Objective: This study examined the neuromuscular responses to 60 min of self-paced high intensity exercise punctuated with 6 x 1 min -all-out- sprints at 10 min intervals in moderate (19.8 ± 0.3 -°C) and warm (33.2 ± 0.1 -°C), humid (~ 64% relative humidity) conditions with either complete hydration (CF) or without hydration (NF). Design: Seven subjects (mean ± SE; age 20.6 ± 1.1 yr, mass 73.8 ± 4.5 kg, peak power 288 ± 11.3 W) performed the time trial on four separate occasions which were differentiated by ambient temperature and fluid ingestion. For each sprint interval, distance, power output and electromyographic (EMG) data from the rectus femoris and vastus lateralis muscles were recorded. Results: The NF trials resulted in a reduction in body mass for the moderate and warm conditions of 1.7% and 2.1%, respectively. Final rectal temperatures were not different among conditions (~ 38.7 -°C). Total body sweating was higher in the warm condition (19.1 -€" 16.5 mL/kg/h; P < 0.05) compared with the moderate condition (16.1 -€" 16.5 mL/kg/h; P > 0.05). Neither fluid ingestion nor ambient temperature altered total distance cycled for any of the trials (range 30.1 -€" 32.6 km). The normalised iEMG (as % maximal voluntary contraction) when compared with the first sprint increased from sprint three for the rectus femoris muscle in both NF and CF but decreased for vastus lateralis muscle. However, the mean percentile frequency shift increased for both vastus lateralis and rectus femoris muscles in both NF and CF.Conclusions: These results suggest that the integrity of the neuromuscular system is adjusted according to hydration status and ambient temperatures during intense self-paced cycling.

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Neuromuscular responses to hydration in moderate to warm ambient conditions during self-paced high intensity exercise

Neuromuscular responses to hydration and exercise heat stress

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ABSTRACT

Objective: This study examined the neuromuscular responses to 60 min of self-paced high intensity exercise punctuated with 6 x 1 min “all-out” sprints at 10 min intervals in moderate (19.8 ± 0.3°C) and warm (33.2 ± 0.1°C), humid (~64% relative humidity) conditions with either complete hydration (CF) or without hydration (NF).

Design: Seven subjects (mean ± SE; age 20.6 ± 1.1 yr, mass 73.8 ± 4.5 kg, peak power 288 ± 11.3 W) performed the time trial on four separate occasions which were differentiated by ambient temperature and fluid ingestion. For each sprint interval, distance, power output and electromyographic (EMG) data from the rectus femoris and vastus lateralis muscles were recorded.

Results: The NF trials resulted in a reduction in body mass for the moderate and warm conditions of 1.7% and 2.1%, respectively. Final rectal temperatures were not different among conditions (~38.7 °C). Total body sweating was higher in the warm condition (19.1 – 21.3 mL/kg/h) compared with the moderate condition (16.1 – 16.5 mL/kg/h; P < 0.05). Neither fluid ingestion nor ambient temperature altered total distance cycled for any of the trials (range 30.1 – 32.6 km). The normalised iEMG (as % maximal voluntary contraction) when compared with the first sprint increased from sprint three for the rectus femoris muscle in both NF and CF but decreased for vastus lateralis muscle. However, the mean percentile frequency shift increased for both vastus lateralis and rectus femoris muscles in both NF and CF.

Conclusions: These results suggest that the integrity of the neuromuscular system is adjusted according to hydration status and ambient temperatures during intense self-paced cycling.
Previous studies have shown that differences in environmental temperature result in differences in fixed intensity exercise duration consistent with the development of hyperthermia and fatigue with alterations to the physiological and subjective responses [1,2]. Fluid ingestion is a practical intervention strategy used to improve exercise in the heat by reducing thermoregulatory strain [3] by offsetting the effects of dehydration, which at levels of less than 2% reduction in body mass has been shown to compromise exercise performance [4]. In addition, time to exhaustion has increased when fluid was ingested to offset sweating related reductions in body mass [5].

Exercise termination in the heat is thought to be associated with a critical limiting core temperature of ~ 39.5 °C [6]. The mechanism/s for this remain unclear; however, there is general consensus that exercise-induced hyperthermia is associated with a reduction in central motor drive [7,8]. Moreover, even when a maximal conscious effort was observed subjects experienced a reduction in efferent drive to the active muscles during high intensity cycling in the heat [3]. These findings suggest that high internal temperatures are associated with de-recruitment of muscle fibres mediated by the central nervous system (CNS). However, it remains to be determined how the application of intervention strategies, such as fluid ingestion might influence this relationship given that exercise performance in the heat is improved when appropriate fluid is ingested [9].

Typically, the benefits of fluid ingestion have been shown during fixed intensity protocols [10,11] where the rise in rectal temperature is attenuated and exercise time is improved. Conversely, fatigue during self-paced exercise between 60 and 150 minutes duration is regulated by some other mechanism so that the demands of exercise are adjusted enabling subjects to finish the exercise bout and avoid cellular catastrophe [12-16]. This phenomenon is not easily identified during fixed intensity exercise as the physiological responses are driven by an externally imposed load [17].

In our previous companion paper we showed that maintenance of body mass by adequate hydration during self-paced exercise did not enhance exercise performance in the heat compared to restricted fluid ingestion [12]. We speculated that this was due to the subjects’ ability to adjust their pacing in such a way as to complete the exercise bout with similar terminal physiological responses. The mechanism for such a response is unknown but could reside in the muscle recruitment strategy applied throughout the exercise bout. In the present paper we report the neuromuscular responses and the hydration parameters which are thought to influence exercise performance.

METHODS

Subjects and experimental design

The methods and experimental design including cycling performance criteria and fluid ingestion methods have been previously reported in detail [12]. Seven healthy, moderately trained individuals (five males, two females) volunteered to participate in the study (mean ± S.E.M.; age 20.6 ± 1.1 years, height 1.75 ± 0.04 meters, mass 76.9 ± 3.8 kg, $V_{O2peak}$ 3.8 ± 0.2 l.min$^{-1}$, peak power 288 ± 11.3 W). The experiment was approved by the Ethics in Human Research Committee of the University and all participants signed a letter of informed consent. Prior to the experimental trials participants attended the laboratory where they were familiarised with procedures and equipment. During this session descriptive measurements were taken after which participants performed an incremental cycle test for the determination of peak oxygen consumption ($V_{O2peak}$) and peak power output (PPO) [12]. Participants who were not familiar with the exercise protocol then completed a familiarization ride following
their $V_{O2peak}$ test. Some subjects had previously participated in experiments utilising this exercise protocol and thus were familiar with the protocol, procedures and expectations.

During the initial visit to the laboratory, participants were provided with a nutritional information sheet which recorded their dietary (food and fluid) intake and physical activity pattern for 48 h before the first experimental trial. Upon arrival for the first trial this record was copied and returned to the participant who was instructed to repeat this regime before all remaining trials. Subjects were asked to refrain from undertaking any exhaustive exercise and to abstain from the ingestion of alcohol, caffeine and tobacco for the 48 h period preceding each trial. The order of the trials was randomized and all sessions were performed at the same time of day to control for circadian variation, with trials conducted at least one week apart to allow sufficient recovery between trials and minimise acclimation to ambient conditions.

**Experimental procedure**

On arrival at the laboratory participants voided, nude body mass was recorded and rectal thermistor inserted. Electromyography electrodes and heart rate transmitter strap were secured and participants performed a maximal isometric contraction on an isokinetic dynamometer. Skin thermistors were attached and participants then entered the temperature controlled chamber and mounted their bicycle in preparation to cycle. On completion of the trial, participants were weighed nude for the determination of total body sweating. Throughout all trials participants were dressed in shorts and shoes, while females added a lycra top.

**Performance trial and hydration**

Our previous studies have shown the test-retest coefficient of variation for this cycling time trial following the completion of at least one familiarization trial to be 1.34% [18]. The performance trial required participants to undertake a 60 min cycling time trial, with the aim to complete the greatest distance possible within the allotted time. Each participant completed four self-paced cycling time trials differentiated by environmental conditions (moderate 19.8 ± 0.3°C, 65.0 ± 0.3% relative humidity, (rh) or warm 33.2 ± 0.1°C, 63.3 ± 0.4% rh) and fluid ingestion regimen. During the experimental trials, participants received either no fluid (NF) or complete fluid replacement (CF), intended to replace all body fluid losses and negate any change in body mass during exercise. When the NF trial was conducted first the rate of fluid ingestion for the subsequent trials was determined from the change in body mass during this trial. In the situation where the CF trial was performed first, fluid ingestion rates were estimated to be 16 ml/kg/hr based on previous data [19] for the moderate condition, and 18 ml/kg/hr for the warm condition from previous work conducted in warm humid conditions in our laboratory. This volume was divided into 12 equal portions where cold (4.5°C) distilled deionised water was provided at 5 min intervals, with participants given 1 min to consume the fluid.

Participants performed the trial using their own bicycle mounted to the electromagnetic cycle trainer altering gear selection and cadence as required. The stochastic nature of cycle racing and performance was simulated with 6 x 1 min sprints at the 10th, 20th, 30th, 40th, 50th and 60th min. Participants were encouraged to perform a maximal effort for each 1-min sprint whilst in a seated position. Power output (W), distance cycled (km) and heart rate (Vantage NV, Polar Electro Oy, Kempele, Finland) were constantly monitored and recorded at 5 min intervals, immediately prior to, and at the midpoint of all sprint intervals. Thermoregulatory variables (rectal temperature, $T_{m}$; mean skin temperature; total body sweating) were monitored and recorded as previously described [12,20]. Raw EMG data were sampled from the rectus femoris and vastus lateralis muscles for a duration of 5 s at the midpoint of each sprint interval.
Determination of fluid balance

As experiment required either complete fluid replacement or no fluid ingestion, blood samples were drawn before and after the trials for the determination of fluid balance parameters. Blood was collected from a 21-gauge catheter inserted in a superficial forearm vein. Patency was maintained with saline (0.9%) solution; the catheter was flushed with 2 mL of blood before each sample was drawn. Aliquots were collected in vacutainers (Starsdet, Germany) containing either SST gel for serum separation or EDTA. Following collection the tubes were gently inverted and placed in ice. At the completion of each experimental session samples were centrifuged at 1233 x g for 15 min and the supernatant transferred to storage containers and frozen at –25 °C until analysis. Blood samples from EDTA tubes were assayed for haemoglobin (Hb) within 2-3 hours of collection using the cyanmethaemoglobin method.

Percent changes in blood and plasma volume were calculated from Hb concentration and red cell volume, respectively as described previously [21]. Serum osmolality was measured by freezing-point depression (Model 3MO Advanced Micro-Osmometer, Advanced Instruments, Norwood, MA, USA). The instrument was calibrated before and after analysis using standards of known osmolality and all samples were analysed in duplicate with the mean values reported. The distribution of body fluid between extracellular and intracellular compartments from pre to the end of exercise were determined using equations 1 and 2 [22]:

\[
ECF_2 = ECF_1 + [ECF_1 \times (\Delta BV\% \cdot 100^{-1})]
\]

Eq. 1

\[
ICF_2 = ICF_1 + [ICF_1 \times (\Delta Osm\% \cdot 100^{-1})]
\]

Eq. 2

Where ECF\(_1\) is initial extracellular fluid in litres and calculated as 0.375 (0.57 x body mass (kg)) and ICF\(_1\) is initial intracellular fluid in litres as 1.666 x ECF\(_1\), \(\Delta BV\%\) is percent change in blood volume and \(\Delta Osm\%\) is percent change in plasma osmolality.

Assessment of muscle function

Muscle function was assessed before the first cycling time trial using a Kin-Com™ isokinetic dynamometer (Chattanooga Group Inc., USA.). The participant was secured to the dynamometer with a shoulder and waist strapping. To avoid interference with the EMG electrode placement, the active leg was not stabilized. The axis of rotation of the dynamometer was visually aligned with the lateral femoral epicondyle with the lower leg attached to the lever arm at the level of the lateral malleolus.

A maximal voluntary contraction (MVC) determining isometric function of the knee extensor muscle group was performed by each subject prior to the cycling protocol. Participants performed a series of 4 x 5 s MVC throughout which EMG and torque data were recorded. The knee was positioned at 60°, with 0° being full knee extension. The trial with the highest value was then retained for analysis.

Electromyography

Prior to exercise, EMG electrodes with bandwidth of 20-450 Hz were attached to the belly of the rectus femoris and vastus lateralis muscles of the right limb. The skin overlying these muscles was carefully prepared. Hair was shaved off, the outer layer of epidermal cells abraded and thoroughly cleaned with alcohol. Differential surface electrodes (Delsys, Boston, MA, U.S.A.) were then placed on the muscle site as described above and linked via insulated cable to the signal acquisition apparatus (Bagnoli-4, Delsys, Boston, MA, U.S.A) and host
computer equipped with data acquisition software (Delsys, Boston, MA, U.S.A.). EMG data were sampled at 1024 Hz for the duration of all tests, thus yielding raw signals. No notch filter was applied. Electrodes were of a parallel bar configuration with an inter-electrode distance of 10 mm. The site for the reference electrode, consisting of a gel adhesive electrode, was prepared as described above and positioned over an electrically neutral and mechanically stable site.

EMG data during the isometric MVC were obtained over a 5 s period. EMG data during cycling were obtained during the middle 5 s of each 1 min sprint. For quantitative data, raw EMG signals were full wave rectified and movement artifact removed using a high-pass second order Butterworth filter with a cut off frequency of 15 Hz. Data were then smoothed with a low-pass second order Butterworth filter with a cut-off frequency of 5 Hz. This was performed using MATLAB™ gait analysis software (The Mathworks Inc., USA). Finally, mean integrated EMG (iEMG) was subsequently determined by calculating the area under the EMG-time curve and dividing by the number of data points. The iEMG data for the isometric MVC was the used to normalize the subsequent cycling EMG data using the following equation: Sprint Interval (x) value = absolute value / isometric MVC value x 100. As such, cycling iEMG data are presented as a percentage of the isometric MVC value. This method of EMG normalization has been used previously by others [16] and shown to be reliable and valid [23] in addition to iEMG responses during high intensity cycling in the heat being reproducible [24].

The frequency spectra of each interval of the raw EMG data were analyzed using a Fast Fourier Transformation algorithm. The frequency spectrum analysis was restricted to frequencies in the range 5-500 Hz, as the EMG signal content outside of this range consists mostly of noise. The frequency spectrum from each interval of data was compared with that from the first interval, and the amount of spectral compression was estimated. This was performed using the technique previously described [25-27]. The spectrum of the raw signal of each interval was obtained and the normalized cumulative power at each frequency was calculated. The shift in each percentile frequency (i.e. at 0%...50%...100% of the total cumulative), was examined. The frequency shift was then estimated by calculating the mean percentile frequency shift (MPFS) throughout the mid-frequency range, i.e. 5-500 Hz. This method has been suggested as a more accurate estimate of spectral compression than median frequency analysis, which only uses the value of a single (50th) percentile frequency [25,26].

Statistics

Power calculations were determined a-priori using G*Power software (v3.0.10). Power (1 - β) was set at 0.95 revealing a total sample size of n = 4 with actual power = 0.98. Therefore, we recruited seven subjects that met the criteria for performance as previously determined. Descriptive data were generated for all variables and presented as the mean ± S.E.M. A repeated measures ANOVA (hydration x ambient temperature x time) was used to analyze data. Once main effects were identified individual differences between means were located using Tukey’s HSD post hoc procedure. Where a significant interaction between trial x time was identified, a one-way ANOVA for repeated measures was applied to determine the source of differences. Statistical significance was accepted when P < 0.05.
RESULTS

Cycling Performance

The total distance completed after 60 min self-paced cycling was not altered by either hydration or environmental conditions ($P > 0.05$; table 1). Distance cycled during the maximal effort sprint intervals was not different amongst treatments ($P > 0.05$; table 1). However, there was a within trial difference for the last two sprint intervals for the moderate CF trial when compared with the initial sprint ($P < 0.05$; table 1).

Table 1. Distance (km) for cycling performance during moderate and warm ambient conditions with complete fluid replacement (CF) and without hydration (NF).

<table>
<thead>
<tr>
<th>Trial</th>
<th>Moderate-NF</th>
<th>Moderate-CF</th>
<th>Warm-NF</th>
<th>Warm-CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total distance (km)</td>
<td>32.6 ± 2.3</td>
<td>30.8 ± 2.0</td>
<td>30.5 ± 1.7</td>
<td>30.1 ± 1.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sprint (km)</th>
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</thead>
<tbody>
<tr>
<td>9-10min</td>
<td>0.81 ± 0.07</td>
<td>0.83 ± 0.06</td>
<td>0.81 ± 0.07</td>
<td>0.80 ± 0.07</td>
</tr>
<tr>
<td>19-20min</td>
<td>0.76 ± 0.05</td>
<td>0.76 ± 0.04</td>
<td>0.80 ± 0.06</td>
<td>0.76 ± 0.05</td>
</tr>
<tr>
<td>29-30min</td>
<td>0.80 ± 0.06</td>
<td>0.77 ± 0.06</td>
<td>0.74 ± 0.03</td>
<td>0.76 ± 0.06</td>
</tr>
<tr>
<td>39-40min</td>
<td>0.79 ± 0.06</td>
<td>0.79 ± 0.07</td>
<td>0.70 ± 0.04</td>
<td>0.80 ± 0.06</td>
</tr>
<tr>
<td>49-50min</td>
<td>0.76 ± 0.06</td>
<td>0.71 ± 0.07 *</td>
<td>0.73 ± 0.06</td>
<td>0.76 ± 0.05</td>
</tr>
<tr>
<td>59-60min</td>
<td>0.81 ± 0.06</td>
<td>0.66 ± 0.09 *</td>
<td>0.81 ± 0.07</td>
<td>0.77 ± 0.06</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Self-Paced (km)</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>0-9min</td>
<td>4.61 ± 0.41</td>
<td>4.47 ± 0.37</td>
<td>4.43 ± 0.31</td>
<td>4.43 ± 0.30</td>
</tr>
<tr>
<td>10-19min</td>
<td>4.64 ± 0.36</td>
<td>4.51 ± 0.34</td>
<td>4.54 ± 0.28</td>
<td>4.40 ± 0.26</td>
</tr>
<tr>
<td>20-29min</td>
<td>4.70 ± 0.33</td>
<td>4.44 ± 0.31</td>
<td>4.47 ± 0.28</td>
<td>4.27 ± 0.32</td>
</tr>
<tr>
<td>30-39min</td>
<td>4.69 ± 0.32</td>
<td>4.27 ± 0.30</td>
<td>4.20 ± 0.25</td>
<td>4.09 ± 0.29</td>
</tr>
<tr>
<td>40-49min</td>
<td>4.63 ± 0.32</td>
<td>4.23 ± 0.33</td>
<td>4.07 ± 0.22</td>
<td>4.10 ± 0.30</td>
</tr>
<tr>
<td>50-59min</td>
<td>4.61 ± 0.33</td>
<td>4.36 ± 0.34</td>
<td>4.14 ± 0.25</td>
<td>4.13 ± 0.33</td>
</tr>
</tbody>
</table>

*P < 0.05 compared with sprint distance at 9 – 10 min.
Hydration

The effect of hydration on body mass is given in table 2. Fluid ingestion during the CF trials successfully maintained body mass during exercise. The volume of ingested fluid during the moderate and warm conditions was 1.31 ± 0.13 and 1.52 ± 0.15 liters, respectively, a difference of 0.21 l. The NF trials resulted in a negative change in body mass of 1.27 ± 0.13 kg for the moderate condition and 1.61 ± 0.18 kg for the warm condition. The changes in body mass are equivalent to reductions of 1.7% for the moderate condition and 2.1% for the warm condition.

The pre-exercise serum osmolality was similar for each condition (range: 270.4 – 276.7 mOsm/kg H₂O) which increased at the end of each trial (range: 283.3 – 295.5 mOsm/kg H₂O; P < 0.05) but was not different among conditions (table 2). There were no significant differences between conditions for %Δ plasma volume, ECF or ICF compartments (table 2).

Heart rate and Thermoregulatory Responses

Mean heart rate over the entire trial for the moderate-NF, moderate-CF, warm-NF and warm-CF trials were 161 ± 1, 155 ± 1, 160 ± 1, and 160 ± 1 beats/min, respectively, indicating a similar effort amongst trials. The only significant difference among trials was the reduced heart rate for moderate CF (P < 0.05). No difference in heart rate was observed among conditions during the sprint intervals (P > 0.05). Thermoregulatory responses are presented in table 2. As expected ambient temperature had a significant effect on skin temperature (P < 0.05); however, the combination of fluid ingestion and ambient temperature did not influence Tₑₑ responses during exercise (P > 0.05). Total body sweating was higher for the warmer compared to the moderate condition.

Neuromuscular Responses

In the moderate ambient condition normalised iEMG for the rectus femoris muscle was similar for both NF and CF. However, iEMG decreased for both fluid conditions at sprint intervals 3 - 5 to be between ~ 0.10 and 0.15% of MVC after which the iEMG was restored to initial values for the last sprint (figure 1). In contrast, iEMG in the warm condition displayed a different pattern so that iEMG for CF was somewhat depressed compared to NF, although this was not statistically significant. The MPFS for the rectus femoris muscle in the moderate ambient condition was identical for both ingestion treatments. However, in the warmer condition MPFS increased from about sprint 3 at 1.05% of MVC until the end of exercise reaching ~ 1.15% of MVC (P < 0.05) for both CF and NF. Interestingly, the MPFS for the vastus lateralis muscle displayed similar characteristics to the rectus femoris muscle in the warmer ambient conditions but not in the moderate ambient condition. For all trials iEMG for the rectus femoris and vastus lateralis muscles appeared to track power output, displaying a similar pattern across the six sprint intervals (figure 1).
Table 2. Thermoregulatory responses and body mass changes in moderate and warm ambient conditions with complete fluid replacement (CF) and without hydration (NF). Δ is change in body mass from pre – exercise to end – exercise. * P < 0.05 compared to moderate ambient condition, a P < 0.05 compared to pre-exercise value. Values are means ± S.E.M.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Moderate NF</th>
<th>Moderate CF</th>
<th>Warm NF</th>
<th>Warm CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-exercise T&lt;sub&gt;re&lt;/sub&gt; (°C)</td>
<td>37.47 ± 0.04</td>
<td>37.50 ± 0.09</td>
<td>37.46 ± 0.06</td>
<td>37.49 ± 0.04</td>
</tr>
<tr>
<td>Final T&lt;sub&gt;re&lt;/sub&gt; (°C)</td>
<td>38.90 ± 0.30</td>
<td>38.60 ± 0.40</td>
<td>38.90 ± 0.50</td>
<td>38.70 ± 0.40</td>
</tr>
<tr>
<td>Δ T&lt;sub&gt;re&lt;/sub&gt; (°C)</td>
<td>1.43 ± 0.1</td>
<td>1.10 ± 0.1</td>
<td>1.54 ± 0.2</td>
<td>1.21 ± 0.2</td>
</tr>
<tr>
<td>Final Mean Skin Temperature (°C)</td>
<td>28.90 ± 0.34</td>
<td>28.82 ± 0.53</td>
<td>34.10 ± 0.35*</td>
<td>34.34 ± 0.21*</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre–exercise</td>
<td>77.31 ± 3.67</td>
<td>77.46 ± 3.68</td>
<td>76.64 ± 3.68</td>
<td>77.46 ± 3.43</td>
</tr>
<tr>
<td>Post-exercise</td>
<td>76.04 ± 3.57</td>
<td>77.52 ± 3.72</td>
<td>75.03 ± 3.61</td>
<td>77.49 ± 3.40</td>
</tr>
<tr>
<td>Fluid Ingestion (water), litres</td>
<td>0</td>
<td>1.31 ± 0.13</td>
<td>0</td>
<td>1.52 ± 0.15</td>
</tr>
<tr>
<td>Δ Body mass (kg)</td>
<td>-1.27 ± 0.13</td>
<td>+0.07 ± 0.10</td>
<td>-1.61 ± 0.18</td>
<td>-0.03 ± 0.09</td>
</tr>
<tr>
<td>Total Body Sweating (mL/kg/h)</td>
<td>16.5 ± 1.3</td>
<td>16.1 ± 1.2</td>
<td>21.3 ± 2.1*</td>
<td>19.1 ± 2.1*</td>
</tr>
<tr>
<td>Pre-exercise Osmolality mOsm/kg/H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>270.4 ± 2.5</td>
<td>271.5 ± 3.6</td>
<td>275.9 ± 2.7</td>
<td>276.7 ± 2.6</td>
</tr>
<tr>
<td>End-exercise Osmolality mOsm/kg/H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>284.0 ± 4.6*</td>
<td>283.3 ± 2.7a</td>
<td>296.5 ± 2.6*</td>
<td>283.8 ± 2.6a</td>
</tr>
<tr>
<td>Δ Plasma volume %</td>
<td>-8.4 ± 1.6</td>
<td>-8.2 ± 2.7</td>
<td>-11.8 ± 2.2</td>
<td>-6.9 ± 2.0</td>
</tr>
<tr>
<td>ECF, (litres)</td>
<td>27.2 ± 1.3</td>
<td>27.0 ± 1.6</td>
<td>25.8 ± 1.3</td>
<td>27.8 ± 2.7</td>
</tr>
<tr>
<td>ICF, (litres)</td>
<td>50.4 ± 2.8</td>
<td>50.1 ± 2.2</td>
<td>50.7 ± 2.3</td>
<td>49.8 ± 2.7</td>
</tr>
</tbody>
</table>
DISCUSSION

The novel finding of the present study is that fluid ingestion *per se*, in either moderate or warm ambient conditions did not improve performance of high intensity cycling compared with complete fluid restriction in the same ambient conditions. Using the same exercise protocol we have previously shown that glycerol hyperhydration does not improve performance in the heat [13]. At present, there is no clear explanation regarding the mechanisms responsible for such a result when many previous studies have shown fluid ingestion to improve exercise performance albeit during fixed intensity exercise [9,10,28]. The present study extends our previous findings [12,13,29] as it examines the neuromuscular responses to hydration in moderate and warm ambient conditions. This is a salient point as there is evidence that premature fatigue during exercise heat stress may be related to alterations in CNS drive and evident in neuromuscular profiles with similar changes suggested to occur with dehydration [30].

In elevated ambient temperatures the response of the neuromuscular system as determined from surface iEMG during high intensity cycling is thought to be repeatable, and that alpha motor neuron firing and neuromuscular propagation remain intact for the duration of the exercise [24]. St Clair Gibson *et al.* [31] utilising a protocol that employed a series of 1 and 4 km sprints as part of a 100 km time trial showed that only 20% of available muscle was recruited during maximal effort cycling when normalised against a pre-exercise MVC. These authors also observed considerable depletion of muscle glycogen following exercise. Although in the present study we did not measure muscle glycogen, the iEMG data are consistent with those reported by St Clair Gibson *et al.* [31] as only about 20 – 40% of available muscle was recruited throughout exercise (see figure 1). The progressively decreasing iEMG signal combined with the apparent reduction in neuromuscular drive is evidence for CNS alterations in the recruitment patterns of muscles during the time trial.

However, a limitation of previous work [24,31] was that the EMG signal was sampled from the rectus femoris muscle only and although synergistic muscles have been shown to demonstrate similar neuromuscular fatigue profiles during sustained isometric contractions [32], it remains unclear if a similar response occurs during normal dynamic activities. The present investigation captured EMG data from both the bi-articular rectus femoris and mono-articular vastus lateralis muscles. The findings indicate that during variable intensity cycling these muscles display similar recruitment patterns which may be influenced by hydration and/or environmental temperature or changes in body temperature. In a previous study, it was shown that the normalised iEMG of the rectus femoris muscle declined from the initial sprint during self-paced cycling but returned to initial values at the end of the trial. Figure 1 shows a similar pattern for the rectus femoris muscle where the normalised iEMG is reduced during sprints 3, 4 and 5 and returns to initial values in the final sprint. This “re-recruitment” of muscle when the conscious effort is the same throughout the test, as evidenced by heart rate, represents an anticipatory regulation of exercise intensity. In the present study each sprint interval produced identical peak heart rates, and thus it is difficult to argue that subjects did not produce a maximal conscious effort. This is evidence that subjects paced themselves accordingly as terminal rectal temperatures were similar irrespective of heart rate during the sprint intervals. This finding could possibly explain the results of Tatterson *et al.* [15] who showed that elite cyclists pace themselves to prevent an excessive rise in body temperature and self-select a power output which allows the maintenance of core temperature below a critical limit.

The MPFS increased with respect to time in the warm condition for both muscles. This alteration in spectral compression may have resulted from several factors. For instance
an increasing core temperature, which causes the spectral component of the EMG signal to shift toward a higher frequency is a possibility [33]. Peripheral factors such as increasing skin temperature have also been demonstrated to influence the frequency of the EMG signal [34,35]. However, whereas the magnitude of the change in rectal temperature was higher in the NF treatments, the final rectal temperatures were the same amongst all trials, suggesting that the rising core temperature did not influence the MPFS in this instance as MPFS was only higher for the warmer ambient condition. If rising core temperature was a determinant of MPFS shifts in this instance, we should have observed the shift to higher frequencies across all trials. In the present study skin temperature was higher during the warmer than the moderate condition. Given that skin temperature was different then this might have contributed to the peripheral contribution to the changes in MPFS; although Hunter et al. [23] found that during fixed intensity exercise performed at 15°C and 35°C the iEMG signal was not altered throughout exercise at different intensities. The authors suggested that the control of thermoregulatory responses which maintain a stable core temperature allowed for the integrity of the neuromuscular system to be stable. Similarly, others have found that despite considerable increases in core temperature, heart rate and percent dehydration during treadmill running, the surface EMG determinants of neuromuscular function remained relatively unchanged [2].

In addition to the possible influence of temperature changes on the EMG signal, changes in muscle fibre recruitment strategy may have also contributed to this effect, with the selective recruitment of larger Type II fibres later in the trial [36]. Although the evidence for a change in muscle recruitment pattern is scant, Ray and Gracey [37] have shown that exercise with heating promotes a higher muscle sympathetic nerve activity which is thought to convey nociceptor information and in turn induce force inhibition. If this were possible, then as force generating capacity begins to attenuate, sequential recruitment of Type II fibres might increase to compensate for the reduction in force.

A surprising but not uncommon result from the present study was the similar rectal temperature response amongst hydration treatments and ambient conditions. This supports previous findings [13] but is in contrast to studies where high intensity exercise without fluid ingestion in warm conditions resulted in premature fatigue [4,28]. One explanation may be that during self-paced exercise subjects are able to control intensity and regulate pacing irrespective of the environmental conditions. Other investigations utilising a cycling time trial have also demonstrated no difference in core temperature response despite the use of intervention strategies designed to influence thermoregulatory responses [38]. The mechanism for this is unclear but may include the intervention of a centrally mediated command [24,31] which is thought to allow down-regulation of efferent output to account for the changes in physiological demands required by different conditions at various stages throughout exercise.

In contrast to findings under moderate conditions only two previous investigations [4,28] have found fluid ingestion to enhance high intensity exercise in the heat (~32°C). That is, a reduction in body mass of 1.8% decreased time to exhaustion at 90% VO_2peak following 60 min of cycling at 60% VO_2peak [4]. While similar changes in body mass (~ - 2%) were observed in the present study during self-paced exercise in the heat, the prevention of a reduction in body mass failed to improve the total distance cycled or influence power output during the maximal effort sprint intervals. Notably, the serum osmolality increased for each condition and as expected the osmolality was highest in the warm environment with restricted fluids although this was not statistically different. Additionally, the fluid compartments (ECF, ICF) were maintained for each condition. From these data we can only conclude that the fluid ingestion protocol in the present study did not influence the thermoregulatory responses compared to the trials where fluid was restricted. The reason for this is most likely related to the fact that both osmolality and fluid compartments were kept within physiological range.
However, others [28] have attributed the improved exercise performance with fluid ingestion to the attenuated $T_r$ and heart rate response during exercise which was thought to be due to the fluid ingested before rather than during exercise. Perhaps under the present conditions the inability of fluid ingestion to exert influence on these variables ($T_r$ and heart rate) contributed to the similar outcomes in each of the trials. It is important to note; however, that regardless of the intervention strategy, peak and terminal heart rate were similar among conditions. It remains unclear why these subjects would finish exercise under very different conditions with similar terminal heart rates. Perhaps these terminal heart rates are indicative of a physiological limit during prolonged exercise. The possibility that fluid ingestion would contribute to a 'heat sink' has been previously suggested [3,9], but others suggest that fluid ingestion has minimal effect on heat storage and cardiovascular strain at least within the timeframe for the present experimental protocol.

In conclusion, the present findings acknowledge the importance of cortical level, or forced conscious, de-recruitment of muscle following integration of feedback from all physiological systems involved with the exercise. Such a mechanism would balance the desired outcome and requirements of the exercise bout within the constraints of the environment, regulating metabolic activity in order that premature fatigue is abated and optimal physiological function is preserved.

**Information Box**

'What is already known on this topic'

The assumption that fluid ingestion *per se* improves exercise performance in the heat is based largely on experiments where the exercise was at a fixed intensity. The effect of fluid ingestion on neuromuscular recruitment is not fully known.

'What this study adds'

This study shows that fluid ingestion in either warm or moderate ambient conditions does not improve high intensity self-paced exercise performance over 60 min. The findings suggest that the neuromuscular system adapts in order to allow the completion of the trial by altering muscle recruitment strategy.

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The authors declare that there are no competing interests.

**Figure legend**

**Fig. 1.** Values for IEMG and MPFS of the *rectus femoris* and *vastus lateralis* muscles, power output (W) and sprint distance (km) during 60 min of high intensity cycling. Ambient conditions are either moderate or warm with complete fluid replacement during cycling (CF) and without hydration (NF). Values were obtained at the midpoint of each sprint interval with EMG data normalized as a percentage of the pre-exercise isometric MVC *different from first sprint, $P < 0.05$; values are means ± S.E.M.*
References


