A 4-d Water Intake Intervention Increases Hydration and Cognitive Flexibility among Preadolescent Children

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ABSTRACT

Background: Hydration effects on cognition remain understudied in children. This is concerning since a large proportion of US children exhibit insufficient hydration.

Objective: This study investigated the effects of water intake on urinary markers of hydration and cognition among preadolescents.

Methods: A 3-intervention crossover design was used among 9- to 11-y-olds [$n = 75$ (43 males, 32 females); 58.2 ± 28.5 BMI percentile]. Participants maintained their water intake [ad libitum (AL)] or consumed high (2.5 L/d) or low (0.5 L/d) water for 4 d. The primary outcomes were performance on cognitive tasks requiring inhibition, working memory, and cognitive flexibility assessed using a modified flanker, go/no-go, and color-shape switch tasks, respectively. Secondary outcomes included urine hydration indices [i.e., color, urine specific gravity (USG), osmolality] assessed using 24-h urine collected during day 4 of each intervention. Repeated-measures ANOVAs were used to assess intervention effects.

Results: There was a significant difference in hydration across all 3 interventions. Urine color during the low intervention [median (IQR): 6 (2)] was greater than during AL [5 (2)], and both were greater than during the high intervention [18 (0)] (all $P \leq 0.01$). Similarly, osmolality [low (mean $\pm$ SD): 912 ± 199 mOsmol/kg, AL: 790 ± 257.0 mOsmol/kg, high: 260 ± 115 mOsmol/kg] and USG [low (mean $\pm$ SD): 1.023 ± 0.005, AL: 1.020 ± 0.007, high: 1.005 ± 0.004] during the low intervention were greater during AL, and both were greater than during the high intervention (all $P \leq 0.01$). USG and osmolality AL values were related to switch task measures (β: 0.21 to -0.31, $P < 0.05$). Benefits of the high intervention were observed during the switch task, whereby participants exhibited 34% lower working memory cost relative to the low intervention. No significant changes in cognition were observed for the flanker and go/no-go tasks.

Conclusions: The water intervention improved urinary markers of hydration and had selective benefits during task switching. Furthermore, children's cognitive flexibility selectively benefits from greater habitual hydration and water intake. This study is registered at clinicaltrials.gov as NCT02816450. *J Nutr* 2019;00:1–10.

Keywords: working memory, cognitive flexibility, inhibition, hydration, urine, water intake, children

Introduction

Emerging evidence suggests that insufficient hydration is increasingly prevalent among children across the globe. Epidemiological examination of urine osmolality, a marker of hydration, suggested that over half of children in the United States have highly concentrated urine (≥ 800 mOsmol/kg), indicative of insufficient hydration (1). While there are limited representative data on hydration in children in other countries, data on fluid intake from a large cross-sectional survey of children from 13 countries indicated that >60% of children did not meet the adequate intake for water from fluids (2). Moreover, it has also been reported that in some countries,

up to 50% of children have limited or no free access to water while at school, where they spend a large proportion of their waking hours (3). However, the health implications of modulating hydration via water intake in children are unclear. Dehydration, defined as the process of uncompensated water loss resulting in reductions in total body water below the basal value necessary to maintain bodily functions (4), has been linked to diminished attention, short-term memory, and psychomotor function among adults (5–7). However, only a handful of studies has examined hydration effects on cognition in the pediatric population (8, 9). This gap in knowledge is concerning since children may be at higher risk for insufficient

hydration as they depend on adults for regular access to water and have a higher daily water requirement relative to body mass (10).

Earlier work in this area suggested that elevated urinary markers of hydration—such as urine osmolality—are associated with poorer short-term memory performance in children (11, 12). Although limited, a handful of interventions also indicate that providing children with water, without prior induction of dehydration, may result in acute benefits for memory and visual attention (12–16). For example, Perry et al. (17) used a 2-condition crossover design to examine changes in working memory and attention among 9- to 12-y-olds following provision of either 750 mL of water throughout the morning hours compared with no water (17). Interestingly, the results indicated that drinking water had a differential impact on task performance as a function of the child's ad libitum (AL) urine osmolality (i.e., ≥800 mOsmol/kg) (17). Therefore, benefits derived from water consumption on cognitive function may be moderated by usual hydration. However, a significant gap in the previous intervention work has been the lack of a cognitive assessment under habitual or AL hydration, which is necessary to fully understand how changes in water intake affect changes in cognition. Furthermore, previous interventions have used acute provision of water, typically within a couple of hours of cognitive testing (14, 15); therefore, the effects of prolonged changes in water intake over several days on children's cognitive function have not been directly investigated.

Accordingly, the present work aimed to investigate the effects of changes in hydration—via water intake intervention—on cognitive tasks designed to tap multiple domains of cognitive control. Cognitive control, often referred to as executive function, comprises a set of interrelated, yet dissociable, higher-order cognitive processes including inhibition (the ability to resist distractions and maintain focus and the ability to inhibit a dominant response in favor of a lesser learned response), working memory (the ability to hold information in our mind and manipulate it for later usage), and cognitive flexibility (the ability to dynamically shift attention and alter response strategies to changing demands) (18, 19). These processes have ecological validity and are important for the performance of numerous daily living activities, including regulating mind wandering, health and safety, and social adjustment (20, 21). Characterizing factors that influence cognitive control is important because greater performance on cognitive control tasks during development is predictive of later academic achievement and has been linked to greater educational attainment, higher income and socioeconomic status (SES), and better access to health care (22). We hypothesized that insufficient hydration—assessed using urine specific gravity (USG) and urine osmolality (UOsm)—would be inversely associated with cognitive control. In addition, we anticipated that improving hydration—via a water intake intervention—would significantly improve performance during cognitive control tasks among preadolescent children.

TABLE 1 Demographic characteristics of children participating in the trial[1]

Characteristic	Value
Age, y	9.8 ± 0.6
Sex, male/female, *n*	43/32
Pubertal timing	1.4 ± 0.5
IQ	113.8 ± 15.9
Socioeconomic status,[2] *n* (%)	
Low	20 (26)
Middle	23 (31)
High	32 (43)

[1]Data presented as mean ± SD unless otherwise indicated, *n* = 75. IQ, Intelligence Quotient.
[2]Socioeconomic status determined by a trichotomous index based on *1)* participation in free or reduced-price meal program at school, *2)* the highest level of education obtained by the mother and father, and *3)* number of parents who worked full-time.

Methods

Participants

Children aged between 9 and 11 y were recruited to participate in the Water Intervention for Thinking in Kids study. Participants were recruited from the east-central region of Illinois via flyers posted in public buildings and disseminated to local schools. All guardians and children provided written informed consent and assent prior to participating in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the University of Illinois. The study is registered at clinicaltrials.gov as NCT02816450. Children's families received a small payment incentive for participation. Participants were included based on age (9–11 y at the time of enrollment) and excluded if they had prior diagnosis of cognitive or learning disabilities, history of chronic kidney disease, diabetes, or use of medications that may alter water balance or mood and anxiety. Regarding SES, the proportion of children with low (26%), medium (31%), and high (43%) SES is outlined in Table 1. Thus, a relatively larger proportion of the children was from more affluent families. However, almost one-third of the participants were of poorer SES, as indicated by qualification/participation in free/reduced school lunch and parents without college education.

Figure 1 illustrates the participant CONSORT (Consolidated Standards of Reporting Trials) diagram and final sample size. In total, 128 participants were screened for participation. Following screening, 91 participants enrolled in the study. Among those enrolled, 82 participants completed all 3 interventions and provided complete data. Seven participants were excluded using a combination of 2 a priori defined criteria urinary values that, together, suggested a very high probability of poor compliance with the water intake protocol. Six participants were excluded on the basis of urine markers suggesting noncompliance with the high condition [both a 24-h urine volume (UVol) <1000 mL and 24-h UOsm >650 mOsm/kg]. One additional participant was excluded on the basis of urine markers suggesting noncompliance with the low condition (both a 24-h UVol >1000 mL and 24-h UOsm <500 mOsm/kg). This double requirement for probable noncompliance (both urine volume and osmolality out of range) was deliberately chosen to ensure that the largest number of participants possible remained included in analyses. Following removal of participants with poor performance and outliers, the final study sample comprised 54–70 participants depending on the cognitive task. Individual participant data were inspected for outliers [reaction time (RT) >3 SDs] and poor performance (accuracy <40%), and instances that violated this criterion were removed from analyses. This resulted in the removal of 5 participants from the flanker task (3 outliers, 2 performance), 19 from the go/no-go task (8 outliers, 11 performance), and 12 from the switch task (12 performance). In addition, 2 participants did not complete all blocks of the go/no-go task and 1 participant did not complete both switch task blocks.
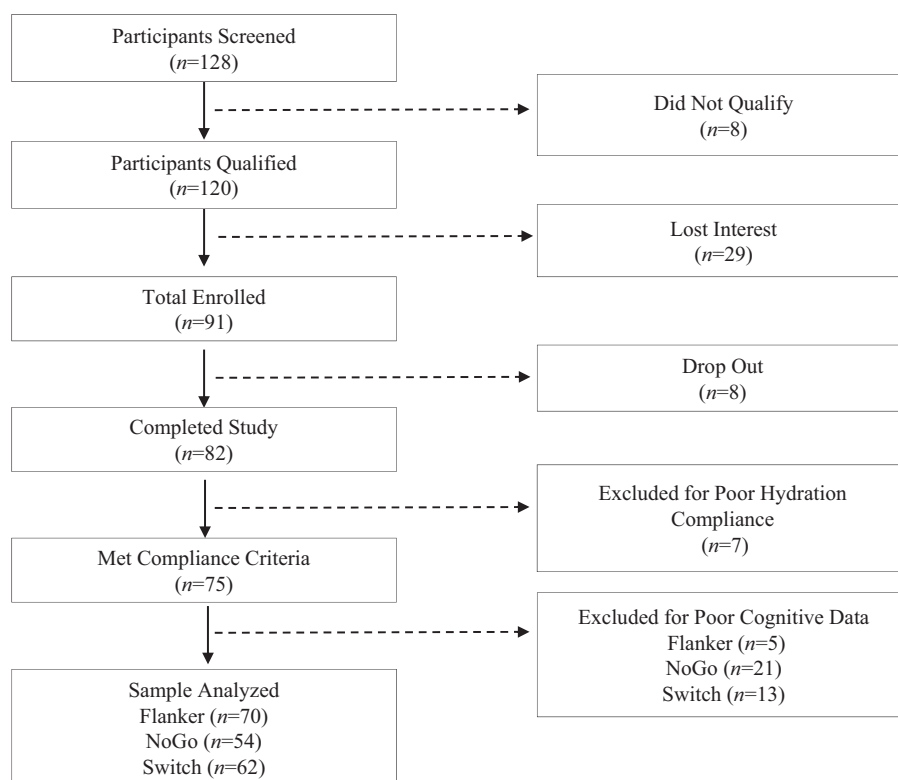
FIGURE 1 CONSORT figure illustrating participant enrollment and final sample analyzed.

This resulted in final sample sizes of 70 participants for the flanker task, 54 for the go/no-go task, and 62 for the switch task. Participant characteristics are summarized in Table 1. Analyses of the urinary indices were conducted among the largest participant sample with the complete task data (i.e., 70 participants).

Study design

A 3-intervention crossover design was employed whereby all participants underwent an AL intervention [4 d of habitual fluid intake (AL)] as well as 2 experimental interventions wherein participants either restricted their total fluid intake to 0.5 L of water/d for 4 d (low) or consumed a higher total fluid intake of 2.5 L of water for 4 d (high). Participants were asked to limit their beverage intake to only the prescribed water throughout the low and high interventions, with the exception of milk with cereal at breakfast. All interventions were administered in a counterbalanced manner. During the fourth day of each intervention, participants were asked to perform a 24-h urine collection using the following procedure: the day before the study visit, upon waking in the morning, participants voided and discarded this first morning void. They then collected all subsequent voids in separate containers, including voids produced during the night. On the morning of the study visit, upon awakening, participants collected their first morning void, thus completing the 24-h collection. The urine samples were stored in 16-oz sealable containers within a plastic cooler with freezer packs provided by the researchers and were brought back to the laboratory during each study visit. Response to the study intervention was assessed as the change in urine color, USG, and osmolality. Participants also underwent a cognitive battery during the mornings when they returned to the laboratory following each 4-d intervention, which included a modified flanker task, a go/no-go task, and a color-shape switch task.

Urinary hydration assessment

All urine samples collected over the 24-h period were pooled and analyses were performed on fresh (i.e., nonfrozen) samples. Urine color was determined in a well-lit room by placing the sample in a clear container against a white background and scoring the sample according to a validated 8-point urine color scale (23). USG was assessed using a digital handheld pen refractometer (ATAGO Co.). Urine osmolality was assessed using freezing point depression (Advanced 3320 Micro-Osmometer; Advanced Instruments).

Cognitive control tasks

To assess cognitive control, participants completed 3 different computer-based tasks. A modified flanker task (24), a go/no-go task (25), and a color-shape switch task (26) were performed to assess inhibition and cognitive flexibility. However, the switch task also affords the ability to examine task performance under different working memory loads. In all tasks, participants were instructed to respond as quickly and accurately as possible. Measures of RT and accuracy were collected.

During the flanker task, participants were instructed to respond to the directionality of a central target arrow amid 2 flanking arrows on either side (e.g., a left thumb press for a left-facing arrow and a right thumb press for a right-facing arrow). Flanking arrows could either be congruent (>>>>> or <<<<<) or incongruent (>><>> or <<><<) to the target arrow. Congruent trials require lower amounts of attentional inhibition (i.e., perceptual interference) as all arrows point in the same direction and only 1 response mapping is activated. However, incongruent trials require comparatively higher amounts of attentional inhibition because perceptual interference is elevated via the flanking arrows pointing in the opposite direction of the central target arrow, which activates multiple response mappings. This latter condition results in longer RT and lower accuracy. Before the task, participants completed a practice block of 24 trials. The practice blocks were followed by 2 experimental blocks of 108 trials. Congruent and incongruent trials were equiprobable (i.e., 50% of each trial type) and in random order. Stimuli were presented for 167 ms with a variable interstimulus interval of 1500, 1700, or 1900 ms. In addition to accuracy and RT, differences between congruent and incongruent trials were presented as interference scores (i.e., RT interference = incongruent − congruent; accuracy interference = congruent − incongruent). Interference scores index the degree of modulation in accuracy and RT necessitated by increasing

inhibitory control demands; therefore, smaller interference values are favorable and indicate maintenance of performance as inhibitory demands are increased during the incongruent trials.

The go/no-go task was composed of 2 blocked conditions (go and no-go tasks). In both conditions, participants were presented either a cartoon lion (target, 0.2 probability) or a cartoon tiger (nontarget, 0.8 probability). The stimulus was presented for 167 ms with a variable intertrial interval of 1400, 1500, 1600, 1700, or 1800 ms. Participants completed 35 practice trials before completing 2 experimental blocks consisting of 125 trials. In the go task, participants were instructed to respond to the lion stimulus with a right thumb press. In the no-go condition, participants were instructed to respond to each tiger stimulus and withhold their response during the lion stimulus. Given that the go task was always completed first, a prepotent response toward the rare stimulus was developed. Thus, during the no-go task, participants were required to inhibit this prepotent response, necessitating greater amounts of inhibitory control to prevent committing the prepotent response.

A color-shape switch task assessed aspects of working memory and cognitive flexibility (26). During this task, participants were required to learn a set of response mappings arbitrarily assigned to a set of colors (blue and green) and shapes (circle and square), then use a ruleset cue (the direction of the character's arms) to flexibly shift visuospatial attention toward the correct feature set and execute the correct response mapping. During 2 separate homogeneous blocks, participants were first asked to respond to color (e.g., a left thumb press to green and a right thumb press to blue) followed by a block of trials where participants were asked to respond to the shape (e.g., a left thumb press to the square and a right thumb press to the circle) of the stimuli (26). Participants completed 20 practice trials before completing 2 experimental blocks of 60 homogeneous trials. Next, a heterogeneous condition was completed in which participants had to flexibly switch between the color and shape tasks based on a cue (i.e., the direction of the character's arms with arms up cuing the shape task and arms down cuing the color task).

During the heterogeneous condition, participants were required to hold both the color and shape rulesets in working memory and flexibly activate the correct ruleset based on a cue. The heterogeneous block consisted of 2 trial types: nonswitch trials, where the previous trial ($n – 1$) and the current trial (n) did not change rulesets, and switch trials, where the previous trial ($n – 1$) and the current trial (n) changed rulesets. Participants initially completed a practice block of 18 trials before completing 2 blocks of 128 trials. Homogeneous and heterogeneous stimuli were presented on the screen for 200 ms with an intertrial interval of 2000 ms. In addition to RT and response accuracy, several switch costs were calculated. First, global switch cost was calculated as the difference between homogeneous and heterogeneous conditions and captured the cost in performance due to maintaining multiple rulesets in working memory and switching between these rulesets. Second, local switch cost calculated the cost in performance due to switching between rulesets during the heterogeneous block and was calculated as the difference between nonswitch and switch trials. Last, working memory cost was calculated as the cost in performance due to maintaining multiple rulesets in working memory in the absence of switching between rulesets (i.e., nonswitch trials) and was derived from the difference between nonswitch trials in the heterogeneous condition and the homogeneous condition trials. Therefore, a larger positive number indicates a larger cost.

Additional variables

In addition to primary independent and dependent variables of hydration and cognitive function, we measured pertinent covariates including SES, Intelligence Quotient (IQ), and pubertal timing. A trichotomous index was used to determine SES based on *1*) participation in free or reduced-price meal program at school, *2*) the highest level of education obtained by the mother and father, and *3*) number of parents who worked full-time (26, 27). Participants completed the Kaufman Brief Intelligence Test (28) to assess IQ and a Tanner Staging System questionnaire to assess pubertal status (29). AL water consumption was assessed by using the average of 7-d food records. The record was completed by the child with assistance from the parent. Both

child and parent received instructions on how to correctly fill out the food record. In addition, the record contained written instructions for recording food intake (including how to describe food preparation methods, added fats, brand names, and ingredients of mixed dishes and recipes). Trained staff entered food records into the 2014 Nutrition Data Systems-Research (Nutrition Coordinating Center) software. Water intake analyses were conducted by using the intake properties file to determine mean macronutrient intake.

Statistical analyses

The Statistical Package for the Social Sciences (SPSS, version 24; IBM) software was used for all statistical analyses. An α of 0.05 was used as a cutoff for statistical significance.

Repeated-measures ANOVA was conducted to examine differences in USG and urine osmolality across interventions (secondary outcomes). Given the ordinal nature of the urine color measure, a Wilcoxon signed-rank test for related samples was conducted to determine paired differences in urine color between interventions.

Hierarchical linear regressions were used to investigate relations between hydration markers and cognitive function under AL intervention. First, significant covariates were investigated using Pearson correlations to determine which demographic variables (age, sex, IQ, pubertal timing, and SES) correlated with the dependent measures and hydration outcomes. These covariates were entered in step 1 of the regression model. Hydration was entered into step 2 to determine the degree of variance hydration accounted for within the dependent variable after accounting for the covariate measures.

Repeated-measures ANOVA was used to investigate the intervention effects on the cognitive variables (primary outcomes). Flanker task performance was assessed using a 3 (hydration intervention: AL, high, low) × 2 (condition: congruent, incongruent) model for accuracy and RT. Repeated measures for the 3 hydration interventions (AL, high, low) were assessed for the interference effects using a 1-factor repeated-measures ANOVA. Go/no-go task performance was assessed using three 1-factor repeated-measures ANOVA with AL, high, and low performance (RT and accuracy for go target and accuracy for no-go target). Switch task performance was assessed using a 3 (hydration intervention: AL, high, low) × 2 (condition: homogeneous compared with heterogeneous, nonswitch compared with switch, homogeneous compared with nonswitch) repeated-measures ANOVA. In addition, switch costs were assessed using a series of 1-factor ANOVAs with intervention (AL, high, low) and the different switch cost performance measures (i.e., global switch cost, local switch cost, working memory cost) for RT and accuracy. Post hoc tests were Bonferroni corrected for multiple comparisons.

Results

Urinary hydration markers

Only 67 participants returned food records following the AL. The average AL total water/moisture in diet (i.e., moisture in foods + fluids) consumed (mean ± SD) was 1000.2 ± 621.5 mL. Regarding hydration changes, the difference/change in osmolality (mean ± SD) between the AL and high intervention was 507.0 ± 275.4 mOsm/kg with >75% of the participants exhibiting a reduction in osmolality of >287.4 mOsm/kg. On the other hand, the average increase in osmolality (mean ± SD) was comparatively lower and had a higher degree of variability (i.e., only 159.3 ± 254.1 mOsm/kg) with ~30% of participants not exhibiting an increase in osmolality between the AL and low intervention.

Statistical analyses comparing the intervention effects on hydration indices revealed that all urinary biomarkers differed significantly between interventions (all $P < 0.05$). Urine color during the low intervention [low: 6 (median) and 2 (IQR)] was significantly higher than AL [5 (median) and 2 (IQR)], and
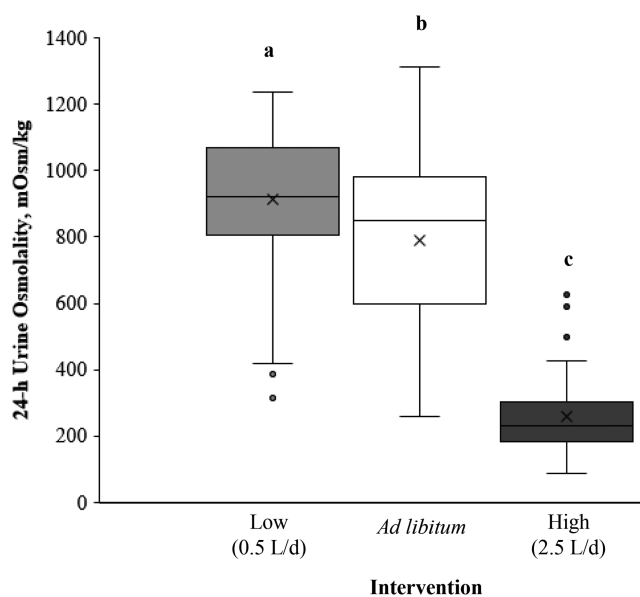
FIGURE 2 Values are 24-h urine osmolality (mOsm/kg) across interventions, $n = 70$. Treatment means without a common letter differ, $P < 0.05$.

both were significantly greater than the high intervention [3 (median) and 0 (IQR), all $P \leq 0.01$]. A significant effect of intervention [$F_{(1, 69)} = 411.0$, $P < 0.001$; $\eta^2 = 0.92$] for 24-hour urine osmolality was observed. Osmolality (mean ± SD) during the low intervention (912 ± 199 mOsmol/ kg) yielded significantly higher ($P < 0.001$) osmolality (mean ± SD) than during the AL (790 ± 257 mOsmol/kg) and high (260 ± 115 mOsmol/kg) ($P < 0.001$) intervention (**Figure 2**). Similar effects were observed for 24-h USG [$F_{(1, 69)} = 289.2$, $P < 0.001$; $\eta^2 = 0.90$], whereby the low intervention (1.023 ± 0.005) yielded significantly higher USG ($P < 0.001$) than the AL (1.020 ± 0.007) ($P < 0.001$) and high (1.005 ± 0.004) ($P < 0.001$) intervention. Furthermore, USG was lower during the high intervention compared to AL (**Figure 3**). The Wilcoxon
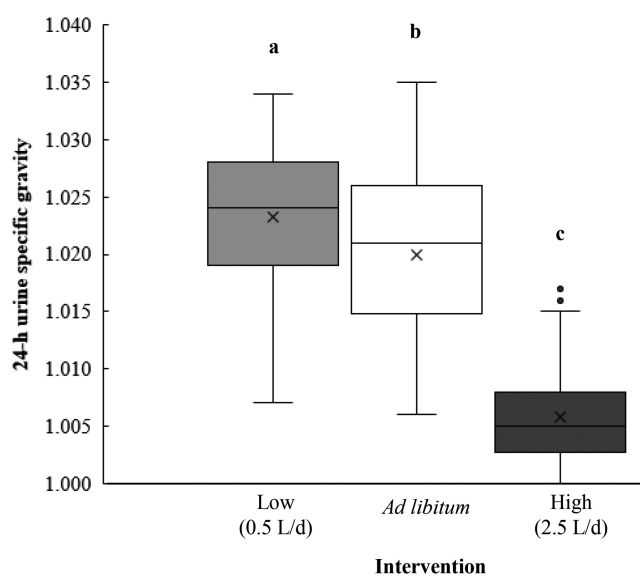

FIGURE 3 Values are 24-h urine specific gravity across interventions, $n = 70$. Treatment means without a common letter differ, $P < 0.05$.
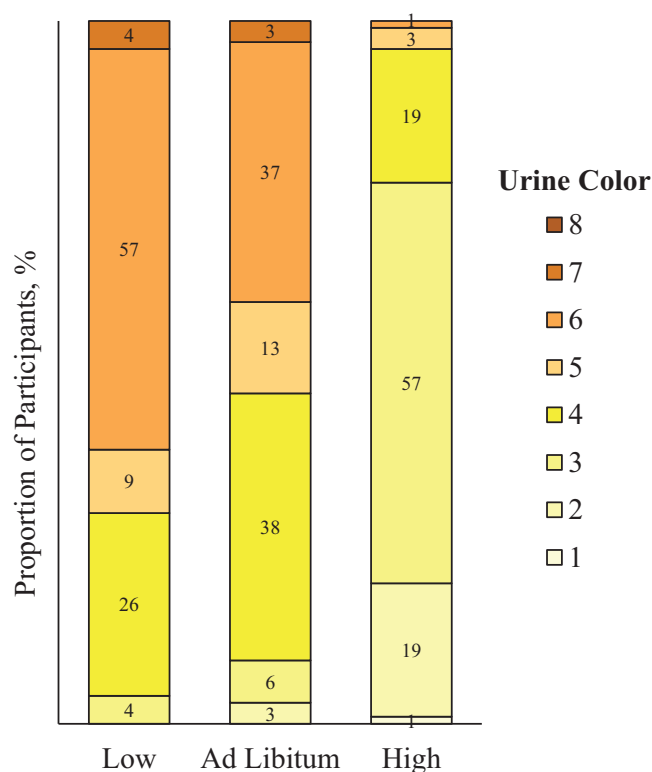

FIGURE 4 Values are proportion (%) of participants within each urine color category across interventions, $n = 70$.

signed-rank test indicated that the urine color score during the low intervention was significantly higher than during the AL ($z = -2.57$, $P = 0.01$) and high ($z = -6.93$, $P < 0.01$) intervention (**Figure 4**). In addition, the urine color score in the high intervention was significantly lower than in the AL intervention ($z = -6.75$, $P < 0.01$).

AL hydration and cognitive function

There were no associations between flanker or go/no-go performance outcomes and AL hydration (all $P > 0.05$). However, regression analyses for the switch task revealed an inverse association between accuracy and hydration (USG) during the homogeneous trials (β: -0.29, $P = 0.02$), indicating that higher accuracy was associated with better hydration. These relations persisted even after adjusting for the demographic factors of age (β: 0.24, $P = 0.05$) and SES (β: 0.29, $P = 0.01$) that were also observed to be related to accuracy during the homogeneous condition. Partial regression plots reflecting switch task relations with AL USG are illustrated in **Figure 5**. Heterogeneous accuracy followed a similar pattern (β: -0.25, $P = 0.05$) even following adjustment for age (β: -0.25, $P = 0.05$). Furthermore, there was an inverse association between switch trial accuracy within the heterogeneous condition and hydration, demonstrating higher accuracy with lower USG (β: -0.28, $P = 0.03$) even following adjustment for age (β: 0.27, $P = 0.03$). In fact, this effect was selective for the most difficult condition (i.e., switching between rulesets) since no such effect was found for nonswitch accuracy (β: -0.21, $P = 0.10$). All other associations were nonsignificant. No significant relations were observed between AL USG and RT outcomes (all $P > 0.05$).
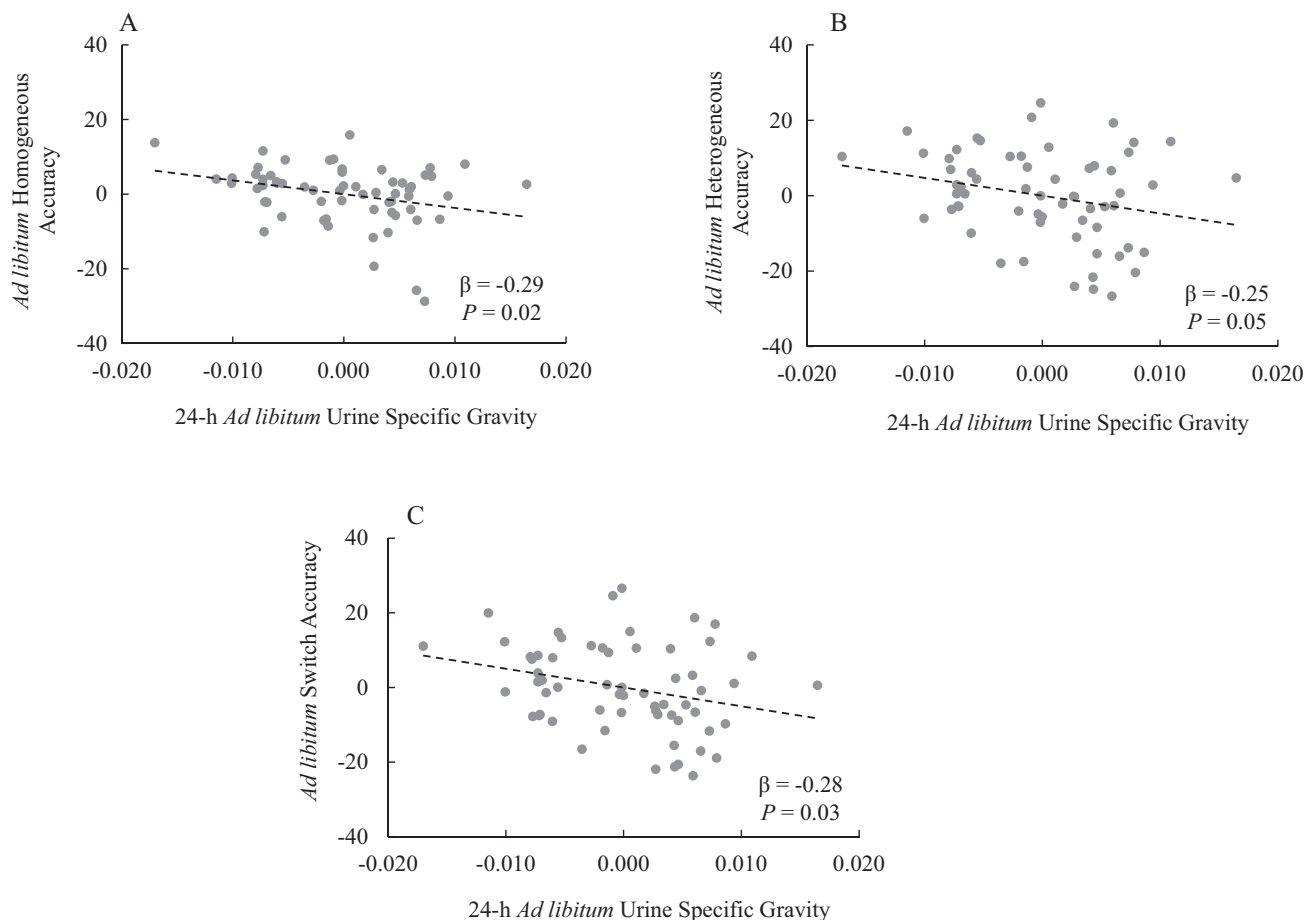
FIGURE 5 Partial regression plots illustrating inverse relations between baseline urine specific gravity and baseline switch task accuracy for homogeneous (A), heterogeneous (B), and switch trials (C) within the heterogeneous condition, *n* = 62.

Urine osmolality during the AL intervention was inversely related to accuracy during the homogeneous trials (β: −0.24, $P = 0.05$) following adjustment for the demographic factors of age (β: 0.28, $P = 0.02$) and SES (β: 0.30, $P = 0.01$). While there was not a significant relation between osmolality and overall accuracy during the heterogeneous trials (β: −0.20, $P = 0.11$), higher osmolality was selectively related to accuracy on the switch trials within the heterogeneous condition (β: −0.24, $P = 0.05$) following adjustment for age (β: 0.31, $P = 0.02$), whereas no significant relation was observed for nonswitch trials (β: −0.15, $P = 0.23$). Finally, there were no associations of flanker and go/no-go performance outcomes with AL osmolality (all $P > 0.05$).

Intervention effects on cognitive function
Descriptive information on the main cognitive task outcomes based on AL and intervention are summarized in **Table 2**. Statistical analyses for cognitive task performance are summarized in **Table 3**.

Flanker task
Neither the low nor the high water intake intervention significantly affected flanker task performance. Analyses revealed a main effect of congruency for RT, indicating the expected congruency manipulation whereby longer RT and reduced response accuracy were observed for incongruent trials. No such main effects were found for the water intervention for RT or accuracy. The analyses also did not reveal a hydration

intervention × congruency interaction for RT or accuracy. No effect of the hydration intervention was revealed for the interference effects for RT or accuracy.

Go/no-go task
Reaction time on the go task was not affected by low or high water intake. A trend for go target accuracy was observed. There were no effects for no-go target accuracy.

Switch task
Low or high intake did not affect RT or accuracy in the homogeneous compared with the heterogeneous condition. Analyses revealed a main effect of condition for RT and accuracy, indicating the expected decrease in performance during the more difficult heterogeneous condition (i.e., longer RT, lower response accuracy). No effect was found for the hydration intervention on RT or accuracy. There was no intervention × condition interaction for either RT or accuracy. Global switch cost analyses indicated a similar pattern with no effect of the intervention on RT or accuracy.

RT was slower in the low than in the high intervention for the switch compared with nonswitch conditions. Analyses revealed a main effect of condition for both RT and accuracy, indicating the expected decrease in performance (i.e., longer RT, lower response accuracy) in the more difficult switch condition. No such effect was found for the hydration intervention on accuracy. However, analyses revealed a trend for hydration intervention on RT. Post hoc analysis indicated that the low

TABLE 2 Cognitive task reaction time and accuracy among children during each of the high (2.5 L/d), low (0.5 L/d), and ad libitum interventions[1]

Characteristic	Flanker			Go/no go		Global switch			Local switch		
	Congruent	Incongruent	Interference[2,3]	Go target	No-go target	Homogeneous	Heterogeneous	Global cost[4,5]	Nonswitch	Switch	Local Switch[6,7]
RT, ms											
Ad libitum	606.1 ± 12.9	683.6 ± 16.0	77.6 ± 6.6	600.6 ± 11.7	—	676.5 ± 16.5	854.4 ± 23.2	177.9 ± 22.6	819.7 ± 20.4	889.1 ± 26.8	69.4 ± 10.6
High	600.2 ± 12.7	670.9 ± 14.8	70.8 ± 5.9	595.1 ± 13.8	—	678.9 ± 16.6	855.7 ± 22.3	176.8 ± 22.4	809.9 ± 23.5	891.7 ± 29.3	81.8 ± 10.4
Low	614.5 ± 13.4	681.4 ± 14.5	66.9 ± 5.5	606.4 ± 15.2	—	675.8 ± 16.4	870.5 ± 21.2	194.7 ± 22.0	851.9 ± 21.8	921.3 ± 25.6	69.4 ± 11.7
Accuracy, %											
Ad libitum	88.7 ± 1.3	79.7 ± 2.0	9.0 ± 1.1	94.8 ± 0.9	76.2 ± 2.2	88.2 ± 1.1	68.0 ± 1.7	20.2 ± 1.4	70.5 ± 1.8	65.5 ± 1.6	5.0 ± 0.8
High	88.6 ± 1.4	81.9 ± 1.8	6.7 ± 1.1	96.4 ± 0.6	79.0 ± 1.8	88.8 ± 1.0	67.9 ± 1.5	21.0 ± 1.4	69.8 ± 1.9	65.3 ± 1.6	4.5 ± 0.8
Low	88.2 ± 1.2	80.2 ± 1.8	8.0 ± 1.2	94.3 ± 1.1	77.6 ± 1.8	86.4 ± 1.3	68.4 ± 1.4	18.0 ± 1.6	70.5 ± 1.5	66.3 ± 1.5	4.2 ± 1.0

[1] Data presented as mean ± SEM, n = 70 (flanker), n = 54 (go/no go), n = 62 (switch). RT, reaction time; —, nonresponse trial-type (no RT measures were recorded).
[2] Reaction time interference (incongruent − congruent).
[3] Accuracy interference (congruent − incongruent).
[4] Global cost reaction time (heterogeneous − homogeneous).
[5] Global cost accuracy (homogeneous − heterogeneous).
[6] Local switch react time cost (switch − nonswitch).
[7] Local switch accuracy cost (nonswitch − switch).

intervention had a slower RT than the high intervention, but this did not survive a Bonferroni multiple comparisons correct P_{crit}. There was no hydration intervention × condition interaction for either RT or accuracy. Local switch cost analyses indicated a similar pattern with no effect of intervention for RT or accuracy.

Slower reaction time also occurred in low compared with high in the most challenging condition (homogeneous compared with nonswitch), suggesting selective reduction in performance when holding multiple rulesets in working memory. Analyses revealed a main effect of condition for both RT and accuracy, indicating the expected decrease in performance (i.e., longer RT, lower response accuracy) in the more difficult nonswitch condition. No such effect was found for the hydration intervention on RT and accuracy. There was no effect of hydration intervention × condition interaction for accuracy. However, there was a significant interaction of hydration intervention × condition for RT. Follow-up Bonferroni-corrected t tests indicated that all interventions were significantly different in the homogeneous condition, $t(1, 61) \geq 6.5$, $P < .001$. However, within the nonswitch condition, the low intervention demonstrated longer RT than the high intervention, $t(1, 61) = 2.8$, $P = 0.006$, $d = 0.29$, indicating a selective reduction in performance when holding multiple rulesets in working memory. All other comparisons were nonsignificant, $t \leq 1.9$, $P \geq .060$, $d \leq .21$. In addition, working memory cost analyses found a similar pattern of effects such that the hydration intervention was not significant for accuracy, but a significant effect was observed for RT. Post hoc pairwise comparisons indicated that the high hydration intervention indicated a significantly lower working memory cost than the low hydration intervention, $t(1, 61) = 2.8$, $P = 0.006$, $d = 0.29$. All other comparisons were nonsignificant, $t \leq 1.9$, $P \geq .062$, $d \leq 0.21$.

Discussion

Converging epidemiological evidence indicates that a large proportion of adult and child populations across the globe consumes less than the adequate intake for water from fluids set by health authorities (2, 30). While limited, biomarker data based on urinary indices of hydration also indicate that insufficient hydration is evident even among pediatric populations (1, 31). Whereas the implications of mild to moderate dehydration on cognition including mood state have been widely documented in adults (32–34), the impact of high urine concentration on cognitive health, particularly among children, has received comparatively limited attention.

The present work aimed to elucidate the effects of changes in hydration—via a water intake intervention—on cognitive tasks designed to tap multiple domains of cognitive control. As expected, the water intake intervention resulted in significant changes in urinary markers of hydration. However, it is difficult to compare our findings with previous work because deliberate water intake manipulations among children are rare. To our knowledge, this study signifies the first clinical trial that deliberately manipulated water intake over several days among preadolescent children. An important finding from the present study is that all urinary measures of hydration (i.e., color, osmolality, USG) were sensitive to modulation of water intake. Interestingly, AL hydration markers were very similar to the low intervention in which total fluid intake was restricted to 0.5 L/d: we observed that the reduction in urine osmolality, following the high intervention, was ~4-fold greater than the increase

TABLE 3 ANOVA statistics for cognitive task performance comparisons for interventions [high (2.5 L/d), low (0.5 L/d), and ad libitum] among participating children[1]

Characteristic	Reaction time				Accuracy			
	df	F	P	η^2	df	F	P	η^2
Flanker								
Intervention[2]	(2, 68)	0.93	0.40	0.03	(2, 68)	0.28	0.75	0.01
Congruency (congruent compared with incongruent)	(1, 69)	274.12	<0.01	0.80	(1, 69)	80.82	<0.01	0.54
Intervention × congruency	(2, 68)	1.15	0.32	0.03	(2, 68)	1.75	0.18	0.05
Interference[3]	(2, 68)	1.15	0.32	0.03	(2, 68)	1.75	0.18	0.05
Go/no go								
Go target intervention[2]	(2, 53)	0.52	0.60	0.02	(2, 53)	2.57	0.09	0.09
No-go target intervention[2]	—	—	—	—	(2, 53)	0.99	0.38	0.04
Global switch								
Intervention[2]	(2, 60)	0.40	0.67	0.01	(2, 60)	0.73	0.49	0.02
Condition (heterogeneous compared with homogeneous)	(1, 61)	80.01	<0.01	0.57	(1, 61)	237.44	<0.01	0.80
Intervention × condition	(2, 60)	0.87	0.42	0.03	(2, 60)	2.35	0.10	0.07
Global switch cost[4]	(2, 60)	0.87	0.42	0.03	(2, 60)	2.35	0.10	0.07
Local switch								
Intervention[5]	(2, 60)	3.02	0.06	0.09	(2, 60)	0.15	0.86	0.01
Condition (nonswitch compared with switch)	(1, 61)	79.78	<0.01	0.57	(1, 61)	73.15	<0.01	0.55
Intervention × condition	(2, 60)	0.97	0.39	0.03	(2, 60)	0.19	0.83	0.01
Local switch cost[5]	(2, 60)	0.97	0.39	0.03	(2, 60)	0.19	0.83	0.01
Working memory								
Intervention[6]	(2, 60)	1.82	0.17	0.06	(2, 60)	0.38	0.69	0.01
Condition (nonswitch compared with switch)	(1, 61)	76.21	<0.01	0.55	(1, 61)	182.61	<0.01	0.75
Intervention × condition	(2, 60)	4.21	0.02	0.12	(2, 60)	1.34	0.27	0.04
Working memory cost[6]	(2, 60)	4.21	0.02	0.12	(2, 60)	1.34	0.27	0.04

[1]*Indicates a significant intervention effect ($P < 0.05$). —, nonresponse trial-type (no reaction time measures were recorded).

[2]Intervention (ad libitum compared with high compared with low).

[3]Accuracy interference (congruent – incongruent); reaction time interference (incongruent – congruent).

[4]Global cost accuracy (homogeneous – heterogeneous); global cost reaction time (heterogeneous – homogeneous).

[5]Local switch accuracy cost (nonswitch – switch); local switch reaction time cost (switch – nonswitch).

[6]Working memory cost (homogeneous – nonswitch); local switch reaction time cost (nonswitch – homogeneous).

in osmolality following the low intervention, relative to AL hydration. Similarly, the difference in the decline in USG values following the high intervention was ~4.5-fold greater than the increase in USG following the low intervention. Our findings are consistent with the results of a previous nationally based sample of US children that indicated that many children habitually exhibit highly concentrated urine, suggestive of insufficient hydration (1). Furthermore, we observed a similar urine profile during the AL compared with fluid restriction. This is somewhat alarming since it suggests that children's habitual fluid intake is not likely to be different from the restrictive dose relied upon by our trial. Consistent with Kenney et al. (1), this suggests that many children are habitually in a state of antidiuresis/water saving, with a negative free water clearance and approaching the upper limit for euhydration set by Manz et al. (35).

Furthermore, the results of this work extend the potential implications of changes in hydration to cognitive function among preadolescents. While limited, a handful of previous intervention studies has assessed cognitive function following the provision of water, without prior induction of dehydration (12–14). Benton and Burgess (13) observed improvements in short-term memory following the provision of 200 mL of water among 8- to 9-y-olds. Edmonds and Jeffes (14) observed that children who were allowed access to water, up to 500 mL, outperformed their fluid-deprived counterparts on visual attention tasks but not visual memory or visuomotor tasks. Furthermore, Fadda et al. (12) observed that improving hydration during the school day was positively correlated

with short-term memory among 9- to 11-y-olds. However, to date, only 1 study has investigated the cross-sectional relation between usual or AL hydration and cognitive function among children (11). Specifically, Bar-David et al. (11) separated 10- to 12-y-olds into hydrated or dehydrated groups based on hydration assessed by urine osmolality. The dehydrated group exhibited lower working memory performance using a number span task and showed trends toward poorer performance on semantic flexibility and pattern identification, suggesting that children's cognitive performance is affected by dehydration in a similar manner to that observed in adults. Consistent with our *a priori* hypothesis, we observed that poorer hydration at AL, as indicated by higher urine osmolality and USG, was related to lower cognitive flexibility. This finding is congruent with previous work indicating an inverse association between hydration and children's cognitive function (11). However, a significant strength of the current work is that we relied on 24-h urine osmolality rather than a spot urine sample since previous work has indicated that urinary markers of hydration are subject to circadian variation (36). Given that no significant relations were observed for other cognitive tasks, our findings indicate that the negative influence of insufficient hydration may be domain specific rather than generalized across cognitive domains.

Examination of the intervention effects provided further support for the potentially selective effects of inadequate hydration among preadolescent children. During the switch task, the low hydration intervention demonstrated longer RT

relative to the high intervention, indicating a selective reduction in performance when holding multiple rulesets in working memory. In support of this finding, working memory cost analyses found a similar pattern of effects such that the high intervention had a significantly lower working memory cost than the low hydration intervention. Given that no significant intervention effects were found for the inhibitory control tasks, our findings selectively implicate cognitive flexibility and working memory failures due to poorer hydration.

The magnitude of changes in cognitive performance observed was numerically small. However, these effects are consistent with the findings from other behavioral interventions (e.g., physical activity/exercise), where small to moderate effects sizes are consistently shown across the literature. In previous exercise work by our team, we have observed 3–9% changes based on the type of task (37–39). Most physical activity/exercise meta-analyses support a small to moderate effect (40). Cognitive flexibility has been previously studied using switch tasks, similar to the paradigm used in the present work, to cross-sectionally link markers of diet quality to childhood executive function (41). Although physical activity interventions have been shown to improve cognitive flexibility (39), knowledge of dietary effects on children's cognitive flexibility has been limited (42). Given that previous nutritional interventions using the set of cognitive tasks used here are limited (43), it is difficult to directly compare the magnitude of effect sizes with other nutrition studies. This reflects the comparatively limited literature examining nutrient effects on cognitive function in school-aged children. Therefore, additional studies examining the influence of dietary factors are necessary to address this gap in knowledge.

Given the known importance of water intake and hydration for physiological function, surprisingly little is known regarding the mechanisms by which hydration may influence cognitive function. Nevertheless, several potential mechanisms may underlie the implications of inadequate hydration on brain and cognition. First, moderate unreplenished fluid loss (dehydration) may directly lead to shrinkage of extracellular volume, leading to reduced brain perfusion (44). Alternative hypotheses propose that cognitive dysfunction due to mild dehydration may result from the central effect of alterations in the hormonal profile. For example, dehydration is linked to higher circulating cortisol (45, 46), which contributes to cognitive decrements as well as dendritic atrophy in brain tissue, including the hippocampus (47). Indeed, findings from animal studies indicate that elevated corticosteroids worsen active learning and compromise short-term memory (48). Insufficient hydration may also affect cognitive function via alterations in neurotransmitters. For example, *in vitro* studies have observed that cellular dehydration increases glutamine (49), a major brain amino acid and precursor to neurotransmitters glutamate and γ-aminobutyric acid. Finally, MRI studies also indicate that acute dehydration affects brain structure and function. Following an exercise protocol, Kempton et al. (50) observed a correlation between loss in body mass and third ventricular volume increase, indicating that ventricular expansion occurs following acute dehydration. In a subsequent study among adolescents, Kempton et al. (51) demonstrated that dehydration following a thermal exercise protocol led to a significantly stronger increase in frontoparietal blood oxygen level dependent response during an executive function task (Tower of London) than the control condition, whereas cerebral perfusion during rest was not affected. Since this increase in blood oxygen level dependent response after dehydration

was not accompanied by a change in cognitive performance, it was proposed that an inefficient use of brain metabolic activity was observed following dehydration (51). However, these aforementioned neuroimaging studies are confounded by the exercise regimens, which also have known effects on the brain (52) and make it difficult to isolate the dehydration effects on cognition and brain.

While the present work provides valuable information pertaining to the role of water intake in influencing hydration and cognitive function among preadolescent children, there are several limitations worth noting. First, future research relying on larger study samples is necessary to apply a more comprehensive set of covariates that may have relevance to either hydration and children's cognitive function, including counterbalancing, or order effects and habitual diet quality. In addition, the administration of a broader range of cognitive tasks (e.g., spatial memory, pattern separation, and sustained attention, as well as mood) may provide further insight into the effects of changes in water intake on global or specific aspects of cognitive function in children. Finally, future research in this area is needed to determine the optimal dose of water for cognitive function and the extent to which intervention duration may affect benefits of water intake for cognitive function in children.

In conclusion, modulating water intake for a period of 4 d influenced urinary markers of hydration among preadolescent children. In addition, provision of higher water was selectively related to benefits in working memory and cognitive flexibility rather than inhibitory control. These positive relations were also evident for AL hydration and cognitive flexibility. Collectively, these findings support the benefits from 4-d water-based hydration for selective aspects of cognitive function and provide insights into the importance of habitual hydration. This work has relevance to public health given the emerging epidemiological evidence indicating that most children in the United States exhibit elevated urine concentration, suggestive of insufficient hydration.

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