The effect of exercise induced hyperthermia on muscle fibre conduction velocity during sustained isometric contraction

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INTRODUCTION

It is likely that a major cause of fatigue during endurance exercise in a hot environment is reduced power output which is regulated by the central nervous system (Hunter et al., 2002c; Nybo 2007). It has also been suggested that this fatigue acts as a protective mechanism to protect the body from **extreme** damage which could occur with excessive **heat retention**. In support of this it has been shown that voluntary muscular activation is reduced during hyperthermic conditions and a greater rate of fatigue is observed during a sustained isometric contraction (Nybo and Nielsen 2001). This increased fatigue was suggested to be caused by a reduction in global **neural activation** as shown by a decline in EMG amplitude. However, an additional important neuromuscular control factor is muscle fibre conduction velocity (MFCV) which is a **potential** indicator of both **central factors such as motor unit recruitment** and **peripheral factors such as fibre membrane properties** (Andreassen and Arendt-Nielsen 1987). It has been previously demonstrated that passively heated muscle results in elevated MFCV (Farina et al., 2005; Gray et al., 2006). It has been proposed that this occurrence is as a result of the higher temperature accelerating opening and closing of the voltage-gated Na^+ channels which allows less Na^+ to enter the cell (Rutkove et al., 1997). Consequently, action potential amplitude and duration declines resulting in an increased capacity for the commencement of depolarization which produces faster MFCV. However, during prolonged submaximal exercise **at a fixed intensity** in the heat there will be an

increased accumulation of lactate (Galloway and Maughan 1997) from an increase rate of muscle energy metabolism (Edwards et al., 1972; Febbraio et al., 1996). This increase in lactate concentration will result in a greater decline in extracellular pH (Fitts 1994) which will result in a concomitant decline in MFCV (Brody et al., 1991). Recently, we (Hunter et al., 2009) manipulated pH by inducing alkalosis and following prolonged submaximal exercise showed an increase in MFCV during a sustained isometric contraction when compared to placebo ingestion. Therefore, it is likely that accumulation of lactate from submaximal exercise in the heat will indirectly attenuate the increased muscle temperature effect on MFCV.

It would therefore appear that there are inherently contradictory responses in MFCV during exercise in the heat which may cause it to increase but with a greater accumulation in lactate will conversely produce slower values. This is an important factor to consider when inducing hyperthermia by exercising in a hot environment as opposed to just passive heating. Todd et al (2005) induced hyperthermia by submerging subjects in a warm bath and found that the elbow flexors produced less **absolute** force **with** a **greater decline** during a sustained **2 minute maximal** isometric contraction. The authors concluded that greater central fatigue was observed **during this contraction** despite **a** faster **rate of motor neuron discharge into the** muscle. Therefore, exercise associated changes will have implications for neuromuscular control strategies, which would need to take into account the level of change in MFCV when delivering activity-

controlling coded action potentials to the peripheral muscle. The situation is made more complex from a control perspective in that, as described above, there are two different strategies available for regulating peripheral muscle activity namely; 1) peripheral alterations in the conducting properties of the muscle fibres; and/or 2) altering global recruitment strategy to all or some of the motor units controlling skeletal muscle function. As far as we are aware no study has explored the effect of exercise induced hyperthermia on the relationship between RMS, MFCV and force during isometric fatigue.

Accordingly, the aim of this study was to determine the effect of inducing hyperthermia by using a submaximal cycle protocol in a hot environment which will increase lactate concentration and heat storage. We therefore used **two main** interventions to determine this effect: 1) cycling in a hot environment; and 2) cycling in a thermoneutral environment. **In addition to control for the effect of cycling in a hot environment we had a third intervention which was** resting in a hot environment. Following this, the relative change of both RMS and MFCV during a sustained maximal isometric fatiguing contraction was observed; in order to determine which neuromuscular recruitment strategy operates principally in controlling peripheral muscle activity in a hyperthermic environment.

METHODS

Seven, healthy, well trained club level cyclists volunteered for this study. The mean (± SD) age, $\dot{V}O_{2max}$, height and **mass** of the subjects were 35 ± 9.9 years, 57.4 ± 6.6 ml $kg.^{-1}.min^{-1}$, 178.6 ± 6.6 cm and 78.4 ± 9.6 kg respectively. All subjects gave their written informed consent. The study was performed according to the Declaration of Helsinki and was approved by the local research ethics committee.

Preliminary testing

To determine peak power output (PPO), a modified protocol as described by Hawley and Noakes (Hawley and Noakes 1992) was used. Subjects performed a 10-minute warm up on an electrically braked cycle ergometer (Lode, Groningen, Netherlands). The starting power output was determined by multiplying the subject's body weight by 2.5 W. The load was subsequently increased every 150s by first 50 W and then 25 W until the subjects were unable to maintain force output or pedaling frequency dropped from 90 to < 50 revolutions. $min.^{-1}$. PPO was defined as the last completed work rate in watts plus the fraction of time spent in the final non-completed work rate multiplied by 25 W.

Experimental Procedure

After the preliminary testing each subject reported to the laboratory on **four** separate occasions one week apart, and were instructed to record their dietary intake and physical activity 24 hours before the first visit. The subjects were then

instructed to replicate these conditions for the subsequent visits. During the first visit, subjects familiarized themselves with the equipment and laboratory conditions. Thereafter, they completed a familiarization trial by performing muscle function tests before and after the 50 minute cycle ride at 60% of their PPO (Figure 1). The cycle ride during the familiarization trial was in the hot condition of 40°C and 35% humidity to ensure that all subjects could complete the full 50 minute duration. The subjects then had to return to the laboratory on three subsequent occasions where in random order they were required to either cycle (HOT) or rest (PASS) in 40°C and 35% humidity or cycle in 19°C and 20% humidity (NEUTRO) (Figure 1). At each visit the subjects arrived at the laboratory at the same time of day and their nude body weight was recorded, resting blood samples taken and rectal thermometer (Mon-a-therm, Mallinckrodt, OH, USA) inserted 10cm beyond their anal sphincter. Following this, the subjects were then prepared for the recording of muscle temperature by lying them down in the supine position and injecting 5ml of local anesthetic into the mid distal section of the Vastus Lateralis muscle with the flexed lower limb. After allowing a 5 minute period a needle temperature probe was inserted (Fluke 80PK-5A Type K, Fluke Corporation, USA) 5cm into the same position to record the temperature (Fluke 52, Series II thermometer recorder, USA). A surface thermistor (YSI 400, Yellow Springs, OH, USA) was then attached to the Vastus Lateralis muscle for the recording of skin temperature. The subjects then completed three maximal voluntary contractions (MVC), the highest of which was used to normalize subsequent EMG amplitude (RMS) recordings. Following this a further blood

sample was taken before embarking on a 50 minute cycle on the electrically braked ergometer (Excalibur Sport, Lode, The Netherlands) at 60% of PPO, during which heart rate, sEMG, $\dot{V}O_2$ and rating of perceived exertion (RPE) where recorded every 10 minutes. Upon completion of the cycle ride **the muscle temperature recording procedure was then repeated followed** by a 100 second sustained isometric contraction (SMC) (Figure 1). **The total time taken from completion of the cycle ride to the start of SMC was approximately ~4 minutes**. **To undertake this contraction protocol**, subjects were instructed to contract maximally at the commencement of producing force and attempt to sustain it for the full duration of the 100s trial.

Torque Measurement

The strength of the subjects' right knee extensors was measured on an isokinetic dynamometer (Biodex Medical Systems USA) as described previously (Hunter et al., 2002b). Subjects sat on the dynamometer with their hips, thighs and upper bodies firmly strapped to the seat. In this position their hip angle was 100° angle of flexion. The right lower leg was attached to the arm of the dynamometer at a level slightly above the lateral malleolus of the ankle joint and the axis of rotation of the dynamometer arm aligned with the lateral femoral condyle. The dynamometer arm was set at angle of 60º from full leg extension. Each subject performed 3 x 5 second MVC's with a minute recovery in between. The highest torque recorded from these 3 MVC's was used for subsequent analyses.

Following the cycle the subjects then performed the SMC using the same torque measurement positions for all three conditions.

Electromyography analyses

Four Ag-AgCl EL258S shielded electrodes (Biopac, USA) were inserted into a hard plastic mould in a straight line next to one another. This allowed a distance of 12.5mm between each electrode from the signal detection area and was configured to record 3 parallel EMG signals as described by Lowery et al (Lowery et al., 2002). The electrode array was then positioned on cleansed and shaven skin along the major axis of the muscle fibres half way between the main belly of the Vastus Lateralis and its distal end. Initially the electrodes were inserted with dry round silver inserts for ease of multiple placements. A variety of different locations on the muscle was used until there was a clear propagation in one direction of the action potentials without change in shape all of the 3 sEMG signals. Then the electrode array position was marked with a permanent marker pen, after which the dry silver inserts were subsequently removed and filled with conductive gel (20-30 µl) and returned and attached to the marked section of skin. The electrode array was firmly secured with 2 sections of Tegaderm. This electrode array was linked to the BioPac EMG apparatus (Biopac Systems, USA) and host computer. The EMG data **were** automatically anti-aliased by the hardware (Biopac Systems, USA). Each activity was sampled at a 2000 Hz capture rate. The raw signal was processed to give root mean square (RMS) of

the sEMG power, which was used for subsequent analyses. All post cycle RMS signals were normalized to the pre intervention MVC.

MFCV was estimated by applying a cross-correlation function between the temporal sEMG signals measured at the three electrodes as previously described (Lowery et al., 2002). The cross-correlation of two signals x and y is given by

$$R_{xy}(m) = E\{x_{n+m}y^*_n\}=E\{x_n y^*_{n-m}\}$$

where E{.} is the expectation operator .The cross-correlation function of two similar signals will peak where the two signals are maximally similar. In our case the signals are pulsed signals where one signal is a delayed and path distorted version of the other signal, so that their cross-correlation should peak at a number of samples equivalent to the time-delay between them. We make use of this property to estimate the delay between the signals measured at the electrodes 1 and 2, 2 and 3 and 1 and 3. With the distance between the electrodes known, we found the velocities of the signals from:

$$\text{velocity}_{nm} = \text{electrode pair distance}_{nm} / \text{estimated time delay}_{nm} \quad (\text{m/s})$$

The 3 sEMG signals were first processed through 2 double differential (DD) amplifiers for the final MFCV estimation. Both DD signals were upsampled to

20kHz and an extra 0.5 seconds either side of the epochs are upsampled to avoid possible end-effects from using the Matlab interpft function. MFCV was estimated using the xcorr function of Matlab where xcorr estimates the cross-correlation sequence of a random process. The maximum cross-correlation is noted as the delay for the signal to travel from one electrode to another as determined from the upsampled DD signals. Estimates of MFCV were accepted only when cross-correlation values were higher than 0.8.

Blood sampling

An 18-guage Teflon cannula (Jelco, Johnson and Johnson, Halfway house, South Africa) was positioned in an antecubital vein and connected to a three way stop cock (Uniflex, Mallinckrodt, Hennef-Seig, Germany). This cannula was flushed periodically with 2-3 ml of sterile saline containing heparin (5 IU ml^{-1}) and was used for the collection of venous blood samples (10 ml) at rest and during exercise. Venous blood samples (10ml) were drawn at rest, at the end of each 15 min work rate and at exhaustion. The samples were then divided into aliquots, which were put into an ice-cold tube containing potassium oxalate and sodium fluoride for later determinations of lactate concentrations. The tubes were centrifuged at 3000-x g for 10 minutes at 4ºC immediately after the completion of the trial and the supernatants were stored at -20ºC for later analyses of plasma lactate. Plasma lactate concentrations were measured with spectrophotomeric (Beckman Model 35, Beckman Instruments Inc., Fullerton, Ca, USA) enzymatic assays (Lactate PAP, BioM (rieux, Lyon, France; NEFA half-micro test;

Boehringer Manheim, Germany). This procedure was the same as described previously (Hunter et al., 2002a).

Recordings of heart rate and perceived exertion

Heart rate was recorded at rest and then recorded along with **rating** of perceived exertion (RPE) (Borg 1973) every 10 minutes for the full 50 minute ride (Figure 1).

Statistical Analyses

All data are expressed as means $\pm$ SD. A (time-by-trial) repeated measures ANOVA**s** were performed to evaluate differences between and within trials. **These** data **were** analyzed by: 1) 3 (condition) x 5 (time [**25s epochs]**) for SMC; 2) % delta change for each variable throughout SMC 3 (condition) x 3 (**variables [torque, RMS and MFCV]**) and; 3) a 3 (condition) x 2 (time **[pre and post]**) for the 50 minute intervention. Post hoc analyses of the main effect of time were done using a Tukey's **HSD**. Significance was accepted at $P \leq 0.05$.

RESULTS

Sustained Maximal Contraction

Delta change over the SMC for all 3 conditions and variables showed a significant ($p<0.01$) difference between the conditions with a significant interaction effect (Figure 2). Post hoc analyses showed that within HOT both torque and RMS declined by ~37% but MFCV was reduced significantly ($p<0.05$) less by just ~9% (Figure 2). While within NEUTRO (torque: ~21%; RMS: ~36%, MFCV: ~20%) and PASS (torque: ~10%, RMS: ~20% MFCV: ~17%) no statistical differences were shown between the decline in variables.

Torque significantly ($p<0.01$) declined for all three conditions at significantly ($p<0.01$) different rates with reductions of ~39% for HOT, ~22% for NEUTRO and ~4% for PASS (Figure 3A). This resulted in significantly ($p<0.01$) different final torque values between conditions with HOT being the lowest followed by NEUTRO and then by PASS (Figure 3A). MFCV significantly ($p<0.01$) declined over the 100 s during SMC for all three conditions (Figure 3B). No differences were observed between HOT and NEUTRO and a group effect revealed that PASS was significantly ($p<0.05$) less than HOT (Figure 3B). RMS also significantly ($p<0.01$) declined over the same contraction at the same rate for all three conditions. There was a tendency ($p=0.077$) for a difference between groups with the biggest differences shown from the reduced RMS of HOT compared to NEUTRO (Figure 3C).

50 minute intervention

Core temperature rose significantly ($p<0.01$) over the duration of the cycling intervention for HOT and NEUTRO with PASS remaining unchanged throughout the 50 minute intervention (Figure 4A). HOT rose to significantly ($p<0.01$) higher values than NEUTRO by the end of the cycle (Figure 4A). Skin temperature significantly ($p<0.01$) rose similarly during the 50 minute intervention for HOT and PASS with NEUTRO remaining unchanged (Figure 4B). No differences existed for skin temperature between HOT and PASS (Figure 4B). Muscle temperature significantly ($p<0.01$) rose following the intervention for all three conditions (Figure 4C). Following the intervention HOT was significantly ($p<0.01$) higher than the other two conditions and NEUTRO had a tendency ($p=0.062$) to be higher than PASS (Figure 4C).

Heart rate significantly ($p<0.01$) increased for both HOT and NEUTRO with HOT rising to a significantly ($p<0.01$) higher level than NEUTRO (Figure 5A). PASS also significantly ($p<0.01$) rose to a higher peak value over the 50 minutes at a slower rate than the other two conditions and was significantly lower ($p<0.01$) than NEUTRO by the end of the intervention (Figure 5A). Lactate rose to a significantly ($p<0.01$) higher level for HOT than NEUTRO while PASS remained unchanged at the end of the intervention (Figure 5B).

RPE was significantly ($p<0.05$) higher for HOT than NEUTRO with both conditions rising at a similar and significant ($p<0.01$) rate over the 50 minutes

with PASS remaining unchanged (Figure 5C). Thermal comfort showed a significant ($p<0.01$) main effect for all 3 conditions with HOT increasing the most, followed by PASS and then by NEUTRO (Figure 5D).

DISCUSSION

As expected cycling in the heat resulted in significantly greater reduction in torque output during a sustained isometric contraction than that found in cycling in a **thermoneutral** environment, or sitting passively in hot conditions; The novel finding from this study was that during the hyperthermic conditions MFCV did not decline in proportion to the torque and RMS as it did in the other two conditions.

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It is clear that hyperthermia was induced to a greater degree during HOT as evident by higher core and muscle temperature values compared to NEUTRO and PASS. The elevated heart rate found in HOT could be explained in some part as being caused by thermoregulatory compensation (Gonzalez-Alonso et al., 1999a; Hunter et al., 2002c), where the higher skin temperature observed is representative of an increase in skin blood flow (Nielsen et al., 1993). This increase in skin blood flow would cause reduced cardiac return, therefore decreasing stroke volume (Rowell et al., 1968). Although there was an increase in thermoregulation in HOT, it is evident from the increase in core temperature that this was ineffective in reducing heat storage to similar levels as was found in NEUTRO. Although muscle and skin temperature increased in PASS, thermoregulatory processes appeared to be effective in attenuating an increase

in core temperature **during** passive resting in the heat condition. This clearly demonstrates that it is the combination of exercise in the heat which is the cause of the increased core and muscle temperature in HOT shown in this study.

There were no significant differences in absolute MFCV between the conditions during the sustained isometric contraction between HOT and NEUTRO despite higher muscle temperature and greater fatigue for HOT. In contrast to this finding Gray et al (2006) demonstrated faster MFCV during 6 seconds of maximal sprint in hot conditions. However, our study examined the MFCV response during isometric fatigue following 50 minutes of cycling at 60% of peak power output which resulted in elevated blood lactate concentrations for both HOT and NEUTRO. These blood lactate concentrations were higher following HOT which, combined with a likely lower muscle blood flow (Nybo 2007), should lower extracellular pH to such an extent to slow down MFCV (Hunter et al., 2009) and attenuate regulation of the temperature effect on MFCV described by Gray et al (2006). Therefore it is likely that the altered muscle energy metabolism would have offset any increase in MFCV brought about by higher muscle temperature values.

However, RMS was reduced to a similar level as **the** torque output in the HOT compared to the other two conditions which suggests that the reduction in RMS may explain in part the lower torque production during SMC. Previous studies (Farina et al., 2005; Rutkove et al., 1997) have also shown reduced EMG

amplitude during hot conditions with Nybo and Nielsen (2001) concluding that the decline in recruitment following submaximal exercise in the heat was a result of reduced drive from the CNS. However, Rutkove et al (1997) heated just the lower limb and fatigued it with tetanic stimulation and showed a reduction in RMS with no alteration in neurotransmission, and concluded that peripheral mechanisms such as nerve and muscle ion channel function were partly responsible for these findings. This therefore suggests that there may be both central and peripheral influences associated with the reduction of RMS in HOT. Nevertheless, when **examining** the level of RMS and MFCV decline in relation to torque decrement it becomes apparent that RMS **rather than** MFCV in our study is likely to be mainly responsible for the fatigue observed in HOT. This is an interesting finding given that it is well established that during normal conditions SMC to fatigue both RMS and mean power frequency spectrum (MPFS) will both decline **in a similar fashion** (Moritani et al., 1986). However, the limitation of MPFS measurement is that it is representative of both firing rate and MFCV which makes it difficult to differentiate between neuromuscular recruitment strategies and peripheral mechanisms altering MFCV. As our study measured MFCV we are able to elucidate that RMS declined to similar levels as torque unlike MFCV despite an increase in lactate accumulation. Therefore, global motor unit recruitment, **including the firing frequency and degree of synchronization for single motor units,** as opposed to slowing of MFCV appears to be the main factor responsible for the significantly greater reduction in torque output observed in the HOT.

As a result of exercising in the heat during the HOT it is likely that there was an increase in muscle metabolism (Edwards et al., 1972; Febbraio et al., 1996) as is evident from the higher lactate values. This increase in lactate accumulation is therefore likely to be from an increase in production without any concomitant elevation in lactate clearance (Gonzalez-Alonso et al., 1999a). Generally, previous studies (Edwards et al., 1972; Febbraio et al., 1996) that have measured muscle energy metabolism elevated just muscle and not core temperature. However, Drust et al (2005) elevated both core and muscle temperature by having subjects perform 40 minutes of high intensity intervals followed by 5 maximal 15 second efforts in a hot environment. Interestingly, it was concluded that the impaired performance was not as a result of any increase in metabolites, but rather from CNS down regulation (Drust et al., 2005; Nybo 2007). However, the subjects produced less power over the 40 minutes during the hot condition which will inevitably reduce muscle energy metabolism. It can therefore be proposed that this occurrence is likely a consequence of, rather than a direct cause of the increased core and muscle temperature. This protocol however is unlike our study which used the same submaximal work rate for both conditions during the cycle. Therefore, the greater plasma lactate accumulation during HOT does indicate that there was an increase of metabolic products in the muscle (Febbraio et al., 1996) which may have had direct effects on the MFCV (Hunter et al., 2009) as well as indirect effects on neuromuscular recruitment strategies (St Clair Gibson et al., 2001).

RPE was higher in HOT than NEUTRO which concurs with previous findings (Gonzalez-Alonso et al., 1999b; Nielsen et al., 2001). Nybo et al (2001) also took electroencephalogram (EEG) alongside RPE measures and both showed a linear increase alongside core temperature. Although the authors suggested that these variables were associated they acknowledged that the impact of altered brain activity on RPE was not necessarily causal. Given that RPE is perception of effort caused from a variety of cues (Hampson et al., 2001) there may well be additional mechanisms affecting this perceptual response. Thermal comfort (discomfort) was higher in HOT which were expected given the higher core temperature values. However, it was interesting to note that thermal comfort values were higher in PASS than NEUTRO when the core temperatures did not reflect this. This suggests that despite a higher rate of heat storage in NEUTRO the perception of the environment is unrelated to the effectiveness of the thermoregulatory processes.

It must be noted that, as described above, there were different patterns of torque output, RMS and MFCV changes in the HOT, NEUTRO and PASS. Despite these differences in patterns of changes, or perhaps because of them, all subjects were able to complete the trials and none terminated either the cycling or sustained isometric contraction components of the tests prematurely. This indicates that there must be some degree of intelligent or strategic processing, which takes into account all the peripheral and central effects of the prior cycling

bout and directs the subsequent alteration to the neuromuscular control pathways to maintain the fidelity of the control processes regulating muscle contraction (St Clair Gibson and Noakes 2004).

In conclusion this study has shown that hyperthermia induced by cycling in the heat resulted in exacerbated fatigue during sustained isometric contraction of maximal effort. It is likely that this was caused mainly from decrements in global motor unit recruitment as opposed to slowing of muscle fibre conduction velocity. However the cause of, or control strategies regulating, the different patterns of relative decline in MFCV, RMS and torque for the three conditions is difficult to interpret due to the complex afferent signalling to the CNS resulting in altered efferent responses to the neuromuscular control strategy.

Acknowledgements

Dr Andi Johnson for her help and observations

FIGURES

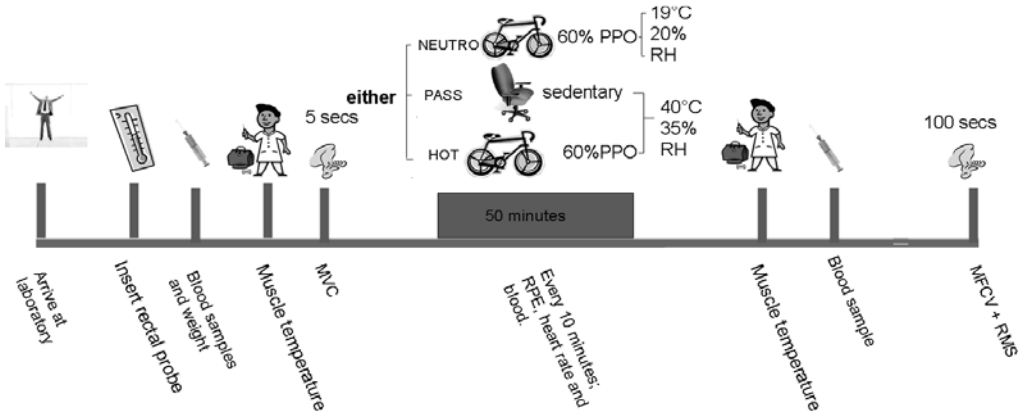

Figure 1. Time sequence of protocol

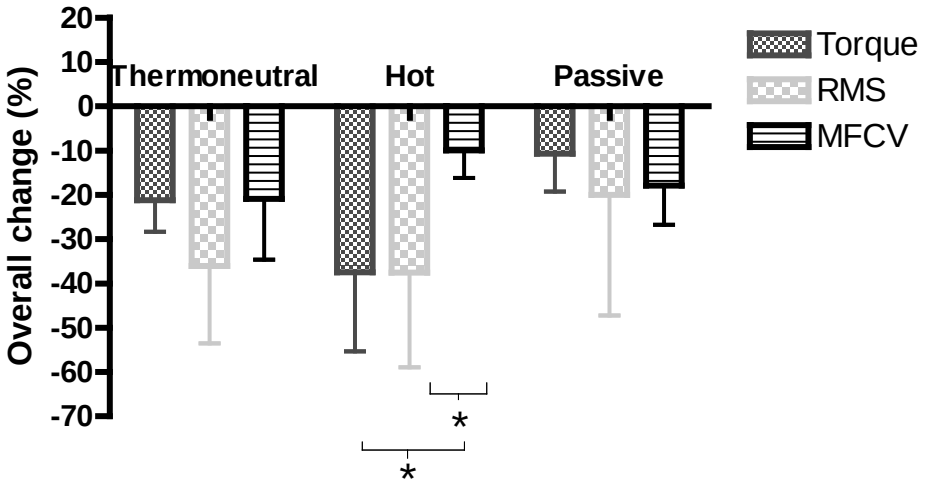

Figure 2. Delta change from peak value to the end of the contraction for torque, RMS and MFCV for the thermoneutral, hot and passive conditions where there was a significant ($p<0.01$) difference between conditions and a significant interaction effect ($p<0.05$). *$p<0.01$

Sustained Maximal Contraction

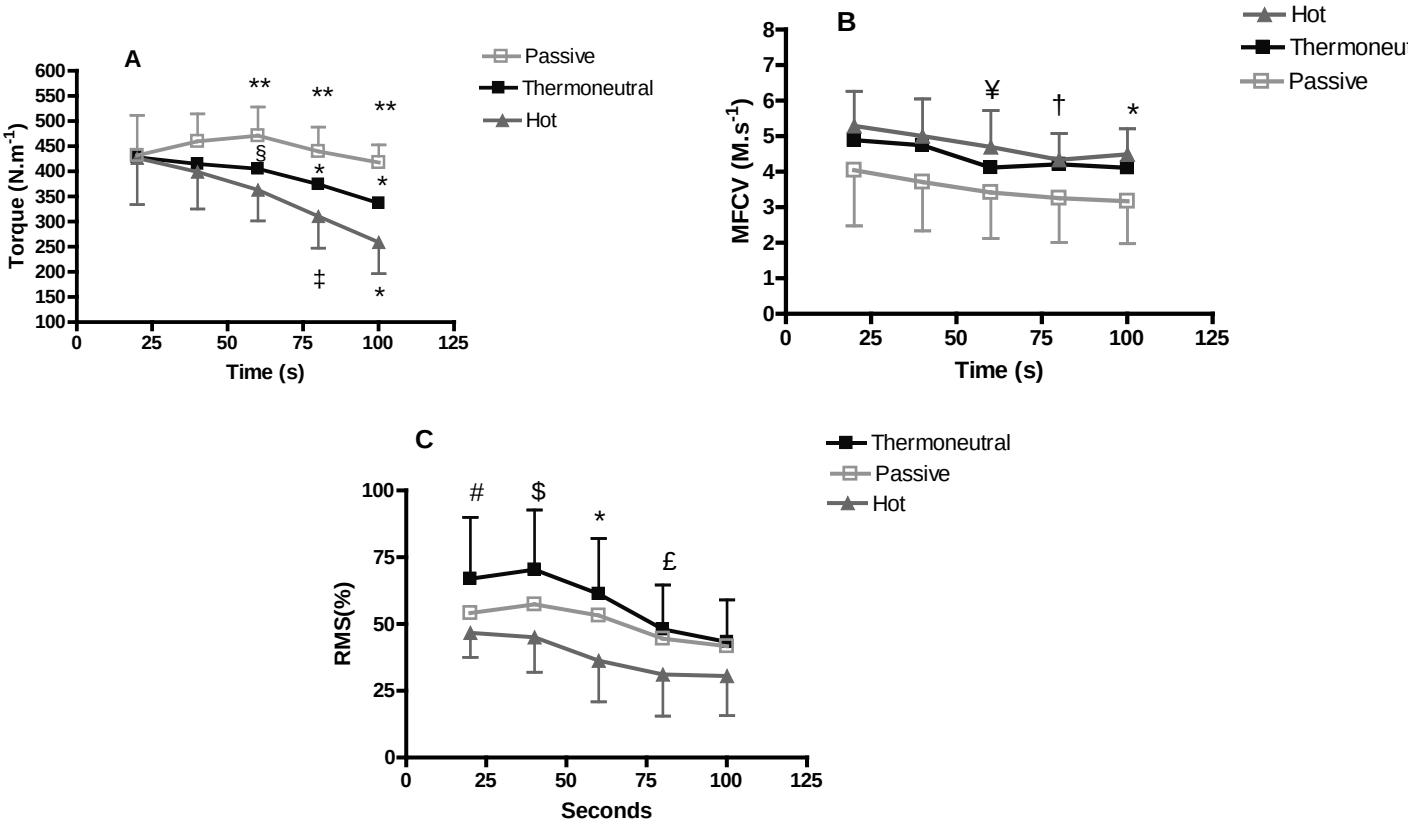

Figure 3 100s MVC post 50minute intervention for; **A**. Torque where all 3 conditions had a significant ($p<0.01$) interaction effect. **B** Muscle Fibre Conduction Velocity where all conditions significantly ($p<0.01$) declined at the same rate. A significant ($p<0.05$) group effect was shown; with differences shown between just hot and passive conditions. **C** RMS where all conditions significantly ($p<0.01$) declined over the contraction at the same rate with a tendency ($p=0.077$) for a difference between groups which was shown between thermoneutral and hot conditions. The top, middle and bottom line of symbols represent passive vs. hot, passive vs. thermoneutral and thermoneutral vs. hot respectively. # - $p=0.083$, ‡ - $p=0.081$, $-$p=0.076$, £-$p=0.063$, §-$p=0.057$, *-$p<0.05$, **$p<0.01$.

50 minute intervention

Body Temperature

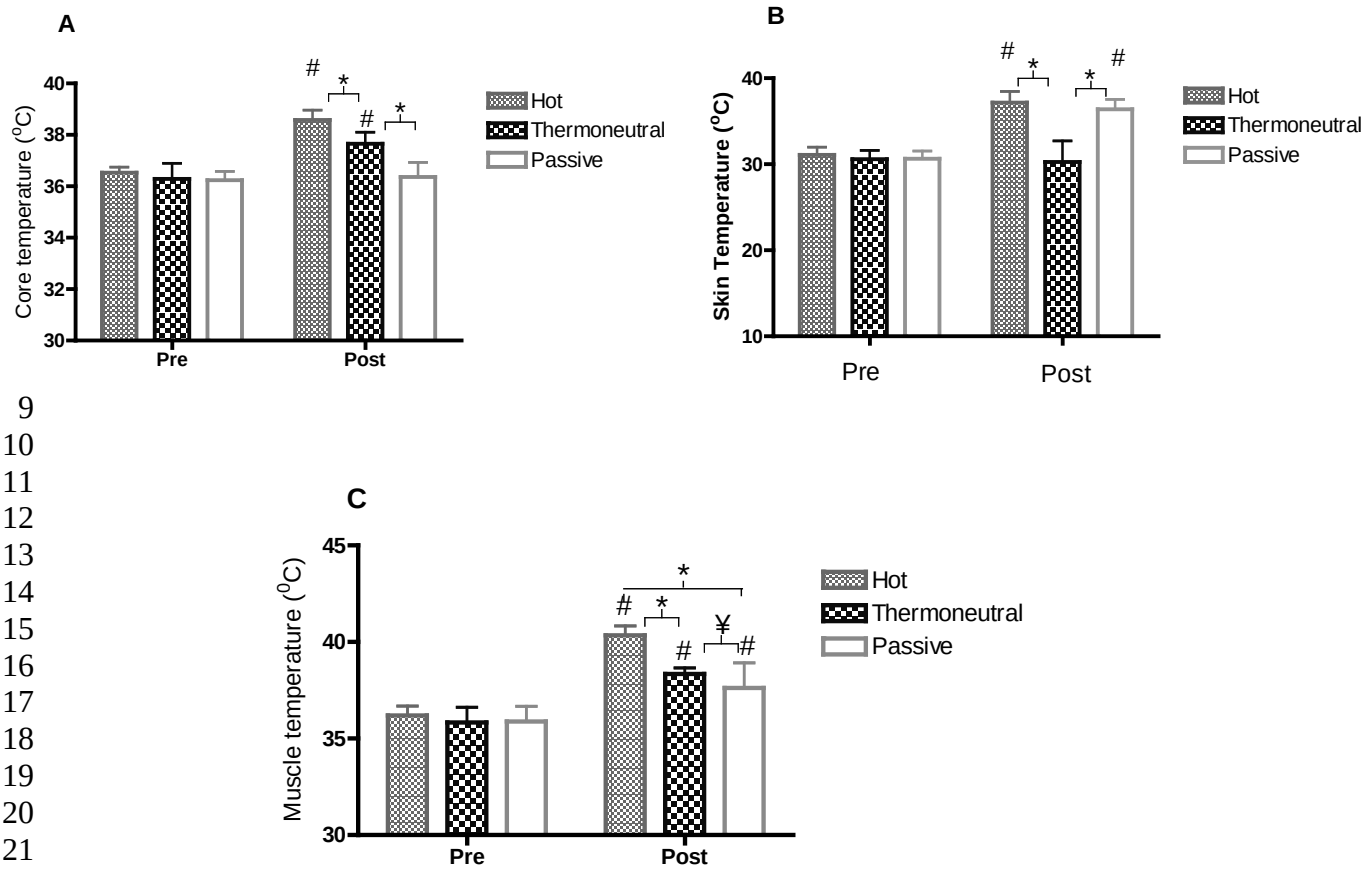

Figure 4 Rectal (A), skin (B) and muscle (C) temperatures taken before and after the 50 minute intervention of cycling in a hot (40°C) and thermoneutral (18°C) environment as well as passively resting in the same environment as hot. All temperatures showed a significant ($p<0.01$) time, group and interaction effect. *=$p<0.01$ and ¥-$p=0.062$ difference between conditions; #=$p<0.01$ difference within condition to pre value.

50 minute intervention

Other physiological and subjective responses

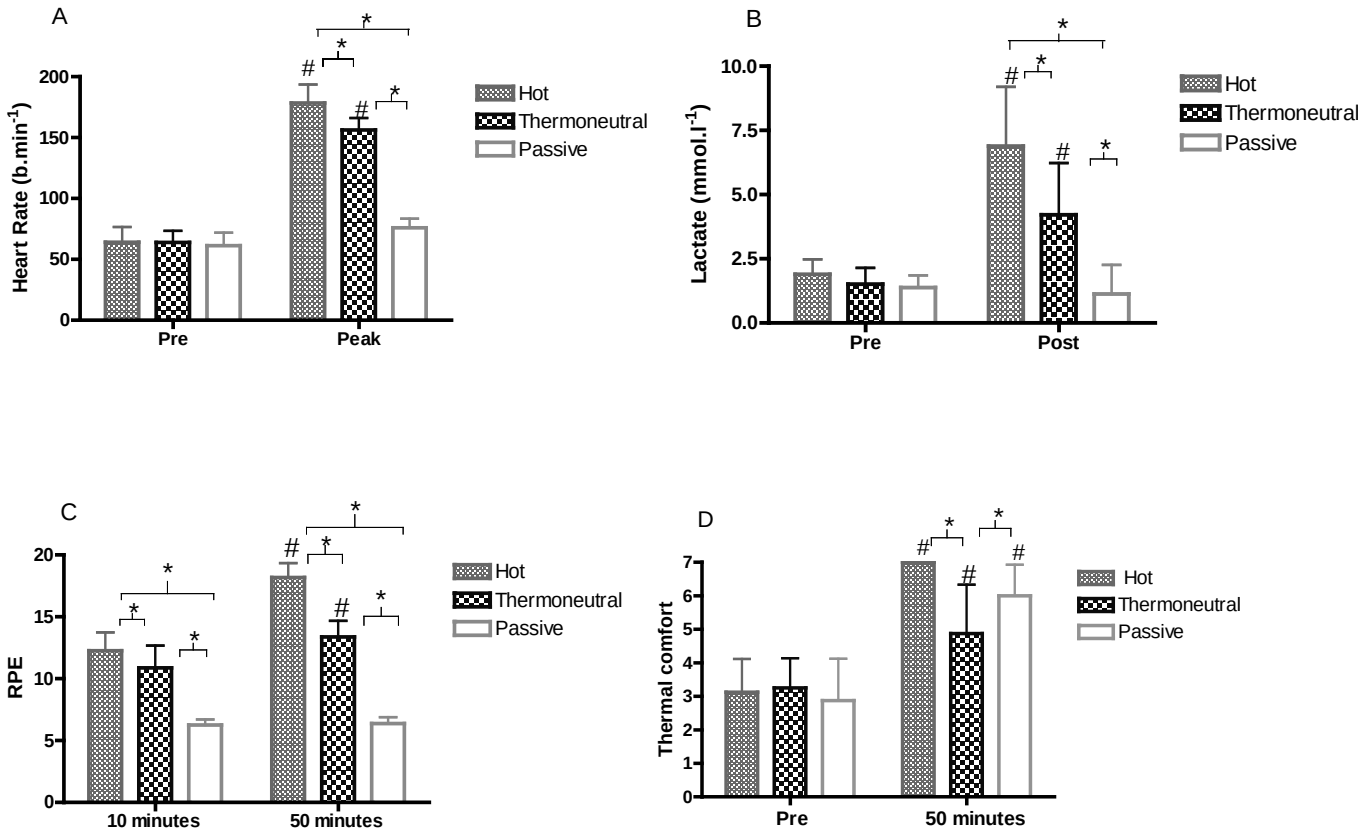

Figure 5. Heart rate (A), Lactate (B), RPE (C) and Thermal comfort (D) taken before and at the peak value (40 minutes); pre and post; 10 and 50 minutes; and pre and 50 minutes of the intervention respectively. All temperatures showed a significant ($p<0.01$) time, group and interaction effect. *=$p<0.01$ difference between conditions; #=$p<0.01$ difference within condition to pre value.

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