Osmotic stimulation of vasopressin acutely impairs glucose regulation: a counterbalanced, crossover trial

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ABSTRACT

Background: Epidemiological studies in humans show increased concentrations of copeptin, a surrogate marker of arginine vasopressin (AVP), to be associated with increased risk for type 2 diabetes.

Objectives: To examine the acute and independent effect of osmotically stimulated AVP, measured via the surrogate marker copeptin, on glucose regulation in healthy adults.

Methods: Sixty subjects (30 females) participated in this crossover design study. On 2 trial days, separated by ≥7 d (males) or 1 menstrual cycle (females), subjects were infused for 120 min with either 0.9% NaCl [isotonic (ISO)] or 3.0% NaCl [hypertonic (HYPER)]. Postinfusion, a 240-min oral-glucose-tolerance test (OGTT; 75 g) was administered.

Results: During HYPER, plasma osmolality and copeptin increased ($P < 0.05$) and remained elevated during the entire 6-h protocol, whereas renin-angiotensin-aldosterone system hormones were within the lower normal physiological range at the beginning of the protocol and declined following infusion. Fasting plasma glucose did not differ between trials ($P > 0.05$) at baseline and during the 120 min of infusion. During the OGTT the incremental AUC for glucose from postinfusion baseline (positive integer) was greater during HYPER (401.5 ± 190.5 mmol/L·min) compared with the ISO trial (354.0 ± 205.8 mmol/L·min; $P < 0.05$). The positive integer of the AUC for insulin during OGTT did not differ between trials (HYPER 55,850 ± 36,488 pmol/L·min compared with ISO 57,205 ± 31,119 pmol/L·min). Baseline values of serum glucagon were not different between the 2 trials; however, the AUC of glucagon during the OGTT was also significantly greater in HYPER (19,303 ± 3939 ng/L·min) compared with the ISO trial (18,600 ± 3755 ng/L·min; $P < 0.05$).

Conclusions: The present data indicate that acute osmotic stimulation of copeptin induced greater hyperglycemic responses during the oral glucose challenge, possibly due to greater glucagon concentrations. This study was registered at clinicaltrials.gov as NCT02761434. *Am J Clin Nutr* 2019;00:1–9.

Keywords: copeptin, oral-glucose-tolerance test, dehydration, hydration, diabetes, glucagon, cellular dehydration, hyperosmolality, underhydration, hypohydration

Introduction

The prevalence of chronic metabolic dysfunction is dramatically increasing worldwide and has become both a major public health issue and a global economic burden (1). Reports from the WHO indicate that the number of people globally with diabetes has risen from 108 million in 1980 to 422 million in 2014, representing 8.5% of adults (2). Therefore, there is an urgent need to identify modifiable risk factors that could contribute to the prevention of metabolic dysfunctions and help to blunt the epidemic of type 2 diabetes (T2D). Evidence suggests that the hormone arginine vasopressin (AVP) could be another modifiable risk factor in the development of diabetes (3–5).

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Supplemental Figures 1 and 2 and Supplemental Tables 1–3 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

Data described in the article, code book, and analytic code will be made available upon request pending application and approval.

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Abbreviations used: ACTH, adrenocorticotropic hormone; AII, angiotensin II; AVP, arginine vasopressin; CRH, corticotropin-releasing hormone; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; HPA, hypothalamic-pituitary-adrenal; HYPER, hypertonic; ISO, isotonic; MAP, mean arterial pressure; OGTT, oral-glucose-tolerance test; PNa, plasma sodium; POsm, plasma osmolality; PRA, plasma renin activity; RAAS, renin-angiotensin-aldosterone system; SBP, systolic blood pressure; T2D, type 2 diabetes.

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AVP is the key hormone in the regulation of body fluid balance. However, stimulation of AVP secretion, for instance, in conditions of low water intake, appears to be a risk factor for the development of diabetes (6). Studies involving vasopressin injection, water intake manipulation, and vasopressin V1a receptor-specific blockade, have demonstrated that vasopressin and hydration play a significant role in glycemia, glucose tolerance, and liver steatosis in Zucker rats (7, 8). Similarly, research in mice has shown that lack of the V1b vasopressin receptor improves insulin sensitivity (9).

Nonetheless, given the instability and low concentration of AVP in blood, the measurement of copeptin, the stable C-terminal cleavage product of the vasopressin precursor secreted in equimolar amounts with AVP, has become a widely used AVP surrogate (10, 11). Epidemiological studies report an independent association of copeptin with hyperglycemia, higher T2D risk, diabetic cardiomyopathy, impaired insulin sensitivity, and death (3, 4, 12, 13).

Yet, studies to test the underlying effect of AVP on metabolic dysfunction remain scarce. Furthermore, many pathways, including the secretion of AVP, the hypothalamic-pituitary-adrenal (HPA) axis, and the renin-angiotensin-aldosterone system (RAAS), are interrelated (14–16) and are implicated in dysregulation of glucose homeostasis (17–19). Additionally, traditional experimental methods of increasing AVP via dehydration (exercise or heat exposure) might independently affect glucose homeostasis. Thus, the independent role of elevated AVP on glycemic control in humans remains unclear.

Therefore, the aim of the present study was to examine the acute and independent effect of osmotically stimulated AVP, measured via the surrogate marker copeptin, on glucose regulation in healthy adults. Intravenous infusion of hypertonic saline was employed to osmotically stimulate AVP; while suppressing RAAS via plasma volume expansion. The primary outcome variables of the study were AUC for glucose and insulin during the 4-h oral-glucose-tolerance test (OGTT). We hypothesized that acute osmotic stimulation of AVP, measured via copeptin, would lead to greater AUC for glucose during the OGTT.

Methods

Experimental design

In this counterbalanced, crossover, and single-blind design study, each participant completed 2 separate trials. To determine the acute effect of osmotically stimulated AVP, measured via copeptin, on glucose regulation, participants received a 120-min saline infusion followed by a 240-min OGTT (**Figure 1**). The 2 trials differed only in saline concentration during the infusion, with each subject receiving 0.1 mL/kg/min of either hypertonic saline (HYPER: 3% NaCl) to osmotically stimulate AVP (copeptin) secretion, or isotonic saline (ISO: 0.9% NaCl) to serve as control (20, 21). Subjects were sequentially assigned to ISO/HYPER or HYPER/ISO based on the order of enrollment, balanced by sex. Hence, 15 males and 15 females underwent the ISO trial first followed by HYPER whereas the rest of the subjects performed HYPER first followed by ISO, to avoid order effect.

Participants

Of 77 volunteers recruited between April 2016 and March 2017, 12 candidates failed to meet inclusion criteria, whereas 5 subjects voluntarily withdrew from the study after enrollment. Sixty adult volunteers (30 females) participated in this study [age: 39.0 ± 8.0 y; weight: 78.2 ± 15.2 kg; height: 1.70 ± 0.09 m; BMI: 26.9 ± 4.0 kg/m^2; glycated hemoglobin (HbA1c): 5.2 ± 0.3%]. The CONSORT flow diagram is shown in **Supplemental Figure 1**. Recruiting and data collection were performed at the Hydration Science Laboratory of the University of Arkansas, Fayetteville.

During the screening process, subjects were required to complete a medical history questionnaire, which was reviewed by an advanced nurse practitioner to exclude patients with diabetes, kidney disease, metabolic disorders, cardiovascular disease, and other potential fluid balance covariates such as habitual use of nonsteroidal anti-inflammatory drugs or serotonin reuptake inhibitors. To avoid enrollment of subjects with undiagnosed diabetes, HbA1c was measured from capillary blood. For female participants, the oral hormonal contraception method was permitted as long as it included a 7-d washout period during their monthly cycle. Pregnancy, lactation, or use of injectable contraceptives, however, were additional exclusionary factors. All participants signed an informed consent statement prior to enrollment. The study was approved by the institutional review board for human experimentation in accordance with the Helsinki Declaration of 1975 as revised in 1983. This trial was registered before the onset of subject recruitment at www.clinicialtrials.gov as NCT02761434.

Study controls

Experimental days were separated by 1 wk for males and postmenopausal or ovariectomized females to ensure sufficient washout (22). Female subjects with a regular menstrual cycle were tested during the early follicular phase of 2 consecutive menstrual cycles, to control for the effect of reproductive hormones on body fluid balance (23). All subjects were instructed to consume ample amounts of carbohydrates (>150 g/d) for the 3 d leading up to test day to improve the accuracy of the OGTT for glucose tolerance classification (24). For the 24 h prior to testing subjects recorded all food and fluid intake in a provided food diary and refrained from exercise, caffeine, and alcohol. In preparation for the second experimental day, subjects received a copy of their first pretrial dietary record and were asked to replicate it. To standardize the dinner prior to each testing day, subjects consumed a provided frozen meal (2 Smart Ones Spaghetti bowls; 506 kcal, 78 g carbohydrates, 10 g fat, 26 g protein), before entering a 10-h fasting period prior to the scheduled trial. During this 10-h fast, subjects were allowed to drink plain water only. Lastly, to ensure adequate hydration, subjects were instructed to consume provided bottled water based on the 80% Institute of Medicine reference values for water intake (2 L for females and 3 L for males) for the day prior to each experiment (25). These pretrial hydration guidelines were based on data from the NHANES, which reported fluid intake to account for ∼80% of total water intake (26).

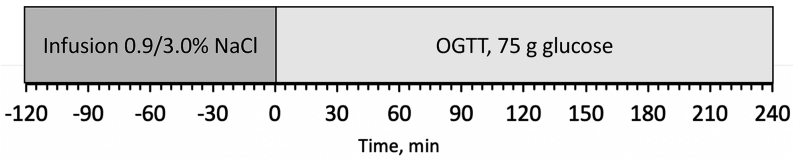

FIGURE 1 Protocol timeline. Infusion protocol started at −120 min, ending at time point 0 min. The OGTT of 75 g was administered at 0 min, sample collection continued until 240 min. OGTT, oral-glucose-tolerance test.

Study protocol

All subjects reported to the laboratory following a 10-h fast and provided a urine sample. Hydration status was assessed by measuring urine specific gravity, with values <1.020 required to proceed with the protocol (16). Subjects then sat in a comfortable chair and were not allowed to stand up or lie down until the end of the experiment to avoid intercompartmental body fluid shifts associated with changes in body posture (27). An intravenous catheter was placed into an antecubital vein and subjects rested for ≥20 min prior to baseline blood sampling. Following the baseline blood sample collection, saline was infused via an intravenous pump (Baxter Flo-Guard 6201; Baxter Healthcare Corporation) for 120 min, at a constant rate of 0.1 mL/kg/min. Following the infusion, a blood sample was drawn, and subjects ingested within 5 min a 239-mL standardized glucose beverage (Azer Scientific) containing 75 g of glucose. Throughout the intravenous infusion (120 min) and subsequent OGTT (240 min), blood samples were taken every 30 min for a total of 13 samples. Blood pressure was recorded right after each blood draw from the noncatheterized arm in duplicate (Tango+; SunTechMedical Inc). Mean arterial pressure (MAP) was calculated based on diastolic (DBP) and systolic blood pressure (SBP) with the following equation: MAP = DBP + [(SBP − DBP)/3] (28, 29).

Biochemical analysis

At each time point, blood samples were analyzed for plasma osmolality (POsm; freezing point depression), plasma sodium (PNa) concentration (ion-sensitive electrodes), and total plasma protein concentration (refractometry). Plasma volume changes were calculated based on hematocrit and hemoglobin concentration with the Dill–Costill equation (30). Copeptin was measured with random access immunoanalysis (BRAHMS Kryptor Compact Plus; ThermoFisher). The range of the standard curve in this assay was 0.7–500 pmol/L. Concentrations detected between 4 and 15 pmol/L had intra-assay and interassay CVs of <8% and <10%, whereas concentrations detected between 15 and 50 pmol/L had intra-assay and interassay CVs of <4% and 5%, respectively. Concentrations of glucagon (intra-assay CV 4.2%, interassay CV 13.1%), insulin (intra-assay and interassay CVs: <4.7%), C-peptide, human corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and cortisol were measured with ELISA. Blood glucose (intra-assay CV 3%, interassay CV 3%), free fatty acids, and triglycerides were quantified by spectrophotometry. Aldosterone and plasma renin activity (PRA) were assessed by LC/tandem MS, whereas angiotensin II (AII) concentrations were determined by RIA. Insulin resistance was assessed with the HOMA-IR at the end of the saline infusion (31), and insulin sensitivity during the first 120 min of the OGTT test based on the Matsuda index (32).

Outcomes and measurements

The primary outcomes of this study were the positive integer of the AUC for glucose and insulin and insulin sensitivity (Matsuda index) during the OGTT. Secondary outcome measures were POsm, PNa, changes in plasma volume, copeptin, glucagon, C-peptide, PRA, AII, and cortisol at the end of the infusion and during the OGTT.

Sample-size calculation

Sample size was calculated on pilot data collected from 4 subjects. To detect differences >10% in glucose AUC, which was 1 of our primary outcomes, a sample of 52 subjects was required to reject the null hypothesis that the means of our subjects are similar between the 2 experimental trials with a probability of 0.8 and type 1 error of 0.05.

Statistical analysis

Data were analyzed by repeated-measures ANOVA to assess within-subject responses across conditions, time, and condition by time. Bonferroni correction was used as post hoc. Baseline data were assessed for normality via the Shapiro–Wilk test. The primary outcomes were the AUC for glucose and insulin, calculated as the positive integer above the postinfusion baseline value (0 min) as previously suggested (33, 34), using MATLAB (MathWorks Inc). All data are reported as means ± SDs unless otherwise noted. Statistical significance was set a priori at an alpha of 0.05. Statistical analysis was performed using the software package JMP Pro 14.1 (SAS Institute Inc).

Results

Subject demographics

Female participants were significantly older, with less lean body mass than males. No overall sex differences in HbA1c, BMI, or trunk fat existed (**Table 1**).

Hypertonic saline infusion

POsm, PNa, and copeptin at the onset of the infusion (−120 min) did not differ between trials ($P > 0.05$). During the HYPER trial, POsm, PNa, and copeptin increased and remained elevated during the entire 6-h protocol (**Figure 2**A,

TABLE 1 Subject demographics[1]

	Females	Males	Total
n	30	30	60
Age, y	41.4 ± 8.3*	36.5 ± 7.0	38.9 ± 8.0
HbA1c, %	5.2 ± 0.3	5.2 ± 0.3	5.2 ± 0.3
BMI, kg/m^2	26.7 ± 4.6	27.1 ± 3.4	26.9 ± 4.0
Trunk fat, kg	13.82 ± 6.3	12.65 ± 5.4	13.24 ± 5.85
Lean body mass, kg	40.7 ± 6.3*	58.6 ± 6.4	49.49 ± 1.10

[1]Independent *t* test was conducted to compare means in demographic variables between genders. Data are means ± SDs ($n = 60$). *Difference between genders, $P < 0.05$. HbA1c, glycated hemoglobin.

B, and D). Plasma volume was significantly expanded in both trials; however, the expansion was greater during HYPER compared with the ISO trial (Figure 2C), despite using the same infusion volume for both trials (938 ± 183 mL), at a rate of 0.1 mL/kg/min. Due to plasma volume expansion in both protocols, measured concentrations were affected. Plasma volume expansion was accounted for by taking the respective concentration value at a given time point and multiplying this value by the sum of 100 and the plasma volume expansion amount at the respective time point, before dividing this product by 100. Results with values corrected for plasma volume expansion are shown in **Supplemental Figure 2** and in **Supplemental Tables 1–3**. Aldosterone, AII, and PRA were within the lower normal physiological range at the beginning of both protocols, but only PRA declined significantly following infusion (**Table 2**). MAP did not change in response to infusion during the HYPER (pre: 86.2 ± 8.6 mmHg; post: 82.7 ± 7.2 mmHg) or the ISO trial (pre: 84.5 ± 7.9 mmHg; post: 83.3 ± 6.85 mmHg; $P > 0.05$).

Glycemic response to the OGTT

Fasting plasma glucose concentrations did not differ between trials ($P > 0.05$) at baseline (−120 min) and remained unchanged during the 120 min of saline infusion for both trials (**Figure 3**). The positive integer for the glucose AUC during the OGTT (primary outcome) was greater in HYPER (401.5 ± 190.5 mmol/L/min) compared with the ISO trial (354.0 ± 205.8 mmol/L/min; $P < 0.05$). Also, ANOVA with repeated measures for glucose concentration showed significant main effect of time ($P < 0.0001$) and trial ($P = 0.049$), but not for interaction ($P > 0.05$; Figure 3). The positive integer of the AUC for plasma copeptin during the OGTT was positively associated with the AUC for glucose ($R^2 = 0.07$; 95% CI: 0.01, 0.49; $P = 0.04$) only in the HYPER, but not in the ISO trial ($R^2 = 0.04$; $P = 0.13$). Baseline serum insulin concentrations did not differ between conditions ($P > 0.05$) and remained unchanged during the saline infusion. The plasma insulin response during the OGTT is shown in Figure 3. The positive integer for the insulin AUC during the OGTT (primary outcome) did not differ between trials (HYPER: 55,850 ± 36,488 pmol/L/min; ISO: 57,205 ± 31,119 pmol/L/min; $P > 0.05$). However, when the positive integer of the AUC was calculated for the first 60 min of the OGTT, it was lower during the HYPER compared with the ISO (11,490 ± 6659 pmol/L/min compared with 15,442 ± 10,295 pmol/L/min; $P < 0.001$). The AUC of copeptin during the OGTT

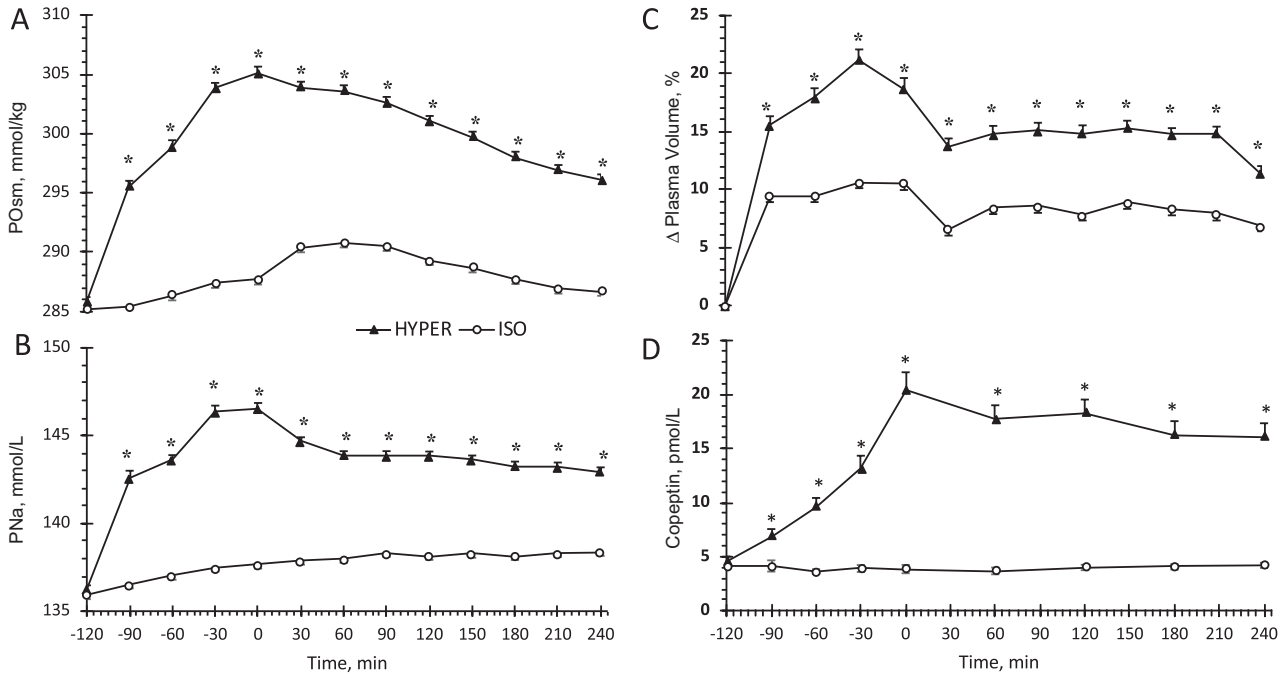

FIGURE 2 Plasma concentrations during the hypertonic and isotonic conditions for: (A) plasma osmolality (POsm, trial × time-point interaction $P < 0.001$); (B) plasma sodium (PNa, trial × time-point interaction $P < 0.001$); (C) changes in plasma volume (trial × time-point interaction $P < 0.001$); and (D) copeptin (trial × time-point interaction $P < 0.001$). Data points are means with error bars depicting SE of the mean ($n = 60$). Repeated measures ANOVA was used to assess main effects, whereas contrast comparisons were used to assess differences between individual timepoints. *Difference between conditions for the same time point, $P < 0.05$. HYPER, hypertonic; ISO, isotonic; PNa, plasma sodium; POsm, plasma osmolality.

TABLE 2 Fluid regulatory hormone responses[1]

	HYPER			ISO		
Time, min	−120	0	240	−120	0	240
Aldosterone, pmol/L	144 ± 96	58 ± 30	55 ± 28	145 ± 95	61 ± 33	70 ± 3
Angiotensin II, pmol/L	22.0 ± 10.7	16.3 ± 6.5	20.8 ± 12.1	23.9 ± 10.8	21.9 ± 9.1	24.0 ± 8.6
Plasma renin activity, ng·mL/h	0.86 ± 0.57	0.43 ± 0.24*,†	0.57 ± 0.49*	0.95 ± 0.50	0.66 ± 0.39†	0.99 ± 0.61

[1]Repeated measures ANOVA was used to assess main effects, whereas contrast comparisons were used to assess differences between individual time points. Trial × time-point interactions were: aldosterone $P = 0.62$, angiotensin II $P = 0.46$, and plasma renin activity $P < 0.05$. Data are means ± SDs ($n = 60$). *Difference between conditions at given time point, $P < 0.05$; †difference from baseline of the same trial (−120 min), $P < 0.05$. HYPER, hypertonic; ISO, isotonic.

was not significantly associated with the AUC for insulin in the HYPER ($R^2 = 0.03$; $P = 0.21$) or the ISO trials ($R^2 = 0.01$; $P = 0.47$). Similarly, BMI was not significantly associated with the AUC of glucose (HYPER: $R^2 = 0.01$; $P = 0.51$; ISO: $R^2 = 0.001$; $P = 0.98$), or insulin (HYPER: $R^2 = 0.01$; $P = 0.50$; ISO: $R^2 = 0.002$; $P = 0.75$). C-peptide values did not differ between the 2 trials during the 120 min of infusion ($P > 0.05$). The positive integer for the C-peptide AUC during the OGTT was smaller in HYPER (305.1 ± 123.5 nmol/L/min) compared with the ISO trial (324.4 ± 120.1 nmol/L/min; $P < 0.05$). During the OGTT C-peptide was not different at 30 and 60 min of the HYPER trial (1.06 ± 0.51 and 1.95 ± 0.65 nmol/L) compared with the ISO trial (1.41 ± 0.66 and 2.19 ± 0.85 nmol/L; $P > 0.05$). Similarly, HOMA-IR (HYPER: 1.58 ± 0.97; ISO: 1.61 ± 1.09; $P > 0.05$) and the Matsuda index of insulin sensitivity (HYPER: 9.2 ± 5.2; ISO: 9.0 ± 4.9) did not differ significantly between trials ($P > 0.05$).

Serum glucagon concentration did not differ before saline infusion onset between the 2 trials (HYPER: 80 ± 17; ISO:

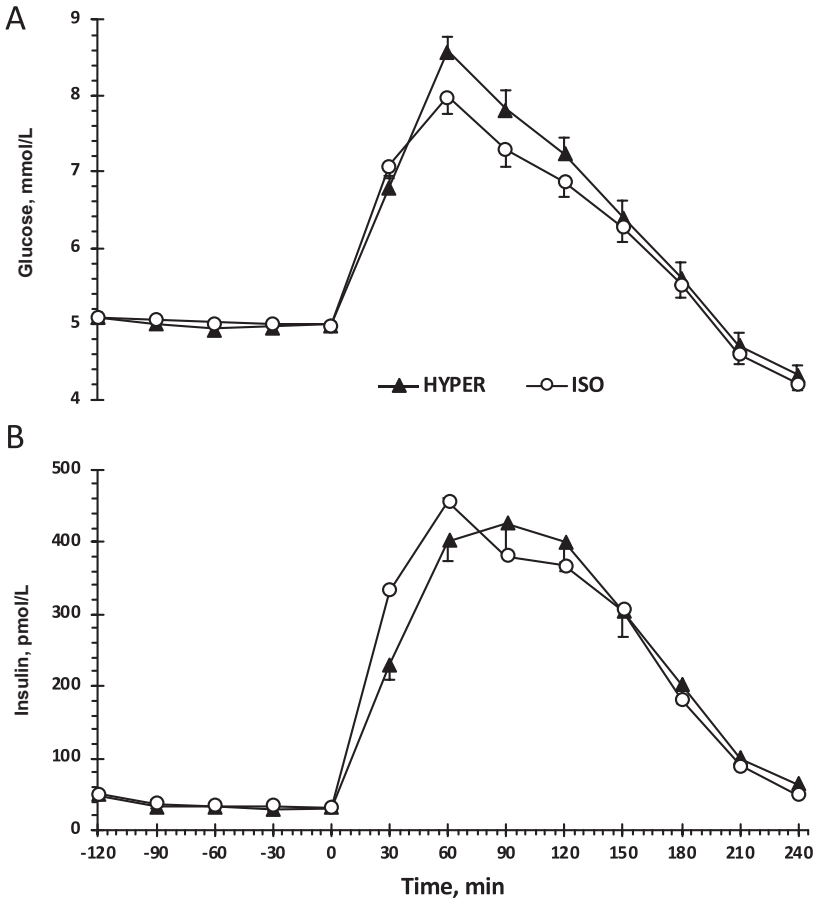

FIGURE 3 Glycemic responses during both conditions for (A) glucose (trial × time-point interaction $P = 0.14$, main effect of time $P < 0.0001$, and trial $P = 0.049$) and (B) insulin (trial × time-point interaction $P = 0.68$, main effect of time $P < 0.0001$, and trial $P = 0.69$). Data points are means with error bars depicting SE of the mean ($n = 60$). Repeated measures ANOVA was used to assess main effects and interactions. HYPER, hypertonic; ISO, isotonic.

TABLE 3 Glucagon and hypothalamic-pituitary-adrenal axis hormonal response[1]

	HYPER					ISO				
Time, min	−120	0	60	120	240	−120	0	60	120	240
Glucagon, ng/L	80 ± 17	75 ± 16	84 ± 18	82 ± 19	78 ± 17	80 ± 16	73 ± 14	79 ± 17	79 ± 17	77 ± 17
CRH, ng/L	518 ± 388	461 ± 363	493 ± 390	505 ± 413	455 ± 338	519 ± 467	484 ± 427	513 ± 461	509 ± 428	523 ± 437
ACTH, pmol/L	4.0 ± 2.5	3.6 ± 2.2	3.3 ± 1.8	3.2 ± 1.6	3.5 ± 2.0	3.8 ± 2.5	2.8 ± 1.6	3.4 ± 2.0	3.0 ± 1.8	3.8 ± 2.2
Cortisol, nmol/L	367 ± 205	259 ± 146	292 ± 163	223 ± 118	292 ± 145	410 ± 186	228 ± 130	248 ± 116	249 ± 124	276 ± 146

[1]Responses of glucagon, CRH, ACTH, and cortisol during the 3% (HYPER) and 0.9% (ISO) trials. Repeated measures ANOVA was used to assess main effects, whereas contrast comparisons were used to assess differences between individual time points. Trial × time-point interactions were: glucagon $P = 0.85$, CRH $P = 0.98$, ACTH $P = 0.34$, and cortisol $P = 0.22$. Data are means ± SDs ($n = 60$). ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; HYPER, hypertonic; ISO, isotonic.

80 ± 16 ng/L). However, it decreased significantly as a response to the isotonic saline (73 ± 14 ng/L; $P < 0.05$), which was not seen during the hypertonic infusion (75 ± 16 ng/L). No statistically significant differences for glucagon at any particular time point were observed between the 2 trials during the OGTT ($P > 0.05$; **Table 3**). However, the positive integer for the AUC of glucagon during the OGTT was significantly greater in HYPER (19,303 ± 3939 ng/L min) compared with the ISO trial (18,600 ± 3755 ng/L·min; $P < 0.05$). BMI was not associated with the glucagon AUC response during the OGTT (HYPER: $R^2 = 0.0002$; $P = 0.92$; ISO: $R^2 = 0.002$; $P = 0.76$). Serum free fatty acids and triglycerides decreased as a response to the 75 g of glucose ingested during the OGTT (see Supplemental Table 3); however, no statistical difference was observed between the 2 trials ($P > 0.05$).

The HPA axis

The HPA axis hormonal responses appear in Table 3. CRH, ACTH, and cortisol did not change during the experiment and no statistical difference was observed between trials ($P > 0.05$).

Discussion

The aim of the present study was to examine the acute and independent effect of osmotically stimulated AVP, measured via copeptin, on glucose regulation in healthy adults. Our main finding was that the glycemic response during the OGTT under the hypertonic condition was significantly greater than the isotonic control. These data suggest that osmotically stimulated AVP (copeptin) could acutely impair glucose homeostasis.

The study design successfully increased POsm during hypertonic infusion, and elevated AVP, measured via copeptin, for the 6-h protocol. Furthermore, our experiment suppressed the RAAS by baroreceptor loading due to volume increase. Hence, we were able to osmotically stimulate the secretion of AVP while keeping the RAAS suppressed, therefore enabling the assessment of its independent impact on glycemic response during an OGTT. Copeptin concentrations observed during both trials were comparable with higher physiological ranges assessed in free-living individuals (3).

The 4-fold increase in copeptin achieved during the present experiment yielded a modest, yet significant increase in glucose and glucagon. Similarly Spruce et al. (35) reported rates of glucose appearance and glucagon concentration to be significantly increased in response to moderate vasopressin infusion, suggesting a link between circulating vasopressin and glucose kinetics. Furthermore, Keller et al. (36) reported increased rates of glucose appearance assessed via stable isotope tracer methodology in a similar experiment employing infusion of hypertonic saline to shrink cell volume. Even though AVP was not measured by Keller et al., we can infer that the achieved >300 mmol/kg POsm stimulated endogenous AVP secretion. In contrast, a recent study by Carroll et al. (37) reported no differences in glycemic regulation assessed by an OGTT in acutely hypohydrated adults. In this study, subjects underwent 2 passive-heating trials followed by either fluid replacement (control) or fluid restriction (hypohydration) to induce the desired testing condition before an OGTT was administered. However, passive heating per se has been reported to affect glucose and insulin concentrations (38). Thus, it is possible that the heat stress to which subjects were exposed for both conditions might have affected the findings.

In the current study the overall AUC for insulin and C-peptide did not differ between conditions during the OGTT. However, a significantly lower AUC for insulin during the first 60 min of the OGTT for HYPER was detected, which could have contributed to the higher glucose AUC. Interestingly, no differences were seen in insulin resistance (HOMA-IR) at the end of saline infusion, or sensitivity (Matsuda index) during the OGTT. These data contrast with previous studies indicating that elevated copeptin and hyperosmolality were factors contributing to insulin resistance (13, 22). One possibility for this difference could be the hyperosmotic hypervolemia that loaded the baroreceptors to deactivate the RAAS. With aldosterone and AII concentrations kept low, their reported effect on glucose uptake at the level of the skeletal muscle could be minimized (39–41), explaining the lack of effect seen in Matsuda and HOMA-IR indices.

Although AVP is well known for its antidiuretic and pressor effects, AVP receptors exist in multiple locations suggesting a broad range of effects. V1a receptor activation increases hepatic glycogenolysis, and gluconeogenesis in vitro and in animal experiments, eliciting an elevation in plasma glucose concentration (7, 8, 42). Moreover, AVP stimulates glucagon and its action on hepatocytes to increase glycemia, whereas AVP can also induce glycolysis independently. Lastly, AVP-specific V1b receptors have been identified within the alpha and beta cells of the islets of Langerhans and have been implicated in stimulating both insulin and glucagon (7, 42–44) secretion. Based on these previous reports, the higher glucose AUC during the OGTT in the AVP-stimulated condition could be the result of the activation of: *1*) the V1a receptors, yielding in hepatic release of glucose; and *2*) the V1b receptors, explaining the increased glucagon concentrations.

Previous studies in humans have suggested an impaired suppression of glucose production induced by cortisol infusion (19). Recently, Johnson et al. (45) reported glycemic impairment following 3 d of decreased water intake in T2D subjects during an OGTT to be associated with elevated cortisol. AVP also has been shown to stimulate ACTH secretion directly, in the absence of CRH stimulus, which in turn elevates cortisol secretion (46). However, in our study no differences in ACTH, CRH, or cortisol were observed suggesting that the AVP-driven stimulation of the HPA axis might not be an acute stress response (47).

Epidemiological evidence has associated low water intake with higher blood glycemia (6, 48, 49), whereas interventional trials report that days or weeks of increased water intake can lower circulating copeptin (50, 51) or AVP (52). Enhorning et al. (50) examined glycemic responses of healthy adults following a 1-wk increased water intake intervention. Here, increased water intake in low-drinkers with high baseline copeptin led to lower fasting glucagon without any changes in insulin. Furthermore, a study by Lundegaard Asferg et al. (53) measured higher copeptin and glucagon concentrations in obese men compared with normal controls, whereas copeptin was associated with glucagon concentrations independent of weight status. As elevated glucagon has been identified as a T2D risk factor, these data might suggest water intake as a potential intervention strategy to lower AVP (50, 54–57).

The present study comes with limitations. In previous reports, both cell shrinkage and vasopressin have been linked to glucose regulatory impairments. The employed protocol does not allow to distinguish whether the observed hyperglycemic effect was driven by cell shrinkage and/or AVP. However, both stimuli coexist during naturally occurring states of dehydration. Data from studies of experimental animals (7, 8) and humans where exogenous vasopressin infusion was employed indicate that AVP seems to have a direct effect on hyperglycemia (35). Another limitation of the osmotic stimulation protocol is that despite the same infusion volume in both trials, hypertonic infusion led to greater osmotic pressure gradients and plasma volume expansion. Subsequently, isotonic fluid could move from the vascular to the interstitial space, whereas the hypertonic fluid drew fluids from the interstitial and intracellular space into the vasculature via osmosis. As a result, the greater plasma volume present in the vasculature could have diluted the concentration of blood measures leading to lower measured concentrations. However, because systemic receptors are sensing concentration and not total content, the uncorrected values are presented in the article. The data corrected for this dilution effect due to plasma volume expansion in both trials appear online as Supplemental Figure 2, presenting similar responses. Because this was a first exploratory study aiming at assessing endogenous AVP stimulation independently of the RAAS, future studies should incorporate gold standard methodologies of glucose metabolic testing, allowing for sensitive assessments of hepatic glucose production, insulin secretion, and insulin resistance. Furthermore, to tease out the effect of AVP on glucose metabolism independent of cellular shrinkage, the application of exogenous AVP via infusion within the presently reported physiological ranges should be considered.

In summary, the present data indicate that acute osmotic stimulation of copeptin, measured as a surrogate marker of AVP, led to a modest, yet significantly greater hyperglycemic response during an oral glucose challenge, potentially associated with a greater glucagon response.

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