromuscular disorders, quantification of muscle relaxation may be useful for diagnostic purposes, for therapy effect monitoring, and in clinical trials (28). Therefore, a reliable diagnostic tool to measure intrinsic muscle relaxation properties (decrease in force that is only caused by muscular factors, e.g., termination of cross-bridge activity) is of clinical and scientific importance.

Currently, measurement of voluntary relaxation time and rate is the most widely used assessment tool for muscle relaxation (4, 37, 46). However, with this method intrinsic muscle relaxation can be underestimated or overestimated because of a slow voluntary decrease of corticospinal drive or antagonist-assisted relaxation, respectively. Transcranial magnetic stimulation (TMS) has the potential to measure muscle relaxation properties with greater repeatability and reliability by abruptly halting neural drive to the muscle. When TMS is applied to the motor cortex during a strong voluntary contraction, it induces a transient neural excitation that results in a motor evoked potential in the muscle and a concomitant small increase in force, known as the superimposed twitch. This short cortical excitation is directly followed by a relatively long period of cortical inhibition with hardly any neural drive to the muscle, also known as the silent period. This results in immediate involuntary muscle relaxation (1, 7, 16, 47). By abruptly eliminating voluntary (corticospinal) influences on muscle relaxation, the use of TMS enables the study of relaxation properties of only the muscle. Previous studies suggested that...
MATERIALS AND METHODS

The study was conducted at the Department of Neurology of the Radboud University Medical Center. Experimental procedures were approved by the institutional ethics committee and performed in accordance with ethical standards laid down in the Declaration of Helsinki.

Subjects. Twenty-five healthy subjects (14 men and 11 women) volunteered for this study. All participants provided written informed consent before the start of the study. Before the start of the protocol, contraindications for TMS (40) were checked, as well as symptoms indicative of neuromuscular dysfunction (e.g., myalgia, cramps, weakness). Subjects were excluded if medication influencing cortical excitability (e.g., antipsychotics, antidepressants, benzodiazepines, or antiepileptic drugs) or muscle relaxation (e.g., verapamil, dantrolene, baclofen) was used. Handedness was determined by the Edinburgh Handedness Inventory (39). For the assessment of physical activity, the General Practice Physical Activity Questionnaire was used, with a possible score between 1 and 4 (1 = inactive, 2 = moderately inactive, 3 = moderately active, 4 = active) (49). Height and body weight were measured on the test day. At the end of the protocol, subjects reported the average numeric (pain) rating scale (NRS) for the entire experience of receiving TMS pulses (0 indicating no pain or discomfort and 10 the worst pain or discomfort imaginable).

Recordings. Relaxation of deep finger flexors was assessed with handgrip dynamometry. The test setup consisted of a forearm support and a handgrip with an incorporated dynamometer. Subjects were seated comfortably with the elbow of their dominant arm in flexion and their forearm stabilized in the support (Fig. 1). Muscle relaxation following an isometric maximal voluntary contraction (MVC) was assessed, defined as the rate of force decline measured during voluntary relaxation and during the TMS-induced silent period.

Force was measured with strain gauges in the handgrip device, recorded via an analog-to-digital converter (NI DAQPad-6015; National Instruments, Austin, TX) with a sampling frequency of 2,000 Hz. Muscle activities of the deep finger flexor and extensor muscles were recorded with surface electromyography (EMG). The electrodes (cloth hydrogel electrodes; Kendall H69P) were positioned with the recording electrode over the muscle belly and the reference electrode over the distal tendon. Surface electrodes were connected to two bipolar channels of an amplifier (Porti System; Twente Medical Systems International, Enschede, The Netherlands) and sampled with a frequency of 2,048 Hz. Force and EMG signals were stored and visualized synchronously with custom in-house developed software. Force data were low-pass filtered at 50 Hz, and a zero force offset was calibrated before each part of the protocol to remove the direct current (DC) component. EMG signals were amplified (20×) and band-pass filtered (10–1,024 Hz).

Experimental protocol. Data was collected on one test day. Skin temperature was checked before the protocol, measured at 1 cm distal to the active electrode on the deep finger flexors with a noncontact infrared thermometer (Fluke 62 MAX, 10:1 spot).

TMS pulses were generated by a Magstim 200 (Magstim, Whitland, UK). A circular coil, 90 mm in diameter, was positioned over the vertex with the direction of current flow (anterior-posterior) corresponding with stimulation of the dominant arm (19). Participants performed three brief MVCs to estimate their maximal strength. Visual feedback was provided by displaying force on a computer screen. Verbal encouragement was given during all MVCs. Next, we determined the stimulation intensity needed to induce a substantial interruption to descending motor drive (i.e., a silent period of ~200 ms, which was visually assessed during the protocol), allowing the muscle to reach its maximal rate of relaxation. To avoid fatigue, all participants performed a sustained voluntary contraction of 10% MVC to determine the effect of TMS intensity on the duration of the silent period. During this contraction, single TMS pulses were given, starting with an intensity of 10% of maximal stimulator output and increased by increments of 10% until a silent period of ~200 ms was reached. When the silent period in the remaining protocol (during a 100% MVC instead of a 10% MVC) was <200 ms, the intensity was raised by 10–20% until a sufficiently long silent period was reached. The minimum adequate duration of the silent period was set at 150 ms, since it was shown in another muscle group that the average time to peak relaxation rate was ~100 ms (36).

The protocol consisted of three parts. During the first part, muscle relaxation was evaluated in a fresh muscle condition. Participants performed six MVCs, separated by 1-min rest, of which three were followed by voluntary relaxation and three by TMS-induced relaxation. During voluntary relaxation, participants were asked to relax directly after an auditory click, which was given when maximal force was reached (visually assessed by the investigator). Participants were instructed to relax only the flexor muscles and not use the extensor muscles to actively open the hand. With TMS-induced relaxation, a single TMS pulse was delivered when maximal force was reached (visually assessed). Participants were instructed to continue their active contraction during and after the TMS pulse, allowing for a reliable estimation of the duration of the silent period.

Fig. 1. Line diagram of the experimental arrangement. EMG, electromyography.
The second part of the protocol evaluated the effects of cooling on muscle relaxation properties. The forearm and hand were covered in a plastic bag and immersed in a warm water bath with cold tap water of 15°C for 10 min to decrease skin temperature below 24°C (mean of 21.8 ± 0.6°C across all subjects). Six MVCs, three followed by voluntary and three followed by TMS-induced relaxation, were performed. Before each MVC skin temperature was measured. If skin temperature was above 24°C, the forearm and hand were again immersed in cold water for 2 min.

Thereafter, the forearm and hand were warmed by immersing them in a warm water bath of ~40°C for 10 min to return skin temperature back to the temperature at the fresh muscle condition (33.0 ± 1.3°C and 32.3 ± 1.2°C, respectively). Subsequently, participants performed three MVCs with TMS-induced relaxation to verify that contractile properties had returned to normal levels, i.e., that no cooling effect remained before the subsequent fatigue protocol.

The third part of the protocol studied muscle fatigue (i.e., the loss of muscle power that results from a decline in both force and velocity (14)]. Participants performed a 30-s isometric contraction of 50% MVC immediately followed by six brief MVCs with 5-s rest in between (3 followed by voluntary and 3 by TMS-induced relaxation) (Fatigue run 1). This part of the protocol was directly repeated to further fatigue the muscle (Fatigue run 2).

Data analysis. Force and EMG signals were analyzed off-line. All variables were calculated with a software routine developed in MATLAB (MATLAB R2014b; The MathWorks, Natick, MA). We defined the following variables:

- Peak force was defined as the start of relaxation. For TMS-induced relaxation this was the peak of the superimposed twitch. For voluntary relaxation this had to be determined manually, since the reaction time after the verbal command differed between measurements and between subjects.

- Relaxation time (RT) interval between 90% and 50% of peak force (90–50% RT) was measured. We chose to use this interim time, and not the half-relaxation time (time between 100% and 50% of peak force), to allow for a more honest comparison between voluntary and TMS-induced relaxation, since the exact start of voluntary relaxation was often difficult to determine (Fig. 2A).

- Peak relaxation rate (pRR) was defined as the steepest negative slope in force during muscle relaxation. pRR was normalized to the peak force (NpRR) to account for differences in strength between subjects (9).

The duration of the silent period was determined manually as the time between the TMS trigger and return of the voluntary EMG activity in finger flexor muscles.

Statistical analysis. The average of three measurements per subject was used to test for differences in relaxation properties between sex and relaxation type (voluntary vs. TMS-induced relaxation) and for the analysis of effects of different muscle conditions (cooling and fatigue). For MVC, the average of all six measurements per muscle condition was used. Results were tested for normality of distribution with the Shapiro-Wilk normality test.

The effect of sex on relaxation properties in the fresh muscle condition was studied with unpaired two-tailed t-tests, and for the effect of relaxation type paired t-tests were used. Repeated-measures ANOVA with pairwise comparison and Tukey’s post hoc test were performed for both sexes separately to identify the effects of different muscle conditions (fresh, cooled, warmed, fatigued) on MVC and relaxation properties. To compare within-subject repeatability in fresh muscle, one-way ANOVA was performed with subject as dependent factor and the different relaxation properties as independent factors to obtain the estimated within-subject standard deviation (SD) on all subjects. This was used to determine the repeatability coefficient (RC), also known as the smallest real difference, which represents the absolute value by which two following measurements in an individual subject will differ in 95% of occasions (2, 51).

In addition, within-subject variability was assessed by calculating the coefficient of variability (CV), defined as the ratio of the within-subject SD to the mean (6, 20). The within-subject CV of all individual subjects was calculated for NpRR and 90–50% RT (separately for different sexes and relaxation types) as the within-subject SD divided by the subject’s mean of the three measurements. The mean of these individual CVs was used as the overall within-subject CV for a given relaxation property.

The intraclass correlation coefficient (ICC) was calculated to determine the reliability of both methods to measure relaxation properties (43). Reliability relates the size of the within-subject measurement error to the inherent variability between subjects. The ICC has a value between 0 and 1, with a value of 1 corresponding to no measurement error and a value of 0 meaning that all the variability in measurements is caused by measurement error (2).

To test for differences in the average within-subject CV between voluntary and TMS-induced relaxation, we used paired two-tailed t-tests on the individual subject’s CVs. It was not possible to perform

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Fig. 2. Force and electromyography (EMG) traces of muscle relaxation in 1 subject. A: 3 force records and corresponding EMG traces of voluntary relaxation from 1 subject. Time 0 ms corresponds to the manually assessed start of relaxation. Note the variation between the force traces and the gradual decrease in EMG activity. B: 3 force records and corresponding EMG traces of transcranial magnetic stimulation (TMS)-induced relaxation from the same subject. Time 0 ms corresponds to the moment of the TMS pulse. Force curves show a small superimposed twitch followed by a force drop with hardly any variability between the 3 measurements. EMG traces show a motor evoked potential shortly after the TMS pulse followed by an EMG silent period of ~200 ms, after which voluntary EMG returns and force increases again.
results indicated no/mild pain/discomfort. The average numeric (pain) rating scale (NRS) for TMS was 1.8
(218.2/11005 219.4/11006 221.2 ms vs. 221.2/11006 ms, with no difference between men and women
For all tests, a two-tailed P value < 0.05 was considered significant. Statistical analyses were performed with Prism (GraphPad
software, version 5.03) and SPSS (IBM SPSS Statistics, version 22). Data are described as means ± SD.

RESULTS
Subject characteristics are provided in Table 1. Except for height, there were no differences between men and women. One female subject was excluded from analysis for all TMS-induced relaxation outcome measures because a duration of silent period > 150 ms could not be reached (103.7 ± 18.3 ms), despite the use of 100% of maximal stimulator output.

TMS intensity ranged from 60% to 100% of maximal stimulator output (78.6 ± 16.1% in men and 86.0 ± 10.7% in women; P = 0.22). This resulted in an average silent period of 219.4 ± 19.5 ms, with no difference between men and women (218.2 ± 21.2 ms vs. 221.2 ± 17.9 ms, respectively; P = 0.72). The average time to peak relaxation rate was 106 ± 10 ms (range 93–128 ms) and 170 ± 56 ms (range 94–313 ms) for TMS and voluntary relaxation, respectively. In all subjects force dropped below 50% of peak force. The average numeric (pain) rating scale (NRS) for TMS was 1.8 ± 1.3 out of 10, indicating no/mild pain/discomfort.

Muscle relaxation properties. All variables showed faster muscle relaxation properties in TMS-induced relaxation compared with voluntary relaxation in both men and women (Table 2).

Men had faster pRR and NpRR than women in both voluntary and TMS-induced relaxation (both P < 0.05). For 90–50% RT, a difference between men and women could only be demonstrated with TMS-induced relaxation (P = 0.002) and not with voluntary relaxation (P = 0.094).

Repeatability and reliability. In Fig. 2, an example of voluntary and TMS-induced muscle relaxation is shown. All relaxation properties showed significantly lower within-subject CV in TMS-induced relaxation compared with voluntary relaxation (Fig. 3A). The RC of TMS-induced relaxation was lower than the RC of voluntary relaxation in all relaxation properties except for NpRR in women (Fig. 3, B and C). TMS-induced muscle relaxation produced higher ICC values compared with voluntary relaxation in all relaxation properties except for NpRR in women (Table 3).

Cooling and fatigue. MVC after cooling was similar to the fresh muscle condition in men (576.3 ± 96.9 vs. 602.7 ± 93.0 N, respectively; P = 0.380) and women (317.2 ± 53.6 vs. 331.9 ± 62.3 N, respectively; P = 0.501). The first fatigue protocol reduced MVC in men (from 602.7 ± 93.0 to

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>M (n = 14)</th>
<th>F (n = 11)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>39.1 ± 12.7</td>
<td>45.3 ± 8.7</td>
<td>0.183</td>
</tr>
<tr>
<td>Height, cm</td>
<td>183.1 ± 5.2</td>
<td>168.4 ± 6.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>83.6 ± 12.4</td>
<td>74.3 ± 11.3</td>
<td>0.065</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.0 ± 3.7</td>
<td>26.2 ± 3.9</td>
<td>0.409</td>
</tr>
<tr>
<td>Handedness (R/L)</td>
<td>12/2</td>
<td>9/2</td>
<td>0.848</td>
</tr>
<tr>
<td>PAI (1–4)</td>
<td>3.9 ± 0.4</td>
<td>3.7 ± 0.9</td>
<td>0.628</td>
</tr>
</tbody>
</table>

Values are means ± SD for n subjects. P values represent the difference between men and women. M, male; F, female; BMI, body mass index; PAI, physical activity index; R, right; L, left. *Significant difference (P < 0.05).

t-tests to look for differences in RC and ICC because these parameters consist of only one value per relaxation type (for all subjects together). Instead, we calculated 84% confidence intervals (CIs), with no overlap in the 84% CI corresponding to P < 0.05 (31).

For all tests, a two-tailed P value < 0.05 was considered significant. Statistical analyses were performed with Prism (GraphPad Software, version 5.03) and SPSS (IBM SPSS Statistics, version 22). Data are described as means ± SD.

Table 2. Results categorized by relaxation type

<table>
<thead>
<tr>
<th>Relaxation Type</th>
<th>PRR, %</th>
<th>NpRR, %</th>
<th>RC of within-subject CV</th>
<th>PRR, s⁻¹</th>
<th>NpRR, s⁻¹</th>
<th>CV of peak relaxation</th>
<th>PRR, ms</th>
<th>NpRR, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voluntary</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>TMS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voluntary</td>
<td>−5.7694 ± 1736.9</td>
<td>−8.1182 ± 1035.3</td>
<td>&lt;0.001*</td>
<td>−2.2858 ± 804.3</td>
<td>−3.9552 ± 930.1</td>
<td>&lt;0.001*</td>
<td>−10.0 ± 2.7</td>
<td>−14.1 ± 2.1</td>
</tr>
<tr>
<td>TMS</td>
<td>−7.6 ± 1.7</td>
<td>−12.1 ± 1.6</td>
<td>&lt;0.001*</td>
<td>−7.6 ± 1.7</td>
<td>−12.1 ± 1.6</td>
<td>&lt;0.001*</td>
<td>−10.0 ± 2.7</td>
<td>−14.1 ± 2.1</td>
</tr>
<tr>
<td>90–50% RT</td>
<td>−7.6 ± 1.7</td>
<td>−12.1 ± 1.6</td>
<td>&lt;0.001*</td>
<td>−7.6 ± 1.7</td>
<td>−12.1 ± 1.6</td>
<td>&lt;0.001*</td>
<td>−10.0 ± 2.7</td>
<td>−14.1 ± 2.1</td>
</tr>
</tbody>
</table>

Values are means ± SD; sample size for each group, including no. of women (F), is reported in parentheses. P values represent difference between voluntary and transcranial magnetic stimulation (TMS)-induced muscle relaxation properties. M, male; F, female; pRR, peak relaxation rate; NpRR, pRR normalized to peak force; RT, relaxation time; 90–50% RT, RT interval between 90% and 50% of peak force. *Significant difference (P < 0.05).

Fig. 3. Comparison of within-subject coefficient of variability (CV; A), repeatability coefficient of peak relaxation rate normalized to peak force (NpRR; B), and repeatability coefficient of relaxation time (RT) interval between 90% and 50% of peak force (90–50% RT; C) between voluntary and transcranial magnetic stimulation (TMS)-induced relaxation, separately for men (M, n = 14) and women (F, n = 11). A: within-subject CV was lower in TMS-induced relaxation for all relaxation properties in men and women. B and C: the repeatability coefficient was lower in TMS-induced relaxation, except for NpRR in women. Bars represent 95% confidence interval. *P < 0.05.

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492.3 ± 74.8 N; P < 0.001) and women (from 331.9 ± 62.3 to 279.8 ± 54.1 N; P = 0.001). MVC was further reduced after the second fatigue protocol in both men (from 492.3 ± 74.8 to 427.1 ± 88.2 N; P = 0.002) and women (from 279.8 ± 54.1 to 250.9 ± 52.3 N; P = 0.043).

Cooling and fatigue decreased NpRR in TMS-induced relaxation in both men and women (Fig. 4). The additional slowing effect of the second fatigue protocol on NpRR could not be demonstrated in men by TMS. With voluntary relaxation, there was a slowing effect of cooling and fatigue on NpRR in both sexes. The additional slowing effect of the second fatigue protocol on NpRR could not be demonstrated with voluntary relaxation.

In voluntary relaxation, there was no difference in 90–50% RT between fresh, cooled, and fatigued muscle conditions in men and women (Table 4). With TMS-induced relaxation, an increase in 90–50% RT after cooling could be demonstrated in both sexes. The slowing effect on 90–50% RT of the first fatigue protocol and the additional slowing effect of the second fatigue protocol could be demonstrated by using TMS in men but not in women. There were no differences in relaxation properties between fresh and warmed muscle conditions, except for NpRR in men (−14.1 ± 2.1 vs. −12.8 ± 2.1, respectively; P = 0.023).

**DISCUSSION**

The present study on muscle relaxation properties in healthy volunteers under different muscle conditions demonstrates that TMS-induced muscle relaxation has greater repeatability and reliability than voluntary muscle relaxation. This is most likely due to the elimination of central influences in TMS-induced muscle relaxation. Consequently, TMS-induced muscle relaxation was more sensitive in detecting the effects of sex, cooling, and fatigue on muscle relaxation properties. In addition, all relaxation properties were faster with TMS-induced relaxation compared with voluntary muscle relaxation.

**Repeatability and reliability.** Our study demonstrates greater repeatability with TMS-induced muscle relaxation compared with voluntary muscle relaxation. This is shown by considerably smaller within-subject CV in all relaxation properties and smaller RC in most relaxation properties in TMS-induced relaxation. A smaller CV indicates less variability in repeated measurements. In general a test with an average within-subject CV < 10% is considered to have high repeatability and a CV < 5% very high repeatability (17). Within-subject CV for TMS-induced relaxation properties were all < 10%, indicating high to very high repeatability. For voluntary relaxation, within-subject CV ranged from 14.3% (for NpRR in women) to 24.9% (for 90–50% RT in men), indicating moderate to poor repeatability. It is most likely that corticospinal influences are more sensitive in detecting the effects of sex, cooling, and fatigue on muscle relaxation properties.
Table 4. Effect of cooling and fatigue on 90–50% relaxation time

<table>
<thead>
<tr>
<th>Relaxation Type</th>
<th>Sex</th>
<th>Fresh</th>
<th>Cooled</th>
<th>Fatigued 1</th>
<th>Fatigued 2</th>
<th>Fresh vs. Cooled</th>
<th>Fatigued 1 vs. Fatigued 2</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voluntary (n = 25, 11 F)</td>
<td>M</td>
<td>73.2 ± 43.4</td>
<td>77.3 ± 19.8</td>
<td>84.1 ± 18.8</td>
<td>84.9 ± 19.5</td>
<td>0.960</td>
<td>0.550</td>
<td>0.487</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>101.0 ± 33.5</td>
<td>109.6 ± 49.1</td>
<td>113.0 ± 54.2</td>
<td>98.2 ± 34.3</td>
<td>0.960</td>
<td>0.902</td>
<td>0.999</td>
</tr>
<tr>
<td>TMS (n = 24, 10 F)</td>
<td>M</td>
<td>37.5 ± 8.1</td>
<td>57.3 ± 12.1</td>
<td>45.6 ± 6.6</td>
<td>53.7 ± 2.3</td>
<td>&lt;0.001*</td>
<td>0.003*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>50.2 ± 9.7</td>
<td>60.3 ± 12.6</td>
<td>45.9 ± 4.8</td>
<td>50.4 ± 7.0</td>
<td>0.046*</td>
<td>0.636</td>
<td>&gt;0.999</td>
</tr>
</tbody>
</table>

Mean ± SD 90–50% relaxation times in different muscle conditions are reported on left. Effect of cooling and fatigue is demonstrated as P values on right. Sample size for each group, including no. of women (F), is reported in parentheses. TMS, transcranial magnetic stimulation; M, male; F, female. *Significant difference (P < 0.05).

the cause of the larger variability seen with voluntary relaxation, since these factors are largely eliminated in TMS-induced relaxation.

Furthermore, reliability was greater in TMS-induced muscle relaxation compared with voluntary muscle relaxation, as indicated by a higher ICC. Greater reliability means that differences measured between subjects are more likely to be due to a genuine difference than due to error in measurement. This leads to a greater sensitivity in detecting differences between subjects (2). Indeed, in the present study TMS-induced muscle relaxation was more sensitive to detect the effects of sex, cooling, and fatigue on muscle relaxation properties than voluntary muscle relaxation.

Muscle relaxation properties. Men had generally faster muscle relaxation properties compared with women, which is similar to previous results in other muscle groups including elbow flexors and knee extensors (22, 36, 52). With TMS, we found a difference in 90–50% RT between men and women (P = 0.002), whereas this could not be demonstrated with voluntary relaxation (P = 0.094). However, a clear difference in (normalized) peak relaxation rate between sexes was demonstrated with both methods of muscle relaxation. Therefore, we are confident that muscle relaxation of the finger flexors really differs between men and women. The lower reliability of voluntary relaxation requires more subjects to demonstrate a sex difference in 90–50% RT.

The faster muscle relaxation properties in men could be due to a greater proportion of fast-twitch muscle fibers. To our knowledge, this has not been studied in finger flexor muscles but has been confirmed in other muscle groups (13, 26, 32, 34, 44, 45). Another explanation could be that men have higher activities of several glycolytic enzymes in finger flexors, as was found in dorsiflexor muscles (41).

With the use of TMS we were able to confirm the previously demonstrated slowing effects of cooling and fatigue on muscle relaxation rate that have been reported in voluntary relaxation (e.g., 4, 37, 46), electrically induced relaxation (e.g., 8, 9), and TMS-induced relaxation (22, 24, 47, 48). Certain slowing effects of cooling and fatigue could only be demonstrated with TMS and not with voluntary relaxation, most likely because of the greater repeatability of TMS-induced relaxation.

Furthermore, TMS-induced relaxation was faster compared with voluntary relaxation, which can be explained by the abrupt interruption of corticospinal drive with TMS that cannot be accomplished with voluntary relaxation. This results in a representation of muscle relaxation properties without voluntary influences on relaxation or the physiological gradual decrease in motor unit firing rate and motor unit recruitment (11). This is illustrated by the abrupt absence of EMG activity after the motor evoked potential in TMS-induced relaxation compared with the gradual decrease in EMG activity in voluntary relaxation (Fig. 2). In other words, TMS-induced muscle relaxation represents only intrinsic muscle relaxation properties (e.g., termination of cross-bridge activity), whereas voluntary relaxation represents the sum of these muscular factors plus all voluntary and involuntary influences of the central nervous system on muscle relaxation properties of both the studied muscles and their antagonist.

Muscle relaxation properties can also be measured by recording the force response (twitch) in a relaxed muscle following peripheral electrical nerve stimulation (8, 9). It is even possible to simulate an MVC with high-frequency tetanic electrical stimulation, inducing a maximal sustained contraction (10). However, the latter is very painful (especially in larger muscle groups) and remains only an approximation of a true voluntary maximal contraction. In voluntary motor unit recruitment, small motor units (typically slow) are recruited first, followed by progressively larger (faster) motor units (21, 25). Peripheral electrical stimulation results in an opposite (nonphysiological) order of motor unit recruitment, with the larger motor units recruited first (3, 18, 50). We argue here that therefore an MVC followed by a TMS pulse is more likely to represent physiological muscle relaxation compared with electrical stimulation.

Clinical and scientific implications. A reliable diagnostic tool to study muscle relaxation is of great clinical and scientific importance. In clinical practice, TMS-induced muscle relaxation could be used as a diagnostic instrument in patients suspected of a myopathy with slowed muscle relaxation. A (specific) abnormal relaxation profile could potentially lead to more targeted genetic testing and the omission of more invasive diagnostic procedures such as a muscle biopsy. Nowadays, next-generation sequencing is a common first step in the diagnosis of neuromuscular diseases (15). However, the pathogenicity of novel mutations is often unknown, which calls for additional in vivo and/or in vitro functional tests (27). As such, TMS-induced muscle relaxation can serve as a reliable tool in proving the pathogenicity of novel mutations in genes involved in muscle relaxation. Furthermore, TMS-induced muscle relaxation can be applied in patients already known to suffer from a myopathy with slowed relaxation, i.e., to monitor disease progression or to study the effects of medication and nonpharmacological interventions such as physical therapy. In clinical scientific research, the greater repeatability of TMS-induced muscle relaxation results in fewer measurements needed per subject and a smaller number of subjects needed.
when studying the effect of an intervention on muscle relaxation. This is demonstrated in the present study, where TMS-induced muscle relaxation could identify slowing effects of sex, cooling, and fatigue with a relatively small number of subjects that were not found with voluntary relaxation. The better reliability results in greater statistical power to detect differences between groups for a given sample size, thus leading to fewer subjects needed, e.g., when studying differences in muscle relaxation properties between different neuromuscular disorders. This would be especially beneficial in trials where small effects are expected and/or few subjects are available, i.e., rare diseases with abnormal muscle relaxation such as myotonic dystrophy, paramyotonia congenita, or Brody myopathy (10, 28).

In the present study, muscle relaxation properties could be well assessed because of an adequately evoked silent period, as previously demonstrated in, for example, elbow flexors (36, 47, 48), thener muscles (42), and plantar foot flexors (35). However, it could be more difficult to elicit an adequate silent period in other muscle groups (30) or in specific patients (e.g., spinally mediated spasticity) because of excessive EMG activity from spinal reflexes during the silent period (5). This could be a limitation when applying TMS to study relaxation in other muscle groups and/or specific patients. In the present study we have only studied relaxation properties of maximally activated muscles. For future studies it would be interesting to additionally test muscle relaxation during submaximal efforts. This could be used to better compare with muscle relaxation in subjects with submaximal central drive to the muscle, e.g., stroke, multiple sclerosis, and spinal cord injury patients or during central fatigue.

**Conclusions.** TMS-induced muscle relaxation demonstrates greater repeatability and reliability compared with voluntary relaxation, represented by the ability to demonstrate typical effects of sex, cooling, and fatigue on muscle relaxation properties that were not seen in voluntary relaxation. Furthermore, the use of TMS results in faster relaxation properties, indicative of a more genuine representation of muscle relaxation, i.e., without influences from the central nervous system. TMS-induced muscle relaxation could be used in clinical practice for diagnostic purposes and therapy effect monitoring. For future studies, fewer subjects will be needed when TMS is used to demonstrate differences in muscle relaxation properties, e.g., the effect of any intervention (medication, exercise, temperature, fatigue), or to study relaxation profiles in different neuromuscular disorders.

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**AUTHOR CONTRIBUTIONS**


**REFERENCES**


