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Title: **Effects Of 22°C Muscle Temperature On Voluntary And Evoked Muscle Properties During And After High Intensity Exercise**

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ABSTRACT

Objective: Investigate the effect of 22°C local muscle temperature of intact human plantar flexors performing fatiguing contractions on evoked and voluntary contractile properties before and after fatigue. **Research Design and Methods:** Twelve subjects were tested on plantar flexor voluntary torque, percent muscle activation derived from twitch interpolation, integrated electromyographic (iEMG) activity, and evoked torque and temporal characteristics of maximal twitch and tetanic stimulations prior to fatigue and 1-, 5-, and 10-minutes after intermittent, high-intensity, isometric fatigue under both normothermic and hypothermic conditions. Hypothermic and normothermic changes between time points were analysed by repeated measures ANOVA. **Results**: Normothermic fatigue induced small to large effects (Cohen's d: 0.29 – 3.06) on voluntary and evoked contractile properties, while most effects of unfatigued hypothermia were limited to rate dependent processes (Cohen's d: 0.78 – 1.70). Most tetanic properties were potentiated 1-minute after normothermic fatigue but remained unchanged by hypothermic fatigue, resulting in significant differences between the two conditions. Soleus iEMG significantly declined 1-minute after normothermic fatigue (-29%) but not hypothermic. Twitch torque was potentiated 29% 1-minute after fatigue while normothermic though 46% while hypothermic; rate of twitch torque development and time to peak twitch were potentiated by 39% and 10% while normothermic but 89% and 28% while hypothermic. **Conclusions**: While voluntary contractile properties are generally impaired soon after normothermic fatigue, most were not with hypothermic fatigue. Furthermore, evoked contractile properties were generally higher one minute after hypothermic fatigue. We conclude that the hypothermic condition slows the recovery of potentiated evoked contractile properties back to baseline values.

Key Words: hyperemia, interpolated twitch technique, hypothermia, maximal voluntary contraction, electromyography

Introduction

Recent studies exploring the effects of hypothermia on the ability of humans to perform work in the cold examine the effects of peripheral cooling in order to isolate if certain effects are centrally or peripherally mediated (Giesbrecht et al. 1995). As manual labor, athletics, and recreation fields expand into cold environments, it is also relevant to begin studying how humans respond to fatigue in the cold. Often the insulation of the torso is adequate to prevent central hypothermia, yet participants continue to experience peripheral muscle cooling (Giesbrecht et al. 1995). While studies have been conducted investigating the rate of recovery from local muscle fatigue under normothermic conditions (Petrofsky et al. 1980; Behm and St-Pierre 1997) none have studied recovery while hypothermic.

The effects of decreased temperature on voluntary and evoked muscle contractile properties have been previously reviewed (Bennett 1984). Decreased temperatures generally decrease voluntary and evoked contractile force (Ranatunga et al. 1987), rate processes (Bennett 1984; Rome 1990), integrated electromyography (iEMG) amplitude and power spectrum (Petrofsky and Lind 1980), and endurance (Bennett 1984; Rome 1990). Similarly, many voluntary and evoked contractile properties have been investigated during fatigue and recovery from fatigue under normothermic conditions (Petrofsky and Lind 1980; Behm and St-Pierre 1997; Pääsuke et al. 1997). Each variable is altered in different ways and to a different extent by hyopthermia and fatigue depending on the muscle group, the intensity and duration of the fatiguing or cooling protocol, and the nature of the muscle contractions (e.g. isometric, dynamic). Although the effects of hypothermia, fatigue, and recovery have been studied independently, interactive effects have yet to be reported.

While the effects of cold environments on muscle activation are reasonably well documented, a distinct lack of human experiments in recovery from fatigue while hypothermic indicates that further research in the area of hypothermic recovery from fatigue is needed. Since many evoked and voluntary properties are slowed during hypothermia, the goal of this research is to investigate differences in rates of change in these properties between recovery intervals. Since it is rate dependent processes that are particularly sensitive to hypothermia (Bennett 1984; Rome 1990), we hypothesize that voluntary and evoked contractile properties will show greater impairment after hypothermic fatigue than after normothermic fatigue.

Materials and Methods

Subjects

Twelve male volunteer subjects of a mean (±SD) age of 23 years ± 6.1, body mass of 82.8 kg ± 8.5 kg and height of 177.5 cm ± 6.3 cm, were recruited. The study protocol was approved in advance by The Human Ethics Committee of the School of Physical Education, Recreation, and Athletics at Memorial University of Newfoundland. Each subject provided written informed consent before participating. Subjects were informed that they could withdraw from the experiment at any time without prejudice.

Overview of Testing

In this study, subjects performed isometric maximum voluntary contractions of the plantar flexors. Subjects were evaluated on the change between time points on a variety of voluntary and evoked contractile properties of the plantar flexors while either normothermic or hypothermic. Muscle properties investigated included percent

muscle inactivation, torque generated from the maximal voluntary contraction (MVC), twitch and tetanic torque, time to peak twitch torque, time to peak tetanic torque, rates of torque development (RTD) during voluntary and evoked contractions, twitch torque half relaxation time, tetanic torque half relaxation time, and muscle compound action potential (M-wave). Each property was measured prior to fatigue (pre-fatigue), and at 1-, 5-, and 10-minutes after fatigue under both normothermic and hypothermic conditions. Subjects performed each testing session (hypothermic and normothermic) separated by at least three days with the order of testing hypothermic or normothermic conditions randomly determined. The time of day that the subject was tested was chosen on the basis of the subject and experimenter's availability, but remained consistent for both testing sessions. All subjects participated in regular physical activity, including progressive resistance exercises, though were instructed not to perform any strenuous activity within 24 hours of testing sessions.

Dependent Variables

Torque: During plantar flexion, subjects were seated with hips and knees at 90^{o}, with their foot in a modified boot apparatus outfitted with a custom designed strain gauge (Figure 1). All voluntary and evoked torque strain gauge signals were sent through a high gain amplifier (Biopac Systems Inc. DA100) and analog to digital converter (Biopac Systems Inc. MP100WSW), and monitored on computer (Sona Phoenix PC). The sampling rate was set at 1000 Hz and all data were stored on computer. Data were recorded and analysed with a commercially designed software program (AcqKnowledge III, Biopac Systems Inc.).

Once the subject was prepared and secured into the modified boot apparatus, the voltage and amperage required to evoke maximal twitch amplitude was

determined. In order to determine the appropriate stimulation intensity for the interpolated twitch technique (ITT), peak twitch torques were evoked with electrodes connected to a high-voltage stimulator (Digitimer Stimulator; Model DS7H+, Welwyn Garden City, Hertfordshire, UK). The amperage (10 mA-1A) and voltage (100-150 Volts) of a 50 μs square wave pulse was progressively increased until a maximum twitch torque was achieved. Stimulation did not exceed 150 volts at 1 amp.

Once a maximal twitch was established, tetanic torque was measured. Tetanus was evoked with 100 volts at the amperage that was used for maximal twitch. A stimulating frequency of 100 Hz for 300 milliseconds was used. The short duration and decreased voltage was chosen to reduce the extent of discomfort for the subject. Thus the tetanic torque may not have been maximal for all subjects.

Integrated EMG: Surface EMG recording electrodes were placed over the mid-belly of the tibialis anterior, mid-belly of the lateral gastrocnemius, and distal portion of the soleus in the mid-sagittal plane immediately distal to the gastrocnemius. Ground electrodes were secured to bony landmarks on the patella, medial malleolus, and lateral malleolus. Stimulating electrodes, 2-3 cm in width, were constructed in the laboratory from aluminium foil and paper coated with conduction gel (Aquasonic) and immersed in an aqueous solution. The electrode length was sufficient to wrap the width of the muscle belly.

EMG activity was sampled (Biopac Systems Inc. MP100WSW) at 2000 Hz, with a Blackman -61 dB band-pass filter between 10-1000 Hz, amplified (bi-polar differential amplifier, input impedance = 2MΩ, common mode rejection ratio $\geq$ 110 dB min (50/60 Hz), gain x 1000, noise $\geq$ 5 μV), and analog-to-digitally converted (12 bit) and stored on personal computer for further analysis. The computer software

program rectified and integrated the EMG signal. Measurements were monitored over a 500 ms period during an MVC (Behm et al. 1996).

Interpolated Twitch Technique (ITT): The ITT was administered first during an MVC and then at 75, 50, and 25% of MVC, performed in random order to an unfatigued muscle. A doublet (2 twitches delivered at a frequency of 100 Hz) rather than a single twitch was utilized for the interpolated evoked stimulation since it provides a higher signal to noise ratio (Behm et al. 1996). An interpolated twitch (IT) ratio would later be calculated comparing the amplitudes of the superimposed stimulation with the post-contraction stimulation to estimate the extent of inactivation during a voluntary contraction. Since the post-contraction stimulation represents full muscle activation, the superimposed torque using the same intensity of stimulation would activate those fibres left inactivated by the voluntary contraction (Behm et al. 1996). All maximal and submaximal (100%, 75%, 50%, 25% of MVC) torques were correlated with their respective IT ratios in order to generate a second order polynomial equation for all subjects. Second order polynomials using both maximal and submaximal contractions (IT ratios) have been shown to be valid and reliable providing a more accurate estimation of muscle activation than a single IT ratio (Behm and St-Pierre 1997). Once these unfatigued, normothermic (baseline) measures were taken the protocol branched. In the hypothermic protocol, the limb was cooled prior to fatigue and further testing. In the normothermic protocol the cooling protocol was omitted.

Independent Variables

Cooling Protocol: To lower muscle temperature during the hypothermic testing session, active cooling methods were used involving a refrigerating pump circulating

cold (-3°C) liquid glycol (anti-freeze) through 17 meters of plastic (Tygon) tubing of 3/8" diameter with a 1/16" wall (R-3603). The subject's leg was wrapped with the tubing in a coiling fashion to cover the entire lower leg from below the knee to above the ankle. The baseline testing procedures were repeated as soon as possible after the subject's muscle temperature reached 22°C. The cold tubing remained on the subject throughout the fatigue protocol and after task failure testing as well.

Prior to cooling, a myocardial temperature probe (thermistor) was inserted into the lateral gastrocnemius. The subject's leg was first swabbed with 70% isopropyl alcohol and anaesthetized with 2% injectable Xylocaine (Astra Pharma Inc.) that was injected from a 1 ml syringe through a 26G 3/8" intradermal bevel needle to a depth of approximately 2 cm into the calf. While it was the temperature of the gastrocnemius that was measured, a myocardial temperature probe was ideal in design for our purpose. The Xylocaine was injected by a physician, who then inserted the myocardial temperature probe into the belly of the lateral gastrocnemius to a depth of 2 cm carefully avoiding vascular and neurally dense areas, and then secured with tape.

Fatigue Protocol: In both normothermic and hypothermic protocols, the fatigue protocol for the plantar flexors was begun within 5 minutes after baseline voluntary and evoked contractile properties were measured for that condition (i.e. normothermic baseline measures for the normothermic testing session, and unfatigued normothermic and unfatigued hypothermic baseline measures for the hypothermic session). The fatigue protocol consisted of isometric contractions of the plantar flexors at 75% of the subject's MVC. Subjects took 3 seconds to rise to this level, held 75% of MVC for 14 seconds, and took 3 seconds to relax. This intermittent protocol was repeated for as long as the subject could maintain 75% of MVC. For the purposes here, we will refer

to this point of task failure as the point of fatigue, though many other definitions of fatigue exist (Hunter et al. 2004). Verbal encouragement was provided throughout. The subject sat passively between time points after fatigue. Other than the peak twitch that was always elicited first to prevent post-activation potentiation, the order of testing measures (i.e. ITT at 25, 50, 75 and 100% of MVC) was randomised at each testing time (i.e. 1-, 5-, and 10- minutes post fatigue).

Analyses

Data were analysed with a two-way ANOVA with repeated measures. The two factors (2x4) were temperature (normothermic and hypothermic levels) and period between time points before and after fatigue (i.e. pre-fatigue, pre-fatigue to 1-minute post-fatigue, 1-minute post-fatigue to 5-minutes post-fatigue, and 5-minutes to 10-minutes post-fatigue). Of interest were differences between normothermic and hypothermic conditions prior to fatigue, within each condition between time point intervals before and after fatigue, and differences between conditions on the change between time point intervals before and after fatigue. P-values were considered significant at $p<0.05$. A Bonferroni – Dunn's post-hoc test was used to assess significant differences between variables. Results are expressed as either percent change between time point intervals or mean (±SD). Where differences were statistically significant, magnitudes of differences are described with standardised effect sized (Cohen's d) (Cohen 1988).

Results

Temperature

As the subjects began the exercise protocol under normothermic conditions the mean temperature of the subjects' lateral gastrocnemius was 34.7°C ± 0.7, but under the hypothermic condition the mean temperature was 21.3°C ± 0.5. Muscle temperature rose 1.0ºC ±1.4 between pre-fatigue and 1-minute post-fatigue ($p=0.01$) and a further 1.6ºC ±1.5 between 1- and 5-minutes post-fatigue ($p<0.01$) during the hypothermic condition.

Prefatigue Hypothermia

All twitch and tetanic properties were significantly slowed in the hypothermic condition prior to fatigue by a large effect size (Cohen's d: 0.91 to 1.70) except time to peak tetanus and twitch torque, which did not quite reach the threshold for 'large' (Cohen's d: 0.78 and 0.75 respectively). There was also a significant increase in M-wave duration (+46%, $p<0.05$) and a decrease of MVC RTD (-48%, $p<0.01$) and soleus iEMG activity (-37%, $p<0.01$) that were also large (Cohen's d: 0.91, 1.07, and 1.17 respectively) (Table 1, Figure 2 and 3).

Normothermic changes

Within the first minute after fatigue, most voluntary properties were lower by large effect sizes. The overall MVC showed a moderate decline (Cohen's d: -0.65), reflecting the large decrement in inactivation (Cohen's d: -3.06), MVC RTD (Cohen's d: -0.88), and lower iEMG of the soleus (Cohen's d: -0.93), and gastrocnemius (Cohen's d: -1.15). The iEMG of the tibialis anterior also showed a small decrement (Cohen's d: -0.34). None of these properties showed significant changes between 1-

and five-minutes (Table 1). Effects one minute after fatigue on evoked properties were almost all significant, except for tetanic torque, though statistically significant effect sizes ranged from small to large (Cohen's d: 0.29 to 1.03) (Table 1, Figures 2 and 3).

Hypothermic changes

In contrast to normothermic changes, the only voluntary property significantly affected at one minute after fatigue was MVC (Cohen's d: 0.49, Table 1).

By one minute after fatigue, all twitch properties were affected to a large effect size (Cohen's d: 0.81 to 1.23) except the half-relaxation time, which moderately decreased (Cohen's d: 0.69) (Table 1, Figure 2). Tetanic torque was the only tetanic property affected by one minute after fatigue, increasing by a small effect size (Cohen's d: 0.36) (Table 1, Figure 3). Between one and five minutes after fatigue, twitch torque experience a small decrease (Cohen's d: 0.36), tetanic torque and rate of tetanic torque development experienced moderate increases (Cohen's d: 0.69 and 0.73 respectively), as did soleus iEMG (Cohen's d: 0.70) (Table 1, Figures 2 and 3).

Interaction

We further assessed for interaction effects to assess if changes between time points before and after fatigue were different between the same two time points under two different conditions. For example, we observed a significant decrease in MVC before and 1-minute after fatigue of 15% while normothermic, and a significant 12% decrease between the same two time points while hypothermic. The difference between the observed 15% decrease and 12% decrease was not significant (Table 1).

The change before and after fatigue was significantly different in soleus iEMG (1-minute normothermic: decreased 29%, hypothermic: ns; 5-minutes normothermic: ns, hypothermic: increased 36%). All rates of torque development were significantly different, including MVC RTD (1-minute normothermic: decreased 39%, hypothermic: ns), twitch RTD (1-minute normothermic: increased 39%, hypothermic: increased 89%), and tetanic RTD (1-minute normothermic: increased 17%, hypothermic: ns; 5-minutes normothermic: ns, hypothermic: increased 40%). There were also significant differences between time to peak twitch (1-minute normothermic: decreased 9.7%, hypothermic: decreased 28%) and time to peak tetanus (1-minute normothermic: decreased 9.7%, hypothermic: ns; 5-minutes normothermic: ns, hypothermic: ns). Significant differences also existed between the change of twitch half relaxation time (1-minute normothermic: increased 38%, hypothermic: decreased 23%), tetanus half relaxation time (1-minute normothermic: increased 35%, hypothermic: ns), tetanic torque (1-minute normothermic: ns, hypothermic: increased 22%), and a trend ($p=0.09$) and twitch torque (1-minute normothermic: 29%, hypothermic: 46%).

Discussion

The current research was undertaken to investigate the effect of local muscle hypothermia on evoked and voluntary contractile properties at several time points before and after fatigue. The current data indicates that during local muscle hypothermia, there are significant differences in the change before fatigue and one minute after fatigue in most evoked and several voluntary contractile properties. While the rate of voluntary force development and soleus iEMG were significantly impaired one minute after normothermic fatigue, these properties were not

significantly impaired one minute after hypothermic fatigue. Twitch and tetanic properties normally potentiated one minute after normothermic fatigue (i.e. torque, rate of voluntary force development, and time to peak torque) were significantly potentiated by a greater extent one minute after hypothermic fatigue. While responses to unfatigued local muscular hypothermia in the current research were in agreement with previous investigations (Bennett 1984), as were our results one minute after fatigue under normothermic conditions (Behm and St-Pierre 1997), this is the first investigation to compare the response to fatigue under normothermic and hypothermic conditions.

Effect of Temperature

The current investigation illustrates a decline of voluntary and evoked properties that are dependent on rate processes under local muscle hypothermia. We found a significant decline of hypothermic soleus iEMG, a finding in agreement with other studies showing decreased iEMG (Mucke and Heuer 1989; Oksa et al. 1995). Decreases in iEMG activity could suggest a change in muscle activation or a slowing of membrane conduction velocity (Mucke and Heuer 1989), a finding we also support with a 46% increase in M-wave duration. Therefore, the changes in iEMG are suspected to be primarily due to hypothermic membrane changes and not related alterations in muscle inactivation due to the lack of changes in ITT (muscle inactivation). The lack of change of tibialis anterior or gastrocnemius iEMG during hypothermic unfatigued MVC was not surprising due to their modest involvement during seated plantar flexion (Signorile et al. 2002).

Isometric force properties are generally not strongly affected by lowering muscle temperature to ~25ºC (Bennett 1984; Ranatunga et al. 1987; Faulkner et al. 1990). The current study found a trend (p=0.10) towards a decline of MVC that was not quite consistent enough to match the results of those studying temperatures of 25ºC. We did not find any change in percent inactivation with cooling the muscle to 22ºC, though we found significant declines of both twitch and tetanic torque. This is likely related to the well-established slowing of rate processes of voluntary (Davies and Young 1983; Bennett 1984), and evoked (Davies and Young 1983; Bennett 1984; Ranatunga et al. 1987; Faulkner et al. 1990; Oksa et al. 1995) properties with cold application, findings that we also confirmed. Reduced rate of ATP hydrolysis by hypothermia has been proposed (Faulkner et al. 1990) to be responsible for the impairment of twitch and tetanic properties while hypothermic by impairing the rate of cross-bridge cycling. Since twitch and tetanic tension occur within fixed stimulation periods (50μs pulse and 300 ms train respectively), a reduced RTD in a fixed period of time would result in the development of less force. Therefore, the decrements in evoked torque with hypothermia are likely to be related more to a decrease in RTD than the ability to develop maximal torque. Similarly, it is postulated that a reduction in the rate of ATP hydrolysis would also cause slowing of sarcolemmal cation pumps by reducing the activity of Na^+-K^+ ATPase prolonging the duration of the M-wave as found in the present study. Possible slowing of sarcoplasmic reticulum-ATPase would further explain the slower half relaxation times (Zhu and Nosek 1991; Fitts 1996).

Normothermic changes

The current results one minute after normothermic fatigue reflect an impairment of voluntary contractile properties and an enhancement of most evoked properties

(Petrofsky and Lind 1980; Behm and St-Pierre 1997; Pääsuke et al. 1997). Since we found a decline of iEMG (Fuglevand et al. 1993) in both plantar flexor muscle groups under normothermic conditions, our decline of MVC (de Hann et al. 1989; Behm and St-Pierre 1997; Pääsuke et al. 1997), rate of voluntary force development (de Hann et al. 1989), and increase in inactivation (Behm and St-Pierre 1997) are at least partially from declining motor unit excitation. This is reinforced by the potentiation of twitch and tetanic torque properties, indicating the absence of faltering excitation-contraction coupling and myofilament kinetics.

The slowing of calcium reuptake proposed to cause prolonged relaxation time could also be responsible for potentiation of most evoked torque properties. Calcium acts as a second messenger to activate myosin light chain kinase (MLCK), a protein kinase. MLCK will cause phosphorylation of phosphorylatable light chains (P-LC) on the myosin molecule regulating force generation by increasing actomyosin ATPase activity (Grange et al. 1993) and increased sensitivity to calcium (Moore et al. 1990). With the slower removal of calcium, the phosphorylate myosin light chains (PLC) remain phosphorylated longer thereby maintaining (Grange et al. 1993) or potentiating tension, becoming dephosphorylated with a time constant of ~5 minutes (Moore et al. 1990). This impairment of calcium reuptake would explain the prolonging of relaxation times (Zhu and Nosek 1991).

The present normothermic results are not entirely in agreement with previous findings. While fatiguing contractions had no significant effects on M-wave amplitude or duration, some studies have found a reduction in amplitude (Fuglevand et al. 1993; Behm and St-Pierre 1997) and/or prolonged duration (Petrofsky et al. 1980;

Fuglevand et al. 1993). Additionally, Pääsuke and co-workers (1997) found longer twitch contraction times where the present study found shorter. It was also found in the current study that twitch half relaxation time was prolonged by a substantially greater margin than Behm and St-Pierre (1997) (9.7% v 38%). In a review by Fitts (1996) it was indicated that often twitch and tetanic forces are reduced by fatigue. Differences between studies investigating the effect of fatigue on evoked contractile properties likely reflect differences between muscle groups and intensities studied. For example Behm and St-Pierre (1997) compared differences between plantar flexors and quadriceps performing isometric contractions at low intensity (allowing approximately 20 minutes duration) and high intensity (allowing approximately 4 minutes duration).

Hypothermic changes

The main focus of the current research was differences in the rate of change between time points in normothermic and hypothermic conditions. For example, properties such as MVC experienced similar significant decreases 1-minute after fatigue while hypothermic (-12%) and while normothermic (-15%), thus no significant differences existed in the recovery of MVC. Conversely, the soleus iEMG was 29% lower 1-minute after fatigue while normothermic but did not experience any significant change while hypothermic in this same time period. Thus we find the rate of change from pre-fatigue to 1-minute after fatigue significantly different (p-value of difference between -29% vs. -3% <0.01). With the exception of half relaxation times, we found that evoked contractile properties were generally higher while hypothermic than while normothermic.

The result that there were significantly different rates of change in all twitch and tetanic properties 1-minute after fatigue was interesting because the variables that were affected by hypothermia prior to fatigue were also the ones with significant differences in rates of change, except M-wave duration. Generally the net effect by 1-minute after fatigue was a decreased difference between the normothermic and hypothermic conditions. For example, time to peak twitch was significantly slower prior to fatigue while hypothermic (50%) but was significantly ($p=0.05$) less affected by 1-minute after fatigue while normothermic (-9.7%) than while hypothermic (-28%). These significant changes brought the normothermic and the hypothermic values for time to peak twitch closer together by 1-minute after fatigue (i.e. 50% difference pre-fatigue vs. 20% difference 1-minute after fatigue) though still significantly different ($p=0.02$). Similarly, soleus iEMG in which hypothermic iEMG was 37% lower than normothermic iEMG prior to fatigue, the 29% decline of iEMG 1-minute after fatigue under normothermic conditions with no significant change while hypothermic nearly eliminated the difference between the two conditions such that by 1-minute after fatigue the normothermic and hypothermic conditions were similar (0.49 mV ±0.10 normothermic, 0.43 mV ±0.16 hypothermic, $p=0.12$). A further significant 36% increase of hypothermic soleus iEMG between 1 and 5 minutes eliminated any such differences (0.56 mV ±0.12 normothermic, 0.58 mV ± 0.19 normothermic, $p=0.38$).

In order to maintain force output during fatigue, a muscle will have a reduced activity of sarcoplasmic reticulum (SR) ATPase. As previously discussed, a reduction of SR ATPase activity serves to maintain force output by increasing the amount of calcium remaining in he sarcoplasm (Marsden et al. 1983; Grange et al. 1993). In the current research, we observed prolonged relaxation time of evoked torques and

potentiation of evoked peak torque, time to peak torque, and rate of force development one minute after fatigue during both the normothermic and hypothermic protocols. Had we been able to collect this series of evoked measurements immediately after fatigue was reached, we may have seen that under both conditions, the potentiation was even higher immediately after fatigue than was evident one minute after fatigue. If hypothermia impairs muscle enzymatic activity (de Hann et al. 1989), then combining hypothermia and fatigue would explain why these properties are significantly more effected one minute after fatigue in the hypothermic condition than in the normothermic condition: the SR ATPase activity is slower to recover from fatigue induced impairment while hypothermic. Impairment of muscular endurance during hypothermia would then be caused by the hypothermia-induced impairment of other enzymes such as MLCK and thus myosin ATPase.

The 1- to 5-minute interval demonstrated only a few variables were still showing significant differences in rates of recovery (i.e. soleus iEMG, voluntary inactivation, and time to peak tetanus). The equivalent change of most variables may be linked to reactive and functional hyperaemia. Metabolic by-products such as inorganic phosphates and potassium are major contributors to high intensity fatigue (Fitts 1996). The partial or full occlusion of blood flow that occurs under high intensity muscular contractions restricts the clearance of these metabolites (Walloe and Wesche 1988). Hyperaemia, both reactive and functional, increase blood flow in response to mechanical occlusion or through a host of metabolites originating from intense muscle contraction in order to sustain and assist in the recovery of muscle contractions (Delp and Laughlin 1998). While it was initially thought the return of blood flow might be impaired due to hypothermia-induced vasoconstriction, thereby impairing recovery while hypothermic, there was evidence of returning of blood flow

with the rising muscle temperature. Since recovery of many voluntary and evoked properties tends to be rapid in the first two to three minutes after recovery (Bigland-Ritchie et al. 1986; Fitts 1996) the recovery between 1- and 5-minutes is not surprising.

In conclusion, the data demonstrate that properties sensitive to hypothermia are at a higher relative point one minute after fatigue while hypothermic than while normothermic, though there are few relative differences by five and ten minutes after fatigue. It seems likely that differences one minute after fatigue are related to the previously reported slowing of the sequestering of calcium back into the SR due to hypothermia-induced slowing of the activity of SR ATPase. While potentiation of evoked contractile properties occurs even during normothermic fatigue, the recovery is slower immediately after hypothermic fatigue. At five and ten minutes after fatigue, there may have been sufficient time for SR ATPase to return to its normal activity level and fatigue-induced metabolite accumulation has been cleared so differences between normothermic and hypothermic conditions no longer exist. Therefore, we conclude that there is an impairment of recovery from fatigue immediately after fatigue though this impairment is not long lasting.

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Table 1 - Summary of rates of recovery of voluntary and evoked properties during recovery in normothermic and hypothermic conditions and differences in rates of recovery between the two conditions. ns=no significant change between recovery intervals under the given condition. *=rate of change between recovery intervals is significantly different at the same time interval under normothermic and hypothermic conditions. + = voluntary activation is in absolute (percent) units measured. MVC=isometric torque of a maximal voluntary contraction; RTD=rate of torque development.

Variable	**Temperature**		**Normothermic Fatigue**				**Hypothermic Fatigue**				**Normothermic and hypothermic recovery**	
	Normothermic to Hypothermic	p	Fatigue (pre- to 1-)	p	fatigue (1- to 5-)	p	Fatigue (pre- to 1-)	p	fatigue (1- to 5-)	p	Pre to 1-minute	1- to 5-minutes
Gastrocnemius	-16%	ns	-41%	<0.01	19%	ns	-23%	ns	<1%	ns		
Soleus	-37%	<0.01	-29%	<0.01	13%	ns	-3%	ns	36%	<0.01	*	*
Tibialis Anterior	-1.4%	ns	-17%	0.06	9.9%	ns	-4.9%	ns	18%	ns		
MVC	-7.7%	ns	-15%	<0.01	3%	ns	-12%	<0.01	1.9%	ns		
MVC RFD	-48%	<0.01	-39%	<0.01	7.3%	ns	-11%	ns	21%	ns	*	
Voluntary Inactivation^{+}	-0.54%	ns	4%	<0.05	-1.8%	ns	3.3%	ns	3.2%	ns		*
Time to peak twitch	50%	<0.01	-9.7%	<0.05	7.1%	ns	-28%	<0.01	1.3%	ns	*	
Twitch RFD	-50%	<0.01	39%	<0.01	-20%	<0.01	89%	<0.01	-7.7%	ns	*	
Twitch torque	-30%	<0.01	29%	<0.01	-19%	<0.01	46%	<0.01	-13%	<0.05	0.09	
twitch half relaxation time	132%	<0.01	38%	<0.01	-7.8%	ns	-23%	0.08	-13%	ns	*	
Time to peak tetanus	7.0%	<0.01	-9.2%	<0.01	4.5%	ns	3.3%	ns	-4.4%	ns	*	*
Tetanic RFD	-46%	<0.01	17%	<0.05	11%	ns	26%	ns	40%	<0.01	*	
Tetanic torque	-42%	<0.01	<1%	ns	24%	<0.01	22%	<0.05	35%	<0.01	*	
tetanus half relaxation time	119%	<0.01	35%	<0.01	-23%	<0.01	-5.0%	ns	-29%	ns	*	
M-wave amplitude	22%	ns	4.1%	ns	-1.5%	ns	16%	ns	-1.8%	ns		
M-wave duration	46%	<0.05	3.1%	ns	9%	ns	-8.4%	ns	-5.7%	ns		

Figure 1. Custom-built modified boot apparatus designed to measure plantar-flexion torque.

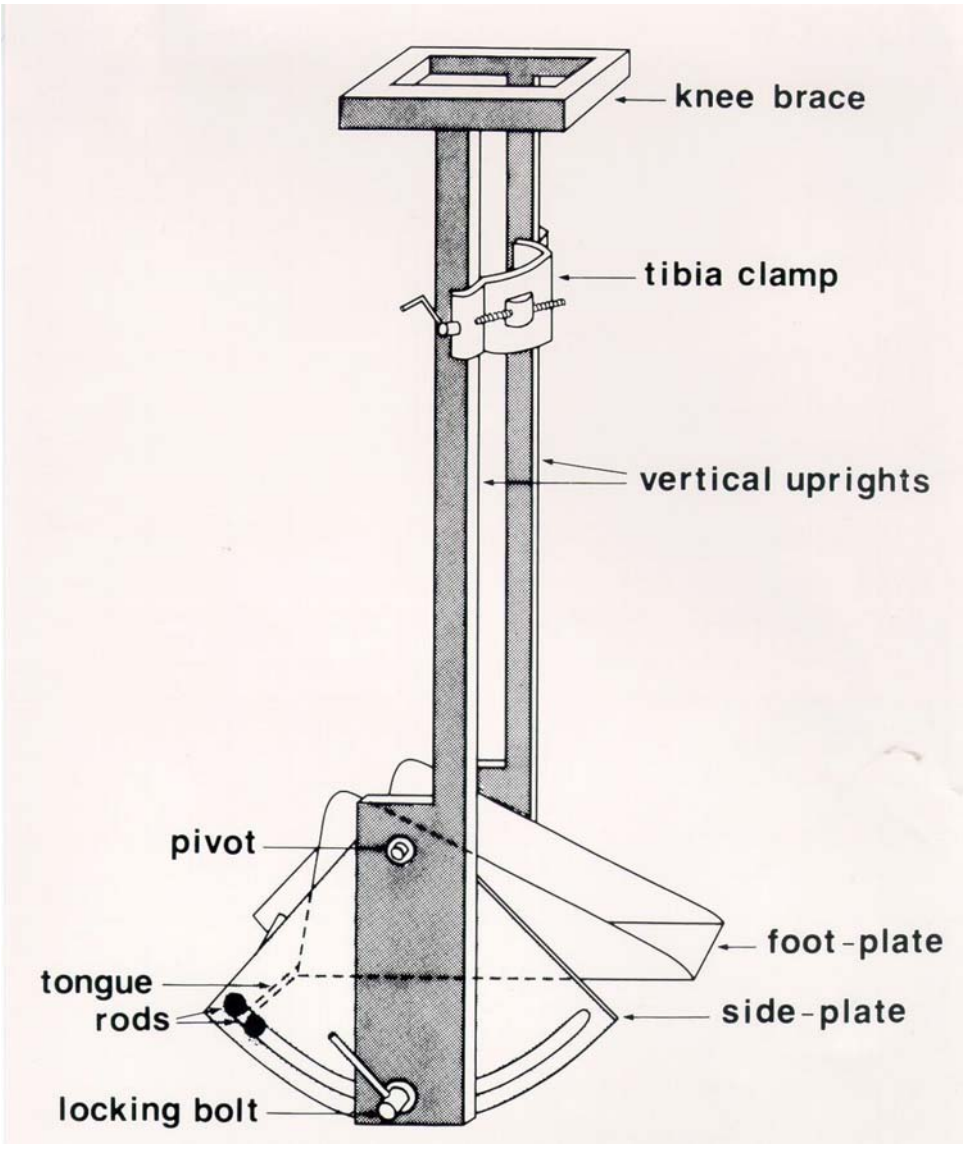

Figure 2 – Summary of twitch properties. ● indicates normothermic recovery. ▼indicates hypothermic recovery. * indicates significant difference between normothermic pre and hypothermic pre. Dotted lines (·····) indicates significant difference between two time points while normothermic. Short dashed lines (– –) indicates significant difference between two time points while hypothermic. + indicates a significant interaction effect of recovery time and temperature between two time points. Note that graph data points are purposefully off-set from the x-axis for the sake of clarity.

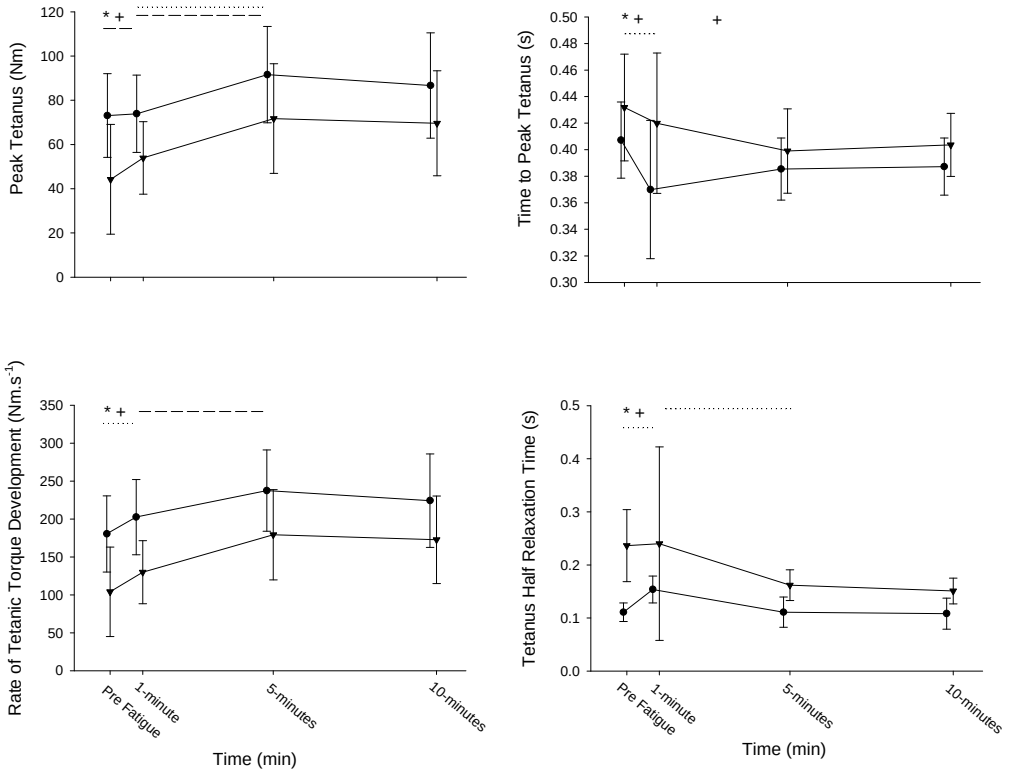

Figure 3 – Summary of tetanic properties. ● indicates normothermic recovery. ▼indicates hypothermic recovery * indicates significant difference between normothermic pre and hypothermic pre. Dotted lines (·····) indicates significant difference between two time points while normothermic. Short dashed lines (– –) indicates significant difference between two time points while hypothermic. + indicates a significant interaction effect of recovery time and temperature between two time points. Note that graph data points are purposefully off-set from the x-axis for the sake of clarity.