Hydration, arginine vasopressin, and gluco-regulatory health in humans: A critical perspective

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Abstract

Gluco-regulatory diseases, such as type 2 diabetes are currently a key public health priority. Public health messages have started to include the addition of water in their dietary guidelines. Such guidelines however are not based on causal evidence pertaining to the health effects of increased water intake, but rather more heavily based upon non-causal or mechanistic data. One line of thinking linking fluid intake and health is that hypohydration induces elevated blood concentrations of arginine vasopressin (AVP). Research in the 1970s and 1980s implicated AVP in gluco-regulation, supported by observational evidence. This important area of research subsequently appeared to stop until this century during which interest in hypertonic saline infusion studies, animal AVP receptor knockout models, dietary and genetic associations, and human interventions manipulating hydration status have resurged. This narrative review briefly describes and critically evaluates the usefulness of the current AVP-gluco-regulatory research. We offer suggestions on how to test the independent gluco-regulatory effects of body mass reductions versus elevated circulating AVP concentrations, such as investigating hydration manipulations using 3,4-Methylenedioxymethamphetamine. Whilst much research is still needed before making firm conclusions, the current evidence suggests that although AVP may only be partially implicated in gluco-regulation; more ecologically valid models using human participants suggests this effect is independent of hydration status. The key implication of this hypothesis if confirmed in future research is that manipulating hydration status to reduce circulating AVP concentrations may not be an effective method to improve gluco-regulatory health.

Key words: vasopressin, copeptin, hydration, health, metabolism, glycemia, type 2 diabetes, diabetes insipidus, syndrome of inappropriate anti-diuretic secretion, MDMA
Introduction

Historically, research in hydration focused on the extreme ends of hydration status. Specifically, the ill-effects of severe hypohydration in soldiers under extreme conditions was investigated, resulting in guidelines for optimal sports performance\textsuperscript{1}. Following this, public health guidelines started to incorporate hydration recommendations; a more detailed description of this shift has been reviewed previously\textsuperscript{1}. Briefly, such recommendations may have oversimplified the complex relationship between fluid intake and health. One of the most prominent examples is the Institute of Medicine guidelines which noted that serum osmolality stays within a well-defined range across a multitude of fluid intakes; subsequent guidelines were therefore based on median intakes of self-reported fluid ingestion (‘adequate intake’)\textsuperscript{2}. Thus, to date, fluid intake guidelines have not been based on evidence pertaining to improved health, such as lower risk of gluco-regulatory diseases (e.g. type 2 diabetes [T2D]).

Understanding the true causal role of hydration status in health and disease is important in order that guidelines are based on the best possible evidence. Increasing fluid intake (particularly from water) as a means to manipulate hydration status (and therefore potentially health) represents a low cost and easy to understand intervention. Further, the addition of water to the diet does not remove hedonically rewarding foods or beverages which may contribute to poor adherence when implementing a dietary intervention, though more research is certainly warranted to understand adherence to recommendations surrounding increasing fluid intake.

One key mechanism linking hydration status to gluco-regulatory health is arginine vasopressin (AVP) which is a hormone implicated in body water regulation. This hormone is typically known for its impacts on blood pressure regulation, whereby hypohydration (as detected by a 1-2 % increase in serum osmolality) is met by an increase in circulating AVP. The result of this is V2 receptor binding in the collecting ducts of the kidney, signalling an increase in aquaporin expression and redistribution to the luminal membrane. This increases water reabsorption, therefore preventing a reduction in blood volume when water intake is low. However, it is important to acknowledge that many factors can alter circulating AVP concentrations, including genetics, ambient temperature, recent fluid intake, and stress. Thus,
the pathway from low fluid intake to high circulating AVP to poor gluco-regulatory health is
difficult to examine as the high AVP may be due to other extraneous variables.

Nonetheless, high plasma concentrations have been associated with poorer cardiometabolic
and gluco-regulatory health. Water intake almost immediately reduces plasma copeptin
concentrations (a surrogate marker of AVP) for > 4 h. This has led some to hypothesize that
interventions to reduce copeptin via increasing fluid intake may facilitate positive health
outcomes (e.g. Perrier et al.; Enhorning et al.). This line of thinking may, however,
oversimplify the relationship between hydration, AVP, and health. The aim of this narrative
review is to briefly discuss early and current human research in hydration, AVP, and health
(focussing on gluco-regulatory health) and provide a critical perspective as to how to advance
the field, focusing on uncoupling the effects of hydration status and AVP.
History

Much research investigating the role of AVP in water balance was conducted in the 1970s (e.g. Robertson et al., Robertson et al.) subsequently resulting in data suggesting AVP may be linked to gluco-regulation. In 1979 Zerbe et al. found elevated plasma AVP concentrations in patients with uncontrolled diabetes (i.e. hyperglycemia). This seemed counterintuitive as hyperglycemia is typically accompanied by polyuria which acts to help maintain euglycemia. Rather, these patients had severe hypovolemia sufficient enough to induce AVP secretion. It therefore appeared that hyperglycemia caused polyuria, resulting in hypovolemia. Consequently, plasma AVP concentrations increased to counteract the loss in blood volume, which may have been potentiated by the osmotic effect of high blood glucose concentrations. Such findings were key in introducing the idea that AVP may have a role in gluco-regulation.

Following this, in 1985, Spruce et al. advanced this research, along with theory from research in rodents and dogs (e.g. Bergen et al., Ma et al.), by infusing AVP into healthy adults and measuring gluco-regulation (including glucose kinetics using labelled glucose). Plasma concentrations of AVP reached 22.3 ± 5.4 pmol·L⁻¹ during 30 min low dose (25 pmol·min⁻¹) infusion, and 112.3 ± 18.4 pmol·L⁻¹ during a subsequent 60 min high dose (75 pmol·min⁻¹) infusion, without altering plasma osmolality. Arterialized-venous blood glucose concentrations increased from 4.9 ± 0.1 mmol·L⁻¹ to 5.2 ± 0.2 mmol·L⁻¹ after the low dose infusion and to 5.7 ± 0.2 mmol·L⁻¹ after the high dose. Such changes were not found after saline infusion (though no details were given regarding the saline so it is assumed this was iso-tonic). No effects from any treatment were found for plasma insulin concentrations, though plasma glucagon concentrations were ~41 pg·L⁻¹ higher during the low dose AVP infusion, which remained throughout the high dose infusion.

Such studies offered insights into potential mechanisms by which AVP might be implicated in gluco-regulatory health. Firstly, as can be seen in from the work of Zerbe et al., there is a complex interplay between hyperglycemia and AVP, potentially mediated by hypovolemia induced by glucosuria. Although the study was unable to determine the temporal direction of the hyperglycemia-AVP relationship, considering the elevated AVP was accounted for by
hypovolemia, it is likely that the hyperglycemia drove higher AVP, rather than *vice versa* (Figure 1). Whilst not discussed in the paper, the AVP-induced hyperglycemia may have also created an osmotic stimulus further facilitating AVP secretion (Figure 1).

**Figure 1.** The relationship between diabetes and increased arginine vasopressin, as per the findings of Zerbe et al. (1979). Dotted lines represent a feedback loop which aims to maintain homeostasis. Dashed line represents a theoretical cycle whereby hyperglycemia induces an osmotic stimulus for greater AVP secretion, thereby facilitating the hyperglycemic-effects of AVP.

Secondly, Spruce *et al.* demonstrated an increase in glucose production but not disposal; this was likely driven by glycogenolysis, rather than gluconeogenesis as there were no changes in gluconeogenic precursors such as lactate. The increase in glycemia was therefore likely due to the greater plasma glucagon concentrations during AVP infusion. The divergence in mechanistic findings of these two studies provides a strong rationale to separate patients with diseases from healthy participants when making comparisons between theories and studies.
Beyond the direct role of AVP on gluco-regulation, theoretical implications have also been hypothesized involving the hypothalamic-pituitary-adrenal (HPA) axis, which were highlighted in the 1980s. The HPA axis is implicated in the stress response; upon experiencing stress, the hypothalamus, both AVP and corticotropin releasing factor (CRF) are synthesized and secreted. These hormones regulate adrenocorticotropic hormone (ACTH) secretion from the pituitary gland, resulting in cortisol secretion from the adrenal cortex. In the 1980s, there was sufficient evidence, primarily from animal models regarding the role of AVP in this response\textsuperscript{11}. Specifically, during physical stress, CRH (a precursor to ACTH) is produced. In vivo, AVP can potentiate the effect of CRF on ACTH production. As AVP is elevated during physical stress, there remains a clear pathway between AVP and the stress response, via CRF, ACTH, and ultimately cortisol secretion\textsuperscript{11}. Accordingly, it may be that increasing water intake to reduce AVP could mitigate excessive cortisol secretion, thus reducing hepatic glucose output (\textbf{Figure 2}).

\textbf{Figure 2}. Theoretical relationship between hydration status, arginine vasopressin, and the hypothalamic-pituitary-adrenal axis. Dashed lines represent a mediating relationship (i.e. hydration status directly influences arginine vasopressin, and hydration status may influence stress); dotted lines represent a moderating relationship (i.e. arginine vasopressin determines the propensity of corticotropin releasing factor to be cleaved into adrenocorticotropic hormone).
Current research

The studies conducted in the 1970s and 1980s provided fascinating insights into the role of hydration and AVP in gluco-regulatory health. Yet, despite the broad ranging implications of the work, interest in AVP waned until the 2000s. Recently, research has expanded the early work and helped provide some critical perspective.

Water intake can be used as a crude proxy for hydration status. Few studies have investigated the relationship between water intake and markers of gluco-regulatory health. In a French cohort of men and women, higher water intake has been associated with lower risk of hyperglycemia. This relationship was replicated in a small UK sample of men and women but not in a sample of US female nurses. Following these studies, a representative UK sample also found an inverse relationship between plain water intake and gluco-regulatory health, though upon further analysis, this association was only found in men. This latest study may explain the null relationship found in US nurses, due to the sample being exclusively women, compared to the other studies which used a mixed sex sample. Regarding AVP, this could make sense due to fluctuations in the osmolality set-point for AVP secretion during by the menstrual cycle, which may cloud any associations.

Further observational evidence has more directly implicated AVP in gluco-regulatory health. In a Swedish sample, higher plasma copeptin (as a surrogate marker of AVP) concentrations were associated with higher prevalence of T2D and insulin resistance, both cross-sectionally, and longitudinally. Advancing these associations are studies investigating variations in AVP receptor genes. Participants with the variation in AVP1a receptor gene (specifically, rs1042615 T allele) had a higher prevalence of T2D in those with a high fat diet or with overweight; similarly AVP1b receptor genes were tentatively associated with increased T2D risk.

Of course, observational evidence has limited causal inference due to well-known problems such as reverse causality and residual confounding. Particularly in the case of water intake, beyond issues surrounding misreporting, water intake is part of a cluster of other healthful behaviors such as higher fiber intake, which may confound any associations. In the case of...
the genetic observations, these could be chance associations, or AVP receptor genes may be
collinear with other genes which directly cause disease, thus representing a marker of a
different mechanistic process. Nonetheless, the genetic work shows some agreement with
rodent models which have used knockout models, improving the confidence in these
findings\textsuperscript{20}. Alternatively, as the outcome for these studies was related to gluco-regulatory
health, there may have been confounding from unmeasured hypovolemia, in line with the
work of Zerbe et al.\textsuperscript{7}. In other words, the findings may not be due to changes in hydration
status or AVP \textit{per se}, but rather represent a response (symptom) to poor gluco-regulatory
health resulting in glucosuria; AVP then responded to maintain blood volume in response to
excessive urinary water losses.

Following these studies, interest has started to focus on understanding the causal relationship
between hydration status, AVP, and gluco-regulation. One study has investigated the acute
and medium-term effects of water ingestion on gluco-regulation. In this study, participants
consumed one liter of water, after which their copeptin reduced within 30 min and remained
suppressed by ~39 % throughout the full test period of 4 h\textsuperscript{5}. Furthermore, after one week of
increased water ingestion, copeptin was reduced by 15 % compared to a control week\textsuperscript{5}. This
study further split participants into ‘responders’ (i.e. copeptin reduced significantly after
water ingestion; typically those with habitually low water intake, high copeptin and elevated
urine osmolality) and ‘non-responders’ (i.e. water ingestion did not meaningfully impact their
copeptin; typically those with habitually higher water intakes and low copeptin)\textsuperscript{5}. In
‘responders’, increasing water intake did not result in changes in plasma glucose or insulin
concentrations, but glucagon concentrations did reduce. Such work builds on that of Spruce
\textit{et al.}\textsuperscript{8}, by demonstrating a reverse effect. In other words, Spruce \textit{et al.} (1985)\textsuperscript{8} induced higher
glucagon concentrations via AVP infusion, whereas Enhorning \textit{et al.} (2017)\textsuperscript{5} induced lower
glucagon concentrations by reducing AVP concentrations (via increasing fluid intake). Taken
together, there appears to be a dose-dependent effect of AVP on circulating glucagon
concentrations.

Arginine vasopressin secretion can be induced by small increases in serum osmolality\textsuperscript{6a}.
Accordingly, some studies have investigated the role of hypertonic saline infusion on gluco-
regulation. After hypertonic saline infusion plus fluid restriction in healthy adults, Keller \textit{et
al.\textsuperscript{21} found higher fasted plasma glucose concentrations, coupled with a longer hyperinsulinemic-euglycemic clamp clearance rate, compared to a hypo-osmotic trial arm. Similarly, hypertonic saline infusion before an oral glucose tolerance test (OGTT) resulted in higher postprandial glucose concentrations 60 and 90 min post-glucose ingestion\textsuperscript{22}. It is unclear from these studies whether the effect seen was directly due to the saline, or indirectly due to hyperosmolality-induced AVP secretion (or another as yet unknown mechanism).

In terms of the gluco-regulatory impacts of direct manipulations to hydration status, there is limited current evidence and no replication studies. In 2001, Burge \textit{et al.}\textsuperscript{23} withdrew insulin in male and female patients (n = 10) with type 1 diabetes and hypohydrated them via fluid restriction (750 mL per 24 h), and both oral and intravenous diuretics, losing on average ~4.1% of their body mass compared to a euhydrated control arm. Eight hours after a set meal, insulin was withdrawn and biochemistry was measured for five hours. Hypohydration resulted in an elevated glycemic response compared to a control group\textsuperscript{23}. In a subgroup of participants, hypohydration was found to result in lower glucosuria compared to the control arm by an amount concordant with the difference in glycemia between the two trial arms. Beyond glucosuria, compared to a euhydrated control arm, hypohydration induced higher plasma glucagon and cortisol concentrations which may also explain the higher glycemia found.

 Similarly, Johnson \textit{et al.}\textsuperscript{24} hypohydrated medication-withdrawn men (n = 9) with T2D (~1.6% body mass loss) via fluid restriction. In both a fasted and postprandial state, hypohydration resulted in higher serum glucose concentrations, though this was only significantly different towards the end of the OGTT. No differences in plasma insulin were found between trial arms. Mechanistically, there was no difference in the renin-angiotensin-aldosterone system (RAAS) according to hydration status, and the authors were unfortunately unable to measure AVP. However, plasma cortisol concentrations were lower 30 min post-glucose ingestion during the euhydration trial arm\textsuperscript{24}. The time course of change in cortisol concentration does not clearly correspond to that of the glycemic response. Thus it may be that there is an interaction between hydration status and nutritional status, which mediated a cortisol response, rather than cortisol playing a role in hydration-induced alterations in gluco-regulation. Alternatively, this may be a response to the medication withdrawal, since the
The cortisol trend is similar (though a more rapid onset) to the work in insulin-withdrawn participants with type 1 diabetes\textsuperscript{23}. Unfortunately, the authors did not measure glucosuria for comparison with Burge \textit{et al.}\textsuperscript{23}.

Considering that early work suggested differential mechanisms between those with glucose dysregulation and those who are healthy, we conducted a pilot study in healthy adults. In this study, we hypohydrated participants using a sauna and fluid restriction protocol and subsequently conducted an OGTT\textsuperscript{25}. As per the research in people with diabetes, we also found a higher glycemic response during hypohydration compared to euhydration. The difference in glycemia emerged after 30 min, contrary to Johnson \textit{et al.} (2017)\textsuperscript{24}. As per Spruce \textit{et al.} (1985)\textsuperscript{8} we also did not find a difference in lactate, though it tended to be higher during hypohydration 60 min post-glucose ingestion. However, this pilot study lacked rigorous control (e.g. verbal compliance only to the 24-h pre-trial standardisation), had a small sample (n = 5), and was unable to measure mechanisms. The consistency and clarity in the results seemed somewhat incredible, thus warranting a tightly controlled follow-up study.

Therefore our follow-up study used much more rigorous pre-trial standardization (four days of food, fluid, and physical activity replication) and measured a multitude of mechanisms\textsuperscript{26}. In this study, participants lost \textasciitilde{}1.9 \% body mass during hypohydration, serum osmolality increased by \textasciitilde{}9 mOsm·kg\textsuperscript{-1}, and their plasma copeptin concentrations increased from levels typically seen in healthy adults to levels seen in those with diabetic ketoacidosis \textsuperscript{27}. Thus, we are confident that we induced meaningful changes in both water balance and copeptin concentrations, with the increase in copeptin in line with those achieved by Spruce \textit{et al.} (1985)\textsuperscript{8}.

Despite this, we did not find a difference between trial arms in the arterialized-venous serum concentrations of glucose or insulin (neither fasted, nor postprandial)\textsuperscript{26}. At 45 and 60 min post-glucose ingestion, there was a small divergence between the trials, similar to our pilot study, though non-significant and non-meaningful (unlike our pilot). Importantly, there were no differences in fasting or postprandial ACTH or cortisol concentrations, contrary to Johnson \textit{et al.} (2017)\textsuperscript{24} and Burge \textit{et al.} (2001)\textsuperscript{23}. This may suggest that the interaction
between hydration status and cortisol responds differentially during medication withdrawal in diabetes compared to healthy adults. Additionally, as our results in healthy adults are divergent to those in diabetes, it seems likely that glucosuria during euhydration provides a better explanation of the lower glycemic responses in diabetes. This has been suggested to be tested via comparing those with diabetes during medication withdrawal and prescription.
Current and critical perspectives and future research

Current rhetoric surrounding hydration is such that the addition of fluids, primarily water, in the diet is good for health. This brief narrative review focuses on gluco-regulatory health which has links to overall metabolic health and disease (such as T2D). It should be noted that the perspectives herein are solely related to the addition of fluids to improve hydration status (i.e. not substitution of energy containing beverages) and the impact on gluco-regulatory health; other outcomes or contexts may be altered differentially and therefore may not be applicable to the perspectives presented. For example, growing evidence suggests higher fluid intake to reduce the concentration of urine may aid in kidney health or reduce the risk of urinary tract infection recurrence, and that hydration status may influence endocrine responses to exercise.

This section therefore aims to: critically discuss the differences found between participants who are health and who have diabetes; critically evaluate the role of the HPA axis in hydration-mediated AVP secretion; clarify the purpose of interventions that claim to manipulate hydration status (e.g. reduce urine concentration versus increase body water); and provide suggestions for future research directions, including methods to uncouple the effects of manipulating hydration status (i.e. body water) and circulating AVP concentrations. As it stands, there is limited evidence that hydration status directly alters gluco-regulation, particularly in healthy adults. Replicating the current research should therefore be a priority. In those with diabetes, the evidence is clearer (though still only two studies) but is likely an artefact of glucosuria after euhydration, and such effects have only been testing during medication withdrawal. Certainly the glucosuria hypothesis needs to be further examined.

As previously mentioned, direct comparisons between healthy participants and those with diabetes should be made with caution. Of particular interest is the differential postprandial cortisol response found when comparing healthy participants with those with diabetes during medication withdrawal. Mechanisms for this interaction are as yet poorly understood but they are unlikely mediated by AVP or the RAAS. The reason these two mechanisms are unlikely is because: (i) the RAAS was no different between hypohydrated and euhydrated participants in participants with T2D; (ii) AVP concentrations (measured by copeptin) appear to remain elevated at roughly a constant magnitude throughout an OGTT.
though it is unknown if this is the case in diabetes. Thus, this change in cortisol is more likely to be mediated via other pathways and may be part of a complex interaction related to medication withdrawal and perhaps nutritional status.

A key underlying theory is that AVP acts along the stress response; thus if an individual is hypohydrated during stress, higher AVP will result in higher ACTH due to AVP potentiating the effects of CRH. Ergo, in theory, maintaining low circulating AVP concentrations would results in lower CRH cleavage into ACTH, mitigating cortisol-mediated hepatic glucose output (Figure 2). This pathway was determined primarily from theory and animal models. We, however, found no evidence that hydration status induced a difference in ACTH or cortisol concentrations, despite meaningful elevations in copeptin concentrations, and even under physical stress (i.e. muscle biopsies). In healthy adults, a large degree (~5 % body mass loss) might be needed to induce an elevation in fasting circulating cortisol concentrations, which is not representative of daily fluctuations in water balance; at a more achievable level of body mass loss (~2.5 %), Judelson et al. (2008) found no difference in cortisol concentrations, in accordance with our data. Thus, if AVP does potentiate the effects of CRF, this is not via hydration-mediated AVP changes, at least during every day fluctuations in water balance.

If we therefore examine the totality of evidence critically, one of the conclusions that could be made is that AVP is maybe only partially implicated in gluco-regulation, however this is perhaps independent of hydration status. In other words, our hypothesis is that the physiological effects of hypohydration-induced AVP secretion counter-regulate AVP-induced hyperglycemia, or the effects of increased AVP from other (non-hydration related) causes interact to cause hyperglycemia, similar to the potential interaction between nutritional status and cortisol secretion found in those with T2D. Alternatively, it could be that there is a residual factor, as yet unknown, that influences both AVP and gluco-regulation that is context-specific, thereby explaining why there appears to be no direct effect of hypohydration on gluco-regulation, whereas there does appear to be an effect of AVP infusion.
Therefore, we hypothesize that manipulating hydration status in order to reduce AVP is likely to have minimal, if any effect, on gluco-regulation. This has somewhat been demonstrated in the aforementioned one week water intervention (+3 L·d$^{-1}$ added to habitual intake)$^5$. Responders to the intervention had a reduction in fasting plasma glucagon concentrations, but not glucose or insulin concentrations, concordant with the increase in glucagon concentrations found by Spruce et al. (1985)$^8$ during AVP infusion. Such results add credence to the idea that reducing AVP (measured by copeptin) fails to alter gluco-regulation, despite increasing glucagon. This perhaps suggests another counter-regulatory process is occurring to mitigate the hyperglycemic effects of glucagon. Considering the findings of Burge et al.$^{23}$, Enhorning et al.$^5$, and Spruce et al.$^8$, researchers should ensure that glucagon is measured so the effects on primary gluco-regulatory hormones can be captured. It is unclear mechanistically why glucagon increased in these studies but did not always result in higher plasma glucose concentrations.

Our hypothesis is specific to endogenous AVP production mediated by non-compartment-specific hypohydration, however. It has been demonstrated that AVP infusion increases glucose concentrations without increasing serum osmolality (i.e. without necessarily altering hydration status per se)$^8$. Although there has been no replication work to confirm these findings, some studies have shown an increase in glycemia when infusing hypertonic (2-5 %) saline (which will likely raise AVP concentrations) (12 h hypertonic saline infusion at 1 mL·kg·h$^{-1}$ followed by 3 h at 200 mL·h$^{-1}$, Keller et al.$^{21}$; and 2 h hypertonic saline infusion at 0.1 mL·kg·min$^{-1}$, Jansen et al.$^{22}$). Spruce et al.$^8$ did not find an effect after saline infusion, though saline infusion did not alter AVP concentrations, though details regarding the properties of the infused saline were not given; thus as no effect of infusion was found, it is likely they used isotonic saline, concordant with the control groups in the studies by Jansen et al.$^{22}$ and Keller et al.$^{21}$. It is unclear if AVP was altered in these other saline infusion studies$^{21-22}$.

Discordance between AVP-infusion$^8$ and hypohydration-induced AVP elevations$^{26}$ may also be explained by nutritional status, i.e. fasted AVP infusion resulted in greater hepatic glucose output (but no change in glucose disposal), whereas in a postprandial state, glucose disposal is more pertinent. This perhaps suggests that AVP acts specifically to increase hepatic
glucose metabolism, which may be more directly influenced by infusion studies that create intracellular dehydration. Such intracellular dehydration, particularly in hepatocytes, has been shown to increase glucagon secretion and is thus implicated in gluco-regulation and could help explain the aforementioned higher glucagon concentrations.

Taken together, these findings may hint towards AVP being the main factor in gluco-regulation, rather than increased serum osmolality having independent effects (Figure 3). Alternatively or additionally, such studies suggest that both (a) exogenous AVP, and (b) compartmental water distribution changes can result in increased glycemia. Whilst these are important mechanistic insights, they are not necessarily valid for every day fluctuations in water balance in humans. Further, they may represent a physiological condition present in some people due to other factors such as genetic variations (i.e. not hypohydration).

Figure 3. Theoretical relationship between AVP or saline infusion and gluco-regulation.
Dotted line with diamond arrow represents no effect. Greyed out box means unmeasured, but theoretically on the pathway.

A counter argument to our hypothesis may point towards genetic studies. Although these studies highlight genetic variation relating to AVP (and therefore by inference water balance physiology) are correlated to poorer gluco-regulatory and metabolic health outcomes, they lack causality. Specifically, such genetic variations may be collinear with other genetic variations that are detrimental to health. If, for the sake of argument, we assume the
association is causal though, such findings may mean that genetic variation in water balance physiology can cause increased risk of cardiometabolic disease. However, this does not automatically mean that altering water balance behaviors (i.e. increasing fluid intake) will reduce this genetically determined disease risk. This of course should be further investigated as perhaps targeting people with certain variants could increase the likelihood that a water intervention may be efficacious (and in the case of the previously discussed study by Enhorning et al., may explain some variation in the responders and non-responders).

Further considerations should also be taken into account in future research. Firstly, the epidemiology investigating water intake and gluco-regulatory health is at least suggestive of sex differences. Considering much physiology research is based on men (e.g. Johnson et al.), or when women are included they are in their (estimated) follicular phase, post-menopausal, or taking hormonal contraceptives (e.g. Carroll et al.), studies investigating water balance and health outcomes during the luteal phase of the menstrual cycle would be of mechanistic interest. The osmolality set point for AVP secretion changes throughout the menstrual cycle as does carbohydrate and fat oxidation and understanding how these fluctuations influence health would aid in our mechanistic understanding of whether and how AVP influences gluco-regulatory health.

Secondly, pre-trial control of known confounding factors should be emphasized. Our recent study included four days of pre-trial diet, activity, and fluid intake standardisation using weighed food diaries and combined accelerometry and heart rate monitors. Such control to our knowledge has not be utilized in previous hydration and health related research. To demonstrate why this may be important, comparatively, our pilot work used 24 hours of diet and activity standardisation in the form of verbal confirmation. The stark differences in the results from these studies may indicate that lack of pre-trial standardisation at least partially contributed to divergences in gluco-regulation during the OGTT.

Thirdly, another explanation as to why there have been conflicting findings, is the use of venous versus arterialized-venous blood. In our pilot work, we used venous blood and found a large difference in the blood glucose response between hydration states. Contrarily, in our
follow-on study, we used arterialized-venous blood. As arterialized-venous blood more closely represents the glucose concentration that cells are exposed to, whereas venous blood more closely represents the glucose the cells have not taken up, it is reasonable to suspect that this may (at least in part) explain the differences in our findings. Whilst this is a possibility, which warrants further investigation, it is worth noting the use of arterialized-venous blood by Spruce et al., which still demonstrated a difference in glycemia. This of course may also be due to the use of exogenous AVP infusion versus endogenous AVP via dehydration.

Further adding doubt to the arterialisation theory is that in our study, after the OGTT, we measured multiple facets of appetite, including serum glucose and insulin concentrations from (non-arterialized) venous blood after an ad libitum meal test. During this period of testing, plasma copeptin concentrations and serum osmolality remained elevated during hypohydration but blood glucose and insulin concentrations remained remarkably similar to the euhydrated trial arm. Whilst the ad libitum nature of the meal test confounds any definitive inferences, energy intake was (on average) approximately equal between the trial arms, and accordingly, there were no differences in serum glucose or insulin concentrations. If arterialisation was a cause of the disparities between studies, it may have been apparent during this period of testing.

A final consideration is the measurement of hydration status and how this relates to the conclusions of studies. There is currently no gold standard measure of hydration, as each method has its strengths and limitations according to the context. Our recent study, to our knowledge, measured hydration status more extensively than any other research investigating hydration and health. Whilst this level of measurement is unnecessary for all research, future work should consider the appropriateness of the measures taken. A clear distinction needs to be made before starting the trial: is the aim to alter urine concentration, AVP, or body water?

If the aim of the study is to increase urine volume or decrease urine concentration, then measures such 24-hour urine volume, urine osmolality, or urine specific gravity are suitable. However, these measures alone do not indicate that hydration status has been altered, though
they may be sufficient to infer (with caution) that AVP has been manipulated. Changes in these outcomes simply demonstrates that the body has (no) need to reabsorb extra fluid, or fluid has been consumed in a way that is conducive to increased/decreased urinary output such as consuming a large bolus of fluid rapidly (e.g. Shafiee et al.³⁴).

Equally, measuring AVP (or a marker of) alone does not necessarily infer that body water (hydration status) has been altered. Arginine vasopressin is secreted in order to reduce water losses. Thus at least in the early phase of elevated concentrations, it should be effective at maintaining water balance within the body. Acutely, measuring body mass can be effectively used to determine whether hydration has been altered, though this implies energy balance, emphasising the importance of proper pre-measure standardisation of diet and activity. Therefore, it is essential to specify the aim of the study, use the appropriate (combination of) measures, and most importantly to frame conclusions within the correct context (i.e. are the inferences based on reducing urine concentration, circulating AVP, or manipulating body water)?

This raises a wider question regarding how we can improve the measurement of hydration status and accurately assess the contribution of hydration-mediated changes in AVP in glucose-regulatory health. We propose two potential pathways that could help uncouple the independent effects of alterations in body water and circulating AVP concentrations: (i) investigating 3,4-Methylenedioxymethamphetamine (MDMA), and (ii) investigating those with water balance conditions, namely the syndrome of inappropriate antidiuretic hormone (another name for AVP) secretion (SIADH) and diabetes insipidus.

3,4-Methylenedioxymethamphetamine is the psychoactive ingredient in the recreational drug more commonly known as ‘ecstasy’. In terms of hydration, this drug is most fascinating as it gives the symptoms of hypohydration (reduced urine volume, and increased urine osmolality [despite greater fluid ingestion], plasma copeptin concentrations, thirst, desire for fluid, dry mouth, and body temperature), whilst simultaneously causing cell swelling (due to the elevation of AVP resulting in hyponatremia and greater water retention, as well as the greater fluid ingestion)³⁵. Mixed effects have been found for whether plasma osmolality changes
from MDMA administration: two placebo-controlled studies found no effect\textsuperscript{35a, 35b}, whereas another natural study taking pre- and post-clubbing measured in self-administering participants found a post-MDMA reduction in plasma osmolality\textsuperscript{35c}, with similar results in another placebo-controlled trial though this study did not find an interaction between MDMA and AVP\textsuperscript{36}. As studies varied in their levels of control regarding fluid intake, physical activity, and dosage, the effects of MDMA on plasma osmolality need to be clarified in order to fully understand the hydration-AVP-health interactions (described below).

Accordingly, MDMA could (a) help improve our understanding of hydration physiology and measurement, and (b) provide a useful model to help assess the role of AVP in gluco-regulatory health. With regards to the first point (a), if we were able to find a simple measure of hydration that accurately describes those under the influence of MDMA as hyperhydrated, despite the overwhelming symptoms of hypohydration, we may more effectively be able to assess hydration status. Additionally, such symptoms have some sex-differences (specifically that copeptin concentrations increase more in women than men\textsuperscript{35b}), further highlighting the usefulness of an MDMA model in understanding the mechanisms surrounding water balance. An unintended consequence of pursuing this line of research may be a reduction in MDMA-related deaths which are primarily caused by hyponatremia or hyperhydration.

Regarding the latter point (b), considering MDMA results in both elevated AVP and hyperhydration, if AVP alone was the cause of hyperglycemia, this should mean MDMA induces hyperglycemia. Of course this is very difficult to test for several reasons, such as ethical and legal restrictions, and confounding factors in natural settings such as the temperature and activity patterns of users. As such, there is very limited research. In rats, MDMA administration results in hypoglycemia\textsuperscript{37}. However, in humans administered MDMA in a natural setting, but with no controls of food or fluid intake, six out of 21 participants had a non-significant elevation in blood glucose concentrations compared to baseline\textsuperscript{38}.

It is unclear as to whether MDMA increases circulating cortisol concentrations or not; in a club setting, MDMA increased cortisol\textsuperscript{39}, whereas in a placebo-controlled setting it did not\textsuperscript{35a}, meaning it is unclear as to whether euglycemia was maintained in the study by Downing.
despite changes cortisol secretion. It may be concluded from these that MDMA-induced endogenous AVP secretion is at least not associated with gluco-dysregulation.

Using an MDMA model of AVP physiology and gluco-regulatory outcomes may be useful in aiding our understanding of these complex relationships as it enables us to uncouple the mechanistic effects of hydration status and AVP. Nonetheless, caution should still be made when making inferences from such results to the general population as MDMA affects a multitude of metabolic and neuronal pathways which could be implicated in gluco-regulation. However, this does not necessarily detract from the mechanistic understanding such a model can bring.

Following an MDMA model is those with SIADH. This condition is characterized via low serum osmolality (which can be accompanied by cell swelling and hyponatremia), highly concentrated urine, and elevated AVP; thus in some ways this condition mimics the water balance effects of MDMA administration and could also be used as a model to uncouple the effects of body water versus AVP in gluco-regulatory health. Conversely, neurohypophyseal diabetes insipidus is the underproduction of AVP resulting in excessive fluid losses (though there are other forms which can result in elevated AVP, e.g. nephrogenic diabetes insipidus).

We were unable to find data regarding gluco-regulatory health in either of these conditions. If the AVP-induced hyperglycemia model is correct, we would expect to see a greater prevalence of markers of glucose intolerance (e.g. impaired fasting glucose, or higher T2D prevalence) in those with SIADH, and lower prevalence in those with diabetes insipidus. In the absence of clear current data, the relationship between diabetes insipidus and mellitus was previously of interest; although there were some cases of increased glucosuria with diabetes insipidus, there was no evidence that diabetes mellitus prevalence was different from the general population. Epidemiological work could investigate this relationship further in order to discern whether more causal work is warranted. As with MDMA research, inferences from both SIADH and diabetes insipidus models should be made cautiously when extrapolating to the general population; for example, in SIADH, inappropriate secretin
secretion is currently the most likely cause of the condition, rather than problems with the AVP response per se\(^4\). Further, the study of those with diabetes insipidus may help to uncouple the effects of glucosuria and gluco-regulation found in those with type 1 and T2D during medication withdrawal; this may however, additionally reduce the applicability of this model to the general population. Nonetheless, such models provide useful mechanistic understandings which can help drive future hypotheses.

Future research should also consider longer term interventions. Whilst this has ethical implications, there is ample evidence to suggest that some people are chronic low fluid drinkers; understanding the causal implications of this behavior is essential for public health. Although the acute evidence in healthy adults at least suggests that such an intervention will not cause metabolic harm, reducing some initial ethical concerns, important questions remain to be answered as to whether this lack of harm extends beyond a matter of days. In other words, does chronically high AVP induced by fluid restriction eventually fulfil the HPA axis causing elevated cortisol (notwithstanding other potential mechanisms such as changes in cell volume which may influence gluco-regulation\(^3\)).

Finally, much of the mechanistic work relating AVP to gluco-regulatory health has involved isolating a single mechanism (AVP in the case of the focus of this review, but also hepatocyte volume and more recently, adipocyte AVP receptor expression). Such studies are vitally important for understanding the underlying physiology and generating testable hypotheses. However, the more recent work in humans, which has encompassed the full range of the physiological effects of hypohydration, demonstrates that such models are not necessarily applicable to human fluctuations in water balance. Thus in order to understand the gluco-regulatory impacts of AVP, ecologically valid methods should be used in order to make accurate inferences applicable to human health.

**Conclusion**

Overall, this narrative review provided a brief account of the history of AVP-gluco-regulation related research. Whilst the earlier research gave insight into potential mechanisms, backed by observational studies, tightly controlled studies do not at this time appear to support a
causal role for hypohydration-induced increases in AVP in gluco-regulatory health. Although
studies in people with diabetes show euhydration results in lower glycemia, this is likely due
to glucosuria from medication withdrawal; though more research needs to confirm this. Such
findings in their totality suggest that AVP may be implicated in gluco-regulation, but not via
alterations in hydration status. However, research in this field using ecologically valid
methods in both healthy participants and those with diabetes is in its infancy. Several ideas
for further examining mechanisms and outcomes were also discussed, with an emphasis on
replication of current studies.

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References


