Chapter 6

The combined effect of heat stress and hypohydration on pacing pattern during a 40-km cycling time trial

Koen Levels • Robert Lamberts • Jos de Koning • Meriam Berkulo
Susan Bol • Carl Foster • Hein Daanen

Submitted for publication
ABSTRACT

Purpose
To determine the combined effect of hypohydration and heat stress on pacing pattern and performance during a 40-km cycling time trial.

Methods
13 Male cyclists performed 40-km cycling time trials in 25.3 ± 0.3°C and 35.3 ± 0.2°C in a euhydrated (EU25, EU35), and hypohydrated (HYPO25, HYPO35) state. Relative humidity was 55 ± 3% and air velocity was 7 m·s⁻¹. Hypohydration was induced before starting the time trial by 50 min exercise in 30.2 ± 0.2°C and 55 ± 1% relative humidity.

Results
Body mass at the start of the time trial was 1.2% lower in the HYPO trials than in the EU trials. At the finish, hydration levels were -2.1 ± 0.4% (EU25), -2.7 ± 0.3% (EU35), -3.1 ± 0.3% (HYPO25), and -3.8 ± 0.4% (HYPO35) of initial body mass. Heat stress increased finish time whereas a trend was observed for hypohydration. No interaction effect between heat stress and hydration status on finish time was found. Heat storage, skin temperature, thermal sensation/comfort and RPE were higher in the hot trials, whereas RPE and thirst sensation were higher in the hypohydration trials.

Conclusion
The negative effect of hypohydration on exercise performance during a 40-km cycling time trial is similar in hot and moderate conditions, probably because of the provided wind cooling.
INTRODUCTION

The effect of heat stress on self-paced cycling performance has been extensively studied (Peiffer and Abbiss 2011; Tatterson et al. 2000; Tucker et al. 2004). In these studies, subjects generally start cycling in the heat at a similar exercise intensity as in cooler conditions. However, a stronger decrease in exercise intensity is seen over time, especially in medium to long exercise durations (> 20 min) (Peiffer and Abbiss 2011; Tucker et al. 2004). The pronounced reduction in power output with increase of heat stress assures that the human body does not reach critically high temperatures and that the exercise bout can be completed successfully (Noakes 2011). This implies that exercise intensity is regulated by a feed-forward as well as feed-back mechanism, which is influenced by (thermo)physiological heat stress as suggested by Tucker et al. (2004).

When exercise is performed in the heat, the evaporation of sweat becomes the most prominent mechanism for heat dissipation (Gagnon and Kenny 2011). Especially in endurance exercise and when there is inadequate opportunity to replenish the fluid lost by sweating, the fluid deficit (hypohydration) can result in increased cardiovascular and heat strain (Gonzalez-Alonso et al. 1995, 1997) and through this have a negative effect on exercise performance (Cheuvront et al. 2003; Murray 2007). The most recent position stand of the American College of Sports Medicine (ACSM) (Sawka et al. 2007) states that hypohydration by more than 2% of body mass (BM) should be avoided, especially in warm environments. In support of this, several studies have shown that hypohydration levels between 1 and 2% BM might already result in performance decrements (Armstrong et al. 1985; Bardis et al. 2013; Walsh et al. 1994). As many athletes start (middle- to long-duration) exercise bouts mildly hypohydrated, in particular to avoid voiding during the race (Maughan et al. 2005), this might impair their performance. For even smaller fluid deficit levels at the start of exercise (< 1% hypohydration) it still remains questionable if this results in impaired thermoregulation or even decreased exercise performance. This is especially relevant since these small fluid deficits will generally not increase thirst sensation (Fitzsimons 1976), and are therefore not compensated for by fluid intake. Also
for greater levels of hypohydration, not all studies have shown performance decrements. A review by Goulet (2011) showed that dehydration up to 4% BM did not impair exercise performance in moderate-to-warm conditions (20-33°C) when individuals were allowed to drink according to thirst. In addition, factors such as the type of exercise protocol (fixed intensity vs. self-paced exercise), mode of exercise (e.g. running or cycling), the moment of inducing hypohydration (before or during exercise), and the amount of environmental heat stress, seem to influence the effect of hypohydration on exercise performance.

Although the relationship between hydration status and exercise performance has been extensively studied, only a few studies investigated the relationship between hypohydration and pacing pattern during exercise. Stearns et al. (2009) found a decreased ability of hypohydrated runners to maintain an even pacing pattern during a 3 * 4-km loops trail running exercise in a moderate temperature (~26°C). However, Kay and Marino (2003) observed no differences in pacing strategy between euhydrated and hypohydrated athletes during a 60-min cycling time trial with a one-minute sprint every 10 min. These differences can possibly be explained by the hydration status at the start of the trial, as the runners within the study of Stearns et al. followed a strict regime leading to a decrease in BM of > 2% at the start of the trial, while cyclists in Kay and Marino’s study started the trial euhydrated. Therefore, at this moment, the effect of hydration status on the modulation of work rate during self-paced exercise is still relatively unknown. However, as hypohydration is generally accompanied by high body temperature and cardiovascular strain, there is a reason to believe that hypohydration might have a negative effect on pacing strategies and exercise performance.

Although the separate effects of heat stress and hypohydration on exercise performance have been studied extensively, their interactive effects are relatively unknown. This is especially true for self-paced exercise that is conducted in realistic environmental conditions. Kenefick et al. (2010), for example, demonstrated that hypohydration decreased exercise performance (total work completed during a 15-min cycling time trial) to a greater extent with increasing heat stress. Moreover, Sawka et al. (2012) showed in a review that a high skin temperature degrades aerobic exercise performance when
individuals are euhydrated, but that this effect is even greater for hypohydration. However, it remains questionable if these findings can be extrapolated to real-life outdoor events. The absence of a realistic air velocity, and the associated greater heat loss (Saunders et al. 2005), may seriously reduce the impact of hypohydration on exercise performance.

Overall, it can be concluded that the effect of heat stress on pacing pattern during aerobic exercise is known, but the effect of hypohydration remains unclear. Moreover, the interaction effect between heat stress and hypohydration is still unknown. Therefore, the main goal of this study is to investigate the effect of hypohydration on exercise performance and pacing pattern during a 40-km cycling time trial in moderate and hot conditions. We hypothesize that hypohydration will negatively affect pacing pattern and exercise performance during a 40-km cycling time trial in the heat but not in moderate conditions.

METHODS

Participants

Thirteen trained, experienced male cyclists participated in this study (Table 6.1). After receiving detailed information about the study, all cyclists gave their written informed consent and completed a health screening questionnaire and the Physical Activity Readiness Questionnaire (PAR-Q) (ACSM 2007). The cyclists were instructed to avoid strenuous exercise the two days before a visit to the laboratory and to refrain from alcohol and caffeine 24 hours prior to each test. The study was approved by the Human Research Ethics Committee of the University of Cape Town, South Africa, where the data was collected.
Table 6.1 Descriptive statistics of the cyclists (n = 13) including their responses during the incremental exercise test (mean ± SD).

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33 ± 7</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>79 ± 8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>183 ± 7</td>
</tr>
<tr>
<td>$\dot{V}O_2$ max (ml·kg$^{-1}$·min$^{-1}$)</td>
<td>56 ± 6</td>
</tr>
<tr>
<td>HR$_{max}$ (beats·min$^{-1}$)</td>
<td>186 ± 7</td>
</tr>
<tr>
<td>Peak power output (W)</td>
<td>410 ± 34</td>
</tr>
<tr>
<td>Peak power output (W·kg$^{-1}$)</td>
<td>5.2 ± 0.6</td>
</tr>
<tr>
<td>Sum of skinfolds (mm)</td>
<td>52 ± 12</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>14 ± 3</td>
</tr>
</tbody>
</table>

Experimental design

All cyclists completed an incremental exercise test to exhaustion, a familiarization 40-km cycling time trial (TT), and four experimental 40-km cycling time trials. These trials were allocated in a balanced order, at the same time of the day, and at least three days apart. The experimental trials started with a 30-min period of cycling at 50% peak power output (PPO) in which either mild hypohydration was induced, or the cyclists remained euhydrated. After a 30-min recovery period, the 40-km time trial started in either 25°C (moderate) or 35°C (hot). The combination of these two differentiations resulted in the four experimental conditions: 25°C and euhydrated (EU25), 25°C and hypohydrated (HYPO25), 35°C and euhydrated (EU35), and 35°C and hypohydrated (HYPO35). In Figure 6.1, a schematic overview of the experiment is shown.

Incremental exercise test

During the first visit to the laboratory, body mass, height, and seven skinfolds (triceps, biceps, abdomen, calf, thigh, supra-iliac region and subscapular region) were obtained from the cyclists. Body fat was determined as the sum of these seven skinfolds and as percentage of body mass (Durnin and Womersley 1974). After the collection of anthropometric data, the rear tire of the cyclists’ own race bike was inflated to 800 kPa.
and the bike was mounted with the rear axis to a cycle ergometer (CompuTrainer Pro 3D, RacerMate, Seattle, WA, USA). The contact pressure of the load generator against the rear wheel was calibrated to 0.88–0.93 kg to simulate rolling resistance during outdoor cycling. After initial calibration, the cyclists completed a warm-up protocol consisting of 6 min cycling at a target heart rate of 60% of their maximum heart rate (HR_max), 30 s of rest during which the load generator was recalibrated, 6 min of cycling at 80% HR_max, and 3 min of cycling at 90% HR_max (Lamberts and Lambert Submaximal Cycle Test; LSCT). A more detailed description of the LSCT can be found in Lamberts et al. (2011).

**Figure 6.1** Schematic overview of the experimental trials. Arrows indicate 2 mL·kg⁻¹ water consumption in the euhydration trials (EU25 and EU35).

Five minutes after completion of the LSCT, the incremental test to exhaustion was started at an initial workload of 100 W, after which the load was continuously increased at a rate of 20 W·min⁻¹. The end of the test was determined if the cyclists could no longer maintain a cadence higher than 70 revolutions per minute. During the test, heart rate (HR) was recorded at 5-s intervals (Polar s810i, Polar Electro Oy, Kempele, Finland). Respiratory gas exchange was measured breath-by-breath using a mask covering mouth and nose, connected to a gas analysis system (Jaeger Oxycon Pro, Viasys Healthcare, Hoechberg, Germany), and maximal oxygen consumption ($\dot{V}O_{2\text{max}}$) was determined as the highest continuously recorded 15-s oxygen consumption. Peak power output was determined as
the average power output (PO) during the last minute of the test (CompuTrainer Coaching Software 1.6, RacerMate, Seattle, WA, USA).

**40-km cycling time trials**

At least two days after the incremental test, all cyclists were familiarized to the testing protocol and 40-km TT distance. During this trial only heart rate and RPE were measured, while cyclists were only given continuous feedback about the completed distance of the time trial.

Minimal three days after the familiarization session, the first of four experimental trials was performed. Upon arrival at the lab, cyclists were weighted to the nearest 100 g (Model 770, Seca, Bonn, Germany) and euhydration was confirmed by a difference in body mass of less than 200 g with the preceding trials. After mounting the cyclists own bike with the rear axis to the cycle ergometer, the cyclists performed the LSCT as a standardized warm-up protocol (Lamberts et al. 2011). Three minutes after the LSCT, the cyclists were instructed to cycle for 30 min at a fixed workload corresponding to 50% of their PPO in a climatic chamber set at 30°C and 55% relative humidity (RH). In the euhydration trials (EU25 and EU35), cyclists consumed tap water during this period to remain euhydrated. The water was at tap temperature and the volume to be consumed was determined from the body mass loss during a previous trial. However, if no trial was performed before, the total volume to ingest was estimated to be 12 mL·kg⁻¹ BM based on pilot experiments. This volume was divided in six equal portions and given to the cyclists every 10 min (Figure 6.1). After this period, the cyclists left the climatic chamber and were given a 30-min recovery period in an adjacent room before re-entering the climatic chamber. During this period, no fluid was provided. The temperature in the climatic chamber at the moment of re-entering was 25°C (EU25 and HYPO25) or 35°C (EU35 and HYPO35). After entering the climatic chamber, the cyclists performed a warm-up consisting of 6 min cycling at a target HR of 80% HR_{max} and 3 min at 90% HR_{max}. Three minutes after the warm-up the 40-km cycling time trial started. The cyclists were instructed to complete the time trial in the fastest possible time, while only completed
The combined effect of heat stress and hypohydration on pacing pattern during a 40-km cycling TT

distance was given as feedback. Throughout the time trial, the cyclists were free to shift gears and alter their cadence. Four fans with a diameter of 1 m that were part of the climatic chamber created a laminar airflow of 7 m·s⁻¹ over the cyclists’ body, aiming to reflect airflow during outdoor cycling.

Measurements

At least three hours before arrival at the lab, cyclists ingested a disposable core temperature capsule (Jonah, Hidalgo, Cambridge, UK) to measure gastrointestinal temperature \( T_{GI} \) that was recorded at 15-s intervals using the Hidalgo Equivital™ Physiological Monitor system (Hidalgo, Cambridge, UK). Upon arrival at the lab, four iButtons (DS1922L, Maxim Integrated Products Inc, Sunnyvale, CA, USA) were taped to the skin (neck, right scapula, right shin and left hand) and local skin temperature was recorded at 10-s intervals. Mean skin temperature was calculated using equation 6.1 (ISO9886 2004).

\[
T_{sk} \ (°C) = 0.28 \cdot (T_{neck} + T_{right \ scapula} + T_{right \ shin}) + 0.16 \cdot (T_{left \ hand}) \quad (equation \ 6.1)
\]

Mean body temperature \( T_b \) was calculated using the following equation (Eq. 6.2):

\[
T_b = \alpha \cdot T_{GI} + (1 - \alpha) \cdot T_{sk} \quad (equation \ 6.2)
\]

Where \( \alpha \) was set at 0.6 \((T_{sk} < 31.5°C)\), 0.7 \((31.5°C < T_{sk} < 33°C)\) or 0.8 \((T_{sk} > 33°C)\) (Burton 1935).

Average body heat content \( Q_c \) was calculated for every 4-km segment of the time trial using equation 6.3:

\[
Q_c \ (J) = T_b \cdot m \cdot 3.47 \cdot 10^3 \quad (equation \ 6.3)
\]

In which \( T_b \) is mean body temperature in °C, \( m \) represents body mass in kg, and 3.47 \( \times \) 10³ is the specific heat of the human body \( (J \cdot °C^{-1} \cdot kg^{-1}) \).
Total heat storage during the 40-km time trial was calculated using equation 6.4:

$$Q_S (J \cdot g^{-1}) = \frac{Q_{C, km\ 40 - km\ 0}}{m \cdot 1000} \quad \text{(equation 6.4)}$$

In which $Q_{C, km\ 40 - km\ 0}$ is the difference in body heat content in joules between the finish and start of the time trial.

The body mass of the cyclists was determined to the nearest 100 g directly before start of the LSCT, 25 min and 15 min before start of the time trial, and directly after the time trial (Model 770, Seca, Bonn, Germany). Heart rate was measured every 5 s and during the time trial, PO, speed and cadence were measured at 34 Hz. The RPE was measured every 4 km on a scale ranging 6-20 (Borg 1982). Thermal sensation (TS) and thermal comfort (TC) were also measured every 4 km on a 9-point scale with a range from -4 to 4, and a scale ranging 0-5, respectively (ISO10551 1993). Thirst sensation was measured during the preparation period and every 4 km during the time trial on a numerical scale ranging from 1 to 9 with associated word anchors (1 = not thirsty, 3 = a little thirsty, 5 = moderately thirsty, 7 = very thirsty, 9 = very, very thirsty) (Engell et al. 1987).

**Statistical analysis**

Statistical analysis was performed in SPSS statistical software (SPSS 20.0, SPSS Inc., Chicago, IL, USA). Experimental condition (EU25, HYPO25, EU35, HYPO35) was the independent variable, whereas body mass (changes), finish time, PO, $T_{GL}$, $T_{sk}$, $T_{Bu}$, $Q_S$, HR, RPE, TS, TC, and thirst sensation were the dependent variables. Two-way ANOVAs for repeated measurements (hydration status * heat stress) were used to determine the significance of the effects of hydration status and heat stress on body mass changes, finish time, and mean power output. The significance of effects of experimental condition on the dependent variables over time was determined using three-way ANOVAs for repeated measurements (hydration status * heat stress * distance completed). Post-hoc analyses used Bonferroni correction to adjust for multiple comparisons. The relationship between changes in mean body temperature and changes in PO were established with linear
regression analysis and the Pearson product-moment correlation coefficient. Statistical significance was set at the 5% level for each analysis. Values are reported as mean ± SD.

To determine the practical (rather than the statistical) significance of the effect of heat stress and hypohydration on cycling time trial performance, the effect was also expressed as 90% confidence limits (magnitude based inferences). By comparing the overlap of these limits with the smallest substantial and practically meaningful change in TT performance, the chance that the observed effect is beneficial/trivial/harmful could be determined (Batterham and Hopkins 2006). For this analysis, we assume that the smallest practically meaningful change in 40-km TT performance is 0.7% (Lamberts et al. 2009).

**RESULTS**

**Effect of interventions**

The preparation period consisting of the LSCT, the 30-min fixed 50% PPO cycling, and the 30 min of recovery resulted in a difference in body mass at the start of the time trial of -1.1 ± 0.1% BM in HYPO25 and -1.2 ± 0.2% BM in HYPO35. Because the fluid loss was replaced in the euhydration trials, the change in body mass was close to zero during these trials (-0.1 ± 0.3% BM in EU25 and 0.0 ± 0.2% BM in EU35; effect of hydration status: F=267, P<0.001). Since the main goal was to induce mild hypohydration in HYPO25 and HYPO35 and to maintain euhydration in EU25 and EU35, the manipulation of hydration status can be considered successful.

The environmental conditions during the 40-km cycling time trial were 25.3 ± 0.3°C and 58 ± 1% RH for EU25 and HYPO25, and 35.3 ± 0.2°C and 51 ± 1% RH for EU35 and HYPO35 (temperature: F=1234, P<0.001; RH: F=111, P<0.001). The air velocity was kept constant at 7 m·s⁻¹ in all the trials.
Hydration status during the time trial

Sweat loss during the time trial was smaller in the moderate trials than in the hot trials (2.0 ± 0.4 and 2.7 ± 0.3% BM, respectively; F=116, P<0.001), but no effect of hydration status on sweat loss was observed (F=2.30, P=0.16). After the time trial, total body mass loss was lowest for EU25 (-2.1 ± 0.4% BM), followed by EU35 (-2.7 ± 0.3% BM), HYPO25 (-3.1 ± 0.3% BM), and HYPO35 (-3.8 ± 0.4% BM; F=119, P<0.001). Fluid losses before and during the time trial are shown in Figure 6.2.

![Figure 6.2 Fluid losses before and during the time trial. * Greater fluid loss before the time trial in the hypohydrated trails than in the euhydration trials (P<0.001). # A greater fluid loss during the time trial in the hot conditions than the moderate conditions (P<0.001).](image)

Time trial performance and pacing

Finish time and mean PO of the 40-km time trial are presented in Table 6.2. Heat stress had an effect on finish time (F=14.9, P=0.002) and mean PO (F=21.1, P=0.001), whereas a trend towards a difference in finish time (F=4.09, P=0.06) and mean PO (F=4.90, P=0.05) was apparent for hydration status. No interaction effect between heat stress and hydration status on finish time and mean PO was observed (F=0.10, P=0.75 and F=0.01,
The combined effect of heat stress and hypohydration on pacing pattern during a 40-km cycling TT

P=0.98, respectively). The chances that the effects are beneficial/trivial/harmful on the finish time of a 40-km cycling time trial in real-life competition are 0/1/99 % for the heat stress intervention and 0/19/81 % for the manipulation of hydration status.

**Table 6.2** Finish time and mean PO during the 40-km cycling time trials and differences between starting the trials euhydrated (EU) and hypohydrated (HYPO).

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Finish time (mm:ss)</th>
<th>Difference between EU and HYPO (m:ss)</th>
<th>Mean PO (W)</th>
<th>Difference between EU and HYPO (W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EU25</td>
<td>66:33 ± 4:09</td>
<td>1:02 ± 3:04</td>
<td>251 ± 41</td>
<td>-8 ± 3</td>
</tr>
<tr>
<td>HYPO25</td>
<td>67:35 ± 3:44</td>
<td></td>
<td>242 ± 39</td>
<td></td>
</tr>
<tr>
<td>EU35</td>
<td>70:19 ± 3:51</td>
<td>1:30 ± 4:06</td>
<td>219 ± 32</td>
<td>-8 ± 4</td>
</tr>
<tr>
<td>HYPO35</td>
<td>71:49 ± 5:32</td>
<td></td>
<td>211 ± 40</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 6.3** Pacing pattern during the 40-km cycling time trial. * Main effect of heat stress on PO during the time trial (P<0.05). For clarity of the figure, no error bars are displayed.

The pacing pattern during the time trial (average PO for every 10% segment of the distance completed) is shown in Figure 6.3. This pattern was different for the hot trials than for the moderate trials (interaction effect of heat stress x distance completed:
Although the mean power output showed a trend to be lower in the HYPO trials than in the EU trials, pacing pattern was not affected by hydration status (interaction effect of hydration status * distance completed: F=0.70, P=0.71). Moreover, no interaction effect was found between heat stress, hydration status, and distance completed (F=0.72, P=0.70). In all the four conditions, an increase in PO at the end of the time trial (end spurt phenomenon) was found. The magnitude of the end spurt, determined as the difference in mean PO between km 40 and 39, was similar in EU25 (39 ± 30 W), HYPO25 (28 ± 39 W), EU35 (42 ± 21 W) and HYPO35 (44 ± 29 W; F=1.32, P=0.28).

Temperature patterns

GI temperature at the start of the time trials was similar for the four conditions (37.7 ± 0.2°C; F=1.41, P=0.27). The increase in $T_{Gl}$ during the time trial showed a trend to be higher in the hot trials (EU35: 1.1 ± 0.6°C, HYPO35: 1.5 ± 0.4°C) than in the moderate trials (EU25: 1.0 ± 0.3°C, HYPO25: 0.9 ± 0.3°C; F=5.51, P=0.05) but this did not result in a higher final $T_{Gl}$ for EU35 (38.8 ± 0.5°C) and HYPO35 (39.0 ± 0.5°C) than for EU25 (38.6 ± 0.2°C) and HYPO25 (38.8 ± 0.4°C; F=2.01, P=0.20). Hydration status did not influence the increase in $T_{Gl}$ (F=2.06, P=0.19) and final $T_{Gl}$ (F=2.01, P=0.20).

$T_{sk}$ was higher at the start of the time trial for the hot trials (35.3 ± 0.5°C) than for the moderate trials (31.3 ± 1.1°C; F=302, P<0.001) and remained higher during the entire time trial (F=271, P<0.001). No effect of hydration status was observed for $T_{sk}$ during the time trial (F=1.08, P=0.32).

$T_b$ during the time trial was higher for the hot trials (37.8 ± 0.3°C) than for the moderate trials (35.6 ± 0.5°C; F=298, P<0.001). However, no effect of hydration status on $T_b$ was observed (F=0.50, P=0.50). A weak overall correlation ($r = -0.18$, P<0.001) was found between change in mean body temperature and change in power output (Figure 6.4), but no difference was observed between the euhydration and hypohydration trials in slope (F=0.46, P=0.63) and $y$-intercept (F=0.10, P=0.90) of the linear regression. Total heat storage during the time trial was higher (F= 8.48, P=0.015) for the hot trials (3.0 ± 2.0 J·g$^{-1}$
for EU35, 3.3 ± 1.5 J·g⁻¹ for HYPO35) than for the moderate trials (1.8 ± 1.2 J·g⁻¹ for EU25, 1.5 ± 2.0 J·g⁻¹ for HYPO25) but no effect of hydration status was found (F= 0.01, P=0.98).

Figure 6.4 The relationship between change in mean body temperature and change in power output during the 40-km time trial. Δ Power output and Δ mean body temperature are determined per 4-km segment of the time trial. Data from km 0-4 and km 36-40 are not included in the figure and in the linear regression analysis.

Thermal sensation and comfort

TS increased gradually during the time trial and average values were higher for EU35 (2.4 ± 1.1) and HYPO35 (2.7 ± 1.1) than for EU25 (0.5 ± 1.0) and HYPO25 (0.6 ± 1.1) during the entire time trial (F=35.6, P<0.001; Figure 6.5a). TS at the end of the time trial was 1.1 ± 0.1 in the moderate trials, corresponding to a sensation of ‘warm’, whereas in the hot trials final TS was 3.1 ± 0.1 (effect of heat stress: F=180, P<0.001), corresponding to a sensation of ‘hot’. No main effect of hydration status was observed (F=2.25, P=0.16).

TC followed a similar pattern as thermal sensation during the time trial (Figure 6.5b): a main effect of heat stress was found (F=60.1, P<0.001), whereas no effect of hydration status was observed (F=1.39, P=0.26). At km 40, TC was 2.3 ± 0.2 in the moderate trials
and 4.0 ± 0.2 in the hot trials (F=28.0, P<0.001), corresponding to ‘slightly uncomfortable’ and ‘very uncomfortable’, respectively.

**Figure 6.5** Thermal sensation (a), thermal comfort (b), rating of perceived exertion (c), and thirst sensation (d) during the 40-km time trial. * Main effect of heat stress (P<0.05). ** Main effect of heat stress (P<0.001). † Higher RPE during the time trial for HYPO35 than for EU25 (P=0.01). ‡ Main effect of hydration status (P<0.001). For clarity of the figure, no error bars are displayed.

**RPE, thirst sensation, and heart rate**

RPE increased during all the time trials and a main effect was found for heat stress (F=8.41, P=0.01) and hydration status (F=6.07, P=0.03; Figure 6.5c). However, at the finish values were similar for all the experimental conditions (19.2 ± 1.0; F=0.984, P=0.41).

Thirst sensation was lower for the euhydration trials (5.5 ± 1.2) than for the hypohydration trials (7.3 ± 0.9) during the entire time trial (F=50.0, P<0.001; Figure 6.5d). Also, an effect of heat stress on thirst sensation was observed (F=6.10, P=0.03). At the end of the time trial, thirst sensation was 7.9 ± 0.8 for the euhydration trials and 8.9 ± 0.3 for the
The combined effect of heat stress and hypohydration on pacing pattern during a 40-km cycling TT hypohydration trials \((F=24.0, \ P<0.001)\), corresponding to a sensation of ‘moderately thirsty’ and ‘very thirsty’, respectively.

Heart rate during the time trial was similar during the time trials for all the conditions \((F=0.307, \ P=0.82)\).

**DISCUSSION**

The main goal of this study was to investigate the effect of hypohydration on pacing pattern and exercise performance during a 40-km cycling time trial in moderate and hot conditions. Both heat stress and hypohydration negatively affected cycling performance but no interaction effect between heat stress and hypohydration on performance was observed, indicating a similar effect of hypohydration on performance in moderate and hot conditions. Therefore we have to reject our hypothesis that hypohydration only decreases performance during a 40-km time trial in the heat.

To our knowledge, this study is the first to examine the effect of mild hypohydration on self-paced exercise performance in moderate and hot conditions. Although previous studies did investigate the effect of starting mildly hypohydrated on self-paced exercise performance in the heat (Bardis et al. 2013; Stearns et al. 2009), it remained unclear if this effect would still be apparent in situations with considerably less heat stress. One study that did take the level of heat stress in account was conducted by Kay and Marino (2003) who looked at the effect of fluid replacement during a 60-min cycling time trial on performance in a moderate \((19.8 \pm 0.6^\circ C)\) and a hot \((33.2 \pm 0.2^\circ C)\) environment. Cyclists started the time trial euhydrated and fluid loss during the trial was fully replaced or not replaced at all. The resulting hypohydration of 2% BM did not translate into differences in cycled distance and final core temperature. A couple of relevant differences between our study and the study of Kay and Marino are: the euhydration at the start of the time trial, the relatively low levels of hypohydration at the finish, and the ability to drink during the time trial. Moreover, the absence of wind cooling in this study significantly reduced the
ecological validity. With our study design, we were able to investigate the separate effects of heat stress and hypohydration as well as their interactive effect on performance. By using a realistic wind speed, we believe that this study is ecologically valid and that the results can be extrapolated to outdoor cycling events.

The effect of ambient temperature on self-paced exercise performance that we found in this study, characterized by a gradual decline in power output during the time trial, is similar to other studies (Peiffer and Abbiss 2011; Tatterson et al. 2000; Tucker et al. 2004). The difference in power output at the start of the time trial is usually not observed (Peiffer and Abbiss 2011; Tatterson et al. 2000), but can be explained by the warm-up preceding the time trial that was conducted in the same conditions as the actual time trial. The ‘direct’ effect of heat stress on power output is usually contributed to the decreased blood flow to the exercising (leg) muscles caused by a redistribution of blood from core to periphery to facilitate heat dissipation. Moreover, the presence of an end spurt indicates that the cyclists exercised with reserve. Apparently, the heat stress during the remaining part of the time trial is anticipated and power output is down-regulated in order to minimize heat storage and ensure successful exercise completion (Marino et al. 2004; Tucker et al. 2004). Another observation indicating exercise with reserve is the relatively low amount of stored body heat during the time trial. Although the total amount of heat stored was two times greater in the heat (3.2 J·g⁻¹) than in moderate conditions (1.6 J·g⁻¹), this is still well below the 8 J·g⁻¹ that has been found as maximal tolerable for exercise lasting approximately one hour (Iampietro and Goldman 1965).

Not only physiological signals, but also thermal perceptions may play a role in the selection and modulation of power output during the time trial (Schlader et al. 2011a). We observed that thermal sensation and comfort were higher at the start and remained higher during the entire time trial in the hot trials than in the moderate trials. These results are in line with the results from Schlader et al. (2011a), suggesting that thermal perceptions are relevant for as well the selection of the exercise intensity at the start, as for the pacing pattern during the time trial. However, they are in contrast to results from Barwood et al. (2012), who used menthol to create a sensation of coolness without
altering skin temperature at the start of a 40-km cycling time trial in the heat and found no effect on pacing and performance. The differences in TS and TC between the hot and moderate trials found in our study possibly accounted for differences in RPE during the time trial. This observation is in contrast with previous studies showing that RPE is usually independent of environmental and physiological manipulations (Levels et al. 2012; Tucker et al. 2004).

The level of hypohydration that was induced in this study prior to the 40-km cycling time trial was ~1.2% BM. Although this level can only be considered as mild hypohydration, it has been associated with performance decrements (Armstrong et al. 1985; Bardis et al. 2013; Walsh et al. 1994). Also, this mild hypohydration has been linked to increased thermoregulatory and cardiovascular strain (Montain and Coyle 1992). When compared to the effect of heat stress, the effect of hypohydration on pacing pattern was less pronounced and consisted of a slightly lower output from the beginning of the time trial onwards. Although the effect of hypohydration on performance found in this study was (just) not statistically significant, the difference in finish time between the EU and the HYPO trials of 1 min 16 s, corresponding to 1.8%, was greater than the typical error of measurement of 27 s (0.7%) that has been found using the same exercise protocol (Lamberts et al. 2009). This resulted in a chance of 81% that the induced hypohydration at the start of the time trial negatively affects performance in both moderate and hot conditions. Moreover, the observed time difference is considerably larger than differences between cyclists in real-life competition. For example, 1:16 min was similar to the difference between finish position one and twelve during the 40.5 km individual time trial of the 2009 Tour de France (Stage 18, Annecy, France, 23/07/2009). Although cyclists started at similar intensities in the EU and HYPO trials, the power output profile already started to deviate in early stages of the time trial. Albeit hypohydration levels were still relatively moderate at that moment, we believe that body water content can be considered as a relevant physiological signal for the anticipatory regulation of exercise intensity during self-paced exercise, even when there is only mild hypohydration. Since there was a clear effect of hydration status on thirst sensation, which is activated when hypohydration levels > 1% BM (Fitzsimons 1976), this feedback signal may be part of the
regulation of exercise intensity. As suggested by Noakes in a discussion paper (Sawka and Noakes 2007), not the dehydration during exercise, but the sensation of thirst may be a regulator of exercise intensity. Moreover, the psychological effect of the withholding of fluid in the HYPO trials while the cyclists were thirsty may have influenced the pacing pattern and finish time, especially because the cyclists were unfamiliar with the hypohydration protocol (Fleming and James 2013). Interestingly, HR was similar for all the conditions despite the difference in hydration level between the EU and HYPO trials. Although we did not determine changes in plasma volume, the difference in hypohydration of 1.2 % BM likely results in a substantial reduction of plasma volume. This hypovolemia would have resulted in an increased HR to maintain adequate cardiac output and oxygen delivery to the exercising muscles. The slightly lower power output during the time trial in the hypohydration trials probably masked the effect of hypohydration on HR.

An interesting observation in this study is the absence of an interaction effect between heat stress and hypohydration on performance. That is, the difference in pacing pattern and overall performance between the EU and HYPO trials was similar in hot and moderate conditions. A weak relationship was found between the change in mean body temperature and change in mean PO during the time trails (Figure 6.4: r = -0.18), which suggests that cyclists who are showing a greater increase in body temperature also show a greater decrease in mean power during the time trial. This is suggestive of a feed-forward and feed-back regulation system that protects the human body from over-heating as suggested by Tucker et al. (2006), showing that an impaired exercise performance in the heat is associated with an anticipatory reduction in skeletal muscle recruitment. Interestingly, hydration status does not modulate this relationship. The absence of an interaction effect was not only found for performance, but also for heart rate and psychophysiological scores (RPE, TS, TC). Although previous studies showed that the effect of hypohydration on performance got more pronounced with increasing heat stress (Kenefick et al. 2010) and high skin temperatures (Sawka et al. 2012), we did not observe this. A possible contributing factor for the absence of this interaction effect could be the laminar air flow of 7 m·s⁻¹ that was used in our study. The high wind speed, and thereby the greater possibility for evaporative heat loss may explain the difference in results
regarding the interaction effect between hypohydration and heat stress on performance (Saunders et al. 2005). Under these conditions, hypohydration does not appear to limit heat dissipation and performance in the heat is not affected by the reduced body water content to a greater extent than performance in moderate conditions. The applied wind speed is therefore an important aspect to consider when extrapolating results of lab studies to outdoor cycling.

CONCLUSION

The detrimental effect of hypohydration on exercise performance during a 40-km cycling time trial is similar in hot and moderate conditions. Probably, the cooling provided by the high air velocity during the time trial prevented the lower body water content to limit heat dissipation and thereby affect performance in the heat to a greater extent than performance in moderate conditions.