

## Original Article

# The effects of exercise, heat, cooling and rehydration strategies on cognitive function in football players

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Accepted for publication 25 March 2010

We investigated the cognitive effects of exercising in the heat on the field players of two football teams in a series of three matches. Different rehydration and cooling strategies were used for one of the teams during the last two games. Cognitive functions were measured before, during and immediately after each football match, as well as core temperature, body mass, plasma osmolality and glucose levels, allowing an estimate of their differential impacts on cognition. The pattern of results suggests that mild–moderate dehydration during exercise in the heat (up to 2.5%) has no clear effect on cognitive function. Instead, plasma glucose and core tem-

perature changes appear to be the main determinants: higher glucose was related to faster and less accurate performance, whereas core temperature rises had the opposite effect. The 50% correlation between plasma glucose and core temperatures observed during exercise in the heat may help to stabilize cognitive performance via their opposing effects. The glucose-like effects of sports drinks appear to be mediated by increased plasma glucose levels, because drinks effects became non-significant when plasma glucose levels were added to the models. The cooling intervention had only a beneficial effect on complex visuo-motor speed.

Football is a complex sport, placing many demands on the player. The primary focus of scientific investigation has been upon the physical demands, and the cardiorespiratory and metabolic responses of match play have been quantified in a number of studies (Ekblom, 1986; Bangsbo et al., 1991). These show clearly that match play is physically demanding and that fatigue results in a decreased functional capacity in the later stages of the game. The factors contributing to fatigue may include depletion of muscle glycogen, a decline in the circulating blood glucose concentration, elevation of core temperature and changes in the motivation to exercise.

Several of these factors will have effects on the function of the central nervous system. These effects may be important because of their effects on fatigue and the ability – or willingness – to continue exercise at a high intensity. They may, however, also affect other aspects of brain function that are important for performance in a competitive situation that demands skill and judgement. Several aspects of cognitive function have been shown to be influenced by changes in hydration status, by increases in body temperature or by the development of hypoglycemia. Because of the reliance of the brain on glucose for energy metabolism, acute hypoglycemia results in a

prompt impairment of some aspects of cerebral function. Severe hypoglycemia (2.2 mmol/L) was found to cause impairments in all measured aspects of cognitive function [including reaction time (RT) (simple and choice), digit vigilance, trail making, word recall, digit sequence learning and verbal fluency] in otherwise healthy young diabetics (Draeos et al., 1995). Even modest hypoglycemia, at a blood glucose level of 2.6–3.0 mmol/L, however, can lead to some performance decrements, with complex tasks generally being more sensitive to hypoglycemia than simple tasks (Warren & Frier, 2005).

A variety of different methodologies have been used to assess the effects of dehydration and hyperthermia on cognitive function, with variations in both the methods used to induce dehydration and the selection of cognitive tests. Where exercise and heat exposure have been used to provoke sweating, an elevation of body temperature might confound the results. Although severe dehydration will undoubtedly have adverse effects, the varying sensitivity of the different tests used means that there is no agreement on the level of dehydration at which adverse effects might first be apparent. In a review of the literature Wilson and Morley (2003) noted that older individuals are more susceptible to deteriorations in

cognitive function as a consequence of dehydration, but that even mild dehydration impairs function in healthy young adults. Shirreffs et al. (2004) noted that short periods of fluid restriction may increase subjective ratings of fatigue and reduce feelings of alertness. Szinnai et al. (2005) reported that 24 h of water deprivation resulted in a loss of 2.6% of body mass but no loss of cognitive function, although it did increase subjective ratings of tiredness and reduce ratings of alertness and concentration. More recently, Lieberman (2007) has suggested that fluid deficits of as little as 1% of body mass may have adverse effects on cognitive function. Passive exposure to a hot environment (2 h at 50 °C) results in some loss of muscle force-generating capacity and peripheral neural function, accompanied by decrements in memory tests, but not in simple attention tasks (Racinais et al., 2008).

When an exercise stress is combined with a high environmental temperature and restricted fluid intake, it seems likely that the adverse effects on cognitive function and subjective feelings will be amplified. These effects may be mediated by reductions in the distribution of the blood volume (Crandall et al., 2008), leading to reductions in cerebral blood flow (Nybo & Nielsen, 2001b) and alterations in regional brain metabolism (Nunneley et al., 2002). These alterations may be sufficient to have detrimental effects on the performance of a number of physiological functions that are relevant to football match play (Edwards et al., 2007).

The aim of this investigation, therefore, was to examine the effects of football match play in a hot environment on a series of tests of cognitive function. A second aim was to examine whether these effects were influenced by interventions designed to maintain hydration, to provide carbohydrate substrate and to limit hyperthermia. Core temperature and plasma glucose data were collected at regular intervals before and during the last two matches, to construct a detailed picture of the internal metabolic and thermal changes during exercise, and their effect on cognitive function.

Of the specific cognitive functions that were assessed in previous studies, working memory (WM) has been found to be affected most consistently by dehydration, hypoglycemia and hyperthermia (Cian et al., 2001; Lieberman, 2007). Accordingly, two WM function tests were included in this study to gain detailed information on this cognitive domain during exercise in the heat. The Corsi block-tapping task measures WM capacity for spatial locations, which is also likely to be a functionally relevant aspect in soccer performance, where player locations on the field must be tracked and remembered accurately. A visual sensitivity (VS) test, assessing simple and complex visuo-motor RTs, was included based on its

proven sensitivity to effects of exercise and carbohydrate intake (Hogervorst et al., 2008), and because of the relevance of accurate and speedy visual processing to soccer performance. Finally, a simple speeded finger-tapping task was also included because of the importance of fine motor control in sport.

## Methods

### Participants and environment

This study was approved by the University of Çukurova, Faculty of Medicine Ethics Committee. All 20 field players (not including goalkeepers) of two male University teams from Ankara volunteered to participate and gave informed consent. The average (mean  $\pm$  SD) age, height and body mass of the 20 participants were 20.2  $\pm$  2 years, 176.4  $\pm$  5.3 cm and 67.9  $\pm$  6.4 kg, respectively.

All data reported here were collected during three football games at three time points per game: immediately before, during the half-time break and directly after each game. The matches took place in Adana, 480 km south-east of Ankara, in July and August 2008. Descriptive characteristics of the environmental conditions during the matches are shown in Table 1.

Three hours before each game, participants ate a small, standardized meal and drank only water. To ensure good hydration status, they were encouraged to drink water liberally the night before the game. Nearly 45 min before the start of the match, a baseline urine sample was collected before the players were weighed to the nearest 20 g on a digital scale. During the match period, all players had free access to plain water from their individually identified drinking bottles and could consume water *ad libitum*. Body mass measurements were repeated immediately after the game to establish a measure of dehydration via body mass loss. The change in body mass, corrected for fluid intake and urine output, was used to estimate sweat loss.

### Serum osmolality and glucose measurements

Pre-match blood samples were drawn from an antecubital vein approximately 5 h before kick-off. The sample was allowed to clot before centrifugation to obtain serum. Post-match blood samples were collected within 10 min after each game. Serum glucose level was measured with the enzymatic hexokinase method (Roche, Hitachi Modular Analytic Systems Integra 800), and the freezing point depression method was used to measure serum osmolality (Knauer, Germany).

### Cooling and fluid replacement strategy interventions

The cooling intervention involved a rectangular tent (3  $\times$  6 m with 2 m height) that was pitched beside the soccer field. A motorized pump with a flow rate of 1 L/min was used to

Table 1. Environmental conditions during the three matches (mean  $\pm$  SD)

	Match 1	Match 2	Match 3
Date	10 July 2008	24 July 2008	07 August 2008
Temperature (°C)	34.3 $\pm$ 0.6	34.4 $\pm$ 0.6	33.8 $\pm$ 0.5
Relative humidity (%)	64 $\pm$ 2	65 $\pm$ 3	62 $\pm$ 2
Heat index apparent temperature (°C)	45 $\pm$ 2	46 $\pm$ 2	43 $\pm$ 2

pressurize (80 bar) water at a temperature of 3 °C for plumbing through the polyamide tubing placed in the middle part of the tent roof. Twelve brass nozzles (internal diameter 0.2 mm) were used to spray water into the tent. The water temperature at the orifice of the nozzle was measured at about 12 °C. The air temperature under the tent was measured at approximately 25 °C (Testo 615). Four fans were placed at each corner of the tent to increase the air flow during cooling intervention. The tent roof was covered with 6 mm white polyethylene fabric. One side of the tent was open to the atmosphere and the other three sides were closed to keep cold water droplets inside the tent. Soccer players sat under the tent for 15 min before the match and for nearly 10 min during the half-time break. Four buckets filled with icy water were also placed at the different sides of the soccer field, and players had opportunity to wash their body with this water at any time they wanted. None of the players had the opportunity to drink icy water from the buckets.

### Hydration intervention

In the first match, players were given no particular advice on hydration and followed their normal practices before and during the match: this involved drinking only water at these times. In the second match, after the players arrived in Adana, sports drinks and water were given to all players and they were encouraged to increase their fluid intake each day. They were encouraged to consume fluid either water or the commercially available sports drink (Powerade, Coca Cola Corp.) according to their preferences. Fluid intake during the matches was measured by change in mass of the drinks bottles.

### Core temperature recordings

Core temperature ( $T_c$ ) was monitored using a VitalSense<sup>®</sup> telemetric physiological monitoring system (Mini Mitter Co. Inc., Bend, Oregon, USA), comprising a receiver and a thermistor-based, ingestible temperature sensor capsule. As reviewed extensively by Byrne and Lim (2007), the ingestible telemetric temperature sensor represents a valid index of core temperature measurements. Sensors were activated and swallowed approximately 5 h before each game. The sensors would have passed through the stomach well before the football games, and thus the temperature measurements should not be substantially affected by the ingestion of hot or cold liquids. Each swallowed sensor was checked and initial core temperature values were recorded just before each game.

### Cognitive tests

Participants were asked to complete a cognitive test battery, which lasted for approximately 15 min. A shorter, approximately 8-min version of the test battery was used at the half-time test points during the games. The short version did not include the Corsi block design test, and used reduced stimulus numbers for the Sternberg WM test.

All cognitive tests were delivered from a single software package on laptops with touch-sensitive screens that served as input medium for two of the tests (VS and Corsi blocks). The finger-tapping test (FTT) and Sternberg test required the use of a single key and two keys, respectively, for participant input. Timing on all tests had millisecond resolution. Each participant had a dedicated test computer, allowing for simultaneous testing of all participants. Instructions were presented on the computer screen before the start of each test, as well as being provided verbally by the investigator. Participants undertook two complete familiarization trials before the first experimental trial.

### Visual Sensitivity

The Visual Sensitivity test was used to assess visuo-motor RTs and comprised two levels, baseline and complex. On both levels, participants were instructed to detect target triangles on the screen and respond as quickly as possible by touching the target. A very similar version of this test, using the keyboard for responses, has been found previously to be sensitive to the effects of prolonged exercise and carbohydrate intake (Hogervorst et al., 2008). The touchscreen version of the test used in this study disregarded responses placed outside the target stimulus, and targets were displayed until a valid response was made; hence negative or false positive responses were impossible. Hence, there were no errors to analyze, and the analysis will be concerned only with RT changes.

Each target triangle disappeared immediately after a correct participant touch. The next target appeared in a new, random location after a minimum time interval of 500 ms with an added random delay, designed to make the timing of the next target unpredictable. The screen was divided into a grid of four rows and five columns (20 intersection points), with an added point in the center (21 points in total). During each test run, a target was placed exactly once on each of these 21 points; hence, there were 21 stimuli per test level. To make target locations unpredictable, the order of target locations was randomized for each test run. The net effect of these parameters is a standardized test in which the same target locations have been covered in each test run, without being predictable for the participants. There were also three initial, randomly placed practice targets in each level for which data were discarded.

In the baseline level, targets were formed from bright green triangles on a black background. In the complex level, moving random dots covering the entire screen served as background distractors. New target triangles were initially drawn with just a few visible dots of each line, and the density of these points increased linearly with time until a response was registered, i.e. target triangles became denser over time until the participant response. The screen was re-drawn every 250 ms with a new set of distractor dots and target triangle points, inducing the distracting visual effect of a flickering background. The baseline level is designed to assess simple visuo-motor speed, whereas the complex level introduces the additional component of complex visual processing. As a result, the difference in baseline and complex level speed reflects the additional time required for complex visual processing, and interactions with test level indicate effects that are specific to this visual processing component for effects that occur only on the complex level.

### FTT

Fine motor speed was measured using a computerized FTT. Participants were asked to tap the space bar as quickly as possible for a 10 s period, a time interval that has been commonly used in previous studies (Cousins et al., 1998). This was repeated four times. Participants were instructed to use the index finger on the dominant hand first, followed by the index finger of the non-dominant hand. This procedure was repeated a second time to increase the reliability of the data, leading to a total of four tapping trials per test run. A counter in the center of the screen displayed the total number of taps in each trial, making participants aware of their current performance and providing additional motivation for fast tapping. Timed data recording started with the first tap and ended 10 s later.

Rather than analyzing the total number of key presses within the measurement period of 10 s, which would provide only a single continuous outcome variable per test run, the

much more sensitive data of time per key press was analyzed here. This has the advantage of providing a highly sensitive measure of timing variance.

#### *Visual/auditory WM*

The Sternberg test (Sternberg, 1969) was used to assess serial WM scanning function by testing the interaction of the variable(s) of interest with different WM loads on response speed and accuracy. We used three WM loads, including one, three and five items that had to be kept in WM for correct performance. The baseline one-item task, for which the target was always the number “3,” is a measure of basic information-processing speed. For WM loads three and five, the targets were a list of three and five letters, respectively. The slope of 0 (response time to one digit) to three- and five-letter loads gives specific information about the speed of memory scanning (Sternberg, 1966).

For all WM loads, the target item(s) were displayed together with on-screen instructions that asked participants to press the left arrow key if they thought any of the following choice items was a target item, and to press the right arrow key otherwise. In the full-length version, 30 choices (single letters) were presented for each WM load level, containing 15 target items and 15 non-target items. To ensure that response key bias alone could not lead to above-average accuracy, correct responses were counterbalanced between both responses keys. In the shortened version used for half-time testing, only load levels 3 and 5 were assessed, and only 20 counterbalanced choices (including 10 targets) were presented for each level.

In all test versions and WM loads, there were five practice choice items after each target list was displayed, during which feedback on correct/incorrect responses was provided on-screen, to ensure that participants had ample opportunity to rehearse the target items. RTs and errors were recorded for each response after the practice, and no feedback was given during the recorded trials. The inter-stimulus interval between each response and displaying the next choice item was 1 s with a blank screen. Choices remained on-screen until one of the two response keys was pressed.

#### *Visuo-spatial WM*

Spatial WM is likely to be important in team sports like football, where many player positions must be remembered. Visuo-spatial WM capacity was measured here with a computerized version of the widely used Corsi block-tapping test (Berch et al., 1998). This test relies on nine blocks, arranged in a 3 × 3 grid, which are used to display a sequence of  $n$  visuo-spatial locations by changing color. After all  $n$  locations were shown, participants are asked to repeat this sequence on the touchscreen in the same order. Only forward recall (same order) was tested. Recall of the entire sequence was scored as correct only when all locations were given in the correct order.

In a modification of the original procedure to obtain continuous outcome variable (Owen et al., 1999), spatial span was increased by 1 after each correct recall and decreased by 1 after each error. Starting span in each test session was 3, and the initial four sequences were discarded to allow participants to reach a span of 7, larger than the 6.4 items mean spatial memory span. Spans of incorrectly repeated sequences were discarded. Owing to time constraints, data were not recorded on the Corsi block-tapping test at half time.

#### *Data analysis*

Mixed effects models were used to analyze the data, corrected for the repeated measures stratification with a random effect

for each participant. Relevant independent variable effects, e.g. body mass loss and glucose levels, were also analyzed with post-exercise performance corrected for the pre-exercise performance measured on the same day, i.e. analyzing change from baseline for each individual. RT analyses were performed with the nlme package for R (<http://www.r-project.org>), version 2.6. The lme4 package with a binomial outcome data distribution was used to analyze the binomial (correct/wrong) accuracy scores. Plots were generated with the R package Design, using generalized linear models that are not corrected for the repeated measures effects. Non-linear fits were generated via three-point cubic spline interpolation.

RTs on all tests were filtered to exclude those for incorrect responses, then log-transformed to normalize the distribution where necessary. To avoid undue influence of unusually slow or fast responses (outliers) on the analysis, RTs were then further filtered to remove outliers, defined after visual inspection of the distribution plots as responses that were outside the range of a typical normal distribution. All analyses were also conducted with outliers included, which in no case changed any of the significant effects other than very minor alterations in effect size. The transformed RTs were subsequently treated as normally distributed in the mixed effect models. Effect sizes were drawn from the same filtered set of RTs but without applying the logarithmic transformation to retain the milli-second scales.

To investigate learning effects that might obscure the impacts of the other variables, mixed effect models were constructed for all four tests with RT and accuracy as dependent and session number as independent variables, including a linear and a quadratic component of session number to adjust for non-linearities. In most cases, no significant learning effects were found after the first two familiarization trials. Because the following analyses use only post-familiarization data acquired during the football games, no further adjustment for test session number was made in the subsequent RT and accuracy analyses. Adjustments for learning effects are described in the results section only for tests where significant learning effect extended beyond the familiarization trials.

The analysis then follows the same pattern for each of the cognitive tests. First, the effects of the factorial variables exercise (coded as binary with 0 = pre-game and 1 = half-time and post-game test sessions), fluid replacement strategy (water vs sports drink) and cooling strategy were investigated in separate models, which are listed as “factorial” models in the effect summary tables. These summary tables are intended to provide a convenient way to gain a quick overview of the sometimes rather complex effects reported here, and the models and effects listed in them are described in more detail in the text.

In the next step, the effects of the amount of water and sports drink consumed during the game (as continuous variables, using the volume in milliliters per player) were investigated in a separate model that only included post-game test data, because these data were only collected after the games. Plasma glucose values were then added to this model to determine if the sports drink effect remains independently significant. If sports drink volume in milliliters becomes non-significant after adding plasma glucose values, the effects of the sports drink are explained by its effect on plasma glucose levels, indicating that the sugar content of the sports drink is likely to be responsible for the effect on cognitive function. These models are listed with the label “isodrink” in the effect summary tables.

Finally, a complex model that included body mass loss, plasma osmolality, plasma glucose levels, core temperature, exercise, fluid replacement strategy and cooling strategy was constructed for each of the tests to determine the relative

contribution of each of these factors to speed and accuracy. The significant effects found in these models are listed under the label “complex” in the effect summary tables.

## Results

### Visual Sensitivity

In the factorial model, which included cooling strategy and fluid replacement strategy and their interactions with exercise as independent variables, no significant effects on baseline level RTs were found. There was also no significant effect on VS RTs at both test levels of the total amount of sports drink or water consumed per player when both variables were entered as main effect into the “isodrink” model (Table 2).

In contrast, there was a significant interaction of cooling strategy and exercise level for the complex level RTs [ $t(1, 3575) = 2.0, P < 0.05$ ], with cooling leading to faster RTs both at half time and full time, but not in pre-game testing (Fig. 1).

These results indicate a significant speed improvement on the VS complex level with a cool environment after exercise, with an effect size of 84 ms for complex RTs when half-time (exercise = 1 in Fig. 1) and full-time (exercise = 2) data are grouped together. The similar magnitude of the cooling effect over the half-time and full-time measurement points indicates that the effect is consistent and stable.

All other co-variables (body mass loss, plasma glucose and osmolality and core temperature) were then also entered into mixed effects models. Neither body mass loss nor plasma osmolality had any significant effects on VS RTs. The significant effect of sports drink volume disappeared when blood glucose levels were entered into the model, indicating that the sugar contents of the sports drink may be responsible for the effects on VS speed. Significant interactions with test level were found for both plasma glucose concentration [ $t(1, 2744) = -3.6, P < 0.001$ ] and core temperature [ $t(1, 2744) = 2.6, P = 0.01$ ], but there was no significant three-way interaction.

Figure 2 illustrates the interactive effects of plasma glucose concentration and core temperature on visuo-motor response speed, showing that the interaction with test level arises because glucose levels and

temperature affect performance only on the complex test level. This implies that both effects are specific to the more demanding visual processing component on the complex level. Rising core temperatures slow down complex RTs (effect size +40 ms/°C), whereas increasing blood glucose levels counteract this effect and lead to significantly faster RTs (effect size -54 ms/mmol/L). The addition of non-linear effects, modeled via cubic spline interpolation, does not significantly improve model fit, suggesting effectively linear glucose and temperature effects over the observed range of values.

The temperature and glucose effects are significant if both variables are entered into the model. However, if either variable is removed from the models, the other becomes non-significant, i.e. plasma glucose and core temperature are only significant predictors of VS RTs when both are entered into the same mixed effects model. The most likely explanation for this observation is a significant positive correlation between temperature and glucose levels (Pearson's  $r = 50\%, P < 0.001$ ), which is shown in Fig. 3. Analyzing either variable in isolation does not reveal the true magnitude of the effect on complex VS speed.

### Fine motor speed

Visual inspection of tapping speed over the test sessions suggests practice effects that are strong

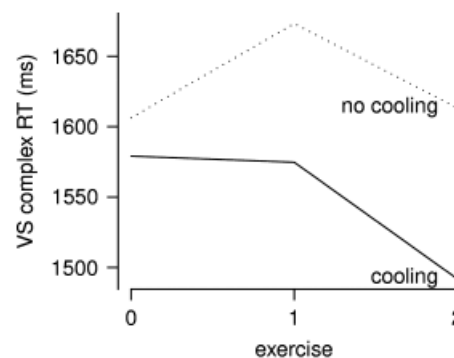


Fig. 1. The effect of cooling strategy on VS complex level before (exercise = 0), during (exercise = 1) and after (exercise = 2) the games.

Table 2. Overview of significant effects on the visual sensitivity test

Model	Outcome	Independent variable	$\beta$	$t$	$P$	Figure
Factorial	VS complex RT	Cooling:exercise	-84 ms	2.0	<0.05	1
Complex	VS complex RT	Core temperature	+40 ms/°C	2.1	0.03	2
		Plasma glucose	-54 ms/mmol/L	2.3	0.02	2

Model types: “factorial” includes only the factorial independent variables; “complex” contains all variables, including the continuous physiological parameters. “:” denotes interactions. Effect sizes ( $\beta$ ),  $t$  and  $P$  values are taken from reduced linear mixed effect models after removing non-significant variables and interactions. Column “figure” lists the relevant effect plots.

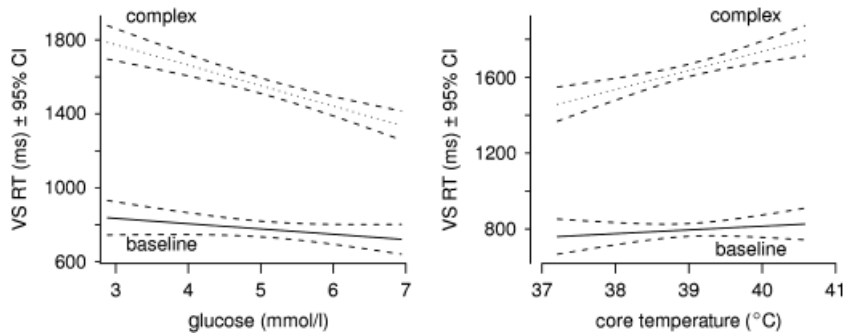


Fig. 2. The interactions of plasma glucose level and core temperature with VS test level. In both cases, there is a stronger effect on the complex level, but glucose also improves baseline speed.

over the first few sessions, then gradually decrease until there is no further practice effect after the seventh test session (Fig. 4). This is confirmed by mixed effect models with FTT RT as a dependent variable and test session, test session squared (to account for decreasing practice effects over time), hand, within-session repeat, exercise (pre- vs post-game) and game vs non-game day testing as independent variables. These independent variables are significant at  $P < 0.0001$  and will thus be included in all subsequent analyses as correction factors.

However, if only test session numbers greater than seven (i.e. after the first game) are included, neither the linear nor the quadratic components of test session remain significant, suggesting that there are no further significant practice effects during the final two games (Table 3).

Factorial mixed effect models with FTT tapping speed as dependent and cooling strategy, drink strategy and their interactions with exercise (pre- vs post-game), as well as corrections for the significant co-variables (test session, game day, hand and repeat) as independent variables were used to analyze the effects of the intervention strategies. There was a significant main effect of encouraging sports drink rather than water consumption [ $\beta = 1$  ms,  $t(1, 64\ 620) = 2.2$ ,  $P < 0.03$ ], and a significant interaction with exercise [ $\beta = 3$  ms,  $t(1, 64\ 620) = 5.0$ ,  $P < 0.0001$ ]. The use of sports drinks thus improved tapping speed by 1 ms overall, and by an additional 3 ms after exercise. This is illustrated in Fig. 5(a). On the other hand, cooling strategy did not show a significant main effect, but instead a significant interaction with exercise [ $\beta = 4.7$ ,  $t(1, 36\ 045) = 6.8$ ,  $P < 0.0001$ ]. This interaction was due to the cool environment effect becoming apparent only after exercise, where it led to 5 ms slower tapping speed [Fig. 5(b)].

The possible benefit of sports drinks over plain water was examined in more detail by entering both the total amount of sports drink and water consumed per player into the mixed effects model. These data

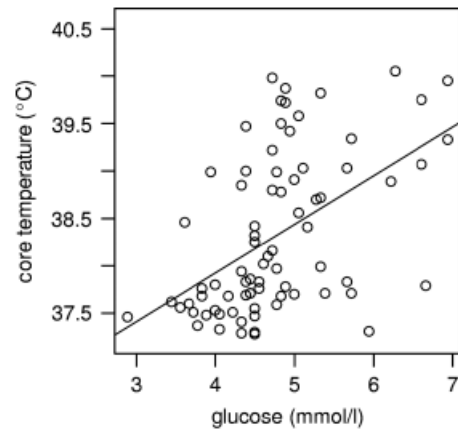


Fig. 3. Core temperature and plasma glucose correlate significantly with 50%.

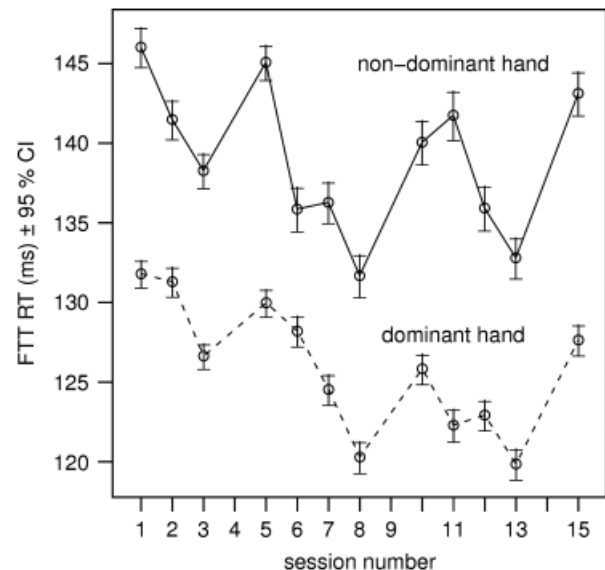


Fig. 4. Practice effects over repeated test sessions on the FTT, split by hand. Some speed increase can be seen up to test session 7 (last practice trial before the final two games) on the dominant hand. Missing data points in sessions four, nine and 14 are due to lack of testing at half time.

Table 3. Overview of significant effects on the finger-tapping test

Model	Outcome	Independent variable	$\beta$	$t$	$P$	Figure
Factorial	FTT speed	Sports drink	- 1 ms	2.2	0.03	5
		Exercise	+ 8 ms	15.8	< 0.0001	5
		Sports drink:exercise	- 3 ms	5.0	< 0.0001	5
		Cooling:exercise	+ 5 ms	8.5	< 0.0001	5
Isodrink	FTT speed	Isodrink volume	- 7 ms/L	6.6	< 0.0001	
		Water volume	- 3 ms/L	5.7	< 0.0001	
Complex	FTT speed	Core temperature	+ 13 ms/°C	13.2	< 0.0001	
		Plasma glucose	- 4 ms/mmol/L	4.7	< 0.0001	
		Body mass loss	- 5 ms/% loss	6.4	< 0.0001	

Model type "isodrink" includes only the effect of volume of sports drink and water consumed during the final two games.

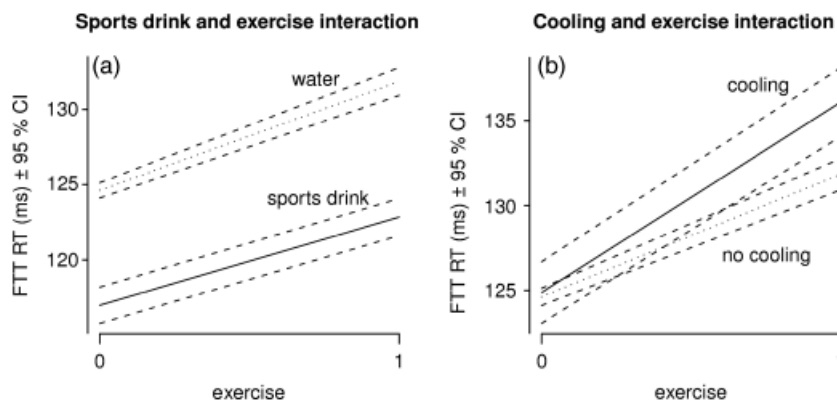


Fig. 5. The effects on finger-tapping speed of using sports drinks rather than water (a), and of providing a cool environment (b), split by exercise (0 = pre-game and 1 = post-game).

were available for the last two games only, where practice effects on the FTT were absent. Accordingly, no correction for test session and game day was included, and only post-exercise data were considered because the fluids were consumed during the game. Significant main effects of water consumption [ $\beta = 3.3$  ms/L,  $t(1, 15\,458) = 5.7$ ,  $P < 0.0001$ ] and sports drink [ $\beta = 6.8$  ms/L,  $t(1, 15\,458) = 6.6$ ,  $P < 0.0001$ ] were observed, indicating about twice the motor speed improvement with sports drinks as compared with water for the same amount of fluid consumption. Again, the beneficial effect of the sports drink appears to be derived chiefly from its sugar content, because it disappears entirely when blood glucose levels are added to the model.

The detailed per-player measurements of body mass loss, plasma osmolality, core temperature and blood glucose levels were also entered into mixed effects models. The cooling environment factor was retained in this model as well, to assess whether the effects of cooling are due to changes in core temperature or other mechanisms, such as an increased perceived comfort. Of the factors entered, only plasma osmolality did not have a significant effect ( $P = 0.2$ ). Higher core temperatures were related to slower RTs [ $\beta = 12.6$  ms/°C,  $t(1, 9038) = 13.2$ ,

$P < 0.0001$ ], while higher plasma glucose levels were associated with faster reactions [ $\beta = -3.7$  ms/mmol/L glucose,  $t(1, 9038) = -4.7$ ,  $P < 0.0001$ ]. Surprisingly, faster RTs were also found with higher body mass loss values [ $\beta = -5.2$  ms/% weight loss,  $t(1, 9038) = -6.38$ ,  $P < 0.0001$ ]. Using a cubic spline interpolation, RTs were found to decrease until a body mass loss of approximately 2.5%, and remain relatively stable for greater losses.

#### Sternberg WM test

The three levels of WM load (one, three and five items) constitute the parametric variation in the WM load variable in this test. The subsequent analyses will thus focus on interactions of all variables with WM load, because such interactions indicate effects that are specific to WM processing. Conversely, main effects affect performance on all WM load levels equally, and are thus not specific to WM function (Table 4).

A mixed effects model to explore the effects of the fluid replacement and cooling strategies, which also included WM load, exercise and their interactions, revealed a significant WM load by exercise interaction [ $t(2, 10\,544) = -4.0$ ,  $P < 0.0001$ ] and a signifi-

cant main effect of fluid replacement strategy [ $\beta = 14$  ms faster with sports drink,  $t(1, 10\,544) = 3.9$ ,  $P < 0.0002$ ]. The main effect of drinks strategy paired with the lack of a significant interaction with exercise indicates that although the use of sports drinks led to a 14-ms improvement in Sternberg RTs, this effect was not specific to the WM component and did not change with exercise. Cooling strategy had no significant main effect or interactions with WM load and exercise. The WM load by exercise interaction arises because response speed slows down more pronouncedly with exercise for the lower WM loads, implying that the exercise effect reduces baseline speed and not specific WM scanning speed. This interaction is included as a correction factor in all subsequent models. Further investigation of the post-game effects of sports drink and water consumption with the more detailed per-player data of fluid consumption in milliliters over the course of the game revealed a significant beneficial main effect of sports drinks [ $\beta = -33$  ms/L,  $t(1, 4158) = -3.1$ ,  $P < 0.002$ ] and a significant slowing main effect of higher water consumption [ $\beta = 27$  ms/L,  $t(1, 4158) = 4.6$ ,  $P < 0.002$ ]. Neither of these fluid intake variables interacted significantly with WM load, again indicating that these effects apply equally to all WM loads. As with the other cognitive tests, the inclusion of plasma glucose levels in the models causes the sports drink volume effect to disappear entirely, implying that the glucose content of the sports drink may be responsible for the reduction in RTs.

The effect of the detailed continuous variables on Sternberg test speed was analyzed with models that included body mass loss, plasma osmolality, plasma glucose, core temperature and exercise as independent variables. There was no significant main effect or interaction of plasma osmolality ( $P > 0.5$ ), and no significant main effect for body mass loss ( $P = 0.76$ ). However, there was a significant interaction of body mass loss with WM load [ $\beta = -9.5$  ms per 1% weight

loss per extra WM load level,  $t(1, 8409) = -3.6$ ,  $P < 0.001$ ]. This interaction arose because more body mass loss was related to slower performance on low WM loads and faster performance at high WM loads (Fig. 6), implying that body mass loss impairs performance only on aspects of the Sternberg test that are not related to WM scanning speed.

The three-way interaction between plasma glucose, core temperature and WM load was also significant in this model [ $t(2, 4809) = 2.6$ ,  $P = 0.01$ ], as well as all two-way interactions and main effects ( $P < 0.02$  for all). Because the three-way interaction best explains the data, it is plotted in Fig. 7 and explained in detail here. Higher temperatures led to slower reactions across all WM load levels when glucose was low [low glucose line in Fig. 7(a)–(c)]. High glucose levels counteracted this effect for lower WM loads, where there was no increase in RTs with rising core temperatures when glucose was high [high glucose line in Fig. 7(a)–(b)]. However, when demands on WM were maximal, performance improved even at low temperatures when more glucose was available [high glucose, WM load 3, Fig. 7(c)]. Hence, glucose appears to be able to counteract the slowing effects of higher core temperatures on the test components that are not related to the speed of WM scanning. The specific WM component benefits from higher glucose levels even at low temperatures, but higher glucose levels cannot completely counteract the slowing effects of rising core temperature on WM.

The Sternberg test also included the possibility of wrong answers, and hence response accuracy was also analyzed. When data are averaged per person, WM load and test session, no speed accuracy trade-off was found for WM load 5 (Pearson's  $r = 0.09$ ,  $P = 0.15$ ) and WM load 3 ( $r = 0.008$ ,  $P = 0.89$ ). There was a significant speed accuracy trade-off at the lowest WM load ( $r = 0.13$ , more errors with faster RTs,  $P < 0.05$ ), suggesting that participants may have traded accuracy for speed in the Sternberg

Table 4. Overview of significant effects on the Sternberg test (STB)

Model	Outcome	Independent variable	$\beta$	$t/z$	$P$	Figure
Factorial	STB speed	WM load	+38 ms/item	25.1	< 0.0001	
		Exercise	+52 ms	4.9	< 0.0001	
		Sports drink	-14 ms	3.9	< 0.0001	
		WM load:exercise	-16 ms	4.0	< 0.0001	
Isodrink	STB speed	Isodrink volume	-33 ms/L	3.1	0.002	
		Water volume	+27 ms/L	4.6	< 0.0001	
Complex	STB speed	Body mass loss:WM load	-9 ms	3.6	0.0003	6
		Temp:bmi:WM load		2.8	0.01	7
Complex	STB accuracy	WM load	-0.7%/item	3.3	0.001	
		Sports drink volume	-2%/L	2.1	0.04	

Main effects and two-way interactions contained in the three-way interaction among core temperature, body mass loss and WM load ("temp:bmi:WM load") are not reported for the complex model as they are contained in the three-way interaction. Refer to text and Fig. 7 for more detail on this three-way interaction;  $t$  values are listed for RT analysis (normal distribution),  $z$  values for accuracy (binomial distribution).



test only when demands on WM function were minimal.

Of all the variables, only WM load and volume of sports drink consumed had significant effects on accuracy. Error rates increased with higher WM loads ( $\beta = 1.4\%$  errors per additional load level,  $z = -3.3$ ,  $P = 0.001$ ), and consuming more sports drink during the games also increased error rates ( $\beta = 2\%$  more errors per liter of sports drink,  $z = -2.1$ ,  $P < 0.04$ ). Although the effect of sports drink on accuracy is not very strong and disappears when water consumption is also included in the model, it nevertheless indicates that the speed improvement on the Sternberg test found for sports drinks (33 ms/L) may come at the cost of increased error rates. Because the interaction between WM load and sports drink volume was not significant

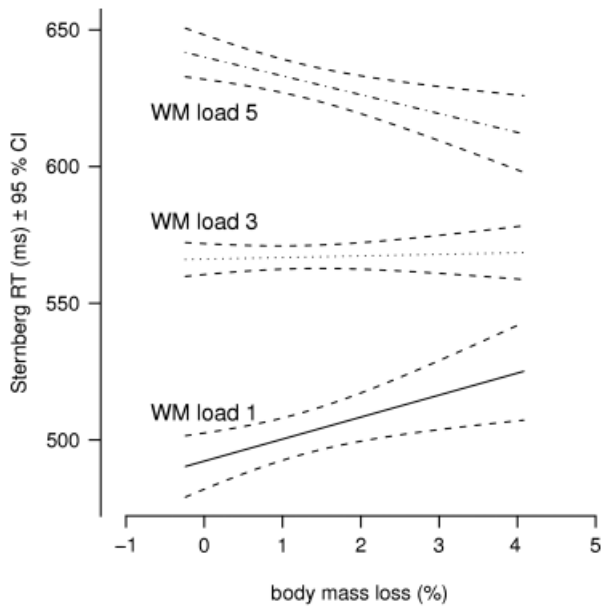


Fig. 6. Sternberg test RT changes with increasing body mass loss, split by WM load. Body mass loss seems to affect test components not related to WM function.

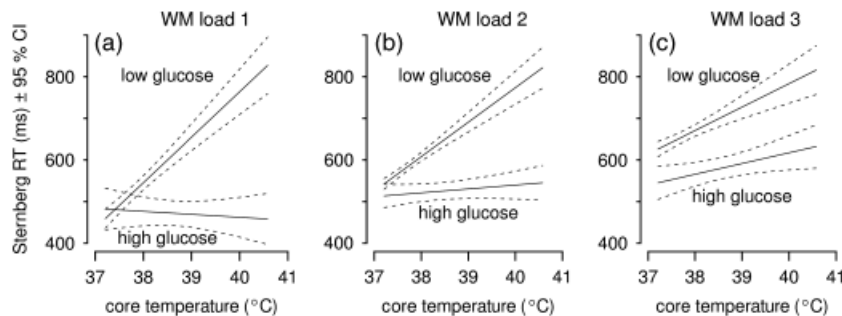


Fig. 7. The three-way interaction between WM load, temperature and plasma glucose levels on Sternberg test speed. High temperatures impair speed for all WM loads when glucose levels are low (model adjusted to 3.9 mmol/L). With high glucose levels (6.7 mmol/L), high temperatures slow down reactions only for the highest WM load [(high glucose line in (c))]. The glucose main effect with WM load 3 implies that glucose can improve WM speed also independent of temperature.

( $P = 0.85$ ), this effect applied equally across all WM loads and is thus not specific to WM function.

### Corsi block-tapping test

When a constant correction for exercise effects (pre- vs post-game) is included in the complex models, no significant effects for the fluid replacement and cooling strategies, body mass loss and plasma osmolality were found (Table 5).

Significant main effects were found for exercise [ $\beta = -0.8$  locations post-game,  $t(1, 239) = -2.1$ ,  $P < 0.04$ ], plasma glucose levels [ $\beta = -0.4$  items/mmol/L glucose,  $t(1, 239) = -2.6$ ,  $P < 0.01$ ] and temperature [ $\beta = 0.52$  items/°C,  $t(1, 239) = 2.7$ ,  $P < 0.01$ ], with all variables in the same model. The three-way interactions and the interaction between glucose and exercise, as well as the glucose by temperature interaction, were not significant ( $P > 0.5$  in all cases).

However, the interaction between exercise and core temperature was significant [ $t(1, 239) = -2.1$ ,  $P < 0.04$ ], driven by a positive relationship between temperature and spatial span before exercise [ $\beta = 2.3$  items/°C,  $t(1, 116) = 3.0$ ,  $P = 0.003$ ], but not after ( $P > 0.2$ ) exercise (Fig. 8). The span-increasing effect of temperature may be spurious because it disappears when glucose levels are left out of the model, but the negative correlation between glucose and spatial span remains a significant factor without including temperature data, when even the exercise effect disappears. Glucose effects are also significant on their own in both the pre- and post-game data. This suggests that higher plasma glucose levels are detrimental to performance on a spatial WM test, while higher core temperatures at rest (observed range 37.28–38.02 °C) may benefit spatial WM span.

### Discussion

Effects of exercise in the heat were found across all four cognitive tests used in this study, and the results

Table 5. Overview of significant effects on the Corsi visuo-spatial WM test

Model	Outcome	Independent variable	$\beta$	$t$	$P$	Figure
Complex	WM span	Exercise	-0.8 lc	2.1	0.04	8
		Plasma glucose	-0.4 lc/mmol/L	2.6	0.01	
		Core temperature	0.5 lc/ $^{\circ}$ C	2.7	0.01	
		Core temperature:exercise	-1.4 lc	2.1	0.04	

Effect sizes are given in the number of correctly recalled locations (lc).

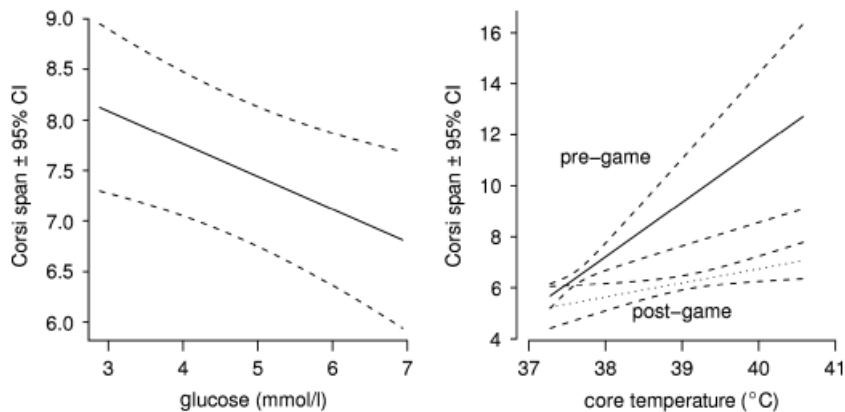


Fig. 8. Glucose and core temperature effects on spatial span in the Corsi block-tapping test. The glucose effect is independent of exercise, unlike the temperature effect.

reveal a complex interplay between a number of physiological changes during exercise, such as changing plasma glucose levels, rising core temperatures and dehydration that appear to be driving these effects. The following discussion will initially focus on cognitive function changes in response to these physiological variables, because this allows a detailed view of how cognition might be affected by the physiology of exercise in the heat. There is also a relatively high degree of confidence in the effects of plasma glucose, core temperature and dehydration in this set of results, because these variables were measured repeatedly in each player before and during the last two football matches. This resulted in a specific data point acquired at essentially the same time as the cognitive test session, with per-player detail, rather than just factorial group-level information. Unlike ANOVA-based methods, the mixed effects models used in the present analyses can make full use of these high-resolution data.

#### Dehydration effects

The cognitive effects of dehydration, assessed via body mass loss and plasma osmolality, were inconsistent across the four cognitive tests, in keeping with the inconsistent and often contradictory results reported in the literature to date (Lieberman, 2007). Plasma osmolality changes did not have significant effects on any of the cognitive tests. Body mass loss

had a significant effect on only two of the tests, FTT and the Sternberg WM test. Finger-tapping speed appears to actually increase, at least up to a body mass loss of approximately 2.5% when a non-linear fit is used. On the Sternberg test, simple RTs with the lowest WM load increased with more body mass loss, and the opposite effect was found for high WM loads (Fig. 6). Hence, WM scanning speed does not appear to suffer with dehydration, only the basic response times that are not specific to WM function. The lack of a clear effect of dehydration on cognitive function is consistent with other studies that investigated aspects such as concentration (Edwards et al., 2007), choice RTs (Serwah & Marino, 2006), perceptual discrimination and WM (Cian et al., 2001).

#### Effects of core temperature elevation

In contrast, higher core temperatures after exercise had significant negative effects on speed on all the cognitive tests. Specifically, there were significant decreases in speed on the FTT, on the VS complex (but not baseline) level (Fig. 2), as well as the Sternberg test, where the temperature effect was most pronounced at low glucose levels with high WM loads (Fig. 7). Because of the slowing effect on all levels of the Sternberg test, and slowing on the FTT as well as the VS complex level, it appears that significantly elevated core temperatures during exercise exert a global, non-specific slowing effect on

psycho-motor response speed, which is counteracted by the observed concurrent rise in plasma glucose levels. Elevated sympathetic nervous system activity associated with core temperature increases (Crandall, 2008) and an increased central fatigue (Nybo & Nielsen, 2001a) may be some of the reasons for this general slowing effect.

In addition to the general slowing effect, rising core temperatures also seem to negatively affect specific cognitive domains such as visual processing (temperature effect only on the complex level, Fig. 2) and WM (high plasma glucose levels cannot completely counteract the slowing temperature effect at the highest WM load, but can do so at lower WM loads, Fig. 7). Hence, both general and domain-specific temperature effects combine to slow response speeds across a range of cognitive domains, indicating that core temperature also exerts domain-specific effects on the brain in addition to the more general explanations presented above. Mechanisms behind these effects may include alterations in dopaminergic and serotonergic function, changes in blood-brain barrier permeability (Maughan et al., 2007) and regional blood flow changes in the brain (Nunneley et al., 2002).

The only other temperature effect detected in this study was a positive correlation with WM spatial span as measured by the Corsi block-tapping test. This effect is found only at rest, where the highest observed temperature was 38.2 °C. This effect was significant only when blood glucose levels were also entered into the model, suggesting that temperature effects can explain some of the performance variance once glucose levels have already been accounted for. Although not robust without glucose levels, it seems plausible that higher core temperatures at rest may be beneficial to WM function, because presumed optimum temperatures of around 38 °C are at the top end of the observed resting temperature range (37.28–38.02 °C) in this study. After exercise, when the observed temperature range was 37.21–40.05 °C, no beneficial effect of higher temperatures on spatial WM span was observed. Thus, while higher temperatures at rest, up to around 38 °C may have a weak positive effect on WM function, the considerably higher core temperatures reached after exercise in the heat do not relate to improved spatial WM capacity.

#### Impact of plasma glucose levels and interactions with core temperature

The effects of changes in plasma glucose concentration in this study were almost exactly the opposite of the temperature effects: higher glucose levels were related to faster performance on all cognitive tests where speed was measured, counteracting the effects

of rising core temperature, and higher glucose levels were also associated with a lower spatial WM span on the Corsi test. On the VS test, the speed-increasing effect of glucose was observed only on the complex level (Fig. 2). The glucose effect on the VS test thus appears, like the temperature effect, to be specific to the complex visual processing component. Similarly, on the FTT, higher plasma glucose levels were associated with faster performance, counteracting the opposite temperature effect.

A more complex picture emerged for the Sternberg test, where at low WM loads, glucose had a beneficial effect on speed only when core temperatures were high (Fig. 7(a) and (b)). At the highest WM load, high glucose levels could increase reaction speed even at low temperatures, but performance did slow down with rising temperatures even when glucose was high [Fig. 7(c)]. In contrast, high plasma glucose levels were able to preserve maximal response speed even at very high temperatures at the lower WM loads [high glucose lines in Fig. 7(a) and (b)]. This indicates that a speed-increasing effect of plasma glucose is observed on Sternberg test components not related to WM function, where it is mediated by counteracting the slowing effect of rising core temperatures. The WM-specific effect of plasma glucose appears to be independent of core temperature in that it also occurs at low temperatures, but glucose cannot entirely counteract the temperature effect on this WM-specific component.

In sum, high plasma glucose levels appear to be related to faster fine motor speed, faster complex visual discrimination and faster WM scanning, as well as more general faster psycho-motor speed. Considering the opposite cognitive effects of rising core temperatures listed above, cognitive functions during exercise in the heat may be stabilized at least in part by the significant 50% positive correlation between plasma glucose levels and core temperatures (Fig. 3) as observed in this study. It is possible that metabolic demands in the brain, which is partially dependent on glucose metabolism, increase in line with core temperatures, and that the concurrent increase in plasma glucose levels serves to meet these demands, thus counteracting the slowing temperature effect. While the negative cognitive effects of acute hypoglycemia are well documented (Warren & Frier, 2005), few studies have investigated plasma glucose effects in the normal physiological range on cognitive function.

However, while higher plasma glucose levels may allow the preservation of response speed, especially at high core temperatures, they had negative effects on accuracy in the two tests that measured accuracy in this study, the Sternberg and Corsi tests for WM function. On the Corsi test, there was a significant negative relationship between plasma glucose levels

and spatial WM span, indicating a decreased spatial WM capacity at high glucose levels. Although plasma glucose had no direct significant relationship with accuracy on the Sternberg test, there was a negative correlation between the amount of sports drink (containing carbohydrates) consumed and response accuracy, for which the sugar contents of the drink is the most likely explanation.

Although the negative effects of elevated plasma glucose on accuracy may be specific to WM function because they were found on the WM tests, the fact that there was no WM load by glucose interaction on the Sternberg test suggests that this effect is more general. It thus appears that high glucose levels within the normal physiological range are able to increase response speed, but this may come at the cost of increased error rates, especially on complex and cognitively demanding decisions. Similar observations with carbohydrate supplementation during prolonged exercise were made in a previous study, which also found that caffeine supplementation was able to counteract this downside of increased glucose levels (Hogervorst et al., 2008).

#### Sports drink and cooling intervention effects

Carbohydrate supplementation was achieved through the use of a sports drink rather than water for rehydration, and the detailed data of the volume consumed by each player during each game allow for a quite precise estimate of the resulting effect. However, the sports drink differed from water not only in the carbohydrate content but also in the inclusion of sodium, other electrolytes and flavoring components, so that the sports drink effect may also be related to those components. Nevertheless, in all analyses where sports drink volume was significant (except for error rates on the Sternberg test), this effect disappeared when plasma glucose levels were also entered in the model, suggesting that the sports drink effects were chiefly mediated by its carbohydrate content. Further support for this view rests on the observation that on the response speed measures on the FTT and Sternberg tests, sports drink volume had a significant positive relationship with response speed, mirroring the glucose effects. The negative effect of sports drink ingestion on Sternberg test accuracy was already mentioned above, also indicating that similar to plasma glucose levels, high carbohydrate loads derived from sports drinks may actually decrease accuracy on demanding cognitive tasks. In sum, the carbohydrate contents appear to be the most active component of the sports drink in the cognitive tests, and it mirrors the plasma glucose effects.

Finally, the cooling intervention also had significant effects, but not on the two WM tests used here. Although cooling did not cause a significant decrease

in core temperatures, complex responses on the VS test were faster after exercise, with little difference between the effect size at half time and full time (Fig. 1). This indicates that the cooling strategy effect was consistent, and that it seems to rest at least partly on the visual complexity component. Given that core temperature changes are not responsible, explanations may include an increased perceived comfort in the cooling tent, allowing participants to respond faster, although this should have affected both test levels similarly. However, there may also have been more scope for improvement through motivational effects on the generally much slower responses on the complex level.

In contrast, manual dexterity seems to actually have decreased slightly in the cooling condition, where finger-tapping speed was significantly slower after exercise [Fig. 5(b)]. While this may again be related to perceived comfort and motivational aspects, it is difficult to see why response speed should increase on one test (VS) and decrease on another (FTT) due to the same reason. A more likely explanation might rest in the observation that cooling of the hand and fingers leads to decreased manual dexterity, even when core temperatures are not affected, although this effect is strongest at ambient temperatures below 20 °C (Havenith et al., 1995). Overall, the cooling strategy effects in this study were ambivalent, and no clear benefit other than faster complex level VS response times could be detected in the cognitive test results analyzed here.

#### Perspectives

The present study found no clear effects of mild–moderate dehydration on several cognitive functions in athletes playing football in the heat. In contrast, higher plasma glucose levels were related to faster performance, traded off with decreased accuracy. Carbohydrate supplementation helped to preserve fast RTs, but only at the expense of decreased accuracy for cognitively complex tasks. On the other hand, core temperature rises were related to slower but also more accurate performance.

Given their opposite effects, the observed 50% correlation between plasma glucose and core temperature measurements may help to maintain optimal cognitive function during exercise throughout the physiological temperature range. This may explain some of the discrepancies in the literature on effects of dehydration on cognitive function: many studies did not measure plasma glucose levels, which may have more impact on cognition than dehydration. The methods of dehydration induction also vary widely, and one may reasonably expect different plasma glucose changes in response to active and

passive heating. A clearer picture of the cognitive effects of dehydration and related changes in core temperature and plasma glucose levels may result from studies that measure all of these variables simultaneously.

**Key words:** exercise, heat, dehydration, reaction times, cognitive function, working memory, core temperature, plasma glucose.

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## Acknowledgements

The authors would like to thank the players of University of Ankara Soccer Team for playing in these matches. Also, Adana Football Club and its staff are thanked for allowing use of its pitch and facilities, and Coca Cola, Turkey, for providing the Powerade drinks for the second match. The assistance of Nuri Yildiz and Ercan Yeldan of Çukurova University is also acknowledged.

*Conflicts of interest:* The authors have no potential conflicts of interest.