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Relationship between fluid intake, hydration status and cortisol dynamics in healthy, young adult males

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ABSTRACT

Background: Previous studies have identified links between fluid intake, hydration related hormones and cortisol measured at one timepoint but have not considered how hydration may influence cortisol dynamics throughout the day. This study assessed associations between hydration status (copeptin, urinary osmolality, urine volume) and habitual fluid intake with cortisol dynamics.

Methods: The day before (DB) a 6-h laboratory visit, 29 male participants (age, 23±4y; BMI, 25.5 ± 4.3 kg/m^2; body fat, 17.3 ± 9.3 %) provided 24-h urine samples and a fasted blood sample for hydration status assessment, recorded their 24-h fluid intake for three days prior, and provided 10 saliva samples to assess cortisol dynamics from DB into the evening of the laboratory visit. Calculated indices of cortisol dynamics included: nocturnal cortisol rise (NCR – salivary cortisol rise from bed to awakening), peak salivary cortisol (peak S_{CORT} – highest cortisol of all samples), cortisol awakening response (ΔCAR – difference between high morning sample and awakening sample), area under the curve with respect to ground (AUCG) and increase (AUCI), and diurnal cortisol slope (DCS – rate of change in cortisol from awakening to bed). The relationships between fluid intake or hydration status and cortisol dynamics were analyzed by separating participants into fluid intake tertile groups and by regressing cortisol dynamics on the continuous variables of total fluid intake (TFI) or hydration biomarkers.

Results: There were no between-group differences for ΔCAR ($p = 0.89$), AUCG ($p = 0.57$), AUCI ($p = 0.48$), peak S_{CORT} ($p = 0.14$), NCR ($p = 0.95$), DCS ($p = 0.22$), or serum cortisol ($p = 0.61$). TFI was not associated with log (peak S_{CORT}) ($p = 0.49$), ΔCAR ($p = 0.61$), AUCG ($p = 0.76$), or AUCI ($p = 0.56$). Copeptin was not associated with log (peak S_{CORT}) ($p = 0.99$), ΔCAR ($p = 0.22$), AUCG ($p = 0.69$) or AUCI ($p = 0.18$). Urinary hydration markers were not associated with any measures of cortisol dynamics ($p > 0.05$). These null effects were consistent when controlling for physical activity, sleep, and body fat percentage.

Conclusion: In the absence of dehydrating stimuli, measures of fluid intake or hydration status may not be associated with cortisol dynamics in young healthy males.

1. Introduction

Low habitual fluid intake, known as underhydration [1], activates hormonal pathways responsible for conserving available fluids, including the secretion of arginine vasopressin (AVP). Prolonged activation of AVP-stimulated pathways have been associated with increased risk of obesity, diabetes, and cardiovascular disease [2]. Further

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Abbreviations:

ΔCAR – cortisol awakening response
DCS – diurnal cortisol slope
NCR – nocturnal cortisol rise
AVP – arginine vasopressin
TFI – total fluid intake
peak S_{CORT} highest salivary cortisol sample
AUCG - area under the curve with respect to ground/zero concentration
AUCI – area under the curve with respect to the increase in cortisol post awakening

clarifying the mechanisms by which underhydration contributes to these risks may better inform the development of intervention strategies designed to increase fluid intake for health promotion and disease prevention.

AVP receptors (specifically V1b) are expressed in the hypothalamic-pituitary-adrenal axis (HPA axis) and contribute to increased cortisol production, both directly in the adrenocortical cells [3] and indirectly by increasing adrenocorticotropic hormone (ACTH) production in the anterior pituitary [4]. Correlations between an increased number of V1b receptors for AVP in the pituitary and increased ACTH responsiveness suggest that AVP is a major regulator of ACTH during prolonged stress [5], thus increasing cortisol release.

Given the mechanisms linking AVP and cortisol, some studies have explored the effect of changes in hydration status on cortisol [6,7]. In humans, acute hypohydration achieved through exercise, heat exposure, or a combination of these stressors consistently increases cortisol concentrations [8]. By contrast, studies have also shown that hypohydration achieved via acute fluid restriction and heat exposure [9] or hyperosmolality induced by hypertonic saline administration [7] do not influence cortisol. Similarly, the role of low fluid intake without a dehydrating stimulus (i.e., removing both exercise and heat stress as an acute intervention) on cortisol dynamics is mixed. Based on limited data in humans, low fluid intake has been associated with higher basal cortisol concentrations in one study [10] but not another [11], while some acute interventions to increase water intake have decreased ACTH [12] and cortisol [6].

Prior cross-sectional research linking fluid intake or hydration status with cortisol have relied only on a single plasma cortisol sample [10,11]. Yet the circadian profile of cortisol is well defined, with higher concentrations in the early morning, which peak soon after awakening (i.e., the cortisol awakening response, [CAR]), and typically decrease into the evening prior to sleep (diurnal cortisol slope, [DCS]) [13] before rising into the morning time (nocturnal cortisol rise [NCR]) [14]. AVP, and its surrogate marker copeptin, also tend to follow a circadian pattern in individuals with higher copeptin concentrations, peaking between 4h00 and 6h00 and troughing between 17h00 and 19h00 [15]. Based on the pathways linking AVP and cortisol release, it is plausible that differences in AVP/copeptin may impact measures of cortisol dynamics. Both an elevated and depressed CAR have been associated with adverse health effects [16,17], and a flatter DCS has been significantly associated with adverse health outcomes, including obesity [18]. Similarly, associations have been observed between copeptin and adverse health outcomes [19], suggesting individuals with habitually low fluid intake, and thus greater copeptin concentrations, may also exhibit HPA axis dysfunction. Yet, no studies have examined associations between habitual fluid intake or hydration status and cortisol dynamics. The purpose of this cross-sectional study was to examine the relationships between fluid intake, biomarkers of hydration status and cortisol dynamics. We hypothesized that lower habitual fluid intake, urinary hydration markers indicative of underhydration, and higher copeptin would be associated with greater peak salivary cortisol, a blunted salivary nocturnal cortisol rise and cortisol awakening response, and a flatter salivary diurnal cortisol slope.

2. Materials and methods

2.1. Experimental design

Thirty male participants (age, 23±4y; height, BMI, 26.0 ± 4.7 kg/m^2; body fat, 17.4 ± 9.4 %) visited the Exercise Endocrinology laboratory for one screening visit and one Experimental Trial. Participants also participated in a Pre-Trial period where they collected their urine and recorded their food and fluid intake the day before (DB) the Experimental Trial, and a Post-Trial period where they repeated collection of their urine and recording of their food and fluid intake for the day after (DA) the Experimental Trial (Fig. 1). Participants were screened to ensure they had 1) no chronic health conditions or diseases which would alter body water regulation, 2) no previous surgery of the gastrointestinal tract that could impact body water regulation, 3) no pharmacologic drug treatment in the previous 15 days, 4) not exercising more than 10 h per week, 4) no known or suspected sleep pathologies (Pittsburgh Sleep Quality Index (PSQI) > 5), and 5) no food allergies or severe dietary restrictions. Only males were included in this study given the independent influence of changes in female sex-steroid hormones throughout the menstrual cycle on both fluid regulatory hormones [20] and cortisol dynamics [21]; although, it is prudent that future work continues whereby females are studied to explore the impact of hydration on cortisol dynamics. To ensure a range of hydration statuses were obtained, participants were also screened using an electronic version of the Brief 15-Item Beverage Intake Questionnaire (BEVQ-15) [22] to estimate habitual fluid intake over the previous 30 days, with a goal of including 10 participants within each of the following fluid intake categories: <1500 mL/day, 1500–3000 mL/day, >3000 mL/day.

One participant displayed cortisol values considerably higher than the values of the remaining participants (peak S_{CORT} = 435 ng/mL, 2000 % higher than the mean of 21.2 ng/mL) and was therefore excluded from further analyses (Fig. 2). Further investigation revealed this participant was taking isotretinoin, a medication commonly used for the treatment of acne, which may also be associated with alterations in HPA-axis activity [23]. As described in Fig. 2, one participant forgot to collect their 45-min saliva sample; this participant was not included in AUCI and AUCG analyses. Blood was not obtained for two participants; these participants were excluded from models containing copeptin but retained for other analyses. With the additional exclusion of participants who did not record or lost their fluid intake log, 27 participants were included for copeptin and cortisol analyses, and 26 participants were included for AUCG and AUCI analyses (Fig. 2). During the screening visit, participants were measured for height using a wall-mounted stadiometer, nude body mass using a standard digital scale (Tanita WB-800s plus Digital Medical Scale, TANITA, Tokyo, Japan), and body fat percentage using air-displacement plethysmography (BODPOD, COSMED USA Inc, Chicago, IL).

2.1.1. Day before and day after

Participants received two 3-liter containers to collect all urine produced for 24 h the day before (DB) and day after (DA) the experimental trial (Fig. 1). Participants were also provided instructions on recording their fluid intake for the three days prior to the experimental trial. Fluid intake was reported using a validated log (Liq.In.7) [24]. Upon awakening on DB, participants began urine collection and wore an accelerometer on their wrist to monitor their physical activity participation and sleep duration that day. Participants were also provided with containers to collect saliva samples on DB and the day of the experimental trial.

2.1.2. Experimental trial

Participants arrived at the laboratory fasted (at least 8 h) within 1–2

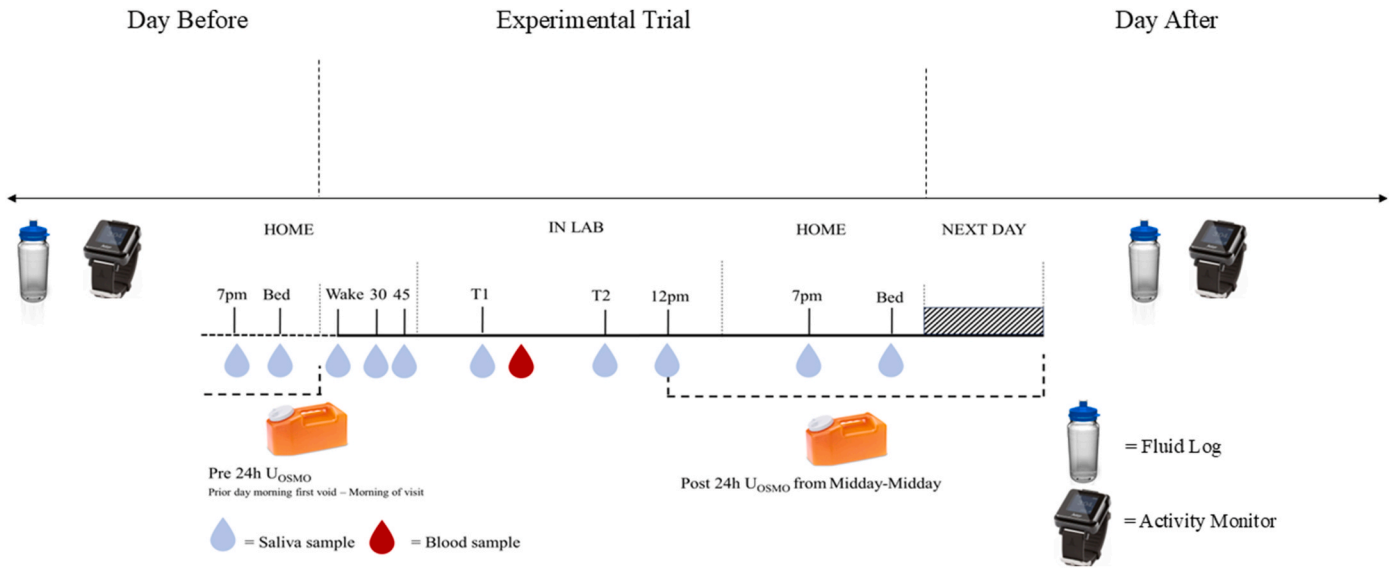

Fig. 1. Timeline of experimental protocol. T1 = 30 min post-breakfast, T2 = 2 h post-breakfast.

h of their usual wake time (0600–0900h) after the 24h of urine collection (DB) and were asked to refrain from exercise the morning of the laboratory visit. Following a 1 h resting period, participants provided a fasting blood sample and then were asked to consume, in its entirety, a standardized breakfast that included 78g of 100 % whole wheat bread, 21g of mild cheddar cheese, 17g of strawberry jam, 18g of peanut butter, 225 mL of orange juice (~546 kcals, 19g protein, 77g carbohydrate, 18g fat). Participants were not permitted to consume additional fluids prior to receiving a provided lunch at the conclusion of the visit to avoid confounding the salivary measures collected during this time. For the purposes of the data presented herein, participants provided repeated saliva samples throughout the experimental trial and for the remainder of the day (see – "Salivary" below). Following the experimental trial, participants were instructed to collect their urine for the following 24 h (DA). During this time, participants were permitted to resume their normal physical activity behaviors.

2.2. Measures

2.2.1. Urinary hydration measures

Each 24-h urine collection container was measured for urine osmolality (U_{OSMO}) and urine volume (U_{VOL}). U_{OSMO} was measured in duplicate via freezing point depression (Model 3320, Advanced Instruments, Norwood, MA). U_{VOL} was measured to the nearest 0.001g with a digital scale (Ranger 3000, Ohaus, Parsippany, NJ).

2.2.2. Saliva

Saliva samples were collected using the "passive drool" technique [25] at 19h00 and immediately before bed the night before, as well as upon awakening (S1), 30- and 45-min post-awakening the morning of the experimental trial. Participants were asked to refrain from brushing their teeth, eating, or drinking during the morning collection period. Additional saliva samples were collected at approximately 30 min (T1), and 120 min (T2) post-breakfast, and at 12h00, 19h00 and immediately before bed the day of the experimental trial for all participants regardless of start time of the experimental trial. Samples were stored at −80 °C upon receipt. Most elements of the updated consensus guidelines for the assessment of the cortisol awakening response were captured in the present design [26] (see supplementary file: CAR checklist).

Salivary samples were assessed via commercial ELISA for salivary cortisol (IBL America, Minneapolis, MN). These samples were used to estimate the cortisol awakening response (CAR), diurnal cortisol slope (DCS), nocturnal cortisol rise (NCR) and peak salivary cortisol (S_{CORT}, defined as the highest salivary cortisol sample) across participants [13]. Saliva samples were thawed and centrifuged at 18.8g for 15 min before completing the assays. All samples were assayed in duplicate, with samples reanalyzed if the coefficient of variation exceeded 25 %. All participant samples were analyzed on the same day and same plate where possible. The intra-assay CV was 7.69 %; the inter-assay CV was 18.7 %.

Individual circadian cortisol profiles were calculated for NCR, CAR, and DCS. To determine the ΔCAR, the change was calculated as the difference between the first awakening sample and the greatest concentration during the awakening period (time 30 or 45 post-awakening). Morning cortisol exposure was also calculated using the area under the curve with respect to a '0' concentration (AUCG), as well as area under the curve relative to the awakening sample (AUCI), per the methods of Pruessner et al. [27]. NCR was calculated using a mixed effects model assessing change in cortisol concentration across the 19h00 and bedtime samples measured at DB and the awakening sample the day of experimental trial. This model included a quadratic effect of time to capture the expected decline in cortisol from 19h00 to bedtime, followed by the rise in cortisol expected from bedtime to awakening. DCS was determined using a linear mixed model with individual salivary observations at level-1 and participant at level-2, similar to methods of Doane et al. [28], using the awakening, 12h00, 19h00, and bedtime sample for each participant to estimate the random slope coefficient for time via restricted maximum likelihood estimation.

2.2.3. Hematologic

Whole blood samples were allowed to clot for 20 min at room temperature before being centrifuged at 2 °C for 15 min at 1912g. Serum osmolality (S_{OSMO}) was measured in duplicate immediately using the freezing point depression method (Model 3320, Advanced Instruments, Norwood, MA). Serum samples were individually aliquoted and stored at −80 °C for further analysis. Samples were later assayed for copeptin and cortisol. Prior to analysis, samples were thawed at room temperature (~21 °C) and vortexed prior to assaying. The copeptin assay was completed via chemiluminescence sandwich immunoassays using TRACE Technology (BRAHMS Copeptin proAVP, Kryptor Compact Plus, Thermo Fisher). Samples were assayed singularly according to the manufacturer's instructions, where the automation of this method eliminates a substantial amount of inter-assay variability. The serum cortisol assay was completed on one plate via competitive ELISA (IBL

America, Minneapolis, MN). The intra-assay CV was 10.1 %.

2.2.4. Physical activity

Participants wore a wrist-worn activity monitor (GTX9 Link; Actigraph, Pensacola, FL) during DB, the experimental trial, and DA. Participants were instructed to wear this device during all activities and to only remove the device in situations where it would be submerged in water (i.e., showering or swimming). Data was downloaded and processed in Actilife software (Version 6.13.5, ActiGraph, Pensacola, Florida, USA). Raw physical activity counts captured at a rate of 30Hz were used for analyses. The activity monitor was also used to capture sleep using the Cole-Kripke algorithm [29].

2.3. Statistical analyses

Spearman correlations were used to compare the pre-screening fluid intake from the BEVQ-15 with the daily reported fluid intake from the Liq.In.7. Participants were then divided into tertiles based on average fluid intake from the 3 days of the Liq.In.7 (Table 1). Between group differences in hydration biomarkers and cortisol dynamics were analyzed using one-way ANOVA or Kruskal Wallace test, depending on variable normality assessed via the Shapiro-Wilks test and if there was heteroskedasticity between groups assessed via Levene's test. Where significant group differences were observed, Tukey HSD post-hoc tests were used for pairwise comparisons between groups.

Outcome variables of interest were also analyzed as continuous variables. Separate ordinary least squares regressions were used to predict salivary ΔCAR, AUCG, AUCI and peak S_{CORT} from fasting copeptin that morning and each 24-h urinary hydration biomarker (U_{OSMO} and U_{VOL}) from DB. In the event of a violation of normality of the residuals for these models, the outcome variable was log-transformed. To determine whether changes in cortisol throughout the day (NCR and DCS) were related to hydration biomarkers, separate mixed effects models tested the interaction effect between each hydration biomarker (copeptin, U_{OSMO} from DB, or U_{VOL} from DB) [18] and time on salivary cortisol using the sampling timepoints listed above for NCR and DCS, respectively. Time was re-coded in these analyses to represent total hours of the day after 19h00 (for NCR) or after midnight (for DCS). If awakening time or bedtime was not captured by the Actigraph accelerometer, clock times were imputed using multiple imputation by chained equations using the 'mice' package in R using predictive mean matching [30]. Ten imputations were run for these models, and parameter estimates were pooled to obtain the overall model coefficients to account for the added variability from imputation [30]. Additional analyses separately controlled for raw physical activity counts assessed via accelerometry on DB, total sleep time, awakening time, or body fat percentage on cortisol measures, given the influence of vigorous exercise participation [31] and/or altered sleep patterns [31] and body fat on cortisol dynamics.

To determine if copeptin or additional hydration biomarkers from DB or DA (U_{OSMO} and U_{VOL}) were associated with any of the individual salivary timepoints, exploratory Spearman correlations were run between each timepoint and hydration, physical activity, and sleep variables.

To ensure hydration status was not substantially impacted by the brief fluid restriction included in the lab, hydration status (U_{OSMO} and U_{VOL}) from DB and DA were compared via paired *t*-test.

Statistical significance was set at $\alpha < 0.05$ for all analyses. Data are presented as median (interquartile range)). All data were analyzed using the statistical software R (v. 4.3.1).

To the authors' knowledge, no prior investigations have assessed the relationship between fluid intake/hydration status and cortisol dynamics. As this was a secondary aim of a larger research study, no formal power analysis was conducted a priori for this specific aim. However, a power calculation was later conducted using G*Power, which suggested a sample size of 26 was sufficient to achieve ~81 % power for detecting

Table 1
Demographic characteristics of participants divided by three days of self-reported fluid intake using the Liq.In.7 survey, excluding participants with missing fluid record data (n = 3).

	Low (N = 9)	Moderate (N = 9)	High (N = 8)	Overall (N = 26)
Fluid and Hydration Biomarkers				
Average Fluid Intake (mL)				
Median [IQR]	1782 [1628.33, 1926.67]	2514.83 [2443.67, 2757.67]	4758.83 [4145.83, 5809.58]	2511.58 [1933.71, 3794.58]
Urinary Osmolality DB ($mOsm*kg^{-1}$)				
Median [IQR]	733 [600, 899.5]	485.5 [444, 751.5]	346.5* [284.25, 505.88]	542.75 [400.38, 746.88]
Urine Volume DB (L)				
Median [IQR]	0.88 [0.78, 1.36]	1.78 [1.32, 2]	1.98 [1.71, 2.36]	1.53 [0.86, 2.08]
Copeptin ($pmol*L^{-1}$)				
Median [IQR]	6.93 [5.74, 7.9]	4.36 [3.99, 4.99]	4.34 [3.76, 5.31]	4.86 [3.96, 7.23]
Serum Osmolality ($mOsm*kg^{-1}$)				
Median [IQR]	293 [289, 296.33]	293.33 [288, 295]	293 [289.25, 294.25]	293 [288.5, 295]
Serum Cortisol (ng/mL)				
Median [IQR]	154.93 [128.31, 182.84]	197.06 [149.44, 231.3]	182.44 [158.66, 198.75]	182.44 [149.44, 197.06]
Δ CAR (ng/mL)				
Median [IQR]	6.48 [3.11, 12.3]	10.15 [4.1, 15.74]	8.45 [1.32, 17.91]	7.48 [3.32, 15.27]
AUCI				
Median [IQR]	32.48 [1.17, 254.43]	163.99 [21.5, 186.64]	202.3 [-2.04, 422.13]	103.75 [1.17, 237.86]
AUCG				
Median [IQR]	483.69 [341.73, 509.51]	415.54 [203.59, 675.74]	619.45 [543.2, 776.24]	492.37 [341.73, 679.09]
S1 (Wake)(ng/mL)				
Median [IQR]	6.99 [4.97, 14.37]	8.3 [3.78, 9.84]	10.43 [6.38, 14.67]	8.37 [4.44, 11.4]
Peak SCORT (ng/mL)				
Median [IQR]	17.27 [15.19, 21.39]	18.45 [13.74, 25.66]	23.02 [20.01, 34.41]	21.12 [14.18, 25.08]
NCR (random slope coefficient)				
Median [IQR]	−1.55 [-6.34, 9.07]	−1.31 [-4.3, 1.26]	0.08 [-0.29, 3.43]	−0.4 [-5.27, 2.14]
DCS (random slope coefficient)				
Median [IQR]	0.08 [-0.08, 0.24]	0.04 [-0.1, 0.17]	−0.02 [-0.26, 0.03]	0.01 [-0.11, 0.16]
Demographic Characteristics				
Age				
Median [IQR]	22 [21, 23]	24 [21, 27]	21.5 [19.75, 26.5]	23 [20, 25.75]
BF (%)				
Median [IQR]	16.1 [12.1, 23.8]	16.1 [6.4, 19.8]	21.1 [13.9, 23.9]	16.5 [9.88, 22.65]
Race				
African American	4 (44.4 %)	0 (0 %)	2 (25.0 %)	6 (23.1 %)
Asian	1 (11.1 %)	0 (0 %)	1 (12.5 %)	2 (7.7 %)
Caucasian	4 (44.4 %)	8 (88.9 %)	3 (37.5 %)	15 (57.7 %)

(continued on next page)

Table 1 (*continued*)

Hispanic	0 (0 %)	1 (11.1 %)	2 (25.0 %)	3 (11.5 %)
BMI (kg/m^2)				
Median [IQR]	22.74 [21.07, 27.16]	23.45 [22.82, 26.38]	27.75 [24.54, 30.85]	24.52 [22.56, 28.41]
Physical Activity Counts				
Median [IQR]	153985 [141421, 323876]	223006 [197417, 588647]	747358* [544718.5, 1111017]	318970 [149479, 945237]

*$p < 0.05$ compared to 'Low'.

a bivariate regression with a slope of 0.5 between two variables with similar standard deviations.

3. Results

Demographic characteristics of the sample are presented in Table 1. After Tukey post-hoc adjustment, U_{OSMO} was significantly lower in those with high fluid intake compared to low fluid intake (MD [95%CI]; −331 [−578, −85] mOsm*kg^{-1}, p = 0.01), but there were no significant differences between high and moderate (−207 [−454, 40] mOsm*kg^{-1}, p = 0.11) or moderate and low (−124 [−363, 115] mOsm*kg^{-1}, p = 0.41) fluid intake groups. Although there was a significant main effect of group on urine volume (p = 0.03), after post-hoc adjustment, there was no statistically significant difference in U_{VOL} between those with high fluid intake and low fluid intake (p = 0.06), moderate and low fluid intake (p = 0.06), or high and moderate fluid intake (p = 0.99). There were no significant group differences for copeptin (p = 0.15), S_{OSMO} (p = 0.34), or total sleep time (p = 0.73). However, the group with high fluid intake had significantly higher activity counts compared to the low fluid intake group (MD [95 % CI]; 548.108 *10^3 [44.135*10^3 1052.082*10^3] counts, p = 0.03), but the high vs. moderate groups and low vs. moderate groups were similar (p = 0.26, p = 0.46, respectively). There were no between-group differences for ΔCAR (p = 0.89), AUCG (p = 0.57), AUCI (p = 0.48), peak S_{CORT} (p = 0.14), NCR (p = 0.95), DCS (p = 0.22), or serum cortisol (p = 0.61).

3.1. Fluid intake and hormones

Continuous average total fluid intake was not associated with log (peak S_{CORT})(β = 2.06 * 10^{-5}, [−10.56 * 10^{-5}], p = 0.739), ΔCAR (β = 8.09 * 10^{-4}, [−0.004, 0.002], p = 0.61), AUCG (β = −0.011, [−0.083, 0.061], p = 0.76), or AUCI (β = 0.017, [−0.042, 0.075], p = 0.56). Similarly, there were no significant Spearman correlations between fluid intake as a continuous variable and any individual cortisol samples (ps > 0.05) or with serum cortisol (ρ = 0.19, p = 0.37) (Figure A.1). Higher average fluid intake was significantly associated with a lower concentration of copeptin (ρ = −0.439, p = 0.03).

3.2. Urinary markers and cortisol

U_{OSMO} from the DB was not associated with log (peak S_{CORT})(β = 4.70 * 10^{-4}, [−12.43*10^{-3}, 3.0 * 10^{-4}], p = 0.22), ΔCAR (β = 5.86 * 10^{-4}, [−0.020, 0.019], p = 0.95), AUCG (β = −0.14, [−0.58, 0.30], p = 0.51), or AUCI (β = −0.120, [−0.484, 0.244], p = 0.50). U_{VOL} from the DB was not associated with log(peak S_{CORT}), (β = 3.86 * 10^{-3}, [−0.18, 0.18], p = 0.97), CAR (β = −0.77, [5.21, 3.67], p = 0.73), AUCG (β = 37.99, [−57.96, 133.93], p = 0.42), or AUCI (β = −41.10, [−120.18, 37.98], p = 0.30). U_{OSMO} and U_{VOL} from DB and DA were not correlated with cortisol at any of the individual time points or with serum cortisol (all ps > 0.05, Figure A.1).

3.3. Copeptin and cortisol

Morning copeptin was not associated with log (peak S_{CORT}) (β = 0.00, [−0.07, 0.07], p = 0.99), ΔCAR (β = 1.06, [−0.69, 2.81], p = 0.22), AUCG (β = 7.79, [−31.46, 47.05], p = 0.69) or AUCI (β = 21.29, [−10.17, 52.75], p = 0.18). Higher fasting serum copeptin was associated with lower salivary cortisol at T3 (12h00) (ρ = −0.41, p = 0.046). However, there was no significant correlation between copeptin and the other salivary cortisol values (all ps > 0.05) or between fasting serum copeptin and fasting serum cortisol (ρ = −0.13, p = 0.52). Serum cortisol was significantly correlated with salivary cortisol sampled 45 min after awakening (ρ = 0.402, p = 0.04) and 30 min post-breakfast (ρ = 0.676, p = 0.0002).

3.4. Nocturnal cortisol rise

There was a significant linear and quadratic change in cortisol from night into the morning across all NCR models (all p < 0.05); cortisol concentrations declined from 19h00 to bedtime before rising in the morning (Fig. 3). However, there was no significant interaction between time and average fluid intake (linear: β = 0.002, [−0.004, 0.008), p = 0.578; quadratic: β = −0.003, [−0.010, 0.003], p = 0.31), U_{OSMO} (linear: β = −6.40, [−17.01, 4.20], p = 0.23; quadratic: β = −0.003, [−0.010, 0.003], p = 0.45), or U_{VOL} (linear: β = 6.43, [−3.60, 16.47], p = 0.20, quadratic: β = 3.68, [−8.55, 15.91], p = 0.55).

3.5. Diurnal cortisol slope

Salivary cortisol declined significantly throughout the day in all linear mixed effects models examining the interaction between average fluid intake or hydration biomarkers from DB and cortisol concentrations (all ps < 0.01, Fig. 3). However, there was no interaction effect between average fluid intake and time (β = −0.0001, [−0.0002, 0.0001], p = 0.22), copeptin and time (β = 0.018, [−0.050, 0.087] p = 0.60), U_{OSMO} and time (β = 0.0002, [−0.001, 0.001],p = 0.70), or U_{VOL} and time (β = 0.12, [−0.10, 0.34], p = 0.30). Hydration status was similar between DB and DA based on a non-significant difference between each day for U_{OSMO} (p = 0.50), and U_{VOL} (p = 0.32), suggesting the brief fluid restriction within the lab was unlikely to impact this relationship.

Regression model results were similar when controlling for total sleep time, awakening time, physical activity counts from DB, or body fat percentage (results not shown).

4. Discussion

In this study, we examined associations between fluid intake, hydration biomarkers and cortisol dynamics. Our findings do not support our hypotheses that lower fluid intake and lower hydration status are associated with higher peak cortisol, blunted CAR, and a flatter NCR and DCS. These findings were consistent regardless of whether the predictor of cortisol dynamics was fluid intake tertiles or the continuous variables of fluid intake or urine and serum hydration biomarkers.

Cortisol dynamics were similar across fluid intake tertiles, which may be partially explained by surprisingly similar copeptin concentrations among these tertiles. Perrier et al. compared two groups ("Low Drinkers" and "High Drinkers") and found significantly higher fasting plasma cortisol concentrations among Low Drinkers (545 vs 459 nmol*L^{-1}) but did not directly assess the relationship between hydration status (U_{OSMO}: 767 mOsm*kg^{-1} vs 371 mOsm*kg^{-1}) or copeptin (2.4 vs 1.5 pmol*L^{-1}) between these groups on cortisol values [10]. By contrast, Zhang et al. observed no difference in fasting plasma cortisol or copeptin between Chinese athletes classified as "Low Drinker" or "High Drinker" [11]. The latter study aligns with our results, where copeptin was similar among fluid intake tertiles, despite differences in total fluid intake and urinary osmolality. Based on the ability of AVP to stimulate cortisol

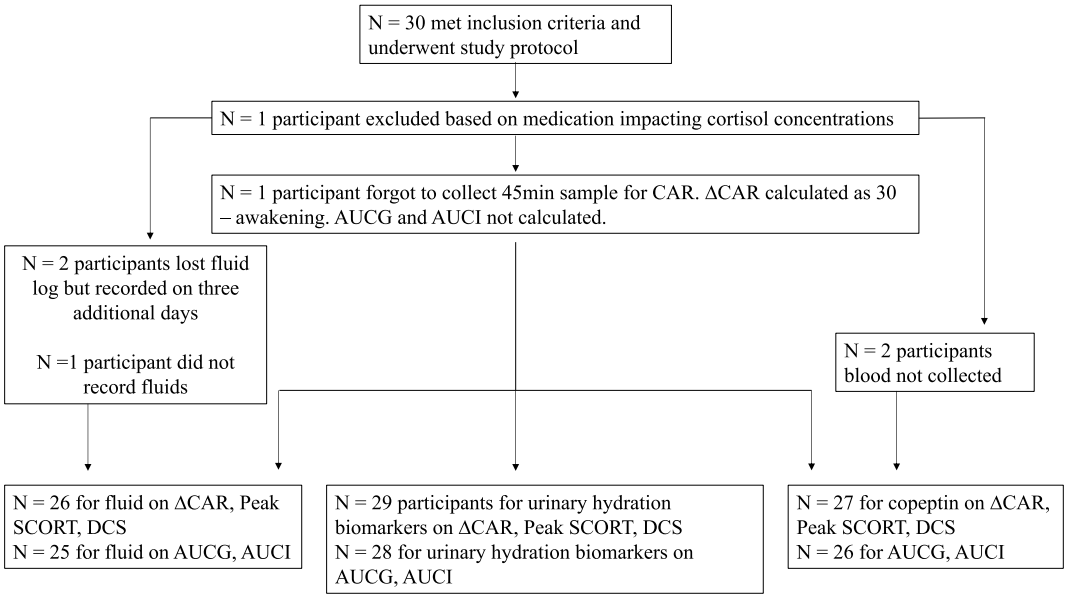

Fig. 2. Flow chart for sample size for each analysis.

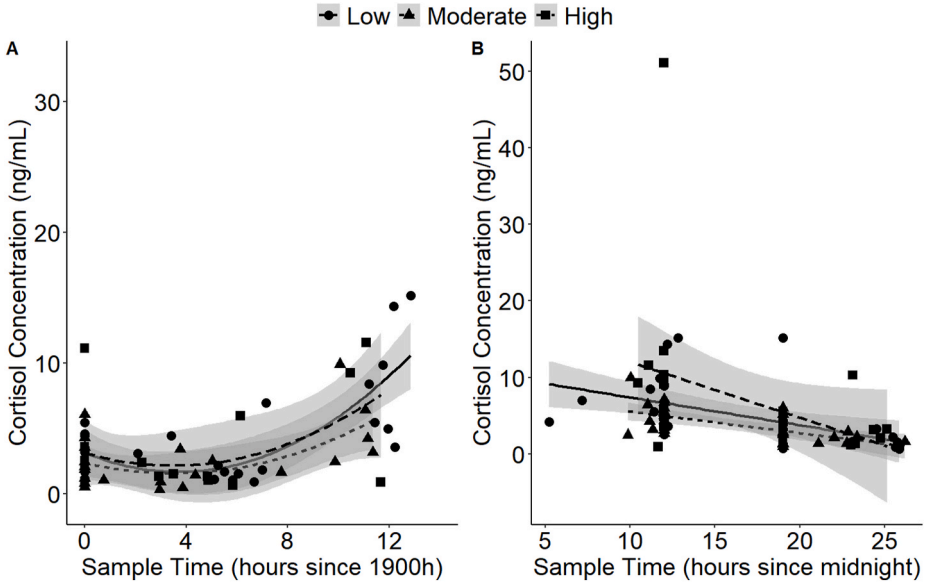

Fig. 3. NCR (A) and DCS (B), stratified by fluid intake tertile.

release both directly in the adrenal gland [3] and indirectly by enhancing ACTH release, previously observed differences in cortisol between fluid intake categories are likely dependent on corresponding differences in AVP [32]. Thus, it may be that between group differences in cortisol dynamics requires a significant difference in AVP/copeptin that may not solely driven by fluid balance. In fact, our highest observed copeptin concentration of 15.6 pmol/L^{-1} was in the "Moderate" fluid consumption tertile, further supporting the notion that factors beyond hydration (i.e., stress or physical activity) could be important contributors to the relationship between AVP/copeptin and cortisol. However, while copeptin appears to increase more in response to physiologically stressful stimuli when hypohydrated compared to euhydrated, cortisol does not always seem to follow this pattern [33]. A potential mechanism for this relationship that has been proposed but not observed is a counterregulatory reduction in the production of corticotropin releasing hormone, a stimulator of ACTH, in the parvocellular neurons of the paraventricular nucleus of the hypothalamus. This mechanism should be further investigated to help clarify the apparent disconnect between AVP and cortisol in response to certain stimuli.

In contrast to our hypotheses, the continuous variables fluid intake and hydration biomarkers ($U_{OSMO,}$ U_{VOL}, and copeptin) were also not associated with cortisol dynamics. However, higher fasting copeptin in the morning was associated with *decreased* S_{CORT} during the middle of the laboratory visit (T3 – 12h00). Perhaps the fluid consumed during breakfast (orange juice) favored a greater reduction in copeptin among participants who arrived at the lab with copeptin (and thus fluid conserving processes) already elevated, which may have driven a concurrent reduction in cortisol, although we did not collect a 12h00 copeptin measurement to confirm this. While participants were allowed to consume fluids freely prior to the laboratory visit, for the duration of the laboratory visit they were not permitted to consume additional fluids following breakfast. It is possible that had participants been given free access to fluids during this time frame that individual cortisol responses could have differed across fluid intake categories for DCS and peak

$S_{CORT.}$ A future study should determine whether *ad libitum* water intake in a similar setting would lead to different results. It has also been suggested that hydration status and nutritional status may interact to influence cortisol and glucose regulation [9]. Thus, the dietary composition of the breakfast, as well as the diet participants followed the rest of the day, may have increased the variability of individual participant cortisol responses, either independently or in conjunction with their hydration status.

Our results suggest that cross-sectional studies examining cortisol dynamics alone may not need to control for the previous day's fluid intake or hydration status among young, healthy male participants. Previous research in individuals with obesity found copeptin was not associated with *long term* cortisol exposure, although copeptin was related to both BMI and waist circumference [34]. Results in our study among healthy young males were consistent when controlling for body fat percentage, with neither copeptin nor body fat being associated with cortisol dynamics. Another study found that copeptin increases during moderate intensity exercise (75 % of VO2max) independently of fluid intake and remains elevated for at least 2 h following exercise [35]. In our study, copeptin was also not associated with cortisol dynamics when controlling for physical activity the day prior and when restricting physical activity the morning of the experimental trial. Perhaps a greater difference in fluid intake and hydration biomarkers, and subsequently AVP/copeptin, is required before cortisol dynamics are altered, as seems to be the case for associations between increased water intake and glucose regulation [36]. Future investigations may seek to expand on these findings by prescribing additional fluid in varying amounts to identify if there is a threshold of additional fluid intake required to induce a change in cortisol.

It may be that the relationship between hydration status and cortisol during the morning period is driven by the volume regulatory hormone aldosterone rather than AVP. This could explain why prior investigations inducing substantial fluid loss (as from exercise and/or heat exposure), which reduce plasma volume and increase aldosterone [37], observed increased cortisol concentrations following these types of stimuli. Perhaps these stressors could also influence the circadian pattern of aldosterone and by extension cortisol, which is supported by Gideon et al. observing a significant correlation between circadian variations in salivary aldosterone and salivary cortisol [38]. Although we did not examine plasma volume changes, the magnitude of plasma volume loss required to increase aldosterone exceeds what would be expected from the non-exercising, thermo-neutral conditions in our present study.

Perhaps the relationship between hydration status and cortisol is also dependent on the type of stressor used to alter hydration status. Jansen et al. found osmotic stress alone was sufficient to induce an increase in AVP but not cortisol [7], while directly inducing dehydration does not always increase cortisol [7,11,33]. Most investigations assessing acute effects of dehydration on cortisol induce significant water loss with exercise, heat exposure, or a combination [8,39,40], suggesting that alterations in cortisol dynamics may require greater perturbances to hydration status. As both cortisol and copeptin are shown to be influenced by acute [33,41,42] and chronic stress [33,43], it may be that previously observed associations between fluid intake and cortisol were driven by a combination of physiologic stressors known to also impact AVP/copeptin (e.g., exercise [35,44], heat exposure [45,46], or a combination [40,45]) *and* body water losses rather than the osmotic stimulation that occurs to maintain normal body water in those in a state of underhydration alone. Further complicating this relationship is the divergent effects of different types of uncontrolled stressors on circadian profiles of cortisol, particularly the CAR, with some forms of stress increasing (job stress and general life stress) or decreasing (fatigue, burnout, or exhaustion) this metric [47]. With these responses, it is possible that other hormones not captured in the present study might influence this relationship, including insulin and ghrelin.

4.1. Strengths

Our study is strengthened by including individuals from diverse ethnic backgrounds, enhancing the generalizability of these findings. This is important since previous investigations observed lower hydration levels among non-Hispanic Black compared to non-Hispanic White young adults [48,49]. We successfully obtained several saliva samples throughout the day, enabling us to capture multiple indicators of cortisol dynamics. Our methodology also allowed participants to collect these samples on their own, minimizing participant burden, enhancing the ecological validity of our results, and providing a measure of the active form of cortisol rather than total cortisol from blood. Although we were reliant on participants' self-reported adherence to the prescribed sample collection timeline, significant correlations between the serum cortisol collected at the laboratory and both the 45 min and T1 salivary cortisol timepoints suggest that participants did adhere closely to the required time course for sample collection.

The administration of the pre-screening survey (BEVQ-15) to create our desired groups allowed us to create a balanced representation from a range of fluid intake behaviors (low, moderate, high) with the appropriate corresponding differences in hydration status among these categories. Interestingly, the BEVQ-15 survey results were not correlated with the Liq.In.7 observations, suggesting some of the participants may have either 1) completed this survey incorrectly or 2) modified their fluid intake behaviors over the course of the study. Because the Liq.In.7 has been validated against doubly labeled water [24], we elected to use this tool for the observed fluid intake in the study. Regardless, for our purposes the BEVQ-15 was a useful tool for recruitment purposes in capturing a range of fluid intake behaviors for the present study.

4.2. Limitations

The cross-sectional nature of our study precludes us from making causal inferences regarding the relationship between fluid intake/hydration and cortisol dynamics. Our modest sample size also increases the risk of type 2 error, particularly for our comparisons between fluid intake tertiles. For this reason, we also ran our analyses as continuous regression models. Our results may also be influenced by the delay between the salivary measurements for cortisol and blood collection of copeptin. However, we did not observe any correlation between serum cortisol and copeptin taken at the same timepoint, although serum cortisol was correlated with the closest saliva collection timepoints (45 min and T1). Thus, we are more confident that the lack of significant findings is not influenced by either sample timing or the different media (serum vs saliva) used for assessing each hormone. Weekday measurement of CAR also tends to be higher in anticipation of stressful days [50]. While we did not control for this in our analyses, the duration of the laboratory visits typically required participants to attend on a day with fewer obligations and so, was less likely to be a factor in the present study. However, we acknowledge the potential for individual variability in psychological stress levels, and the physiological response to these stressors, as a potential contributor to variability in the cortisol response to hydration at the individual level, which may be expanded upon in future investigations by obtaining perceptual measures of stress. Although our results were similar when controlling for sleep time, we did not assess sleep quality in these analyses, though this may enhance our understanding of the relationship between hydration and cortisol dynamics. Our body composition estimates could be improved upon by delineating water compartments or improving accuracy of FFM with bioelectrical impedance or DXA and by assessing plasma volume. Further, our population consisted of young, healthy males which limits generalizability of our findings to other populations, including those with medical conditions often displaying HPA axis dysfunction, such as but not limited to Cushing's syndrome, diabetes insipidus, dysautonomia, and perhaps older adults. Our study is also limited by the exclusion of females, due to the funding source for this study and the availability of

funds to only assess male participants for this investigation. However, we suggest these findings be expanded upon by assessing female participants at the low hormone phase of the menstrual cycle (early follicular phase), where responses are most likely to be similar to male participants. Alternatively, females may be assessed throughout the menstrual cycle to expand upon the currently mixed findings regarding estrogen and progesterone fluctuations on cortisol dynamics in previous literature [51,52].

5. Conclusions

Under free-living conditions, habitual fluid intake and hydration status were not associated with cortisol dynamics in healthy, young males. Our results, combined with prior evidence, suggest that cortisol dynamics are likely robust to individual differences in fluid intake behaviors and hydration status. Provided additional factors previously shown to impact cortisol dynamics are measured or controlled for in the study design (i.e., psychological stress, physical activity, sleep) and no dehydrating stimulus is applied, future research assessing cortisol dynamics in healthy, young adult males may not need to control for habitual fluid intake or hydration status of participants when assessing cortisol dynamics.

CRediT authorship contribution statement

Mitchell E. Zaplatosch: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Laurie Wideman:** Writing – review & editing, Supervision, Resources, Methodology. **Jessica McNeil:** Writing – review & editing, Methodology. **Jesse N.L. Sims:** Writing – review & editing, Visualization, Investigation, Data curation. **William M. Adams:** Writing – review & editing, Supervision, Methodology, Funding acquisition.

Ethics approval and consent to participate

The protocol was approved by the Institutional Review Board at the University of North Carolina at Greensboro (IRB-FY23-14) and carried out in accordance with the Declaration of Helsinki. All participants provided written and informed consent prior to participation in the study.

Availability of data and materials

The datasets used and/or analyzed during the current study are publicly available through Mendeley Data at: https://data.mendeley.com/datasets/g3mn76tzw6/1.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: William M. Adams reports financial support was provided by Emerja Corporation. Laurie Wideman reports financial support was provided by Adams Sports Medicine Consulting LLC. William M. Adams reports a relationship with My Normative that includes: consulting or advisory. William M. Adams reports a relationship with Emerja Corporation that includes: consulting or advisory. William M. Adams reports a relationship with Wu Tsai Human Performance Alliance that includes: consulting or advisory. William M. Adams reports a relationship with Korey Stringer Institute that includes: consulting or advisory. William M. Adams receives royalties from Springer Nature and is also the owner of Adams Sports Medicine Consulting LLC. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cpnec.2024.100281.

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