Effect of temperature on spike-triggered average torque and electrophysiological properties of low-threshold motor units

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Farina, Dario, Lars Arendt-Nielsen, and Thomas Graven-Nielsen. Effect of temperature on spike-triggered average torque and electrophysiological properties of low-threshold motor units. J Appl Physiol 99: 197–203, 2005. First published March 10, 2005; doi:10.1152/japplphysiol.00059.2005.—The aim of the study was to jointly analyze temperature-induced changes in low-threshold single motor unit twitch torque and action potential properties. Joint torque, multichannel surface, and intramuscular electromyographic signals were recorded from the tibialis anterior muscle of 12 subjects who were instructed to identify the activity of a target motor unit using intramuscular electromyographic signals as feedback. The target motor unit was activated at the minimum stable discharge rate in seven 3-min-long contractions. The first three contractions (C1–C3) were performed at 33°C skin temperature. After 5 min, the subject performed three contractions at 33°C (T1), 39°C (T2), and 45°C (T3), followed by a contraction at 33°C (C4) skin temperature. Twitch torque and multichannel surface action potential of the target motor unit were obtained by spike-triggered averaging. Discharge rate (mean ± SE, 7.1 ± 0.5 pulses/s), interpulse interval variability (35.8 ± 9.2%), and recruitment threshold (4.5 ± 0.4% of the maximal voluntary torque) were not different among the seven contractions. None of the investigated variables were different among C1–C3, T1, and C4. Conduction velocity and peak twitch torque increased with temperature (P < 0.05; T1: 3.53 ± 0.21 m/s and 0.82 ± 0.23 N·m, T2: 3.93 ± 0.24 m/s and 1.17 ± 0.36 N·m, T3: 4.35 ± 0.25 m/s and 1.46 ± 0.40 N·m, respectively). Twitch time to peak and surface action potential peak-to-peak amplitude were smaller in T3 (61.8 ± 2.0 ms and 27.4 ± 5.1 μV, respectively) than in T1 (71.9 ± 4.1 ms and 35.0 ± 6.5 μV, respectively) (P < 0.05). The relative increase in conduction velocity between T1 and T3 was positively correlated (P < 0.05) with the increase in twitch peak amplitude (r² = 0.48), with the decrease in twitch time to peak (r² = 0.43), and with the decrease in action potential amplitude (r² = 0.50). In conclusion, temperature-induced modifications in fiber membrane conduction properties may have a direct effect on contractile motor unit properties.

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EFFECT OF TEMPERATURE ON MOTOR UNIT PROPERTIES

MATERIALS AND METHODS

Subjects

Twelve healthy subjects [3 women; age, 25.8 ± 4.3 yr (mean ± SD)] participated in the study. None of the subjects reported symptoms of neuromuscular disorders. The study was in accordance with the Declaration of Helsinki, approved by the local ethics committee (ethical approval no. VN 2003/61), and written, informed consent was obtained from all participants before inclusion.

General Procedures

The subject sat in a reclined position on a chair of adjustable height with the back 30° flexed. The right (dominant) leg was positioned over a support and flexed at ~160° (180° equal to full extension of the leg). The foot was fixed to a foot plate (90° ankle joint angle). The maximal voluntary contraction (MVC) torque in dorsiflexion was recorded three times, separated by 2-min rest. After the MVC measure, intramuscular and surface electromyographic (EMG) electrodes were mounted on the right leg, as described below.

The subject was provided with auditory and visual feedback of the intramuscular EMG signals and was asked to modulate the torque during ankle flexion to identify the activity of a single motor unit (target motor unit). The volunteer was given 10–20 min to train for this task. After the training phase, an infrared heating lamp was located over the tibialis anterior muscle at a distance of ~30 cm from the leg. The lamp covered the entire length of the leg. A temperature sensor was placed next to the recording site of surface EMG signals. A control system (Aalborg University, Aalborg, Denmark) allowed the automatic regulation of heat intensity to maintain a preset temperature, measured by the skin sensor. The skin temperature was then increased to 39°C. After the target temperature was reached, the automatic control system maintained it stable for 20 min, after which the subject performed a contraction identical to the previous ones (T2). The same operations were repeated, increasing the skin temperature to 45°C (contraction T3). After contraction T3, the temperature was decreased again to 33°C by a fan blowing air at adjustable intensity. The decrease in temperature was achieved in 40 min. The same motor unit was used as feedback by the subject in the seven contractions.

Although some investigators suggested 30 min of maintenance of the skin temperature to stabilize the intramuscular temperature in nerve conduction studies (18), the interval of time selected in this study was longer than that indicated by other authors (41). Moreover, in this study, we investigated very superficial muscle fibers due to the need of recording the same motor unit activity with both intramuscular and surface EMG recordings (15). Thus the muscle layer of interest was just below the skin layer. Similar considerations hold for the final decrease in temperature. Finally, the main aim of the study was to compare the relative changes in motor unit force and electrophysiological properties, which were measured concomitantly in the same conditions.

Torque Recordings

Two load cells mounted on a footplate measured the torque around the ankle joint, similar to a previous study (14). The signal from the first load cell was amplified (charge amplifier J011B10, Kistler Instrument, Winterthur, Switzerland), leading to a sensitivity of torque recording of 50 mV/mNm (bandwidth 0.1–100 Hz). This signal was used to extract single motor unit twitch torque by averaging, as described below. The signal from the second load cell was amplified (amplifier unit LAU 73.1, Soemer, Lennestadt, Germany) and used to measure the average joint torque that served for the estimation of recruitment threshold. Both torque signals were sampled at 2,048 Hz and synchronized with the EMG signal recordings.

EMG Signal Detection

Surface and intramuscular EMG signals were recorded simultaneously from the tibialis anterior muscle. The procedure for electrode positioning was described and validated in previous work (15).

Surface EMG. Surface EMG signals were recorded by a linear adhesive array (ELSCH008, SPES Medica, Salerno, Italy) of eight equi-spaced electrodes (interelectrode distance 5 mm, electrodes 5 mm × 1 mm) (14), located along the fiber direction between the most distal innervation zone and the distal tendon. The skin was slightly abraded in the location selected for array placement. To ensure proper electrode skin contact, 20–30 μl of conductive gel were inserted into the cavities of the adhesive electrode array. Surface EMG signals were detected bipolarly and amplified (EMG amplifier, EMG-16, LISIN-Primomed & Sport, Treviso, Italy; bandwidth 10–500 Hz), sampled at 2,048 Hz, and stored after 12-bit analog-to-digital conversion.

Intramuscular EMG. Four-wire electrodes made of Teflon-coated stainless steel (A-M Systems, Carlsborg, WA) were inserted with a 23-G needle, 10–20 mm proximal to the surface array top (15). Approximately 1 mm of the wires was uninsulated at the tip to detect intramuscular EMG signals. The needle was removed with the wire electrodes left inside the muscle. Intramuscular EMG signals were amplified (counterpoint EMG, DANTEC Medical, Skovlunde, Denmark), band-pass filtered (500 Hz–4 kHz), sampled at 10,240 Hz, and stored after 12-bit analog-to-digital conversion.

Signal Analysis

The action potentials of the target motor unit were identified from the intramuscular recordings with a decomposition algorithm previously described (15). Due to the feedback, in most cases, only the activity from one clear motor unit was detected; thus the number of potentials superimposed with each other was negligible. Interpulse interval variability was computed as the ratio between standard deviation and mean interpulse interval and expressed in percentage. The maximum of the cross-correlation function between the average templates of the target motor unit action potentials in the seven contractions was, in all cases, higher than 0.85, which indicated that the target motor unit was most likely the same in the seven contractions.

The intramuscular action potentials were used to trigger the averaging of joint torque and surface EMG signals. For averaging both torque and EMG signals, only discharges distant more than 150 ms from the previous and the next were considered. The twitch torque profile was characterized by peak amplitude and time to peak. Time to peak was defined as the time interval between the minimum and maximum points of the twitch. The recruitment threshold of the target motor unit was estimated as the average torque exerted in the 3-min contractions, since the target motor unit was always activated at the minimum discharge rate.

Conduction velocity was estimated from the averaged multichannel surface potentials by a maximum likelihood technique (17). The channels for conduction velocity estimation were manually selected as those propagating with minor shape changes. The same channels were used for the seven contractions in each subject. From the averaged potentials, mean power spectral frequency and peak-to-peak amplitude were computed. Action potential properties were estimated from the middle channel among those used for conduction velocity estimation.
Statistical Analysis

Data are reported as means ± SE. One-way repeated-measures ANOVA was used to assess the dependency of the variables that described the target motor unit properties on the contraction (seven contractions: C1–C3, T1–T3, C4). When ANOVA was significant, the post hoc Student-Newman-Keuls (SNK) test for pairwise comparisons was applied. Pearson correlation coefficient was used to identify linear relations between the relative temperature-induced changes in the investigated variables. Significance was accepted for \( P \) values of <0.05.

RESULTS

All subjects were able to identify the target motor unit in the seven contractions. In most cases, there was only one main motor unit detected by the intramuscular wires, and in some cases it could be visually identified in the multichannel surface EMG recordings, even without averaging (Fig. 1) (16). The averaging allowed the extraction of the surface motor unit action potentials from the background noise. For all contractions, the peak of the averaged surface potential was more than 10 times larger than the background noise (Fig. 2).

Control Contractions

None of the investigated variables significantly changed among contractions C1–C3, T1, and C4 (Table 1 and Figs. 3 and 4).

Effect of Temperature

Control properties. Discharge rate, interpulse interval variability, and recruitment threshold did not significantly change among contractions T1–T3 (Table 1).

Twitch torque. The number of triggers used for averaging torque and surface EMG (with minimum distance of 150 ms between discharges) was not significantly different among contractions (324 ± 23 per contraction, mean over the seven contractions). The peak twitch torque was different among contractions T1–T3 (ANOVA: \( F = 3.4, P < 0.01; \) SNK: \( P < 0.05 \)), increasing with temperature (Figs. 2C and 3; percent increase between T1 and T3, 94.0 ± 26.1%). Twitch time to peak decreased with temperature and was different between T1 and T3 (Table 1; ANOVA: \( F = 2.5, P < 0.05; \) SNK: \( P < 0.05; \) percent decrease, 11.9 ± 4.2%).

Action potential. The similarity in shape of the average surface potentials recorded at different detection points along the array was assessed by cross-correlation analysis. The maximum of the cross-correlation function between pairs of signals detected by consecutive electrode pairs of the array (i.e., signals detected along the propagation of the action potentials) was 0.87 ± 0.04 (see the representative example in Fig. 2). This condition ensured reliable conduction velocity estimates (34). The number of channels used for conduction velocity estimation was (over the seven contractions) 4.4 ± 0.3.

Conduction velocity increased with temperature (\( F = 9.3, P < 0.001 \)) and was different among T1, T2, and T3 (Fig. 4; \( P < 0.05 \)). Mean power spectral frequency increased with temperature (\( F = 3.5, P < 0.01 \)) and was different between T1 and T3 and between T2 and T3 (Fig. 4; \( P < 0.05 \)). Its relative increase between T1 and T3 (28.9 ± 12.3%) was not significantly different from the relative increase in conduction velocity (26.4 ± 9.4%). Peak-to-peak amplitude decreased with temperature and was different between T1 and T3 (Table 1; ANOVA: \( F = 6.5, P < 0.001; \) SNK: \( P < 0.01 \)).

The relative increase in conduction velocity between T1 and T3 was significantly correlated with the relative increase in twitch peak amplitude (\( r^2 = 0.48; P < 0.05 \)), with the decrease in twitch time to peak (\( r^2 = 0.43; P < 0.05 \)), and with the decrease in action potential peak-to-peak amplitude (20.8 ± 4.7%; \( r^2 = 0.50, P < 0.05 \)).

DISCUSSION

Single motor unit contractile and electrophysiological properties were jointly analyzed during voluntary contractions with varying skin temperature. Twitch torque and conduction ve-
Locity increased with increasing temperature. The relative increase in conduction velocity was correlated to the increase in peak twitch torque and in mean power spectral frequency, and to the decrease in twitch time to peak and in action potential peak-to-peak amplitude. Despite the change in twitch torque, recruitment threshold did not change with temperature.

**Temperature Measurement**

Muscle temperature was varied by heating the skin and monitoring skin temperature. Due to the many recording systems, it was not practically possible to insert an intramuscular probe to directly measure intramuscular temperature. Hopf and Maurer (23) showed that, although there can be a difference between skin and intramuscular temperature, there is an almost linear relation between the two. This also depends on the interval of time during which the skin temperature is maintained. The selected time interval is longer than that suggested to warm the limbs in nerve conduction studies (41). Moreover, in this study, the analyzed motor units were very superficial, because it was

### Table 1. **Mean discharge rate, interpulse interval variability, recruitment threshold, twitch time to peak, and peak-to-peak amplitude of the surface potential of the target motor unit in the seven contractions**

<table>
<thead>
<tr>
<th></th>
<th>Discharge rate, pps</th>
<th>IPI variability, %</th>
<th>Threshold, %MVC</th>
<th>Twitch Time to Peak, ms</th>
<th>Surface Action Potential Peak to Peak, μV</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>7.2±0.4</td>
<td>35.3±9.8</td>
<td>4.4±0.5</td>
<td>70.5±5.9</td>
<td>35.9±6.8</td>
</tr>
<tr>
<td>C2</td>
<td>6.8±0.5</td>
<td>36.1±8.4</td>
<td>4.2±0.6</td>
<td>72.2±5.1</td>
<td>34.2±6.7</td>
</tr>
<tr>
<td>C3</td>
<td>7.1±0.5</td>
<td>38.2±7.7</td>
<td>4.6±0.5</td>
<td>74.6±5.0</td>
<td>31.6±5.2</td>
</tr>
<tr>
<td>T1</td>
<td>6.9±0.3</td>
<td>32.7±10.1</td>
<td>4.3±0.5</td>
<td>71.9±4.1</td>
<td>35.0±6.5</td>
</tr>
<tr>
<td>T2</td>
<td>7.0±0.5</td>
<td>39.1±9.5</td>
<td>4.5±0.3</td>
<td>69.8±4.0</td>
<td>29.9±5.7</td>
</tr>
<tr>
<td>T3</td>
<td>7.2±0.4</td>
<td>36.3±9.8</td>
<td>4.7±0.6</td>
<td>61.8±2.0*</td>
<td>27.4±5.1†</td>
</tr>
<tr>
<td>C4</td>
<td>7.5±0.3</td>
<td>32.2±8.9</td>
<td>4.6±0.3</td>
<td>69.7±4.7</td>
<td>31.9±5.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. IPI, interpulse interval; MVC, maximal voluntary contraction; C1–C4, control contractions; T1–T3, contractions with varying temperature. *Significantly different with respect to T1 (P < 0.05); †significantly different with respect to T1 (P < 0.01). All differences with respect to T1 also hold with respect to C1–C3 and C4. For all variables, C1–C3 and C4 did not result in differences with T1.

Fig. 2. Target motor unit action potentials and twitch torques in the 7 contractions (C1–C4, control contractions; T1–T3, contractions with varying temperature) of a representative subject. A: intramuscular action potentials of the target motor unit (1 action potential every 10 detected is shown for clarity). B: averaged surface action potentials detected bipolarly by the array electrode. Note that the potentials detected by the 7 bipolar systems are similar in shape and delayed between each other. The delay represents the propagation time and is inversely related to conduction velocity (CV). CV values in the 7 conditions are also shown. C: twitch torques obtained by the spike-triggered averaging.
The effect of temperature on membrane properties of excitatory cells is primarily due to alterations in sodium channel function (22). Peak inward/outward ionic current densities (24) and peak-to-peak amplitude of the first derivative of the intracellular action potential (19) are linearly related to temperature. With an increase in peak inward sodium ionic current and in peak outward potassium ionic current, the depolarization of the membrane proceeds faster, with an increase in conduction velocity.

The increase in muscle fiber conduction velocity with temperature has been previously observed in animal preparations (9, 19) as well as in human motor units (36, 42). Further evidence for this effect was provided by the analysis of spectral variables of surface EMG (6, 35) and of the duration of intramuscular action potentials (5, 10, 19).

Conduction velocity influences the duration of the action potential (2, 30); thus mean power spectral frequency of the surface action potential increased with temperature (Fig. 4). Because the relative change in mean frequency was not different or smaller than that in conduction velocity, temperature did not affect the degree of synchronization of the single-fiber action potentials in the motor unit, in agreement with previous studies (5, 21).

Surface action potential peak-to-peak amplitude decreased with temperature, according to the temperature-induced decrease of the evoked muscle potential amplitude (40), and its relative change was correlated to the relative increase in conduction velocity. The peak-to-peak amplitude of the second derivative of the intracellular action potential increases with temperature, but the length of the depolarization zone decreases (19). These two mechanisms have an opposite effect on the surface-detected action potential. Moreover, the decreased conduction velocity in single fiber action potentials increases the degree of signal cancellation in the motor unit action potential (27).

The increase in conduction velocity and the decrease in peak-to-peak amplitude of motor unit action potentials observed in this study partly explain the smaller surface EMG amplitude at a given force level with increasing temperature (e.g., Ref. 3). However, it is not possible to generalize the present results, obtained from low-threshold motor units, to high-threshold units.

Twitch Torque

Although the effect of temperature on the tension produced by single muscle fibers has been established (4), there are no reports that analyzed single motor unit twitch torque in vivo during voluntary contractions at varying temperature. Single motor unit twitch torque can be measured in vivo by artificially activating motor axons (50) or from the average of the joint torque triggered by the motor unit action potentials (44), as done in this study. Although it has limitations (47), the spike-triggered averaging method allows the estimation of motor unit twitch torque during voluntary muscle contraction. On the contrary, stimulation of motor axons activates motor units in an otherwise passive muscle, which attenuates the measured force (49).
The present results indicate that temperature has an important effect on motor unit peak twitch torque, with a relative increase of almost 100% in the range of 33–45°C skin temperature (Fig. 3). Moreover, the twitch time to peak decreased as an effect of increased temperature (Table 1). The observed increase in twitch torque with increasing temperature cannot be due to changed discharge rate, which was the same for all contractions (Table 1). The potential methodological issue of increased degree of motor unit synchronization on the spike-triggered average torque (47) can also be excluded, because this would have increased peak-to-peak amplitude of the averaged surface action potential (26), opposite to what was observed (Table 1).

The duration of the contractions was relatively long, due to the need of obtaining a sufficient number of triggers for extracting single twitches (~300 triggers with 3-min-long contractions). Thus it could not be excluded a priori that the observed changes in twitch properties were due to the repetitive motor unit activation rather than to the change in temperature. The control contractions C1–C3 allowed us to rule out this possibility because no changes in any of the analyzed variables were observed in these contractions.

The main contribution of this study is the joint assessment of contractile, electrophysiological, and control motor unit properties with varying temperature. It has been shown in vivo that the increase in peak twitch torque and the decrease in twitch time to peak are correlated to the increase in conduction velocity. Interestingly, in a previous study (14), a similar increase in single motor unit twitch torque due to fatigue corresponded to a decrease (not correlated to the increase in twitch torque) in conduction velocity, which underlines the different relation between contractile and electrophysiological properties in different muscle conditions.

The correlation between changes in twitch torque and conduction velocity with temperature may be due to both a direct effect of electrophysiological modifications on contractile responses or to a similar effect of temperature on twitch torque and action potential properties through unrelated mechanisms.

The speed of calcium release depends on the speed of propagation of the depolarization zone along the fiber. The observed decrease in motor unit twitch time to peak with increasing temperature is in agreement with animal (4) and human (12) studies on whole muscle twitch force. Our results indicate that the temperature-induced increase in contraction speed is probably the consequence of increased action potential propagation velocity. Accordingly, the motor unit twitch time to peak is correlated to conduction velocity across the muscle motor unit population (1).

The increased peak of the twitch torque may be due to temperature-induced changes in the intrinsic contractile properties of the muscle fibers, such as to increased force produced per cross bridge (20), or to increased calcium sensitivity (31). The force-pCa curve shifts toward lower pCa with increasing temperature (20), as the affinity of troponin for calcium decreases with increasing temperature. These mechanisms are not related to modifications in fiber electrophysiological properties.

Temperature may also change the viscoelastic properties of the muscle (13), thus indirectly affecting the twitch torque recorded at the joint by changing the friction force between muscles fibers (38). This effect is independent of the modifications of electrophysiological fiber properties. Moreover, the twitch forces summate nonlinearly (37, 38), depending on the number of active motor units (37). A superadditive summation occurs with a small number of recruited motor units (37). Although unlikely, since the joint force did not change in the different contractions, it cannot be excluded that the change in temperature affected the nonlinearity in twitch summation.

On the other hand, electrical phenomena may also play a direct role in the increase of twitch with temperature. Although the peak-to-peak amplitude of the surface potentials decreased, the second derivative of the intracellular action potential increases with temperature (19), indicating an increase of the ionic currents, which may be associated with increased calcium release. Accordingly, the increased conduction velocity associated with very close previous discharges (doublets) may be related to an increase in twitch torque (39).

A direct association between conduction velocity and peak twitch torque with varying temperature cannot be fully proven with the present data. It can, anyway, be concluded that temperature caused partly correlated changes in contractility and action potential properties.

**Recruitment Threshold**

Despite the changes in membrane and contractile motor unit properties, there were no changes in recruitment threshold (Table 1). A similar relative increase in peak twitch torque with fatigue (without no modifications in the time to peak) led to an increase in recruitment threshold (14). Moreover, the recruitment threshold of motor units that increase their twitch torque with fatigue increases, whereas the opposite is observed for motor units decreasing their twitch torque (11). Comparing the data on fatigue (14) and the present results on temperature, it can be concluded that the changes in motor unit recruitment threshold are not exclusively related to the modifications in twitch profile. Similar changes in peak twitch torque can indeed induce different alterations in recruitment threshold.

Motor unit discharge rates decrease with fatigue (7), and this was interpreted as an adaptation of the control strategies to the change in twitch torque [the “muscle wisdom” theory (32)]. However, when contractile muscle properties are changed by varying temperature (8), the motoneuron discharge rate does not decrease. Overall, these observations and the present results exclude a short-term adaptation of the control strategy to the modifications in twitch torque, at least at the single motor unit level.

In conclusion, this study shows that, with increasing temperature, low-threshold motor unit peak twitch torque and muscle fiber conduction velocity increase, time to peak of the twitch and action potential amplitude decrease, whereas recruitment threshold is unchanged. Changes in twitch properties are correlated with changes in muscle fiber conduction velocity. Thus some of the temperature-induced modifications in contractile motor unit properties may be mediated by electrophysiological mechanisms.

**GRANTS**

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