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Minor degree of hypohydration adversely influences cognition: a mediator analysis[1]

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ABSTRACT

Background: Because the assumption that small changes in hydration status are readily compensated by homeostatic mechanisms has been little studied, the influence of hypohydration on cognition was examined.

Objectives: We assessed whether a loss of <1% of body mass due to hypohydration adversely influenced cognition, and examined the possible underlying mechanisms.

Design: A total of 101 individuals were subjected to a temperature of 30°C for 4 h and randomly either did or did not consume 300 mL H_2O during that period. Changes in body mass, urine osmolality, body temperature, and thirst were monitored. Episodic memory, focused attention, mood, and the perceived difficulty of tasks were measured on 3 occasions. The data were analyzed with the use of a regression-based approach whereby we looked for variables that mediated the influence of hypohydration on psychological functioning.

Results: Drinking water improved memory and focused attention. In the short-term, thirst was associated with poorer memory. Later, a greater loss of body mass was associated with poorer memory and attention (mean loss: 0.72%). At 90 min, an increase in thirst was associated with a decline in subjective energy and increased anxiety and depression, effects that were reduced by drinking water. At 180 min, subjects found the tests easier if they had consumed water.

Conclusions: Drinking water was shown, for the first time to our knowledge, to benefit cognitive functioning when there was a loss of <1% body mass at levels that may occur during everyday living. Establishing the variables that generate optimal fluid consumption will help to tailor individual advice, particularly in clinical situations. This trial was registered at clinicaltrials.gov as NCT02671149. *Am J Clin Nutr* 2016;104:603–12.

Keywords: attention, cognition, dehydration, hypohydration, memory

INTRODUCTION

Water accounts for ∼50–60% of total body mass with received wisdom being that homeostatic mechanisms maintain the hydration status of individuals who live a sedentary life in temperate climates (1). However, a conference of the European Hydration Institute concluded (2) that "regulation is not perfect and dyshydration, a positive or negative deviation from the state of euhydration, can occur. Transient mild hypohydration is common, and probably of little consequence." However, the assumption that transient mild hypohydration has few consequences has been subject to little experimentation, even though hydration status plays a role in all aspects of bodily functioning and in many chronic diseases (3). Therefore, because the first signs of subclinical nutrient deficiency are typically psychological in nature (4), aspects of cognition were examined in individuals who were hypohydrated.

A review of the psychological consequences of mild dehydration showed that, when body mass fell >2%, there were mood changes; reports of fatigue increased, whereas alertness declined (5). However, the effects on cognition were less consistent. The relevance of such conclusions to individuals who are going about their everyday life is unclear because it is unlikely that a loss of 2% of body mass will occur often. For example, during Ramadan, when no food or liquid is consumed by Muslins from sunrise to sunset, there is typically a loss of only 1% of body mass (6). Nevertheless, to our knowledge, the assumption that small changes in hydration status are readily compensated has not been systematically considered; in particular, the point at which fluid loss affects mental performance has not been established. Brain-imaging studies that have examined subjective thirst have shown increased activation in the anterior cingulate and decreased activation in the parahippocampal gyri (7). Because it is unclear whether these changes have other functional consequences, and these brain areas are associated with both focused attention (8) and episodic memory (9), these variables were examined.

Currently, because there are no adequate biomarkers of hydration status, recommended intakes of water in the United States are based on median intakes (3). These figures can only be valid to the extent that current intakes are optimal and that there are not marked differences in individual needs. In addition, the guidelines simply provide optimal daily intake, which raises the question of whether, within 1 d, there may be times when hydration status is less than optimal. Therefore, the objective of the current study was to consider whether a loss of <1% of body mass influenced psychological functioning with the use

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of a novel statistical technique to explore the role of osmolality, loss of body mass and thirst.

METHODS

Procedure

A total of 101 subjects were recruited from an undergraduate population by a circular e-mail. With the use of a parallel design, 26 men consumed water, and 26 men did not consume water; the comparable numbers for women were 25 and 24, respectively. The sample size was chosen to be similar to that in previous studies in our laboratory that have used this protocol. Inclusion criteria were that subjects were aged between 18 and 30 y, were nonsmokers, were not taking a prescribed medication (except an oral contraceptive), did not report having a major medical complaint that included neurologic or psychological disorders, and had not, in the past week, used a sleeping tablet, antihistamine, decongestant, or pain killer. Seventy percent of the women were taking an oral contraceptive. Data were collected between October and May from 2012 to 2015. A preliminary analysis of findings that were associated with the months when data were collected and the random allocation to the experimental conditions ensured that there was no systematic relation between the time of year and the experimental results.

The average age of participants who consumed water was 20.4 y (range: 18–30 y), and that of subjects who did not consume water was 20.5 y (18–31 y). Participants were exposed to a temperature of 30°C for 4 h, during which they either did or did not drink 300 mL pure water. At baseline, both men and women who subsequently did or did not drink water were well matched; they did not differ in urine osmolality [mean ± SD urine osmolality: no-water condition, 688.5 ± 51.6 mOsm/kg; in subjects who subsequently drank water, 635.4 ± 39.0 mOsm/kg; BMI (in kg/m^2): no water, 24.0 (range: 16.7–33.6); water consumed, 23.6 (range: 16.4–33.3)].

The procedure, which is outlined in **Figure 1**, was carried out in accordance with the Helsinki Declaration and was approved by the local Ethics Committee. This trial was registered at clinicaltrials.gov as NCT02671149. Participants gave written informed consent; they were told that they would be in a room at 30°C and perform cognitive tests, but no mention was made of whether they would be allowed to drink. Eating was not permitted during the procedure. Data were collected in the laboratories of Swansea University. Participants were randomly assigned to receive either water or no water during the procedure. The randomization sequence was created (by HAY) on the basis of a random-numbers table with subjects allocated to conditions in the order that they were recruited. It was naturally not possible for either subjects or researchers to fail to know whether water had been drunk. However, subjects were blind as to the nature of the experiment (they were informed that the experiment was looking at the effect of heat) and unaware that other subjects did or did not consume water. On no occasion did any subject in the no-drink condition ask for water.

To capture the full range of habitual hydration status, on the day of testing, participants arrived having consumed their usual breakfasts. On arrival, body temperature and body mass were measured, and a urine sample was collected. Subjects remained at 30°C and on 3 occasions performed a battery of tests (Figure 1). The temperature of the room varied from 30°C to 31°C with an average humidity of 53% (depending on the testing day, humidity ranged from 43% to 62%). One-half of the participants received two 150-mL glasses of water during the procedure. Participants were weighed at periodic intervals (Figure 1). At the end of the procedure, body temperature was taken again, and a second urine sample was collected. The stated a priori primary outcomes were episodic memory and focused attention with mood as a secondary outcome measure.

Body mass

Body mass was measured with the use of an electronic scale (Kern KMS-TM; Kenr and Sohn GmbH) that, to avoid problems associated with movement, took 50 assessments over a 5-s period and produced a mean value. The scale was sensitive enough to weigh to ≤5 g (17% of 1 oz) and could pick up, over short periods, changes in body mass that were due to breathing and perspiration. Subjects were weighed on arrival and after 50, 140, and 230 min. Finally, after emptying their bladders, subjects were weighed again after 240 min. Changes in mass from baseline to 240 min reflected water loss that was due to perspiration, breathing, and urine excretion.

Preliminary studies examined the possible need to weigh individuals naked because some mass loss might have been masked if perspiration remained in the clothing of subjects. With the use of the current protocol with 32 subjects who did not drink, the

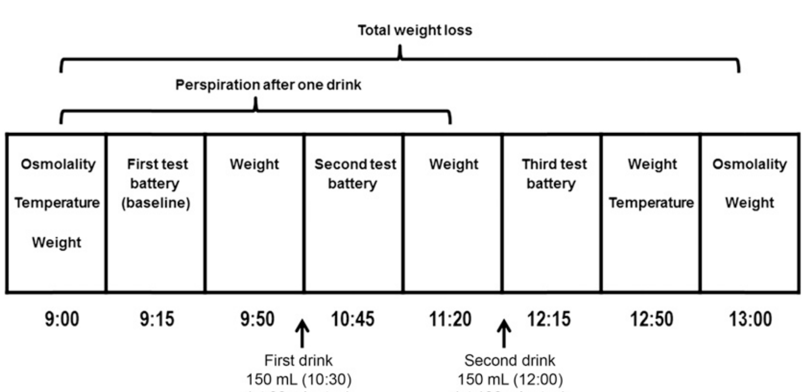

FIGURE 1 The experimental procedure. A total of 101 subjects randomly either did or did not drink 150 mL H_2O on 2 occasions when kept at 30°C for 4 h. Cognitive tests were completed at 3 timepoints.

percentage of change in body mass when weighed naked was 0.26% (SD 0.05) after 230 min and 0.60% (0.33) after urination after 240 min. In the same individuals, these values were virtually identical before light clothing was removed; the comparable percentages of change were 0.26% (0.07) and 0.61% (0.35). Therefore, the data presented were obtained with subjects who wore the same light clothing throughout the procedure.

Osmolality

The osmolality of urine was assessed with the use of an Osmomat 3000 freezing point osmometer (Gonotec GmbH).

Body temperature

Body temperature was measured with the use of a TH8 Infrared Ear Thermometer (Radiant Innovation).

Subjective mood

After subjects completed the cognitive tasks, a visual analog scale was used to rate, on a 100-mm line, subjective scores of energy, depression, and anxiety. The words energetic and tired, elated and depressed, and composed and anxious were written at each end of the lines such that a higher score indicated a more-positive mood.

Difficulty

After subjects completed the cognitive tasks, a visual analog scale was used to rate, on a 100-mm line, the perceived task difficulty. The words "Not at all difficult" and "Very difficult" were written at each end of a line such that a higher score indicated greater difficulty.

Episodic memory: word-list recall

With the use of the MRC Psycholinguistic Database (http://www.psych.rl.ac.uk) (10), 3 lists of 30 words were constructed and matched for the number of syllables, image ability, and frequency with which they occur in English. With the use of a recorder, words were presented at a rate of one word every 2 s. Immediately after the presentation, participants were asked to write down as many words as they could remember (immediate recall). Approximately 20 min later, subjects were asked to recall as many words as possible (delayed recall). The data presented are the changes in performance from baseline such that a minus score indicates a poorer memory.

Focused attention: arrow flanker test

A modified version of the Eriksen and Eriksen (11) flanker task was used to measure the ability to focus attention and ignore peripheral information. Participants were required to indicate whether the middle arrow of 5 arrows was pointing to the right or left by pressing the corresponding arrow on a keyboard. On either side of the central arrow was a distractor that produced a task of increasing difficulty. Congruent arrows [i.e., pointing in the same direction (>>>>>)] provided the easiest version; a neutral version with squares as distractors was of intermediate difficulty (□□<□□); the most difficult version used incongruent arrows [i.e., they pointed in the opposite direction (>><>>)]. A stimulus remained on screen for 1.8 s or until a key was pressed. There was a randomly varying interstimulus interval between 1 and 3 s with an average of 2 s. Sixty stimuli were presented pseudorandomly with congruent, incongruent, or neutral (squares) stimuli, each of which appeared on 20 occasions. The test was scored as the average response time in milliseconds. Difference scores from baseline are reported.

Statistical analysis

The total body mass that was lost because of urination, perspiration, and breathing was calculated as the percentage of change from the beginning to the end of the study. The cognitive tests were completed at baseline and at 90 and 180 min (Figure 1). When possible, 2 sets of measurements were calculated as follows: changes from the first test session (baseline) to after the second test session and changes from baseline to after the third test session. The data reported are change scores compared between the 2 conditions.

The cognitive data were analyzed with a multiple mediation analysis (12) with the use of the PROCESS macro designed for the Statistical Package for the Social Sciences program (IBM SPSS Statistics for Windows, Version 22.0, IBM Corp.). This macro uses bootstrapped sampling, because it imposes no distributional assumptions, to estimate the indirect mediation effect. In the current analysis, 5000 bootstrapped samples were drawn, with replacement from the data set, to estimate a sampling distribution for the indirect mediation pathway. A dummy variable was created for the dichotomous independent variable drink compared with no drink (denoted by X in **Figure 2**). Changes in memory, attention, energy, and ratings of test difficulty were considered dependent variables (Y), and changes in thirst, osmolality, and mass loss were parallel mediators (M). The total effect of X on Y (denoted by c in Figure 2) can be expressed as the sum of the direct (denoted by c') and indirect effects, which is the product of the a and b paths (denoted by ab) such that

$$c = c' + \mathrm{ab} \qquad (1)$$

Indirect effects and 95% CIs are reported. We initially considered whether the effects of drinking differed in men and women; however, because all interactions were NS, sex was subsequently removed from the model.

Cook's distance

When conducting a regression analysis, because it is possible that a particular observation may exert undue influence, Cook's distance (13) was calculated, which reflects the extent to which model residuals would change if a particular datum was excluded. Larger Cook's distance values indicate greater influence. The threshold for determining influential observations was set as $4/N$ in line with previous recommendations (14). On the occasions that a case had a Cook's distance that exceeded this threshold, they were excluded, and the data were reanalyzed. This method resulted in 3 cases that were deleted from the entire flanker task analysis. On inspection, these cases had Cook's distances of >0.2 and appeared not to have engaged with or had misunderstood the task. One case was deleted from the analysis of subjective energy at 90 min because it had a Cook's distance of >0.4 and

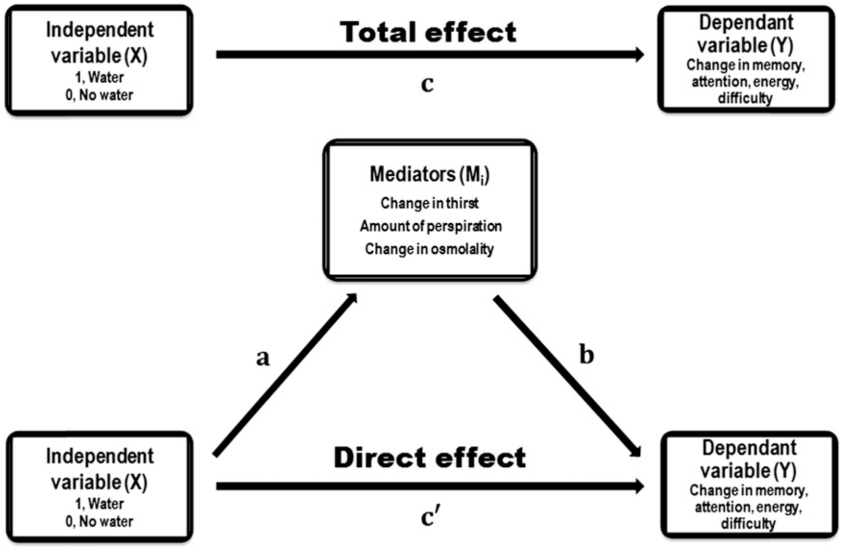

FIGURE 2 Outline of the use of a mediation analysis. Traditionally, we have looked at the relation between an independent variable *X* (e.g., having a drink) and a dependent variable such as memory (*Y*), which is represented in the figure as the total effect. The mediation analysis determines whether there is an in-between step, i.e., whether a mediator (*M*) such as thirst or a change in osmolality is involved. If, after taking into account that the mediator *X* no longer affects *Y*, there is evidence of complete mediation. If the association between *X* and *Y* is only reduced when mediation is considered, there is partial mediation.

was considered an outlier. Two cases were deleted from the delayed memory analysis at 180 min with Cook's distances of 0.15 and 0.32, respectively. All other analyses proceeded with the entire data set.

RESULTS

Total effects

Hydration variables

As expected, participants lost a larger percentage of their body mass if they did not drink than if they did drink [at 90 min: −0.22% compared with 0.05%, respectively; $B = 0.17$ (95% CI: 0.12, 0.21); at 180 min: −0.72% compared with 0.56%, respectively; $B = 0.15$ (95% CI: 0.21, 0.28)]. Similarly, subjects who did not drink had a greater increase in osmolality over the morning ($B = -117.24$; 95% CI: −193.20, −41.28) (**Table 1**). After 1 drink ($B = -6.56$; 95% CI: −12.99, −0.14) and after 2 drinks ($B = -9.79$; 95% CI: −18.02, −1.56), participants who had not drunk were more thirsty. The change in body temperature during the procedure did not depend on whether water had been drunk ($B = -0.08$; 95% CI: −0.23, 0.06).

Memory and attention

At 90 min participants who had not drunk forgot more words with both the immediate ($B = 1.14$; 95% CI: 0.29, 2.00) and delayed memory tasks ($B = 1.255$; 95% CI: 0.31, 2.19). Similarly, at 180 min, memory was poorer both immediately ($B = 1.86$; 95% CI: 0.93, 1.70) and after a delay ($B = 1.09$; 95% CI: 0.26, 1.92) in subjects who did not drink than in those who did (**Figure 3**).

Participants who drank water had faster response times on the arrow flanker task for the incongruent stimuli at 90 min. That is, relative to baseline, subjects who consumed water had better performance, whereas subjects who did not drink water did not have better performance ($B = -27.15$; 95% CI: −52.44, −1.85). However, performance did not change with the easier neutral stimuli ($B = 3.14$; 95% CI: −19.54, 25.83) and congruent stimuli ($B = 2.44$; 95% CI: −16.28, 21.17). A similar effect occurred at 180 min; in the water condition compared with the no-water condition, participants had better response times (i.e., they reacted more quickly) to the incongruent stimuli ($B = -31.05$; 95% CI: −55.70, −7.84) and the neutral stimuli ($B = -22.09$; 95% CI: −43.66, −0.52) although not with the easiest congruent task ($B = 1.00$; 95% CI: −16.86, 18.87) (**Figure 4**). The differential response to the 3 forms of the test

TABLE 1
Changes in hydration variables at 90 and 180 min[1]

	After 1 drink, at 90 min		After 2 drinks, at 180 min	
	No water	Water	No water	Water
Mass lost, %	−0.22 ± 0.02*	−0.05 ± 0.02*	−0.72 ± 0.05***	−0.56 ± 0.05***
Change in osmolality, mOsm/kg	—	—	0.91 ± 32.87*	−115.8 ± 32.2*
Change in temperature, °C	—	—	0.09 ± 0.06	0.02 ± 0.06
Change in thirst	26.31 ± 2.82*	19.58 ± 2.76*	34.77 ± 3.61**	24.64 ± 3.54**

[1]All values are mean ± SE changes from baseline. A higher value denotes greater thirst. Sample size: $n = 101$.
*,**,***Conditions differed significantly: *$P < 0.05$, **$P < 0.05$, ***$P < 0.01$.

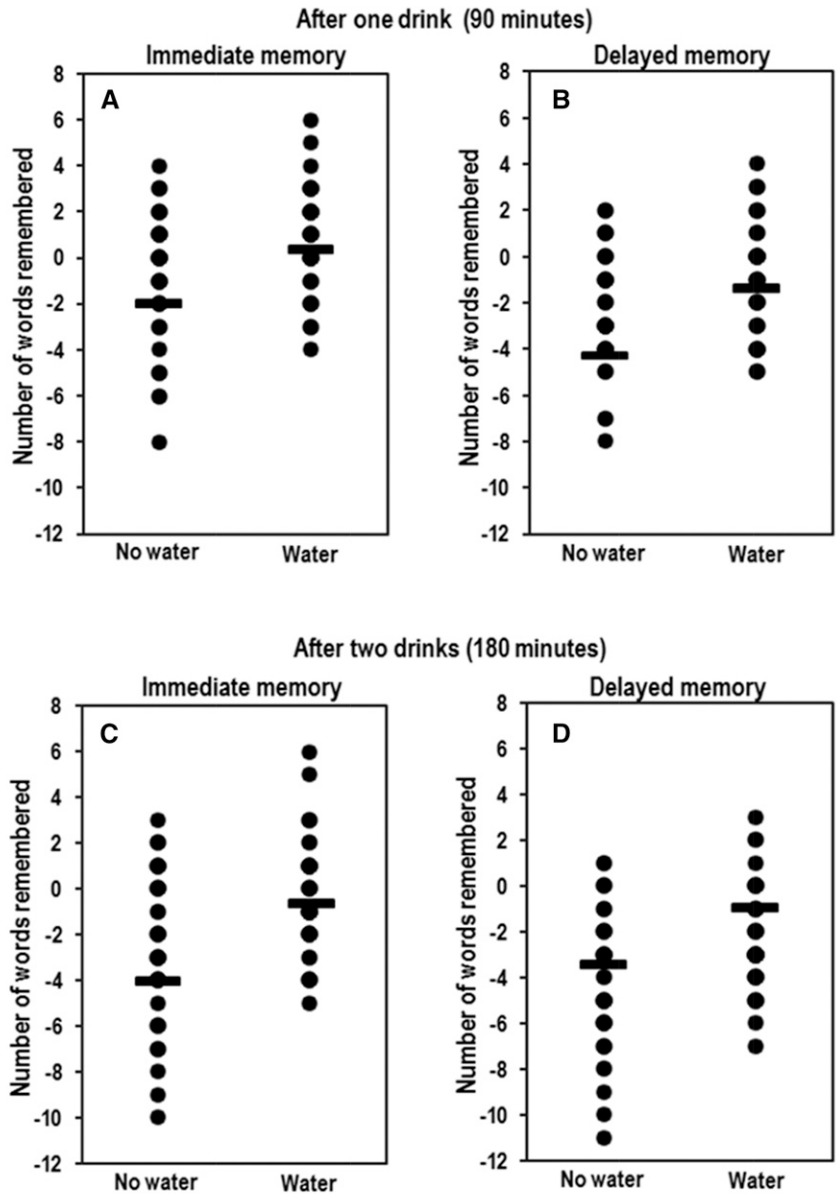

FIGURE 3 Total effect of drinking 1 and 2 drinks on immediate and delayed episodic memory. Data are differences in the number of words remembered compared with the performance at baseline. At 90 min (after 1 drink), participants had better immediate memory (B = 1.14; 95% CI: 0.29, 2.00) (A) and delayed memory (B = 1.25; 95% CI: 0.31, 2.19) (B). Similarly after 2 drinks (180 min), memory was better immediately (B = 1.86; 95% CI: 0.93, 1.70) (C) and also better after a delay (B = 1.09; 95% CI: 0.26, 1.92) (D). n = 101 at 90 min, and n = 101 for immediate memory at 180 min. Cook's analysis reduced the sample to n = 99 for delayed memory at 180 min.

of focused attention suggested that drinking water might preferentially improve reaction times with more-difficult tasks.

Subjective mood and difficulty

Subjective energy did not change substantially from those reported at baseline at either 90 min (B = −2.20; 95% CI: −9.12, 4.55) or 180 min (B = −2.08; 95% CI: −9.60, 5.42) irrespective of whether water had been drunk. Similarly, participant ratings of depression did not depend on whether subjects had drunk [depression ratings at 90 min: B = −0.59 (95% CI: −4.86, 3.67); depression ratings at 180 min: B = −3.52 (95% CI: −9.29, 2.24)]. However, in participants who consumed water compared with those who did not, anxiety decreased from baseline to 90 min, although this effect was not significant at 180 min [anxiety at 90 min: B = −7.28 (95% CI: −14.36, −0.21); anxiety at 180 min: B = −3.92 (95% CI: −10.62, 2.77)]. At 90 min, participants found the test battery equally difficult regardless of whether they had drunk (B = −7.06; 95% CI: −21.92, 7.80). However, at 180 min, the tests were reported to be easier when water had been consumed (B = −16.65; 95% CI: −27.89, −5.63).

Mediator analysis

Possible mechanisms by which drinking water benefitted cognition were examined. Was the degree of fluid loss, change in osmolality, or increase in thirst influential? After the first drink, only

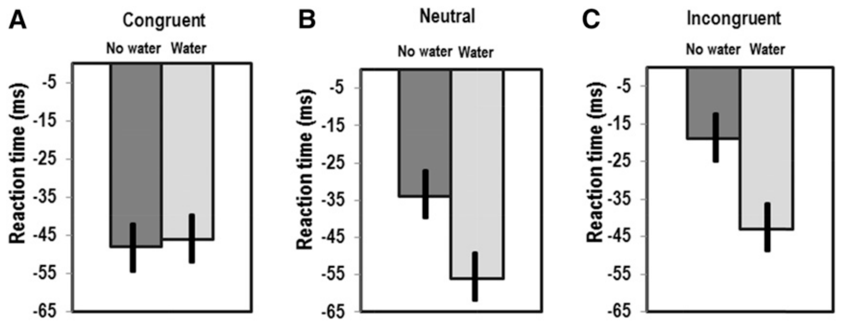

FIGURE 4 Mean ± SE effects at 180 min, after drinking or not drinking a second 150 mL of water, on focused attention depending on the level of difficulty of the task. Data are changes in performance at 180 min (third test battery minus baseline) for reaction times in milliseconds. $n = 98$. Drinking did not influence the performance on the easiest version of the flanker task (congruent stimuli; $B = 1.00$; 95% CI: −16.86, 18.87) (A) but did improve performance with both the more-difficult neutral stimuli ($B = -22.09$; 95% CI: −43.66, −0.52) (B) and the incongruent stimuli ($B = -31.05$; 95% CI: −55.70, −7.84) (C).

the possible mediating effects of a loss of fluid and a change in thirst were considered (**Table 2**) because, at this point, there was no measure of the change in osmolality. Because drinking water did not influence body temperature, it was not included in the analysis.

Immediate memory at 90 min

As shown in Table 2, despite the total effect of drinking being significant ($B = 1.14$; 95% CI: 0.29, 2.00), the direct effect of drinking on memory was NS ($B = 0.59$; 95% CI: −0.41, 1.61), which suggested that the mediators were able to explain a considerable proportion of the total effect. However, the percentage of change in body mass did not mediate the effect of drinking after intake of one drink ($B = 0.35$; 95% CI: −0.14, 0.89) although thirst did ($B = 0.18$; 95% CI: 0.03, 0.54). Subjects who consumed water were less thirsty than those who did not, and in turn, lower thirst was associated with better memory. The effect of thirst accounted for 16% of the total effect [percentage of mediation (P_M) = 0.16].

Delayed memory at 90 min

As shown in Table 2, after the intake of one drink, the direct effect of drinking on delayed episodic memory was NS ($B = 0.72$; 95% CI: −0.29, 1.74), and the percentage of change in body mass ($B = 0.03$; 95% CI: −0.53, 0.56) did not mediate the total effect. However, thirst mediated the effect of drinking on delayed memory ($B = 0.15$; 95% CI: 0.04, 0.98); greater thirst was associated with poorer memory, which was an effect that accounted for 12% of the total effect ($P_M = 0.12$).

TABLE 2
Total, direct, and indirect effects of drinking water compared with not drinking water on memory, attention and mood at 90 min (after the first drink)[1]

Dependent variable	Total effect c	Direct effect c'	Indirect path ab
Immediate memory	1.14 (0.29, 2.00)*	0.59 (−0.41, 1.61)	*T*: 0.18 (0.03, 0.54)* *W*: 0.35 (−0.14, 0.89)
Delayed memory	1.25 (0.31, 2.19)*	0.72 (−0.29, 1.74)	*T*: 0.15 (0.04, 0.98)* *W*: 0.03 (−0.53, 0.56)
Focused attention			
Incongruent	−27.15 (−52.44, −1.85)*	−8.37 (−38.96, 22.20)	*T*: −1.17 (−8.59, 2.39) *W*: −21.48 (−44.86, −2.37)*
Neutral	3.14 (−19.54, 25.83)	24.59 (−2.09, 51.28)	*T*: −2.28 (−12.48, 0.21) *W*: −21.08 (−41.42, −5.28)*
Congruent	2.44 (−16.28, 21.17)	15.66 (−6.8, 38.17)	*T*: −2.96 (−13.31, −0.92)* *W*: −10.28 (−26.72, 5.65)
Energy	−2.20 (−9.12, 4.55)	−5.54 (−13.19, 2.10)	*T*: 2.81, (0.22, 6.93*) *W*: 0.68 (−3.27, 5.72)
Depression	−0.59 (−4.86, 3.67)	−2.28 (−7.34, 2.77)	*T*: 1.04 (0.05, 3.55)* *W*: 0.38 (−2.29, 3.27)
Anxiety	−7.28 (−14.36, −0.21)*	4.25 (−14.36, 0.21)	*T*: 1.82 (0.26, 4.87)* *W*: −0.09 (−3.83, 3.06)
Difficulty	−7.06 (−21.92, 7.80)	−13.32 (−31.94, 5.28)	*T*: −0.52 (−4.39, 1.21) *W*: 6.05 (−4.27, 16.52)

[1]All values are *B*s (95% CIs). Values were determined with the use of a multiple-mediator analysis: *T* is the indirect effect through the change in thirst, and *W* is the indirect effect through the change in body mass. Drinking water prevented declines in memory and attention and reduced anxiety. Thirst mediated the effect of drinking water on immediate and delayed episodic memory, mood, and reaction times with congruent stimuli of the focused attention task. A loss of body mass mediated the effect of drinking water on the incongruent and neutral stimuli of the focused attention task. *Significant effect.

Immediate memory at 180 min

As shown in **Table 3**, after intake of 2 drinks, the direct effect of drinking on immediate memory was still significant ($B = 1.10$; 95% CI: 0.10, 2.11). However, at this time, thirst did not mediate the total effect ($B = -0.09$; 95% CI: −0.46, 0.03) although the indirect influence of both the total percentage of mass lost ($B = 0.18$; 95% CI: 0.01, 0.43) and change in osmolality during the study reached significance ($B = 0.20$; 95% CI: 0.02, 0.54), which accounted for 10% ($P_M = 0.10$) and 16% ($P_M = 0.16$), respectively, of the total effect. Subjects who lost the most mass had poorer memory, and an increase in osmolality was associated with forgetting more words. Because the direct effect of drinking on immediate memory was still significant after controlling for the mediators, it was clear that other unmeasured factors were also involved.

Delayed memory at 180 min

As shown in Table 3, after 2 drinks, the direct effect of drinking on delayed memory was NS ($B = 0.73$; 95% CI: −0.16, 1.64), which suggested that mediators accounted for a large proportion of the total effect. Although the indirect effect of thirst was NS ($B = -0.06$; 95% CI: −0.41, 0.06), the total percentage of mass lost ($B = 0.22$; 95% CI: 0.04, 0.53) mediated the effect of drinking on delayed memory, which explained 20% of the total effect ($P_M = 0$ 0.20). Thus, drinking water prevented a loss in body mass that, in turn, prevented a decline in memory. A change in osmolality ($B = 0.19$; 95% CI: 0.03, 0.55) also mediated the effect of drinking on delayed memory, which explained 17% of the total effect ($P_M = 0.17$). That is, a larger increase in osmolality tended to be associated with poorer memory, which was an effect that was reduced by drinking.

Attention: arrow flanker at 90 min

As shown in Table 2, having consumed 1 drink resulted in faster response times on the arrow flanker task with incongruent stimuli ($B = -27.15$; 95% CI: −52.44, −1.85) although performance was similar for the neutral stimuli ($B = 3.14$; 95% CI: −19.54, 25.83) and the congruent stimuli ($B = 2.44$; 95% CI: −16.28, 21.17).

Although the total effect for the incongruent stimuli was significant, the direct effect of drinking on the incongruent response speed was NS ($B = -8.37$; 95% CI: −38.96, 22.20), which suggested significant mediation. There was an indirect effect of drinking through changes in body mass ($B = -21.48$;

TABLE 3
Total, direct, and indirect effects of drinking water compared with not drinking water on memory, attention, and mood at 180 min (after the second drink)[1]

Dependent variable	Total effect c	Direct effect c'	Indirect path ab
Immediate memory	1.86 (0.93, 1.70)*	1.10 (0.10, 2.11)*	*T*: −0.09 (−0.46, 0.03)
			W: 0.18 (0.01, 0.43)*
			O: 0.20 (0.02, 0.54)*
Delayed memory	1.09 (0.26, 1.92)*	0.73 (−0.16, 1.64)	*T*: −0.06 (−0.41, 0.06)
			W: 0.22 (0.04, 0.53)*
			O: 0.19 (0.03, 0.55)*
Focused attention			
Incongruent	−31.05 (−55.70, −7.84)*	−24.97 (−50.87, 0.92)	*T*: −1.94 (−8.97, 1.56)
			W: −5.01 (−11.49, −1.03)*
			O: −2.76 (−11.49, 1.03)
Neutral	−22.09 (−43.66, −0.52)*	−16.68 (−39.62, 6.25)	*T*: −1.05 (−7.33, 1.14)
			W: −5.32 (−12.08, −1.70)*
			O: −3.73 (−11.71, 0.26)
Congruent	1.00 (−16.86, 18.87)	4.07 (−15.29, 23.44)	*T*: −1.62 (−7.94, 1.11)
			W: −0.94 (−7.73, 2.50)
			O: −3.14 (−10.07, 0.70)
Energy	−2.08 (−9.60, 5.42)	−4.48 (−12.62, 3.66)	*T*: 1.83 (0.54, 3.89)*
			W: −0.04 (−1.42, 1.50)
			O: 0.70 (−0.97, 2.37)
Depression	−3.52 (−9.29, 2.24)	−5.36 (−11.56, 0.84)	*T*: 2.01 (0.28, 4.47)*
			W: 0.09 (−0.93, 2.06)
			O: −0.52 (−1.89, 0.53)
Anxiety	−3.92 (−10.62, 2.77)	−4.13 (−11.37, 3.10)	*T*: 1.37 (0.12, 4.77)*
			W: 0.04 (−2.76, 1.14)
			O: −1.32 (−4.20, 0.14)
Difficulty	−16.65 (−27.89, −5.63)*	−15.27 (−27.63, −2.91)*	*T*: −1.37 (−6.11, 0.43)
			W: 0.03 (−2.07, 3.43)
			O: 0.43 (−2.78, 3.74)

[1]All values are *B*s (95% CIs). Values were determined with the use of a multiple-mediator analysis: *T* is the indirect effect through the change in thirst, *W* is the indirect effect through the change in body mass, and *O* the indirect effect through the change in osmolality. Drinking water prevented declines in memory, attention, and task difficulty. Thirst mediated the effect of drinking water on mood. The loss of body mass mediated the effect of drinking water on the incongruent and neutral stimuli of the focused attention task. The effect of drinking water on immediate and delayed episodic memory was mediated both by a loss of body mass and by an increase in osmolality. *Significant effect.

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95% CI: −44.86, −2.37) whereby a loss in body mass was associated with responding more slowly in this test of focused attention, which was something that was reduced by drinking. The loss of body mass accounted for 59% of the total effect (P_M = 0.59). In contrast, thirst did not mediate the effect of drinking on reaction times with the incongruent stimuli (B = −1.17; 95% CI: −8.59, 2.39). With the neutral stimuli, the indirect effect of drinking water through changes in body mass reached significance (B = −21.08; 95% CI: −41.42, −5.28; P_M = 0.17); a greater loss of mass was associated with slower responding, which was an effect that was reduced by drinking. However, thirst did not mediate the effect of drinking with neutral stimuli (B = −2.28; 95% CI: −12.48, 0.21). When the congruent stimuli were considered, it was the indirect effect of change in thirst (B = −2.96; 95% CI: −13.31, −0.92 P_M = 0.18) rather than of body mass (B = −10.28; 95% CI: –26.72, 5.65) that was significant. Greater thirst was associated with performing the task more slowly.

Attention: arrow flanker at 180 min

As shown in Table 3, with the incongruent stimuli, the direct effect of drinking on the speed of responding was NS (B = −24.97; 95% CI: −50.87, 0.92), which suggested significant mediation; however, the effect was not mediated via either a change in thirst (B = −1.94; 95% CI: −8.97, 1.56) or osmolality (B = −2.76; 95% CI: −11.49, 1.03). In contrast, the effects of drinking were mediated via the total loss of body mass (B = −5.01; 95% CI: −11.49, −1.03, P_M = 0.14); a smaller loss of body mass was associated with quicker responses.

There were no effects with the congruent stimuli the easiest version of the test. With the neutral stimuli, the direct effect of drinking after intake of 2 drinks was NS (B = −16.68; 95% CI: −39.62, 6.25), however, the indirect effects of fluid loss (B = −5.32; 95% CI: −12.08, −1.70) reached significance, accounting for 15% (P_M = 0.15) of the total effect. Drinking prevented a loss in body mass that resulted in quicker response times. However, neither thirst (B = −1.05; 95% CI: −7.33, 1.14) nor the change in osmolality (B = −3.73; 95% CI: −11.71, 0.26) mediated this effect.

Subjective mood at 90 min

As shown in Table 2, at 90 min, although neither the total nor the direct effect of drinking on ratings of energy was significant (B = −5.54; 95% CI: −13.19, 2.10), the indirect effect through thirst reached significance (B = 2.81; 95% CI: 0.22, 6.93; P_M = 0.50). An increase in thirst was associated with a decline in energy ratings, which was an effect that was reduced by drinking water. The indirect effect of fluid loss was NS (B = 0.68; 95% CI: −3.27, 5.72). The same pattern occurred for ratings of depression and anxiety at 90 min; neither direct effects were significant [anxiety B = 4.25 (95% CI: −14.36, 0.21); depression B = −2.28 (95% CI: −7.34, 2.77)]; however, the indirect effects of drinking via changes in thirst were significant (anxiety B = 1.82 (95% CI: 0.26, 4.87; P_M = 0.32); depression B = 1.04 (95% CI: 0.05, 3.55; P_M = 0.41)]. Drinking prevented an increase in thirst, when thirst was associated with becoming more anxious and depressed. The loss of body mass was not associated with ratings of anxiety and depression (anxiety B = −0.09; 95% CI: −3.83, 3.06; depression B = 0.38; 95% CI: −2.29, 3.27).

Subjective mood at 180 min

As shown in Table 3, at 180 min, the total and the direct effect of drinking on subjective energy were NS (B = −4.48; 95% CI: −12.62, 3.66), and the indirect effects of fluid loss (B = −0.04; 95% CI: −1.42, 1.50) and the change in osmolality (B = 0.70; 95% CI: −0.97, 2.37) were without influence. However, the indirect effect of drinking water on subjective energy via changes in thirst was significant (B = 1.83; 95% CI: 0.54, 3.89; P_M = 0.31); drinking water prevented an increase in subjective thirst that, in turn, was associated with a decline in ratings of energy. Again, the direct effect of drinking water on ratings of anxiety and depression were NS [anxiety B = −4.13 (95% CI: −11.37, 3.10); depression B = −5.36 (95% CI: −11.56, 0.84)] although the indirect effects via changes in thirst were significant (anxiety B = 1.37; 95% CI: 0.12, 4.77 P_M = 0.33; depression B = 2.01; 95% CI: 0.28, 4.47 P_M = 0.53) whereby greater thirst was associated with a poorer mood. Fluid loss and osmolality were not related to ratings of anxiety [fluid-loss B = 0.04 (95% CI: −2.76, 1.14); osmolality B = −1.32 (95% CI: −4.20, 0.14)] or depression [fluid loss B = 0.09 (95% CI: −0.93, 2.06); osmolality: B = −0.52 (95% CI: −1.89, 0.53)].

Difficulty ratings

The indirect effects of drinking on difficulty ratings through thirst (B = −0.52; 95% CI: −4.39, 1.21) and fluid loss (B = 6.05; 95% CI: −4.27, 16.52) were NS at 90 min (Table 2). Similarly, at 180 min, osmolality (B = 0.43; 95% CI: −2.78, 3.74), fluid loss (B = 0.03; 95% CI: −2.07, 3.43), and thirst (B = −1.37; 95% CI: −6.11, 0.43) did not mediate the effect of drinking on difficulty ratings (Table 3). However, the direct effect of drinking was significant (B = −15.27; 95% CI: −27.63, −2.91), which suggested the involvement of other unmeasured factors.

Summary of results

Drinking prevented a decline in memory. At 90 min, thirst mediated the effect on memory, whereas at 180 min, a greater loss of body mass and an increase in osmolality were both associated with poorer memory. Drinking was associated with maintaining better attention, and a greater loss of body mass was associated with poorer performance. Drinking influenced ratings of energy, anxiety, and depression through changes in thirst; subjects who were thirstier had a poorer mood. Subjects who did not drink found the tests more difficult at the end of the morning although thirst, osmolality, and fluid loss did not mediate this effect.

DISCUSSION

A clear finding was that, under the same environmental conditions, there were marked differences in the rate at which body mass was lost. After 4 h at 30°C, the loss of body mass in subjects who did not drink averaged 0.72%, but there was a 5-fold difference whereby it varied from 0.29 to 1.5%. How likely is it that such differences influence cognitive functioning? A review concluded that "Being dehydrated by just 2% impairs performance in tasks that require attention, psychomotor, immediate memory and working memory, as well as assessment of the subjective state" (15). However, it has also been concluded that individuals who live in temperate climates will rarely have even a 1% loss of body mass (5) although the point at which a loss of water affects cognitive functioning has been little studied. Armstrong et al. (16) used exercise and diuretics to generate mild dehydration and reported, in a similar manner as in the current study, that a loss of 1.36% of body mass resulted in feeling less active and more fatigued. However, the performance of various cognitive tests was not influenced although, as in the current study, subjective task difficulty was greater. It has also been reported that a loss of 1% of body mass increased errors when driving (17), and the current study showed that drinking water after having lost 0.72% of body mass prevented declines in memory, attention, and mood. In addition, as would be expected, drinking reduced thirst, which raises the question as to whether there was a role for this subjective experience as opposed to an influence on hydration status. It seems possible that the short- and long-term responses reflected different mechanisms. Initially, the psychological response to the subjective experience of thirst was influential, whereas later, when there had been a greater loss of bodily mass, the changes were more physiological in nature.

Cohen (18) proposed that dehydration distracts attention, and because attention has a limited capacity, performance suffers. In support of this proposal, Edmonds et al. (19) showed that individuals with low thirst were not adversely affected by a failure to drink; the current analysis showed that, after 90 min, thirst mediated the effect that drinking water had on memory and energy. In contrast, after 180 min, changes in osmolality and fluid loss meditated the effects of drinking on cognition and mood, which suggests that different factors were involved at different stages. We found it surprising that the first point at which hypohydration was influential was when, on average, participants had lost only 0.22% of their body mass by perspiration. At this point, it was thirst rather than lost body mass that mediated the effect on memory, although a decline in body mass was later influential: after 180 min, changes in osmolality also mediated the effect of drinking on memory and attention.

Additional research is required to establish the precise mechanisms involved. For example, although the current study considered minor changes in hydration status, a role played by the oral stimulation associated with drinking could not be ruled out. There is increasing evidence that preabsorptive reflexes contribute to the regulation of hydration. For example, Shingai et al. (20) showed that infusing water into the oral cavity of anesthetized rats produced an increase in urine flow. Similarly, Knight et al. (21) examined the role of oropharyngeal afferents on the hypothalamic activation in rats with a gastric fistula. Sham water drinking significantly reduced supraoptic nucleus *c-fos* immunoreactivity, which suggested that, during rehydration, vasopressin neurons are regulated by preabsorptive stimuli. Whether such mechanisms have cognitive consequences needs to be studied.

Because the current findings report that a minor degree of hypohydration adversely influenced both memory and attention, future work should consider whether an understanding of factors that improve hydration status will offer clinical benefits. An obvious suggestion is that better hydration will benefit individuals with cognitive problems. People >65 y of age, when deprived of fluid, are less likely to experience thirst, and fluid balance is regained more slowly (22). The significance of these phenomena has been illustrated in a Europe-wide study that showed women in the lowest tertile of fluid intake had a poorer mental state (23). More specifically, there are reasons to suggest that the renin-angiotensin system, which plays a key role in water regulation, may also influence memory and dementia. The taking of angiotensin-converting enzyme inhibitors was associated with reduced vascular cognitive impairment (24). For example, taking angiotensin-converting enzyme inhibitors improved performance of the Mini-Mental State Examination by 50% (25), and the administration of angiotensin receptor blockers was associated with a lower incidence of Alzheimer disease (26). A survey of individuals with dementia reported that 51% of the sample had low fluid intake (27). A review concluded that there was no doubt that the renin angiotensin system "is an important component in the development of dementia. It also appears to play a role in normal memory consolidation and retrieval" (28). Although it is unclear whether the current findings can be generalized to an older population, they suggest that the question should be addressed.

Even before the possibility of a role in clinical problems is considered, the current findings suggest that even a minor degree of hypohydration adversely influences cognition such that optimal hydration may be more generally helpful. Intervention studies have shown that the cognition of children (29–31) and their behavior in school (1) improved when they were given a drink. Thus, in children, an understanding of the influence of the pattern of drinking on school performance would be helpful. However, at all ages, improved hydration is potentially beneficial.

A problem is this area of study is that, inevitably, individuals know whether they have had a drink, and Lieberman (32) noted that it was desirable to exclude the possibility of a placebo response. In this context, a mediation analysis was helpful because it allowed the role of factors to be considered when participants were unaware of their status. Because changes in body mass, thirst, and osmolality mediated the influence of drinking on cognition, these findings offered evidence of both physiological and psychological mechanisms. Because the current design prevented the role of hypohydration as opposed to a response to heat from being distinguished, future studies should consider the relative importance of these 2 factors. In addition, habitual water intake and caffeine and alcohol consumption need to be examined (32). Although memory and attention were influenced by hypohydration, future work should consider other cognitive domains and whether the influence is specific or general. However, because the adverse effects were shown with the more difficult forms of the arrow flankers test, a selective influence associated with more demanding tasks was suggested.

In conclusion, with hydration, a one-size-fits-all recommendation may not give an individual optimal advice. Because even small differences in hydration status have functional consequences, an understanding of the impact of the pattern of consumption will have practical consequences.

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