Effects of Temperature and Angular Velocity on Eccentric Force of an Intrinsic Thumb Muscle

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Temperature and velocity have significant effects on muscle contractile properties. For example, it is well known that changing angular velocity and altering muscle temperature can change the force-producing capacity of muscle. It is typically understood that with increasing shortening velocity and cooler temperature force decreases. However, the influence of temperature and angular velocity on force during lengthening contractions is less understood. The purpose of this study was to determine whether temperature and/or velocity has an effect on the eccentric to isometric force ratio of the adductor pollicis muscle in young males. Ten young (average 25y) male subjects performed lengthening (0-40°) electrically-evoked contractions of the left adductor pollicis muscles at ~50% of maximum voluntary force at angular velocities ranging from 0-320°/s. This procedure was performed initially at room temperature (21°C), and then repeated two more times after a 20min bath, first in a cold (15°C) water and then in a warm (43°C) water bath in order to change the muscle temperature. The eccentric to isometric ratio was significantly greater in the cold compared to the normal condition (P>0.05), but was not different from normal for the warm condition (P>0.05). The eccentric to isometric ratio was significantly greater at 80, 160 and 320°/s (P<0.05) than at 20/s, but was not different at velocities slower than 80°/s (P>0.05). Instantaneous stiffness was measured 1 s after attaining maximum stretch. There was a significant ~38% increase in active stiffness in the cold muscle compared to normal. No significant difference (~15%) in stiffness was observed for the warm compared with the normal condition, and no significant difference (~20%) was found between warm and cold muscles. The findings suggest that there is an increased force per cross-bridge as temperature approaches normal physiological temperature, but decreases when temperature deviates from normal. Keywords: Electrical Stimulation, Eccentric to Isometric Force Ratio, Adductor Pollicis.

Introduction

Eccentric contractions (i.e., active muscle lengthening whereby the external load is greater than the tension produced by the muscle) are a part of everyday life from walking down the stairs to taking a seat in a chair. Eccentric contractions are notable in that they
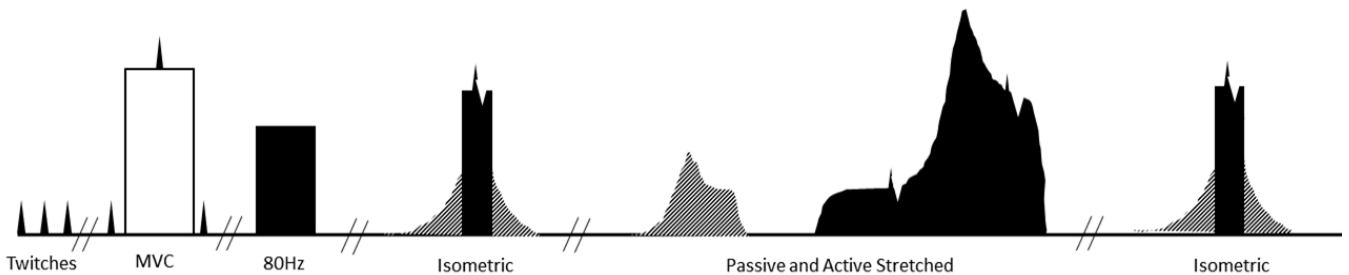
Figure 1:

Experimental Timeline. Contractions conducted within one temperature condition (normal, cold and warm). Open regions are voluntary contractions, grey regions are passive stretches and black regions are electrical stimulation.

have a greater force-producing capacity than purely isometric contractions. Previous work has used the eccentric to isometric force ratio to better understand the properties pertaining to eccentric muscle strength [1]. Stiffness (i.e., increase in force during a fast stretch of a small amplitude) has also been utilized to study the eccentric portion of muscle contractions which can be used to show the approximate proportion of attached cross bridges[2]. However, results with regards to the proportion of attached cross-bridges across temperatures is in contention. For example, Piazzesi et al.[3] found no difference in stiffness across a variety of muscle temperatures, while Lee & Herzog[4] found that the stiffness in single fibres of frog muscles decreases with temperature. Based on the Lee & Herzog[4] study, there was a lack of change in the force seen in the warm compared to the cold muscle. Yet the decrease in stiffness in the warm condition would typically indicate a decrease in force generation. They[4] proposed that this was the result of an increase in the average force produced by each individual cross-bridge via the shifting of the proportion of attached cross-bridges to the strongly bound state.

Within the muscle there is an equilibrium ratio of weakly bound and strongly bound cross-bridge states[5]. The strongly bound are the force producing states, while both the weakly and strongly bound states contribute to stiffness[6]. Cold muscles typically produce less force than warm muscles, with a lack of temperature dependence around 25C in mammalian muscle[7], but may have a similar stiffness, which could be explained by a greater ratio of weakly to strongly bound cross-bridges[5], and a similar proportion of attached cross-bridges in all states[8]. Most studies on temperature effects have been performed in isolated muscle or fibre preparations[5], and little is known

about the behavior of muscles in vivo.

Muscle stretch velocity also plays an important role in the force produced in eccentric contractions[9]. For example, the eccentric to isometric force ratio increases with velocity in adult men[10]. However, it is not known if temperature has an influence on the increasing ratio with increasing velocity. The combination of eccentric velocity and muscle temperature on eccentric force production in an adult population has not been fully investigated.

The purpose of this study was to determine whether increasing or decreasing muscle temperature has an effect on the eccentric to isometric force ratio and muscle stiffness in vivo. It was hypothesized that cooling muscles would result in an increase in the eccentric to isometric force ratio due to an increased ratio of weakly to strongly bound cross-bridges. Conversely, we hypothesized that a warm muscle would show a reduction in the eccentric to isometric force ratio compared to normal because of a decreased ratio of weakly to strongly bound cross bridges.

Methods

Participants

This study was approved by the local ethics committee (REB Number: 15396). Ten healthy young male participants (25 ± 3 yrs, 182.4 ± 7.8 cm, 71.5 ± 5.5 kg) took part after giving written informed consent. The procedures conformed with the Declaration of Helsinki[11]. All participants were recreationally active and free from any known neuromuscular or musculoskeletal disorders. The participants did not undertake any regular hand exercises. The participants visited the lab once during

testing where orientation and data collection took place.

Experimental Setup

The experimental timeline is outlined in Figure 1. Thumb adduction force and carpometacarpal angular displacement were measured using a custom-designed dynamometer, as explained in detail elsewhere[12](Figure 2).

Briefly, the left hand was immobilized with a reusable clinical cast internally lined with a cooling/heating pad extending to the hand which was controlled by a Gaymar T/Pump localized temperature system (Model TP700 series, Gaymar Industries Inc., Orchard Park, NY, USA). A 0° reference angle was defined for each subject as the highest degree of thumb adduction possible before the dynamometer arm came in contact with the cast. Thumb angles increased with abduction, ranging from 0-40°. A thermometer was attached using tape directly on the skin above the location of the adductor pollicis in the left hand.

Electrical Stimulation

All experiments were performed using electrical stimulation of the adductor pollicis muscle via the ulnar nerve, thereby excluding influences from the central nervous system. Two self-adhering Ag-AgCl surface electrodes (2 x 3 cm) were placed over the ulnar nerve to electrically stimulate the adductor pollicis. A computer-triggered stimulator (model DS7AH, Digitimer, Welwyn Garden City, Hertfordshire, UK) was used to increase the electrical current until further increases failed to produce an increase in twitch force (single 100s square-wave pulses). This current was then used to assess voluntary activation (VA) using the interpolated twitch technique for maximum voluntary contractions (MVC)[13].

Protocol

Isometric properties: Peak twitch force amplitude was determined as mentioned above. Participants then performed 1-2 MVC contractions at a thumb angle of 40° to assess individual peak force and voluntary activation (VA). Participants were encouraged verbally during all MVCs and visual feedback of the torque achieved was provided. Only subjects with VA >90% proceeded to further testing. Percent voluntary activation was calculated as:

$$VA = 1 - [\frac{superimposed\ twitch\ force}{resting\ twitch\ force}]x100$$

All testing was performed using an identical electrical stimulation protocol. Current was increased (80Hz; square wave pulses with 100s pulse width) until the evoked force reached a value between 50 to 60% of the participants MVC peak force.

Primary Variables: Participants performed six lengthening contractions at different speeds and three muscle temperatures: 1- "normal" muscle temperature in which the temperature was that occurring at room temperature; 2- "cold" in which the muscle was cooled using a 15°C water bath for 20 minutes; and 3- "warm" in which the muscle temperature was increased using a 43°C water bath for 20 minutes. Muscle temperature was calculated by using a previously established equation developed[14] where muscle temperature = 1.02 (skin temperature) + 0.89; r^2 = 0.98. The angular velocities for thumb abduction were 0°/s, -20°/s, -40°/s, -60°/s, -80°/s, -160°/s and -320°/s followed by a second 0°/s isometric contraction.

The experiment always began with a passive lengthening of the muscle at 10°/s until the thumb was 40° abducted. Once 40° was reached, an active isometric contraction lasting for 500ms was administered to establish a plateau in the force readings. From this point on the testing of the angular velocities was conducted, starting at 0° and ending at 40°, in a randomized order. Once all of the different velocities were completed a second isometric contraction, identical to the mentioned above, was administered. At the end of each temperature condition electrodes were removed from the participant's arm and the left hand was submerged in the cool or warm bath for 20 minutes. The order of temperatures was fixed from "normal" to "cold" to "warm". The order of temperature exposure was fixed for practical reasons, as a pilot study (n=3) showed that if the "warm" tests preceded the "cold" tests, the muscle temperature did not drop sufficiently (Table 1). Once baths were completed the stimulating electrodes were placed at the same location using skin markings, and the thermometer was taped back over the adductor pollicis. The stimulation current for
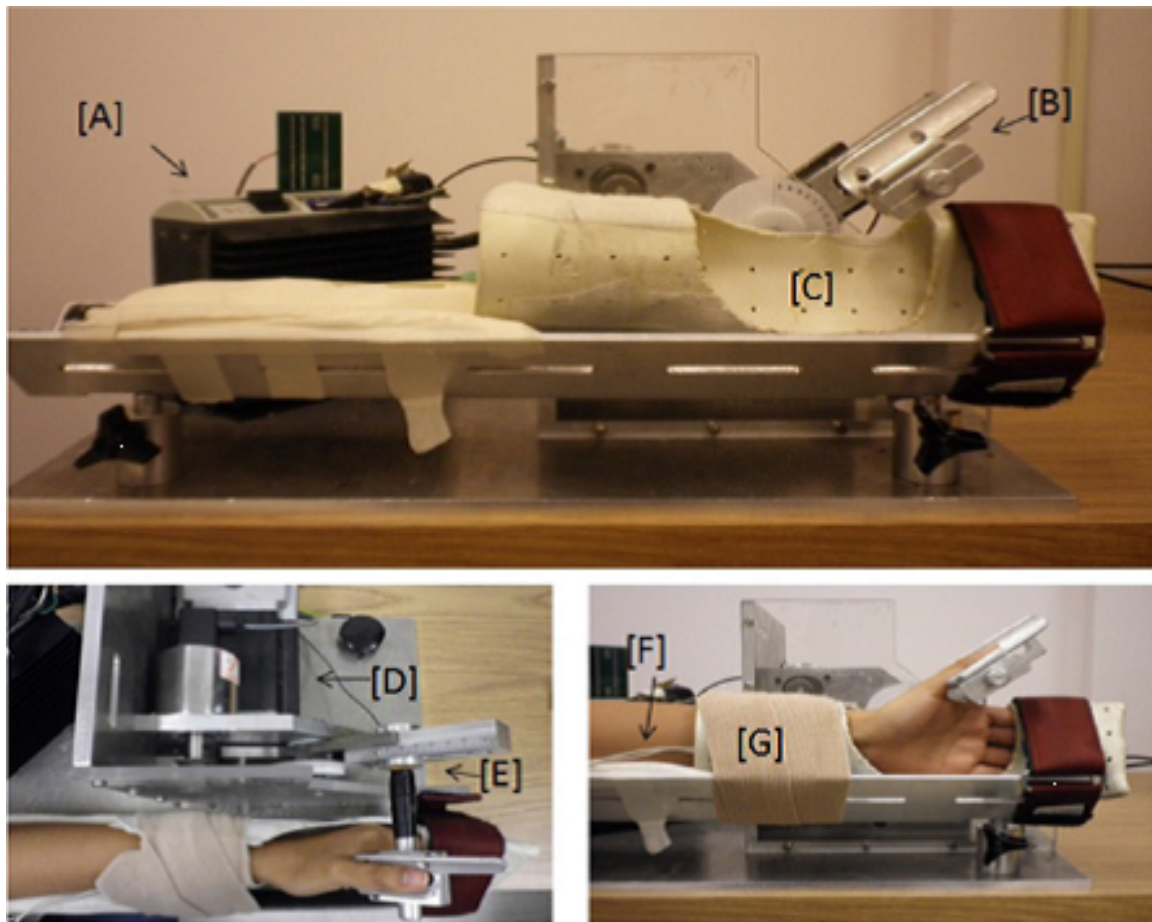
Figure 2:
The apparatus used to conduct the study included a rotary step motor (D) with a digital stepper controller. (E) represents the aluminium rod with pairs of strain gauges and the auxiliary thumb holder (B). The analog encoder is (A). The left arm (F) is fixed by the clinical cast (C) and secured with Velcro straps (G). Adapted from Fortuna, Vaz & Herzog[21].

full activation was then re-established as described above.

Data Analysis

Torque, velocity, and position data were sampled at 2000Hz and collected via an analog-to-digital converter (PowerLab System 16/35, AD Instruments, Bella Vista, Australia). All data were low pass filtered (10Hz). Instantaneous muscle stiffness (Fstiff) was determined via a change in force during a 2° quick-stretch (performed at $500°s^{-2}$) assessed 1s after the thumb position reached 40°. Twitch force was calculated as the maximal force from baseline observed in the third of three twitches applied prior to testing (Figure 1). Twitch time to peak tension[15] and twitch half relaxation time[16] was measured in the third of the three twitches at the beginning of the experimental protocol. Twitch time to peak tension was calculated from the onset of twitch force to the achievement of peak twitch force[15]. Twitch half relaxation time (HRT) was calculated as the time from peak torque occurrence until the torque had dropped to 50% of its peak value[16]. The tetanic HRT was calculated from the last electrical pulse in the 80Hz isometric contraction until force had dropped to 50% of its value at the instant of the last electrical pulse. Voluntary activation was assessed by a maximum voluntary contraction using the interpolated twitch technique (ITT)[13]. Peak eccentric force was determined as the peak force observed during the active stretch. Passive force was determined as the steady-state force following passive stretching. Active eccentric force was determined by

Condition	Pre (Nm)	Post (Nm)
Normal	86.1 ± 25.6	95.1 ± 23.5
Cold	62.8 ± 16.6*	62.9 ± 29.6*
Warm	83.7 ± 27.6	81.1 ± 21.8

Table 1: *Peak isometric force following passive stretch pre and post conditions. Average peak force of the adductor pollicis prior to and following each temperature condition. * indicates significantly different than normal ($P<0.05$). Values represent mean ± SD.*

subtracting the passive eccentric force from the peak eccentric force. Peak isometric force was determined as the peak force at a thumb angle of 40°. Passive isometric force was calculated at 40°, which was subtracted from the peak isometric force taken at 40° to calculate active isometric force.

Statistical Analysis

A one way analysis of variance (ANOVA) was performed to assess changes in peak twitch force, twitch time to peak tension (TPT), peak tetanic force at 80Hz stimulation, HRT for twitch and tetanic stimulation (80Hz) and passive and active eccentric force as a function of muscle temperature. A two way ANOVA was performed to assess changes in the eccentric to isometric ratio and stiffness as a function of thumb abduction velocity and muscle temperature. When significance was observed a *post hoc* analysis using unpaired t-tests was performed with a Bonferroni correction. The level of significance for all tests was set at $P<0.05$. All tables are presented as means ± standard deviations (SD).

Results

Isometric properties: All isometric properties are shown in Table 2. Muscle temperatures for the three temperature conditions were significantly different from each other, with the cold muscle condition being 10.9 ± 2.0°C lower and the warm muscle condition being 5.7 ± 2.0°C higher than the normal muscle temperature ($P<0.05$). Cold muscles had a 33.8% lower twitch force than normal temperature muscles ($P<0.05$), but there was no difference in twitch force between warm and normal temperature muscles ($P>0.05$).

The twitch time to peak tension (TPT) was 142.2% and 111.9% longer for the cold compared to the warm ($P<0.05$) and the normal temperature muscles, respectively ($P>0.05$ for both comparisons),

but there was no difference between the warm and normal temperature muscles ($P<0.05$). Twitch HRT was 316.2% and 214.0% longer in cold compared to warm and normal temperature muscles, respectively ($P<0.05$ for both comparisons), but was not significantly different between warm and normal temperature muscles ($P>0.05$).

The isometric tetanic force for the normal temperature condition was 90.1% higher and 18.0% lower compared to the cold and warm temperature muscles, respectively ($P<0.05$, $P<0.05$). The HRT for the tetanic contraction was 206.7% longer ($P<0.05$) for the cold compared to the normal temperature muscles ($P<0.05$), while there was no difference between the normal and warm temperature conditions ($P>0.05$).

Order of testing:: The isometric forces measured at the beginning and end of each temperature condition, and at the beginning and end of the entire experiment, were similar ($P>0.05$) (Table 1).

Lengthening contractions: Thumb abduction velocity and muscle temperature had significant effects on the eccentric to isometric force ratio. However, there was no significant interaction between these two main effects ($P=0.95$). Post hoc testing showed that in comparison to the 20°/s condition, the 320°, 160° and 80°/s eccentric to isometric force ratios were significantly increased ($P<0.05$, respectively Figure 3). The eccentric to isometric force ratio was 41.1% and 54.0% greater for the cold compared to the warm and normal muscle temperature conditions ($P<0.05$), while there was no difference between warm and normal muscle temperature conditions ($P>0.05$) (Figure 3).

Instantaneous stiffness: Thumb abduction velocity and muscle temperature had significant effects on muscle stiffness ($P<0.05$), but there were no interaction effects ($P>0.05$). The stiffness values were higher for the faster velocities (i.e., 60°, 160°, and 320°/s) as compared with the 20°/s condition

Characteristic	Normal Temperature	Cold Temperature	Warm Temperature
Muscle Temperature (C°)	29.1 ± 2.0	18.2 ± 2.0*	34.8 ± 1.9*
Twitch Torque (Nm)	6.5 ± 1.3	4.3 ± 1.6*	7.5 ± 1.4
Twitch TPT (ms)	90.2 ± 9.0	191.1 ± 36.8*	78.9 ± 3.8
Twitch HRT (ms)	70.3 ± 16.1	220.6 ± 35.1*	53.0 ± 5.1
MVC Force (Nm)	109.5 ± 22.7	-	-
80Hz Torque (Nm)	53.8 ± 9.9	28.3 ± 4.1*	45.6 ± 5.6*
80Hz HRT (ms)	109.5 ± 18.0	245.7 ± 51.7*	80.1 ± 5.4
80Hz Torque (% MVC)	50 ± 10	26 ± 4*	43 ± 6

Table 2: *Forces and times for base line muscle properties conducted in all conditions. Time to peak tension (TPT), half reaction time (HRT), maximal voluntary contraction (MVC). Values are means ± SD. * indicates significance in comparison to normal (P>0.05).*

($P<0.05$) (Figure 4). Across temperatures, isometric stiffness in the cold temperature condition was 37.9% greater than that measured for the normal temperature condition ($P<0.05$). There was no difference in stiffness between the warm and normal, as well as the warm and cold temperature conditions ($P>0.05$) (Figure 5).

Discussion

Muscle Temperature: Temperature and angular velocity effects on the eccentric to isometric force ratios were investigated in young males. Our heating and cooling protocols successfully influenced muscle temperatures and affected muscle contractile properties. Muscles had an increased eccentric to isometric force ratio in the cold compared to the normal condition, but there was no difference between warm and normal. The increased eccentric to isometric force ratio in cold compared to normal was in support of our initial hypothesis. However, our hypothesis was not fully supported for comparisons of force ratios between warm and normal conditions. This latter result may be due in part to the small change in muscle temperature from the normal (29.1 ± 2.0°C) to the warm conditions (34.8 ± 1.9°C), and the reported lack of force dependence on temperature above 25°C in mammalian muscle[7].

Isometric twitch and tetanic forces have been shown previously to increase with increasing temperatures, while instantaneous muscle stiffness has been reported to remain roughly the same across temperatures [3,17,18]. From these observations it can be assumed that there is an increase in the average force per cross-bridge as temperature increases, potentially caused by a shift to a greater proportion of strongly compared to weakly bound cross-bridges with increasing temperatures[3]. In the present study, we found that isometric force (0°/sec) increased with increasing temperature. However once muscle temperature exceeded room temperature, there was no further change in isometric force. Instantaneous stiffness for the isometric contractions was greater in the cold than normal temperature, but was not different between the warm and normal temperature conditions. Changes in stiffness across temperatures were different from those reported by Lee & Herzog[4] who did not observe a change in force across muscle temperatures, but found an increase in stiffness with decreasing temperatures, suggesting that the force per cross-bridge increased with increasing temperatures. The experiments by Lee & Herzog[4] were conducted with frog muscles, which have been shown to adapt to cool conditions to maintain peak force[19], and therefore may demonstrate the same temperature-dependence of muscle properties as mammalian muscles.

Angular lengthening velocities: The eccentric part of the force-velocity relationship[9] is associated with an increase in force with increasing lengthening velocities, up to a certain threshold velocity. At this threshold the muscle does not produce any more force with increasing lengthening velocity[9]. This translates to an increased eccentric to isometric force ratio for increasing stretch velocities up to the threshold mentioned, which has been observed in young and old populations[10,20]. As expected, we found a general increase in eccentric to isometric force ratio at angular velocities ≥ 80°/s compared to the slowest eccentric velocity (20°/s). There was no significant change
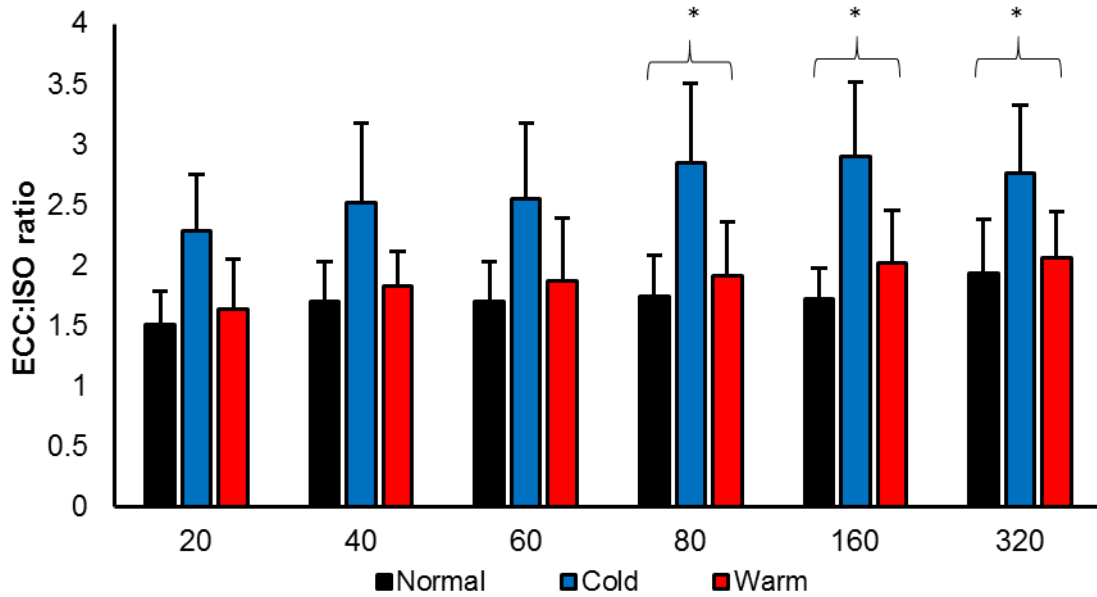
Figure 3:
Average eccentric to isometric force ratios in all three temperature conditions (normal, cold, warm) across six velocities (20°, 40°, 60°, 80°, 160°, 320°sec). Cold was greater than normal and warm across all velocities. * denotes velocity-dependent significance between ratios in comparison to 20°/sec (P<0.05). Values are mean ± SD.
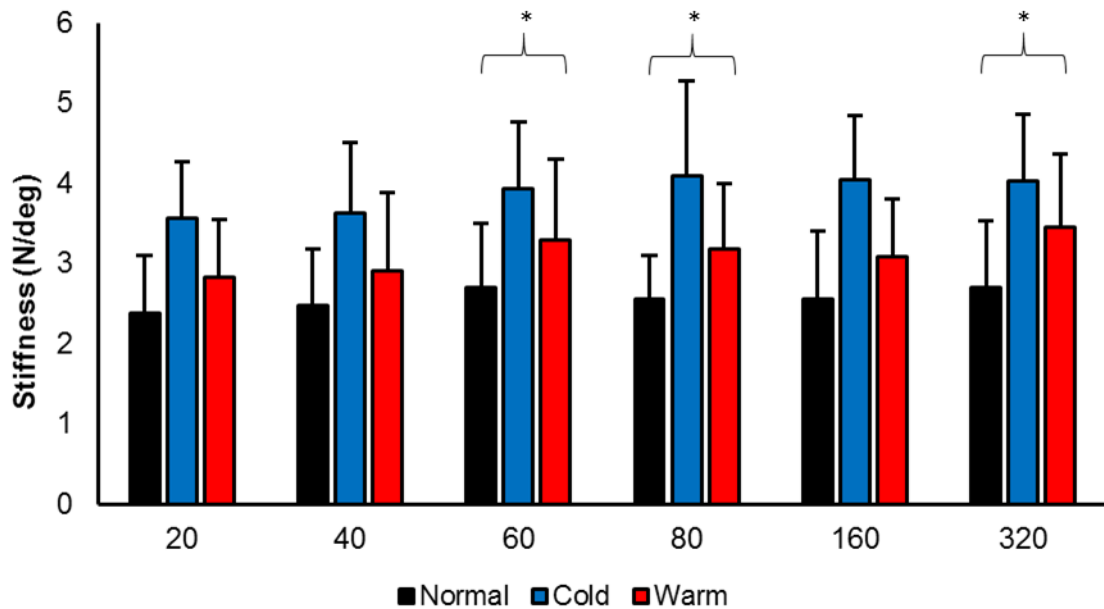

Figure 4:
Average instantaneous muscle stiffness across muscle temperatures (normal, cold, warm) and velocities (20°, 40°, 60°, 80°, 160°, 320°sec). Within each temperature, stiffness was only greater for the cold as compared with normal condition. * denotes significance between grouped velocities stiffness in comparison to 20°/sec (P<0.05). Values are mean ± SD.
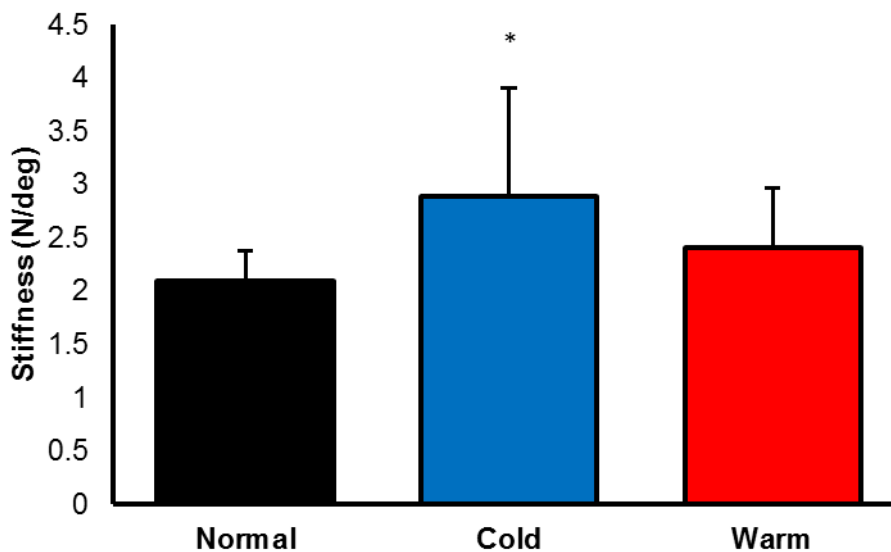
Figure 5:

Average instantaneous stiffness of isometric force following a passive stretch at the beginning of each temperature condition. * denotes significant difference from Normal (P<0.05). Values are mean ± SD.

in the eccentric to isometric force ratio at angular velocities higher than 80°/sec. This observation is consistent with earlier results on the eccentric to isometric force ratio for eccentric contractions [10].

Proposed mechanisms of the temperature and velocity dependence of force: The temperature dependence of eccentric force within the eccentric to isometric force ratio may be explained in part by the altered contractile kinetics in cold compared to warm muscles. In cold conditions compared to warm conditions there tends to be an increase in the eccentric to isometric force and stiffness ratio, possibly because there is an increasing proportion of weakly to strongly bound cross-bridges with decreasing muscle temperature. Since force increased from the cold to the normal temperature conditions with a simultaneous decrease in stiffness, it seems that the force per cross-bridge increased with increasing muscle temperature, as reported in the literature[3,4].

The eccentric to isometric force ratio and the absolute stiffness were not different between the warm and normal temperature conditions. The constant stiffness for the warm and normal muscle temperature conditions suggests that the proportion of attached cross-bridges remains similar for these two temperatures. Since the isometric force between the normal and warm temperature conditions remained the same (Table 1), it seems that the force per

cross-bridge is the same for these two temperatures. This result goes against convention, as it is typically accepted that as temperature rises the force per cross-bridge increases as well[4]. However, due to the sigmoidal relationship of the isometric force and temperature[6], we suspect that there is a temperature dependent theoretical maximum to the equilibrium proportion of strongly to weakly bound cross-bridges which was reached in the present experiments.

Velocity dependence of eccentric force can be explained in part by the slowing of cross-bridge cycling during the active stretch, perhaps owing to a similar rate of detachment and longer time attached[8]. Ultimately, this means the cross-bridges remain attached to the actin filament for a longer period of time. Because the myosin head remains attached for a longer period of time during stretch, the cross-bridge strain associated with the active stretch is greater, thus producing a greater force than during the purely isometric contractions. Further, when the velocity of the stretch is increased the cross-bridge strain increases, on average, as cross-bridges get dragged to greater distances away from their equilibrium conditions[5]. Our results support this increasing force with increased velocity, with a significant difference at 80, 160 and 320°/sec compared to the 20°/sec.

Order of testing: The warm temperature condition experiments were always performed last. This

order of testing could have resulted in residual fatigue or muscle damage for the warm condition experiments, which in turn, may account for the significant decrease in the tetanic force in the warm compared to the normal temperature condition experiments. This loss in peak force for the warm temperature experiments may cause an artificially elevated eccentric to isometric ratio. Assuming this is the case and that the eccentric force was not effected by fatigue or damage, we would have seen a decrease in the eccentric to isometric ratio in the warm condition, and with the accompanied stiffness tests we would have seen the potential for an increase in the force per cross-bridge as mentioned in the literature[4]. However this is unlikely, as we show no reduction in the isometric tests conducted at the beginning and end of each temperature condition (Table 1). Further, there is no difference between the first isometric forces recorded during the testing (first isometric in normal condition) to the last (second isometric in warm condition).

Conclusion

We found that in young men the eccentric to isometric force ratio is increased when the muscle is cooled, and showed no difference when muscle temperature was increased in comparison to the normal temperature condition. The velocity of stretching had an effect on the eccentric to isometric force ratio at velocities of 80°/sec and greater. Based on these findings, we propose that as muscle temperature rises, the force per cross-bridges increases until normal physiological temperature is reached; then, further increases in temperature do not increase force per cross-bridge. Finally, our data indicate that at the warm and normal muscle temperatures, the proportion of strongly bound cross-bridges may reach a limit, thereby preventing a reduction in eccentric to isometric force ratio in warm muscle.

Acknowledgements

Conflict of Interest Disclosure:

The authors have no conflicts of interest to disclose.

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