# Clinical characteristics and novel diagnostic tests in patients with arginine vasopressin deficiency (central diabetes insipidus)

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**ABBREVIATIONS**

| | |
|---|---|
| 5-HT | Serotonin |
| ACTH | Adrenocorticotropic hormone |
| AESI | Adverse Effects of special interest |
| APD | Anterior pituitary dysfunction |
| AQP2 | Aquaporin 2 |
| ASD | Autism spectrum disorder |
| AUC | Area under the curve |
| AVP | Arginine vasopressin |
| BDI II | Beck's Depression Inventory |
| BMI | Body mass index |
| CDI | Central diabetes insipidus |
| CEC | Competent ethics committee |
| CI | Confidence intervals |
| CP | Craniopharyngioma |
| CRH | Corticotrophin-releasing hormone |
| ECLIA | Electrochemiluminescence immunoassay |
| ELISA | Enzyme-linked immunosorbent assay |
| FERT | Face Emotion Recognition Task |
| FSH | Follicle-stimulating hormone |
| GH | Growth hormone |
| GMP | Good Manufacturing Practice |
| HCT | Hydrocortisone |
| HPA | Hypothalamic-pituitary-adrenal axis |
| HPLC–MS/MS | Human plasma liquid chromatography-tandem mass spectrometry |
| i.v. | Intravenous |
| IQR | Inter quartile range |
| ITT | Insulin tolerance test |
| KO | Knockout |
| LC | List-of-Complaints |
| LH | Luteinising hormone |
| M1 | Manuscript 1 |
| M2 | Manuscript 2 |
| M3 | Manuscript 3 |
| MDA | Methylenedioxyamphetamine |
| MDMA | Methylenedioxymethamphetamine |
| MET | Multifaceted Empathy Task |
| MRI | Magnetic resonance imaging |

| | |
|---|---|
| NHS | National Health Service |
| OXT | Oxytocin |
| PP | Primary polydipsia |
| PPS | Polyuria-polydipsia syndrome |
| PPS | Polyuria-polydipsia syndrome |
| PVN | Paraventricular nuclei |
| QoL | Quality-of-life |
| REML | Restricted maximum likelihood |
| RIA | Radioimmunoassay |
| ROC | Receiver-operating-characteristic |
| s.c. | Subcutaneous |
| SD | Standard deviation |
| SERT | Serotonin transporter |
| SF-36 | Short Form 36 Health Survey |
| SGLT-2 | Sodium-glucose transporter 2 |
| SON | Supraoptic nuclei |
| SSRI | Selective 5-HT uptake inhibitor |
| STAI | State-Trait Anxiety Inventory |
| TAS-20 | Toronto Alexithymia Scale |
| THC | Tetrahydrocannabinol |
| TSH | Thyroid-stimulating hormone |
| VAS | Visual analogue scale |
| WDT | Water deprivation test |

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## 1. SUMMARY OF THE PROJECT

**Background:** Arginine vasopressin (AVP) deficiency (central diabetes insipidus) is a neuroendocrine condition characterised by polyuria and polydipsia. The diagnostic evaluation in case of suspected AVP deficiency is challenging and simplified approaches with high diagnostic accuracies are needed. Once correctly diagnosed with AVP deficiency treatment with desmopressin (AVP receptor analogue) is started to control for polyuria. However, desmopressin treatment is accompanied by a high prevalence of hyponatremia. On the other hand, restriction to desmopressin and fluid intake during hospitalisation, partly explained by confusion with 'diabetes mellitus', may lead to severe consequences. Data on methods to counteract the risk of hyponatremia and data on treatment errors during hospitalisations are lacking. Additionally, hypothalamic-pituitary disruptions leading to AVP deficiency could also disturb the oxytocin system with subsequent clinical consequences. However, oxytocin deficiency has never been defined as pituitary entity and no provocation test is available to test for an oxytocin deficiency.

**Objective:** First, to investigate whether the glucagon provocation test might provide a novel diagnostic test in the differential diagnosis of AVP deficiency. Second, to assess patients' perspectives regarding their disease management, psychological comorbidities, and view for renaming 'diabetes insipidus' to avoid confusion with 'diabetes mellitus'. And third, to investigate 3,4-methylene-dioxymethamphetamine (MDMA) as a novel provocation test to reveal an OXT deficiency.

**Methods:** The first study is a double-blind, randomised, placebo-controlled trial including 22 healthy controls, ten patients with AVP deficiency, and ten patients with primary polydipsia (PP). The second study is a cross-sectional, web-based survey including 1034 patients with AVP deficiency. The third study is a randomised, placebo-controlled, double-blind, cross-over trial including 15 patients with AVP deficiency and 15 matched healthy controls.

**Results:** First, in patients with AVP deficiency, copeptin showed no relevant increase in response to glucagon, whereas copeptin was strongly stimulated in patients with primary polydipsia (PP). Glucagon stimulation demonstrated a high diagnostic accuracy in differentiating between both conditions. Second, once diagnosed and treated with desmopressin, a high prevalence of hyponatremia leading to hospitalisations was observed. Patients who routinely omitted or delayed desmopressin to allow intermittent aquaresis had a significantly lower prevalence of hyponatraemia than those unaware of this

approach. Of patients who had to be hospitalised for any medical reason, one in seven reported symptoms of dehydration due to desmopressin restriction or/and wrong fluid management. One in three patients reported psychological changes subjectively associated with their AVP deficiency. In total, 85% of patients supported renaming the disease. Third, in patients, there was only a minimal OXT increase in response to MDMA, while in healthy controls, there was a robust eight-fold OXT increase.

**Discussion:** First, glucagon-stimulated copeptin has the potential for a safe, novel, and precise test in the differential diagnosis of AVP-D. Second, our data show a high prevalence of treatment-associated side-effects, mismanagement during hospitalisation, psychological comorbidities, and clear support for renaming the disease; our data are the first to indicate the value of routinely omitting or delaying desmopressin. Third, these results lay the basis for OXT deficiency as a new hypothalamic-pituitary entity.

## 2. SUMMARY OF THE PROJECT IN GERMAN

**Hintergrund:** Arginin-Vasopressin (AVP)-Mangel (zentraler Diabetes insipidus) ist eine neuroendokrine Erkrankung, die durch Polyurie und Polydipsie gekennzeichnet ist. Die diagnostische Beurteilung bei Verdacht auf AVP-Mangel ist schwierig, und vereinfachte Ansätze mit hoher diagnostischer Genauigkeit sind daher notwendig. Nach korrekter Diagnose wird eine Behandlung mit Desmopressin (AVP-Rezeptor-Analogon) begonnen, um die Polyurie zu kontrollieren. Die Desmopressin-Behandlung geht jedoch mit einer hohen Prävalenz von Hyponatriämie einher. Andererseits kann die Beschränkung der Desmopressin- und Flüssigkeitszufuhr während des Krankenhausaufenthalts, die zum Teil durch die Verwechslung mit "Diabetes mellitus" erklärt wird, schwerwiegende Folgen haben. Daten über Methoden, um dem Risiko einer Hyponatriämie entgegenzuwirken, und Daten über Behandlungsfehler oder Fehlbehandlungen während eines Krankenhausaufenthalts fehlen. Darüber hinaus könnten hypothalamisch-hypophysäre Störungen, die zu einem AVP-Mangel führen, auch das Oxytocin (OXT)-System stören, was wiederum klinische Folgen hätte. OXT-Mangel wurde jedoch nie als hypophysäre Entität definiert, und es gibt keinen Provokationstest, um einen OXT-Mangel nachzuweisen.

**Fragestellung:** Erstens habe ich untersucht, ob der Glukagon-Provokationstest einen neuen diagnostischen Test für die Differentialdiagnose des AVP-Mangels darstellen könnte. Zweitens habe ich die Perspektiven der Patienten in Bezug auf ihre Therapie, psychologische Komorbiditäten und die Meinung zur Umbenennung von "Diabetes insipidus", untersucht. Und drittens habe ich 3,4-Methylendioxy-methamphetamin (MDMA) als Provokationstest zur Identifizierung eines OXT-Mangels untersucht.

**Methoden:** Bei der ersten Studie handelt es sich um eine doppelblinde, randomisierte, Placebo kontrollierte Studie mit 22 gesunden Kontrollpersonen, 10 Patienten mit AVP-Mangel und 10 Patienten mit primärer Polydipsie (PP). Bei der zweiten Studie handelt es sich um eine internetbasierte Querschnittserhebung mit 1034 Patienten mit AVP-Mangel. Bei der dritten Studie handelt es sich um eine randomisierte, Placebo kontrollierte, doppelblinde Cross-over-Studie mit 15 Patienten mit AVP-Mangel und 15 gesunden Kontrollpersonen.

**Ergebnisse:** Erstens ergab sich bei Patienten mit AVP-Mangel kein relevanter Anstieg von Copeptin als Reaktion auf Glukagon, während Copeptin bei Patienten mit primärer Polydipsie (PP) stark stimuliert wurde. Die Glukagon-Stimulation zeigte eine hohe diagnostische Genauigkeit bei der Unterscheidung zwischen beiden Erkrankungen. Zweitens wurde nach der Diagnose und Behandlung mit Desmopressin eine hohe Prävalenz von Hyponatriämie beobachtet, die zu Krankenhausaufenthalten führte. Bei Patienten, bei denen Desmopressin routinemäßig ausgelassen oder verzögert wurde, um eine intermittierende Aquarese zu ermöglichen, war die Prävalenz der Hyponatriämie deutlich geringer als bei Patienten, die diesen Ansatz nicht kannten. Von den Patienten, die aus medizinischen Gründen ins Krankenhaus eingewiesen werden mussten, berichtete jeder siebte über Dehydratationssymptome aufgrund von Desmopressin-Beschränkung und/oder falschem Flüssigkeitsmanagement. Einer von drei Patienten berichtete über psychische Veränderungen, die subjektiv mit seinem AVP-Mangel in Verbindung gebracht wurden. Insgesamt sprachen sich 85 % der Patienten für eine Umbenennung der Krankheit aus. Drittens gab es bei den Patienten nur einen minimalen OXT-Anstieg als Reaktion auf MDMA, während bei gesunden Kontrollpersonen ein starker OXT-Anstieg, um das Achtfache zu verzeichnen war. **Diskussion**: Erstens hat das Glukagon-stimulierte Copeptin das Potenzial für einen sicheren, neuen und präzisen Test für die Differentialdiagnose von AVP-D. Zweitens zeigen unsere Daten eine hohe Prävalenz von behandlungsbedingten Nebenwirkungen, Fehlbehandlungen während des Krankenhausaufenthalts, psychologischen Begleiterkrankungen und eine klare Zustimmung zur Umbenennung der Krankheit; unsere Daten sind die ersten, die den Wert einer routinemäßigen Unterlassung oder Verzögerung von Desmopressin belegen. Drittens legen diese Ergebnisse die Grundlage für den OXT-Mangel als eine neue hypothalamisch-hypophysäre Entität.

## 3. INTRODUCTION AND BACKGROUND

### 3.1. Anatomy and physiology of the neurohypophysis

The **neurohypophysis**, consisting of the hypothalamic supraoptic (SON) and paraventricular (PVN) nuclei, the supraoptic-hypophyseal tract and the posterior pituitary, plays a key role in regulating fluid balance and reproductive function through two nine-amino acid peptide hormones: arginine vasopressin (AVP) and oxytocin (OXT).[1,2]

The **precursors of AVP and OXT** are synthesised and packaged in secretory vesicles in the cell bodies of magnocellular neurons in the SON/PVN projecting to the posterior pituitary (Figure 1).[3] The precursors are cleaved into AVP and OXT, the associated carrier proteins (neurophysin) and copeptin, and stored in the axon terminals.[1] In addition, smaller parvocellular neurons project to the median eminence and co-secrete AVP and corticotrophin-releasing hormone (CRH), regulating the hypothalamic-pituitary-adrenal (HPA) axis.[4] AVP and OXT differ structurally by only two amino acids, have short **circulating half-lives** of a few minutes and are degraded by endo- and amino-peptidases. Copeptin, the C-terminal 39 amino-acid peptide of the AVP precursor, is co-released in equimolar amounts with AVP.[5,6] To date, the exact function of copeptin remains unclear.[6]

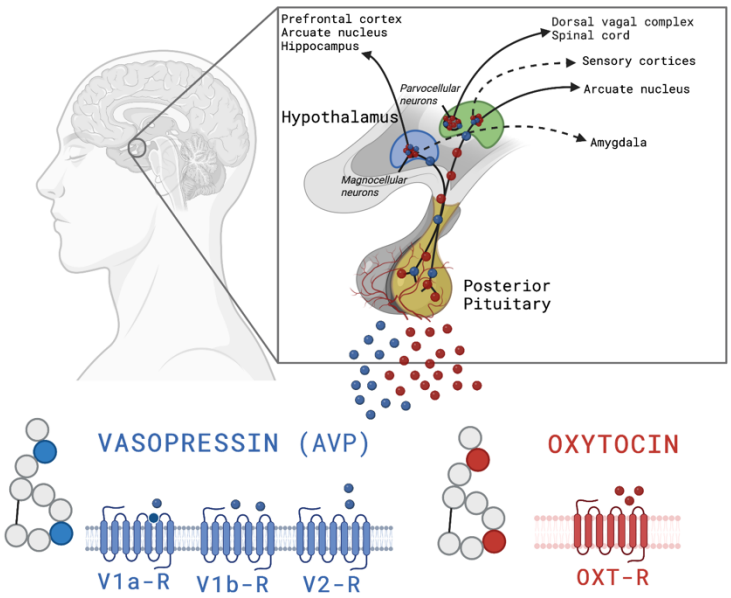


**Figure 1**

**The neurohypophysis**

**AVP** is the main endocrine regulator of renal water excretion and maintains fluid balance by keeping plasma volume and osmolality within the narrow limits of ~285 to 295 mOsmol/kg.[7] The main stimulus for its release is an increase in plasma osmolality.[8] Other stimuli are a reduction in effective blood volume, unspecific stress and nausea.[8] The main target tissues are the kidney collecting ducts (via V2

receptors) and the vascular smooth muscle cells (via V1a receptors).[9] Activation of V2 receptors leads to the expression of specific water channels (aquaporin 2, AQP2) on the luminal surface, allowing the reuptake of free water along an osmotic gradient.[9]

**OXT** is well known for its key role in parturition and lactation; hence, OXT receptors are expressed in reproductive tissues, including the mammary glands and the myometrium.[10,11] High oestrogen levels in late pregnancy increase the sensitivity and expression of OXT receptors in myometrial cells. During labour, OXT is released with increasing frequency, amplitude and duration in pulses to induce uterine contractions.[11] In the post-partum period, suckling induces a pulsatile release in OXT to promote milk ejection via contractive function at the myoepithelial cells surrounding the milk-producing alveoli.[10]

Besides their secretion into the circulation, AVP and OXT are also released dendritically into the central extracellular space and directly projected to other brain regions where they act as neuromodulators or neurotransmitters.[12] These neuropeptides have key roles to play in regulating complex social behaviour. Hence, brain regions involved in behavioural functions, such as the amygdala, hippocampus, cingulate cortex and nucleus accumbens, show high OXT receptor density.[13] OXT promotes prosocial effects such as in-group favouritism,[14] trust and attachment,[15] empathy[16] and emotion recognition.[17] In addition, it also has anxiolytic effects, as it buffers responses to social stress,[18] reduces amygdala reactivity to emotional stimuli[19] and reduces cortisol levels during conflict situations.[20] It is also suggested that it is involved in maintaining relationships by improving conflict resolution and increasing perceived attractiveness.[21]

### 3.2. Polyuria-polydipsia syndrome

Polyuria is defined as > 50 ml urine/Kg/24 hours and is usually accompanied by polydipsia of > 3l/day.[22,23] The three main differential diagnoses of polyuria-polydipsia syndrome (PPS) are AVP deficiency, AVP resistance and primary polydipsia.[24]

**AVP deficiency** (neurogenic, cranial or central diabetes insipidus) results from a partial or complete lack of osmo-regulated AVP secretion resulting from disruptions to the hypothalamic-posterior-pituitary axis. Most causes are acquired, developing when more than 80% of AVP-producing magnocellular

neurons are damaged, whereas inherited/congenital causes account for less than 10% of cases.[25-27] The prevalence is 1:25,000 with an equal gender distribution.[26]

Although non-functioning pituitary adenomas account for 90% of all intra- and parasellar pathologies, even relatively large adenomas do not usually impair AVP-producing neurons. Most cases develop from surgical interventions damaging the AVP-producing neurons, with an estimated postsurgical risk of AVP deficiency of around 30%.[28,29] Non-adenomatous lesions (e.g. craniopharyngiomas or Rathke's cleft cysts) are generally directly present with AVP deficiency, whereas the highest prevalence is reported for germ-cell tumours with up to 76%.[30-33] Less common causes are myelodysplastic syndromes, haematological malignancies, brain metastases (primary sites: lung, breast and myeloma) and systemic and infiltrative disorders (e.g. lymphocytic hypophysitis, Langerhans cell histiocytosis, abscesses, neurosarcoidosis, granulomatosis with polyangiitis and immunoglobulin G4-related hypophysitis).[28,32,34-37] In addition, several drugs have been associated with transient impaired AVP release, including phenytoin, ketamine, temozolomide and opioids.[38-41] New immune checkpoint inhibitors (i.e. anti-PD1/PD-L1 or anti-CTLA4 agents) are known to cause hypophysitis, and published cases usually present with delayed onset of polyuria after treatment initiation.[42,43] Congenital/hereditary forms can be transmitted as autosomal (recessive and, more commonly, dominant) and X-linked, showing individual variations in the clinical onset and manifestation of AVP deficiency.[44-46]

Importantly, despite improvements in laboratory and radiographic diagnostic techniques, approximately 30% of cases remain idiopathic. Literature data suggest that most of these cases might result from autoimmune processes. Accordingly, the detection of specific antibodies (e.g. anti-rabphilin-3a) could improve the diagnostic approach in the future.[34,47-50]

Additionally, placental aminopeptidases can cause transiently increased clearance of AVP, leading to pregnancy-associated polyuria-polydipsia (gestational diabetes insipidus).[51]

**Primary polydipsia** (PP) is characterised by excessive fluid intake despite normal AVP response and function.[52] PP covers several disorders that can be associated with organic structural brain lesions, for example sarcoidosis of the hypothalamus, but also results from drugs that cause a dry mouth. However, in most cases no underlying aetiology can be identified; in this case, the disorder is often associated with

psychological comorbidities.[52,53] In most cases, PP is observed in patients with schizophrenia spectrum disorder, with incidences between 11 and 20%, but also in depression, anorexia nervosa, bipolar disorders and dependency disorders.[13,15–17] Furthermore, polydipsia is prevalent in health-conscious people who voluntarily change their drinking habits to improve their well-being.[54]

**AVP resistance** (nephrogenic diabetes insipidus) is characterised by impairment of urine concentrating capacity owing to renal resistance to AVP.[55] In adults, most of the cases are caused by treatment with lithium or aminoglycosides.[55] Lithium causes dysregulated expression of AQP2 and amiloride-sensitive epithelial sodium channels in the collecting ducts. This may persist after drug withdrawal and may be irreversible.[55] Other causes include hypokalaemia, hypercalcemia and the release of bilateral urinary tract obstruction. Hereditary forms result from V2 receptor or AQP2 gene mutations which are X-linked recessive in 90% of cases and autosomal recessive in 10%.[55] Most mutations lead to a complete loss of function, clinically manifesting in infancy, and only a few are associated with a mild phenotype.[55]

### 3.2.1. Diagnostic workup and evaluation

Diagnosing the correct type of PPS is crucial as potential misdiagnosis and the resulting treatment can have severe consequences. Underlying renal dysfunction and electrolyte abnormalities (e.g. hypokalaemia and hypercalcemia) can result in polyuria and must be ruled out or corrected at the initial investigation.[23,33] Additionally, subjective complaints of polyuria can often misrepresent as symptoms of urinary urgency, nocturia, incontinence or prostatic hypertrophy, which are not associated with an increase in total urine volume and should be assessed using 24-hour urine collection.

Once polyuria is confirmed by using 24-hour urine collection, the next step is to assess for urinary osmolality.[23] A urine osmolality level of > 800 mOsm/Kg indicates normal AVP levels and appropriate renal response and is therefore a criterion for ruling out an AVP deficiency. In most cases, polyuria with isotonic/hypertonic urine is driven by glucose (e.g. uncontrolled diabetes mellitus or the use of SGLT-2 inhibitors), mannitol (e.g. from the treatment of increased intracranial pressure), urea (e.g. high protein intake, tissue catabolism and steroid administration) or medications such as diuretics. In patients with hypotonic polyuria or with a urine osmolality level of ≥ 300 mOsm/Kg and < 800 mOsm/Kg, further evaluation of plasma sodium and osmolality could assist in indicating the underlying type.[23] High plasma sodium (≥ 146 mmol/L) and osmolality (≥ 300 mOsm/Kg) levels point to AVP deficiency or resistance,

while low plasma sodium (≤ 135 mmol/L) and osmolality (≤ 280 mOsm/Kg) levels point to PP. However, in most cases, such a clear constellation is not present and a dynamic test is needed in the next step.

The classic **water deprivation test (WDT)**, also known as the 'indirect water deprivation test', as this test does not involve the 'direct' measurements of plasma AVP, follows the concept that AVP activity is indirectly assessed by measurement of the urine concentration capacity during fluid deprivation.[56,57] Physiologically, an increase in plasma osmolality caused by dehydration stimulates AVP release, leading to free water reabsorption, resulting in an increase in urine osmolality. The type of PPS can be theoretically assessed by the extent of the increase in urine osmolality upon fluid deprivation using cut-offs provided by Miller et al.[58] Dehydration should allow patients with PP to concentrate urine, whereas patients with AVP deficiency or resistance continue to excrete dilute urine. Following dehydration, desmopressin administration is used to distinguish patients with AVP deficiency from resistance, as those with a deficiency should be able to concentrate urine once the deficient AVP effect is replaced by desmopressin, whereas those with resistance should not show a significant response. However, this test protocol was derived from a post hoc analysis of a study involving only 36 patients without prospective validation of these suggested cut-offs.[59] Consequently, the WDT demonstrates a poor overall diagnostic accuracy of only 70% and a much lower accuracy of 40% in cases of PP.[57] Although an additional plasma AVP measurement after fluid deprivation has been proposed to improve the accuracy, these measurements failed to enter clinical practice because of complex preanalytical requirements. In contrast, copeptin, as an AVP surrogate, has the advantage that it is stable for several days at room temperature, does not require preanalytical procedures and provides a strong positive correlation with plasma osmolality.[60,61]

Recently, copeptin-based approaches have been suggested with higher diagnostic accuracy in diagnosing AVP deficiency.[61] An unstimulated basal copeptin level of > 21.4 pmol/l provides 100% sensitivity/specificity in diagnosing AVP resistance, whereas basal copeptin levels in patients with AVP deficiency and PP show considerable overlap.[60] Therefore, stimulated copeptin is needed to further distinguish between these two types of PPS.

One option is stimulated copeptin after a **hypertonic saline infusion test.**[57] This test is characterised by the administration of a bolus of 250 mL 3% sodium chloride followed by a body-weight-adapted infusion rate aiming at a plasma sodium level of ≥ 150 mmol/l. A stimulated copeptin level of < 4.9 pmol/l points to an AVP deficiency with a diagnostic accuracy of 96% (93% sensitivity; 100% specificity). As an alternative to hypertonic saline, copeptin using an **arginine infusion test** has been suggested.[62] A stimulated copeptin level of < 3.8 pmol/l after 60 minutes points to an AVP deficiency with a diagnostic accuracy of 93% (92% sensitivity, 93% specificity).

Although these new copeptin-based tests have significantly improved diagnostic accuracy in the evaluation of patients with PPS, some limitations should be mentioned. Both tests require an intravenous line with an infusion over time, which might cause discomfort (e.g. nausea or vomiting after arginine; vertigo or headache after hypertonic saline), and demand close monitoring under continuous medical supervision (e.g. during the hypertonic saline with a rapid increase in plasma sodium).[63] **Therefore, a new alternative test with comparable high diagnostic accuracy, a simplified protocol and a safe test profile would be of great clinical importance (Manuscript 1).**

Given the wide variety of aetiologies leading to AVP deficiency, the diagnostic evaluation usually includes a magnetic resonance imaging (MRI) of the hypothalamic-pituitary area.[29] The posterior pituitary is usually demonstrated as a hyperintense area on T1 weighted images, referred to as the 'bright spot', which is attributed to the presence of AVP secretory granules.[64,65] The absence of the bright spot is described as pathognomonic for AVP deficiency. However, in a prospective study of 92 patients with PPS, the specificity was low, showing an absence of the bright spot in 70% of patients with AVP deficiency and 39% of patients with PP. The low specificity could be due to an early stage of disease or gradual age-related loss of the bright spot.[66,67] Another reported radiological finding associated with the presence of AVP deficiency, although with low specificity, is a thickened pituitary stalk. However, its presence in combination with an absent bright spot is highly suspicious for neoplastic infiltration or inflammation.[68] Additionally, a sellar- or suprasellar mass or cysts might point to the possibility of craniopharyngioma, Rathke's cleft cyst, granulomatous or inflammatory conditions.

### 3.2.2. Management and complications of AVP deficiency

In most patients, osmoregulated thirst perception is intact; therefore, adequate fluid intake compensates for urinary water loss.[69,70] Nevertheless, desmopressin, a synthetic AVP analogue and selective V2 receptor agonist, is usually initiated after the diagnosis of AVP deficiency.[71,72] Desmopressin differs from AVP by the amino group of cysteine and by substituting L-arginine with D-arginine. These modifications prolong the half-life from 5 minutes (AVP) to 6 to 8 hours (desmopressin) and eradicate the vasopressor potential.[73] Although desmopressin treatment provides immediate symptomatic relief, the main complication of dilutional hyponatremia and its associated risk of cerebral oedema, seizure, coma and even death should be noted.[74] This complication can develop even with modest amounts of fluid intake when the antidiuretic effects of desmopressin prevent free water excretion. Some smaller retrospective studies have reported a hyponatremia prevalence of up to ~30% in routine laboratory controls.[75] **However, data from a larger cohort investigating desmopressin-associated prevalence of hyponatremia in the outpatient setting and the prevalence of hyponatremia leading to hospitalisations are needed** (**Manuscript 2**). To reduce the risk of hyponatremia, some experts recommend delaying a dose of desmopressin up to several times per week until breakthrough symptoms (strong thirst, full bladder, pale urine, frequent urination) occur or omitting a dose once a week.[1] Additionally, a lower risk of hyponatraemia has been suggested with oral desmopressin compared to the intranasal formulation.[76,77] However, **whether delaying or omitting desmopressin doses leads to a lower incidence of hyponatremia compared to a rigid desmopressin dose schedule and whether oral desmopressin carries a lower risk of hyponatremia needs to be investigated or confirmed (Manuscript 2)**.

## 3.3. Oxytocin deficiency

Impaired quality of life (QoL) and psychological changes or comorbidities in patients with anterior pituitary dysfunction are well-recognised.[78,79] By contrast, few attempts have been made to evaluate QoL and psychological changes or comorbidities in patients with AVP deficiency. Available research has primarily focused on patients with craniopharyngioma, a condition accompanied by a high risk of developing AVP deficiency.[80] In these patients, a 10-year follow-up study could reveal personality changes (31%) and increased psychosocial deficits (47%),[81] including anxiety, depression and social

withdrawal.[80,82,83] Interestingly, the age of onset appears to influence the type of socio-behavioural disruptions: while adult onset had higher levels of anxiety and depression, in younger patients more impact was observed within the domains of social isolation and functioning.[84] Additionally, data from smaller studies in patients with other aetiologies of AVP deficiency demonstrated significantly higher trait anxiety, alexithymia and depression levels compared to healthy controls.[85,86] **However, observations from these small studies have not been confirmed in a larger patient cohort, especially comparing patients with isolated AVP deficiency to those with combined anterior/posterior pituitary dysfunction in regard to psychological changes and comorbidities (Manuscript 2)**.

Owing to the close anatomical proximity of the AVP and OXT systems, disruptions leading to AVP deficiency could cause significant collateral damage to OXT-producing neurons, leading to an additional OXT deficiency.[87] It is, therefore, tempting to assume that the increased psychopathology and reduced QoL, as observed in patients, are caused – at least partially – by an additional undiagnosed OXT deficiency.[87] Currently, only limited research has been devoted to the role of OXT in psychological changes in patients with AVP deficiency. Recent smaller studies have shown slightly lower basal or 1-h pooled OXT plasma levels in patients with AVP deficiency compared to controls.[59] In some of those studies, these reduced OXT levels at baseline were associated with reduced empathic abilities or increased anxiety.[88] In support of these data, a study on AVP deficiency/craniopharyngioma with impaired salivary OXT release following physical stress reported higher levels of self-reported autistic traits, lower levels of joy when socialising and worse scores on an emotional recognition task than controls.[89] However, other studies could not confirm these findings or even demonstrated higher basal OXT levels than healthy controls, questioning the validity of baseline OXT levels in evaluating a potential deficiency.[90] This controversy might be explained by difficulties in the measurement of OXT, as it is technically cumbersome, and methods and ideal sampling are controversial. Published studies used different samples, for example blood, saliva, cerebrospinal fluid and urine, different laboratory methods, for example bioassays such as ELISA or RIA, and different measurement methods, for example single basal level or pooled blood analysis.[90] **Importantly, the available cumulative data underline that measurements of basal OXT levels are inadequate for identifying a deficiency**.

**However, to date, no test to investigate whether a potential OXT deficiency exists is available, and therefore, a novel provocation test is urgently needed (Manuscript 3).**

## 4. MAIN OBJECTIVES OF THIS MD-PHD PROJECT

Based on the above, these are the main objectives:

**MANUSCRIPT 1** [the Glucacop study]

Hypothesis: The effect of glucagon on the neurohypophysis and its diagnostic potential to distinguish between AVP deficiency and PP has not been investigated. We hypothesised that glucagon might stimulate the neurohypophysis and, if so, could be used in the differential diagnosis of AVP deficiency.

Aims/Objectives: To investigate the effect of glucagon stimulation on plasma copeptin and assess its diagnostic accuracy to distinguish between AVP deficiency and PP.

**MANUSCRIPT 2** [the DImond survey]

Hypothesis: Data about desmopressin-induced hyponatremia, quality of life and psychological comorbidities, the degree of knowledge and awareness about AVP deficiency among healthcare professionals, and the prevalence of incorrect management/treatment of AVP deficiency (e.g., due to confusion with 'diabetes mellitus') are scarce and restricted to small studies or case series. We hypothesised a high prevalence of desmopressin-induced hyponatremia and psychological comorbidities, low knowledge and awareness about the disease among healthcare professionals, and strong support for renaming "diabetes insipidus" due to frequent incorrect management/treatment.

Aims/Objectives: To investigate patients' perspectives on complications and management as in-patients and out-patients, psychological comorbidities, degree of knowledge and awareness among healthcare professionals, and views for renaming 'diabetes insipidus' to avoid confusion with 'diabetes mellitus'.

**MANUSCRIPT 3** [the OxyMA study]

Hypothesis: For other pituitary hormones, a provocation test to stimulate the respective hormone is often used in case of a suspected deficiency. Yet, no provocation test for OXT is established. 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") is known to stimulate the OXT system. We hypothesised a strong OXT increase in healthy controls in response to MDMA, but blunted OXT increase in patients with AVP deficiency.

Aims/Objectives: To investigate MDMA as a novel biochemical and psychoactive provocation test to reveal an OXT deficiency in patients with AVP deficiency.

## 5. Contribution by the MD-PhD student

### 5.1 Manuscript 1

- Thorough and detailed comprehensive literature research.
- Formulation of the research question
- Writing and preparation of necessary documents (e.g., clinical trial protocol) for submission of the trial to the competent ethics committee (CEC)
    - Correspondence with and submission of amendments to the CEC
    - Contact person for study monitor
- Preparation of study material and elaboration of logistic processes at study centre
- Recruitment of participants and conducting of the study
- Data analysis
- Discussion of the results with primary and secondary supervisor
- Writing of the manuscript.
- Manuscript submission, writing of rebuttal letter to objections raised by reviewers, and publication process.
- Presenting the data at scientific meeting, national and international congresses

### 5.2 Manuscript 2

- Thorough and detailed comprehensive literature research.
- Formulation of the research question
- Writing and preparation of necessary documents (e.g., clinical trial protocol) for submission of the trial to the CEC
    - Correspondence with and submission of amendments to the CEC
    - Contact person for study monitoring.
- Preparation of online platform for the survey
- Planning and overseeing the patient involvement activities
- Recruitment of participants for the survey
- Data analysis
- Discussion of the results with primary and secondary supervisor

- Writing of the manuscript.
- Manuscript submission, writing of rebuttal letter to objections raised by reviewers, and publication process.
- Presenting the data at scientific meeting, national and international congresses.

### 5.3 Manuscript 3

- Successful application for a Young Talents in Clinical Research Grant awarded by the Swiss Academy of Medical Sciences
- Thorough and detailed comprehensive literature research.
- Formulation of the research question
- Writing and preparation of necessary documents (e.g., clinical trial protocol) for submission of the trial to the CEC
    - Correspondence with and submission of amendments to the CEC
    - Contact person for study monitor
- Preparation of study material and elaboration of logistic processes at study centre
- Recruitment of participants and conducting of the study
- Data analysis
- Discussion of the results with primary and secondary supervisor
- Writing of the manuscript.
- Manuscript submission, writing of rebuttal letter to objections raised by reviewers, and publication process.
- Presenting the data at scientific meeting, national and international congresses

## MANUSCRIPT 1 (M1)

# Glucagon-stimulated copeptin measurements in the differential diagnosis of diabetes insipidus: a double-blind randomised placebo-controlled study

the Glucacop Study

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**ABSTRACT**

**BACKGROUND** The differential diagnosis of diabetes insipidus is challenging. The most reliable approaches are copeptin measurements after hypertonic saline infusion or arginine, which is a known growth hormone secretagogue but has recently also been shown to stimulate the neurohypophysis. Similar to arginine, glucagon stimulates growth hormone release, but its effect on the neurohypophysis is poorly studied.

**DESIGN** Double-blind, randomised, placebo-controlled trial including 22 healthy participants, ten patients with central diabetes insipidus, and ten patients with primary polydipsia at the University Hospital Basel, Switzerland.

**METHODS** Each participant underwent the glucagon test (subcutaneous injection of 1mg glucagon), and placebo test. The primary objective was to determine whether glucagon stimulates copeptin and to explore whether the copeptin response differentiates between diabetes insipidus and primary polydipsia. Copeptin levels were measured at baseline and 30, 60, 90, 120, 150, 180 minutes after injection.

**RESULTS** In healthy participants, glucagon stimulated copeptin with a median increase of 7.56 (2.38; 28.03) pmol/l, while placebo had no effect (0.10pmol/l (-0.70; 0.68); $p<0.001$). In patients with diabetes insipidus, copeptin showed no relevant increase upon glucagon, with an increase of 0.55 (0.21; 1.65) pmol/l, whereas copeptin was stimulated in patients with primary polydipsia with an increase of 15.70 (5.99; 24.39) pmol/l. Using a copeptin cutoff level of 4.6pmol/l had a sensitivity of 100% (95%CI 100-100) and a specificity of 90% (95%CI 70-100) to discriminate between diabetes insipidus and primary polydipsia.

**CONCLUSION** Glucagon stimulates the neurohypophysis, and glucagon-stimulated copeptin has the potential for a safe, novel, and precise test in the differential diagnosis of diabetes insipidus.

## INTRODUCTION

The differential diagnosis of central diabetes insipidus (cDI) and primary polydipsia (PP) is challenging. While cDI results from insufficient arginine vasopressin (AVP) release, PP is characterised by excessive fluid intake despite adequate AVP-secretion and -response.[24] The correct discrimination between these entities is essential since the appropriate treatments differ, and an incorrect treatment can lead to deleterious clinical consequences.[91]

The indirect water deprivation test, which has been the diagnostic gold standard for decades, has only limited diagnostic accuracy.[92] Previous attempts to directly measure AVP were not successful, mostly due to the molecular instability and the technical difficulties of AVP measurement.[93] Copeptin, the C-terminal segment of the AVP precursor peptide, is a stable surrogate marker of AVP and can easily be measured with a sandwich immunoassay.[93] Recently, we showed that copeptin measured after a hypertonic saline infusion test can be used as a reliable biomarker for the differential diagnosis between cDI and PP with a high diagnostic accuracy [57]. However, this test requires an intravenous (i.v.) line, often causes discomfort and demands a rapid increase in plasma sodium levels, making it essential to closely monitor plasma sodium levels under continuous medical supervision [63].

Arginine infusion – an established growth hormone stimulation test in children – has been shown to be a non-osmotic stimulus of copeptin, providing a simplified diagnostic tool in the differential diagnosis of cDI and PP, though with somewhat lower potency in stimulating copeptin [60,62]. Whether this test is non-inferior to the hypertonic saline infusion test is currently being investigated (ClinicalTrials.gov NCT03572166). Similarly, to arginine, glucagon stimulates growth hormone secretion [94]. For decades, glucagon injection has been a well-tolerated, reproducible, and safe clinical test for diagnosing growth hormone deficiency in adults and children [95]. In a previous study, Lewandowski et al. showed a copeptin increase upon glucagon stimulation in healthy volunteers but no relevant increase in patients with cDI [96]. In comparison to arginine, glucagon is known to be a more potent growth hormone (GH) stimulus [97]. We, therefore, hypothesised that glucagon injection might be an alternative stimulus of the neurohypophysis and, if so, could be used as a new diagnostic test to differentiate cDI from PP.

## MATERIAL AND METHODS

### Study design and participant

This prospective single-centre, double-blind, randomised, cross-over trial was conducted at the University Hospital Basel, Switzerland, from September 2020 to May 2021. The trial consisted of two parts. In a first step, we recruited 22 healthy participants, eligible if they were ages 18 years or older with a BMI between 18.5 kg/$m^2$ and 25kg/$m^2$, had normal drinking habits, and no history of polyuria. Exclusion criteria were haemoglobin level <120 g/l, acute illness, and vigorous physical exercise within 24 hours before the study participation.

In a second step, we enrolled ten patients with cDI (8 with complete cDI and 2 with partial cDI) and ten patients with PP diagnosed on the basis of the water deprivation test plus hypertonic saline infusion test or arginine test, respectively. Three patients with cDI had an additional anterior pituitary deficiency (two complete, one partial). Patients with cDI and PP were eligible if they were ages 18 years or older with a BMI between 18.5 kg/$m^2$ and 25kg/$m^2$ and evidence of abnormal hydration habits and diuresis defined as polyuria >50ml/kg body weight/24h and polydipsia >3l /24h or regular daily desmopressin medication. Detailed eligibility criteria are listed in the supplementary section (p 2). The local ethics committee, Ethical Committee Northwest and Central Switzerland, approved the study protocol. Written informed consent was obtained from all study participants. The study was registered on ClinicalTrials.gov, identifier NCT 04550520.

### Study procedure

In accordance with the cross-over design, all participants underwent two test days, i.e., one test day undergoing subcutaneous injection of 1 mg glucagon and one test day undergoing 1 ml injection of placebo, i.e., 0.9% sodium chloride, in random order with at least 48 hours between the two days. The study physician, study nurse, and study participant were blinded to the injection contents. The injection was performed by two unblinded study nurses who were not involved in further study procedures. The test day started between 08:00 and 10:00 a.m. after an overnight fast of 8 hours and fluid restriction for 2 hours. Patients on desmopressin treatment discontinued their medication at least 24 hours before the test. A standardised electrocardiogram was performed, and patients with long QT, i.e., >450ms, were excluded from the study. Study participants were settled in a semi-recumbent position 30 minutes before

test started, and a catheter was placed in an antecubital vein. All participants received Ondansetron 8 mg 10 minutes before the test to prevent nausea. At baseline, the first blood sample was collected, and glucagon 1 mg (Glucagen® NovoNordisk - Hypokit) or placebo (0.9% sodium chloride) was injected. Blood was collected at 30, 60, 90, 120, 150, and 180 minutes for analyses of plasma copeptin, prolactin, growth hormone, and glucose. A routine laboratory measurement, including haemoglobin level, was taken at baseline, plasma cortisol at timepoint 0 min and 150 min, and plasma osmolality and plasma sodium at time point 0 min and 180 min. Participants were not allowed to drink during the test procedure.

**Adverse effects and symptom burden**

Adverse effects, i.e., nausea, headache, and dizziness, were evaluated using a visual analogue scale (VAS) ranging from 0, i.e., no discomfort, to 10, i.e., maximum discomfort at baseline and 30, 60, 90, 120, 150, 180 minutes after injection. The overall test burden was evaluated at the end of the test using a VAS ranging from 0, i.e., no test burden, to 10, i.e., maximum test burden.

**Laboratory measurements**

Blood samples for plasma sodium, osmolality, growth hormone, prolactin, and glucose were processed as routine laboratory measurements in the central laboratory of the hospital. Blood samples for plasma copeptin and cortisol analysis were taken in serum tubes, immediately centrifuged at 4 °C and stored at -80°C until central batch analysis. Plasma copeptin concentration was measured in one batch with a commercial automated immunofluorescence assay (B.R.A.H.M.S Copeptin-proAVP KRYPTOR, Thermo Scientific Biomarkers, Hennigsdorf, Germany), and growth hormone levels were measured using an electrochemiluminescence immunoassay (ECLIA) (Cobas8000, Roche Diagnostics GmbH, Mannheim, Germany).

**Study outcomes**

The primary outcome was the maximal increase in plasma copeptin levels within three hours after the injection of a single subcutaneous dose of 1mg glucagon or 1 ml 0.9% sodium chloride. To derive the primary outcome for each participant and trial arm, we determined the maximal copeptin value measured between 30 and 180 minutes after injection ($copeptin_{max}$) and subtracted the corresponding baseline measurement ($copeptin_{baseline}$).

Secondary outcomes were the course of GH, prolactin, glucose, plasma sodium, and plasma osmolality; maximal changes for GH and prolactin; the difference between cortisol values at baseline and 150

minutes after injection and difference between plasma sodium and plasma osmolality values at baseline and 180 minutes among the healthy participants; time from baseline to maximum copeptin value; the number of patients who experienced nausea, headache, or dizziness during the study; maximum/median VAS for those with any symptoms; and number and type of all adverse events for each study group.

**Statistical analysis**

All analyses were predefined in a statistical report and analysis plan and performed in R version 4.0.3 (2020-10-10). Confirmatory analyses were restricted to the primary outcome in healthy volunteers. We tested the null hypothesis that the maximal copeptin change under glucagon and placebo are the same using a two-sided Wilcoxon's signed rank test for paired samples and significance level $\alpha$ of 5%. The primary analysis was performed as an intention-to-treat analysis using all 22 randomised healthy volunteers. Sensitivity analyses are described in the supplementary section.

All further analyses are exploratory without hypothesis testing and were performed on complete cases for healthy volunteers and patients with PP or cDI separately. Summary statistics for all outcomes at each measurement time are provided for glucagon and placebo treatment separately as well as for within-patient treatment-differences (glucagon - placebo). We further derived the individual time to the maximal copeptin value, the maximal change in GH and prolactin, and the changes in cortisol (baseline to 150 minutes) and plasma sodium and osmolality (baseline to 180 minutes). Since glucagon-stimulated copeptin levels separated patients with primary polydipsia and cDI, we further calculated ROC curves for each measurement time and derived the "best" copeptin cut-offs based on Youden's J. We report ROC-AUC, sensitivity, specificity, and overall diagnostic accuracy. We report the frequency and intensity of nausea, headache, and dizziness during the study. The sample size for healthy volunteers was estimated as n = 22 (allowing for 10% drop-out) to be able to show a difference between glucagon and placebo injection in the maximal increase in copeptin within three hours with $\alpha = 0.05$ and$\beta = 0.90$ a statistical power of 90% (for details see supplementary (M1)).

## RESULTS

### Baseline characteristics

Between September 1, 2020, and May 31, 2021, we enrolled 22 healthy participants, 10 patients with cDI, and 10 patients with PP at the University Hospital Basel, Switzerland. In healthy participants the median (IQR) age was 25 (22; 29) years with 45% female participants, in patients with cDI the median age was 31 (27; 44) with 60% female participants, and in PP the median age was 32 (26; 55) with 90% female participants. Baseline characteristics for each study group are presented in Table 1 (M1).

### Effect of glucagon on plasma copeptin levels in healthy volunteers and patients with cDI and PP

In healthy participants, the median (IQR) copeptin at baseline was 4.38 (3.28, 5.63) pmol/l and increased after injection of glucagon to 12.08 (8.17, 30.81) pmol/l resulting in a median increase of 7.56 (2.38, 28.03) pmol/l. For 20 of 22 healthy participants, copeptin levels peaked between 120 and 180 minutes (Table 2 (M1), Figure 1a (M1)). Under placebo, no notable increase in copeptin was observed (Table 2 (MI), Figure 1a (M1)). This resulted in a clinically relevant difference of 7.67 (1.98, 27.09) pmol/l on copeptin change (p-value <0.001; Table 2). Per protocol and sensitivity analyses provide further support for these findings (supplementary (M1)).

In patients with cDI, the median (IQR) copeptin at baseline was 2.10 (1.81, 2.25) pmol/l and increased only slightly after glucagon injection to 2.98 (2.45, 4.12) pmol/L resulting in a median increase of 0.55 (0.21,1.65) pmol/l. Under placebo, only a slight increase in copeptin was observed (Table 2 (M1), Figure 1b (M1)). In contrast to healthy subjects, there was no relevant peak of copeptin between 120 and 180 minutes (Figure 1b (M1)).

In patients with PP, the median (IQR) copeptin at baseline was 4.24 (2.75, 4.94) pmol/l and increased after glucagon stimulation to 20.06 (10.22, 28.82) pmol/L by a median of 15.70 (5.99,24.39) pmol/l, while under placebo, no notable increase in copeptin was observed (Table 2 (M1), Figure 1b (M1)). This resulted in a difference of 0.34 (0.17, 1.05) pmol/l in patients with cDI and 15.42 (5.72, 24.25) pmol/l in patients with PP (Table 2 (M1)). In healthy participants and patients with PP, the median time from baseline to the maximum copeptin level was 150 minutes after injection (Table 2 (M1)).

**Copeptin in the differentiation of cDI and PP**

The highest diagnostic potential of copeptin was observed at 150 and 180 minutes after glucagon stimulation (Table S3 (M1), supplementary (M1)). Specifically, at 150 minutes, the highest diagnostic accuracy was observed at a cutoff level of 4.60 pmol/l (accuracy 0.95 [95% CI 0.76-1.00], sensitivity 1.00 [1.00, 1.00]; specificity 0.90 [0.70, 1.00]) and 3.72 pmol/l (accuracy 0.95 [95% CI 0.76-1.00], sensitivity 0.90 [0.70, 1.00]; specificity 1.00 [1.00, 1.00]; AUC 0.99 (95% CI 0.96-1.00); Figure 2a (M1)). At 180 minutes, the highest diagnostic accuracy was observed at a cutoff level of 5.01 pmol/l (accuracy 0.95 [95% CI 0.76-0.99], sensitivity 1.00 [1.00, 1.00]; specificity 0.90 [0.70, 1.00]; AUC 0.98 (95% CI 0.93-1.00); Figure S4a (M1), supplementary (M1)).

**Effect of glucagon on growth hormone, prolactin, and cortisol levels**

Upon the injection of glucagon, growth hormone (GH) rose after 90 minutes for participants in all study groups and peaked at 150 minutes (Figure S5a/b (M1), supplementary (M1)). The median (IQR) GH increase under glucagon was 44.96 (26.10, 89.96) mIU/l in healthy participants, 12.44 (0.24, 23.65) in patients with cDI, and 20.13 (18.41, 27.56) in patients with PP (Table 2 (M1)). Of note, there was no increase in GH in two patients with cDI known to have GH deficiency.

In all three study groups, there was a slight decrease in prolactin over the 180 minutes after both treatments. The median prolactin change under glucagon was 13 (-91, 70) mIU/l in healthy participants, 13 (-12, 74) mIU/l in patients with cDI, and -33 (-97, 22) mIU/l in patients with PP (Table 2 (M1)). The median cortisol increase from baseline to 150 minutes after injection under glucagon was 45 (-52, 240) nmol/l in healthy participants, 190 (-49, 366) nmol/l in patients with cDI, and 140 (-47, 288) nmol/l in patients with PP (Table 2 (M1)).

**Effect on plasma sodium and plasma osmolality**

During glucagon stimulation, the median (IQR) plasma sodium level slightly increased by 1.0 (0.0, 2.0) mmol/l in healthy participants, by 2.5 (0.25, 3) mmol/l in patients with cDI, and by 0 (-1.75, 1.00) mmol/l in patients with PP (Table 2 (M1)).

No notable increase was observed for plasma osmolality, the median change from baseline to 180 minutes was 0.00 (0.00, 5.00) mosm/kg in healthy participants, 3.50 (0.25, 7.00) mosm/kg for patients with cDI, and 0.00 (-3.75, 1.00) mosm/kg for patients with PP (Table 2 (M1)).

**Effect on glucose levels**

Under placebo, glucose levels remained stable over the 180 minutes, while they increased at 30 minutes under glucagon and decreased until 120 minutes to baseline levels (Figure 3 a/b (M1)). In healthy participants, the median (IQR) baseline glucose was 4.9 (4.6, 5.2) mmol/l, peaked after 30 minutes to 8.1 (7.2, 9.4) mmol/l, and showed the lowest levels at 120 minutes at 3.8 (3.5, 4.5) mmol/l. In patients with cDI, the median baseline glucose was 4.8 (4.6, 5.3) mmol/l, peaked after 30 minutes to 7.4 (6.9, 8.0) mmol/l, and showed the lowest levels at 150 minutes at 4.0 (3.7, 4.3) mmol/l. In patients with PP, the median baseline glucose was 4.6 (4.4, 4.9) mmol/l, peaked after 30 minutes to 7.1 (6.6, 8.2) mmol/l, and showed the lowest level at 150 minutes at 4.2 (4.1, 4.6) mmol/l.

**Safety and tolerability**

Overall, glucagon stimulation was safe and well tolerated with a median (IQR) test burden of 1.5 (1, 4) VAS-points in healthy participants, 3 (1.5, 4.5) in cDI, and 3 (2, 4.5) in PP. In healthy participants, nausea was indicated with a median burden of 2 (1, 3), headache with 2 (1.25, 3), and dizziness with 1.5 (1, 3.25) among those with symptoms. In patients with cDI, nausea was indicated with a median burden of 2.5 (1.75, 3.25), headache with 3 (2.75, 3.25), and dizziness with 2 (2, 2) among those with symptoms. In patients with PP, nausea was indicated with a median burden of 2.5 (2, 4), headache with 3.5 (3, 4), and dizziness with 1 (1, 3) among those with symptoms (Table S4 (M1), supplementary (M1)). In total, 14 adverse events occurred during the glucagon test, 10 in the healthy participants, 1 in patients with cDI, and 3 in patients with PP (Table S5, supplementary (M1)). All events lasted only for a short time and were resolved within minutes. Under placebo, no adverse events were observed.

**Figure 1 a/b (M1) Course of copeptin levels after glucagon stimulation in healthy participants (A) and patients with central diabetes insipidus and primary polydipsia (B).**

Boxes span the interquartile range (IQR); the thick horizontal line is the median. Whiskers are the most extreme values lying within the box edge and 1.5 times the IQR. All other values are considered to be outliers and plotted as individual points. For better presentation, the y axis is shown up to 60 pmol/l; hence, the maximum values by study group were 757.8 pmol/L at 150 minutes for healthy participants, 5.41 pmol/l at 120 minutes for cDI, and 116.7 pmol/l at 150 minutes for PP.

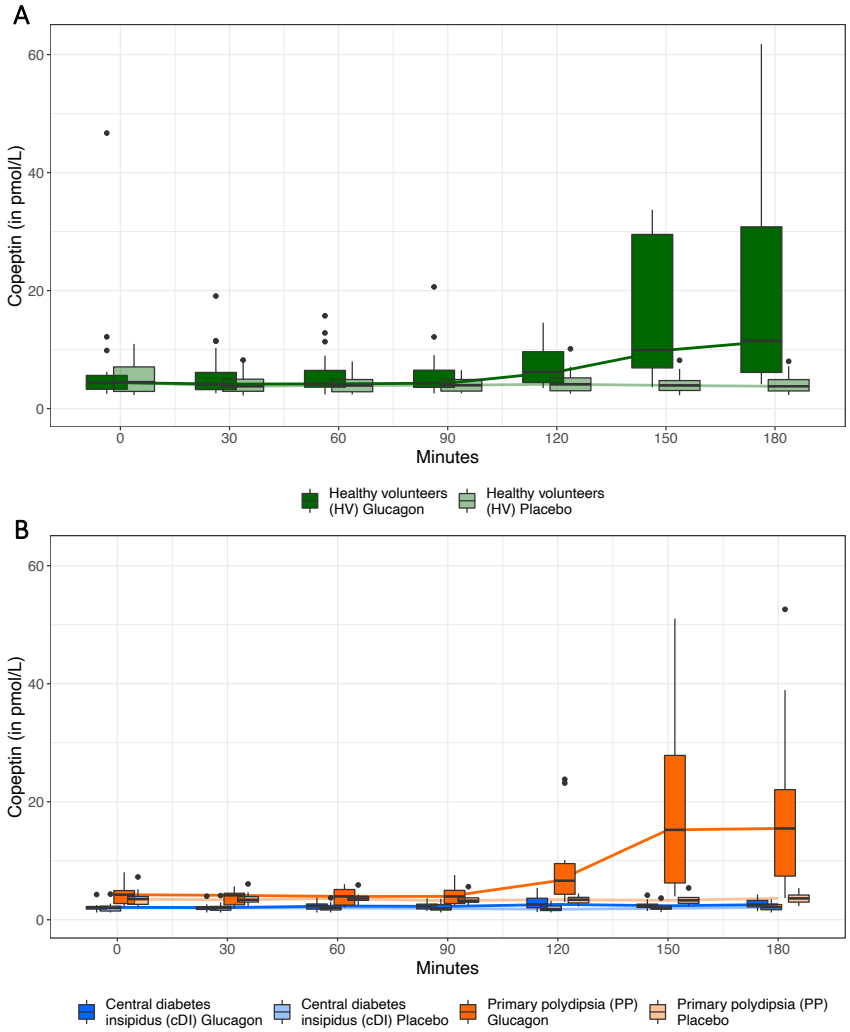

**Figure 2 a/b (M1) Receiver-operating-characteristic area under the curve (ROC) and best copeptin cutoffs at timepoint 150 (A) and individual copeptin levels (B) at timepoint 150 minutes.** (A) ROC curve for copeptin measured after glucagon injection. The red point and text indicate the optimal cutoff (specificity, sensitivity). The red error bars indicate 95% confidence intervals. (B) Individual copeptin level for each patient. The black horizontal lines indicate the proposed best thresholds to differentiate between the patient groups.

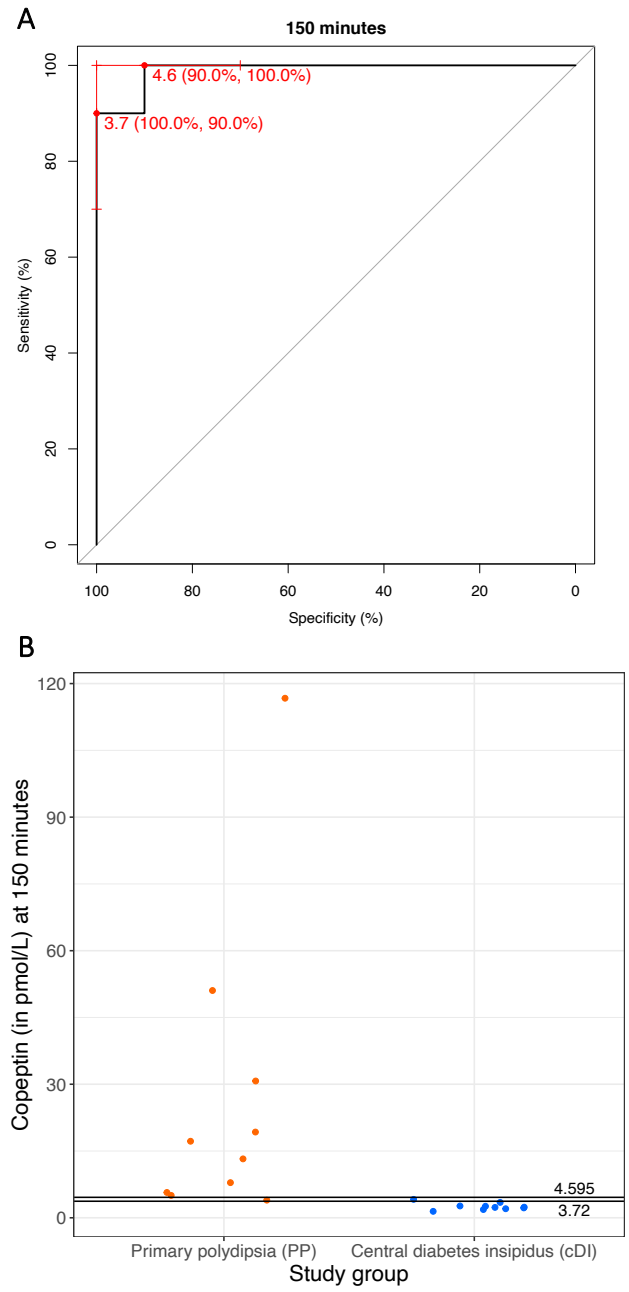

**Figure 3 a/b (M1) Course of glucose levels after glucagon stimulation in healthy participants (A), patients with central diabetes insipidus and primary polydipsia (B).**

Boxes span the interquartile range (IQR); the thick horizontal line is the median. Whiskers are the most extreme values lying within the box edge and 1.5 times the IQR. All other values are considered to be outliers and plotted as individual points.

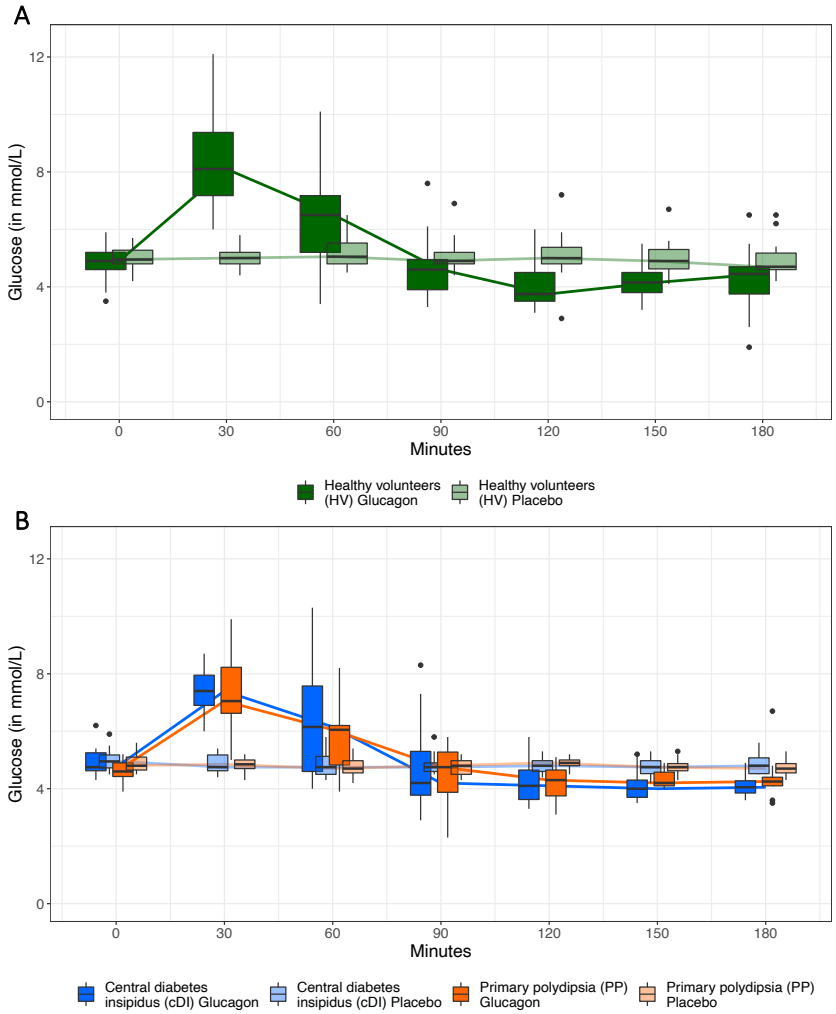

**Table 1 (M1) Baseline characteristics for each study group**

| | Overall | Healthy participants | Diabetes insipidus | Primary polydipsia |
|---|---|---|---|---|
| **n** | 42 | 22 | 10 | 10 |
| **Age (in years)** | 27 [23, 32] | 25 [22, 29] | 31 [27, 44] | 32 [26, 55] |
| **Sex, Male** | 17 (41) | 12 (55) | 4 (40) | 1 (10) |
| **Ethnicity, Caucasian** | 40 (95) | 21 (96) | 9 (90) | 10 (100) |
| **Alcohol consumption (in glasses/week)** | 1.0 [0.0, 2.8] | 1.0 [0.0, 3.0] | 0.5 [0.0, 1.8] | 0.5 [0.0, 2.5] |
| **Current smoking status** | 10 (24) | 5 (23) | 1 (10) | 4 (40) |
| **Body mass index (in kg/m$^{2)}$** | 22.2 (1.8) | 22.4 (1.6) | 22.1 (1.6) | 21.9 (2.4) |
| **Hemoglobin (in g/l)** | 138 [131, 144] | 137 [127, 144] | 142 [137, 149] | 138 [132, 142] |

IQR = interquartile range, BMI= body mass index, SD= standard deviation. Data are presented in median [IQR], mean (SD), and numbers (%).

**Table 2 (M1) Laboratory parameters for placebo/glucagon stimulation in healthy participants, patients with diabetes insipidus and primary polydipsia**

| | Healthy participants | | Diabetes Insipidus | | Primary Polydipsia | |
|---|---|---|---|---|---|---|
| | **Placebo** | **Glucagon** | **Placebo** | **Glucagon** | **Placebo** | **Glucagon** |
| **Copeptin at baseline (pmol/l)** | 4.48 [2.91, 7.07] | 4.38 [3.28, 5.63] | 2.00 [1.48, 2.31] | 2.10 [1.81, 2.25] | 3.50 [2.61, 3.94] | 4.24 [2.75, 4.94] |
| **Copeptin maximum (pmol/l)** | 4.71 [3.44, 6.26] | 12.08 [8.17, 30.81] | 2.23 [1.90, 2.75] | 2.98 [2.45, 4.12] | 3.80 [3.39, 4.33] | 20.06 [10.22, 28.82] |
| **Maximum copeptin change (pmol/l)** | 0.10 [-0.70, 0.68] | 7.56 [2.38, 28.03] | 0.21 [0.04, 0.38] | 0.55 [0.21, 1.65] | 0.14 [-0.40, 0.67] | 15.70 [5.99, 24.39] |
| **Treatment effect * (pmol/l)** | 7.67 [1.98, 27.09] | | 0.34 [0.17, 1.05] | | 15.42 [5.72, 24.25] | |
| **AUC for copeptin** | 747 [528, 896] | 1296 [925, 2156] | 347 [304, 427] | 423 [398, 476] | 609 [567, 664] | 1456 [808, 2322] |
| **Median time from baseline to maximum copeptin level (minutes)** | 120 [38, 150] | 150 [150, 180] | 120 [68, 173] | 120 [90, 173] | 45 [30, 113] | 150 [150, 173] |
| | | | | | | |
| **Maximum GH change (mIU/l)** | 0.00 [-5.85, 3.55] | 44.96 [26.10, 89.96] | 0.66 [-3.72, 1.87] | 12.44 [0.24, 23.65] | -4.81 [-6.06, 0.10] | 20.13 [18.41, 27.56] |
| **Maximum prolactin change (mIU/l)** | -68 [-156, -7] | 13 [-91, 70] | -31 [-55, -4] | 13 [-12, 74] | -65 [-155, -15] | -33 [-97, 22] |
| **Maximum cortisol change (nmol/l)** | -311 [-331, -194] | 45 [-52, 240] | -106 [-265, -13] | 190 [-49, 366] | -136 [-213, -101] | 140 [-47, 183] |
| | | | | | | |
| **Plasma sodium at baseline (mmol/l)** | 139 [138, 140] | 139 [138, 140] | 143 [141, 143] | 143 [141, 144] | 139 [138, 142] | 141 [140, 141] |
| **Plasma sodium at 180 minutes (mmol/l)** | 139 [137, 140] | 140 [138, 141] | 144 [141, 147] | 145 [143, 144] | 141 [140, 141] | 139 [138, 142] |
| **Change in plasma sodium (mmol/l)** | -0.5 [-2.0, 1.8] | 1.0 [0.0, 2.0] | 2.0 [-1.5, 5.5] | 2.5 [0.4, 3.0] | 1.5 [-0.8, 3.0] | 0.0 [-1.8, 1.0] |
| **Plasma osmolality at baseline (mOsm/kg)** | 286 [284, 289] | 286 [282, 291] | 288 [286, 290] | 293 [288, 295] | 286 [285, 286] | 285 [284, 286] |
| **Plasma osmolality at 180 minutes (mOsm/kg)** | 288 [283, 291] | 287 [284, 291] | 292 [287, 298] | 295 [292, 301] | 286 [282, 287] | 284 [280, 287] |
| **Change in plasma osmolality (mOsm/kg)** | 1.0 [-1.8, 2.0] | 0.0 [0.0, 5.0] | 2.0 [-2.3, 14.0] | 3.5 [0.3, 7.0] | 1.0 [-2.8, 3.5] | -0.5 [-3.8, 1.0] |
| **Glucose at baseline (mmol/l)** | 5.0 [4.8, 5.3] | 4.9 [4.6, 5.2] | 5.0 [4.7, 5.2] | 4.8 [4.6, 5.3] | 4.8 [4.7, 5.1] | 4.6 [4.4, 4.9] |
| **Glucose at 30 minutes (mmol/l)** | 5.0 [4.8, 5.2] | 8.1 [7.2, 9.4] | 4.8 [4.6, 5.2] | 7.4 [6.9, 8.0] | 4.9 [4.7, 5.0] | 7.1 [6.6, 8.2] |
| **Glucose at 150 minutes (mmol/l)** | 4.9 [4.6, 5.3] | 4.2 [3.8, 4.5] | 4.8 [4.5, 5.0] | 4.0 [3.7, 4.3] | 4.8 [4.6, 4.9] | 4.2 [4.1, 4.6] |

AUC = area under the curve, GH = growth hormone. Data are presented in median (IQR). *Within-patient treatment differences in maximal copeptin changes (i.e., $Copeptin_{\Delta glucagon} - Copeptin_{\Delta placebo}$) by study group

## DISCUSSION

Our study has two main findings. First, we provide evidence for a strong effect of glucagon on plasma copeptin levels, and second, we show that glucagon-stimulated copeptin measurement has the potential to be used for a safe, novel, and precise test in the differential diagnosis of cDI and PP.

Glucagon is a peptide hormone produced by alpha cells in the pancreatic islets [98]. Besides the possibility of a therapeutic use of exogenous glucagon e.g., in acute treatment of hypoglycaemia, it is used as a safe and reliable test for the diagnosis of GH deficiency in adults and children [99,100]. In addition, exogenous administration of glucagon has been shown to stimulate adrenocorticotropin followed by an acute increase of cortisol levels [101].

In this study we confirm the previously described non-osmotic effect of glucagon on the posterior pituitary gland with release of copeptin as a surrogate marker of AVP. A possible explanation for this effect may be the dynamics of glucose after glucagon injection. During glucagon stimulation, plasma glucose levels increased from baseline and peaked 30 minutes after injection, followed by a rapid decrease to low/normal levels around timepoint 120 minutes. Interestingly, insulin-induced hypoglycaemia is a known stimulus for copeptin [102]. The copeptin peak after glucagon occurred simultaneously with the GH peak, which was at 150 minutes after glucagon injection, suggesting the same stimulatory effect of glucagon on both hormones [99,103]. Accordingly, a rapid decrease of elevated glucose levels might be a potential mechanism for the simultaneous stimulation of copeptin and GH after glucagon injection [104,105]. This hypothesis aligns with the results from earlier studies, showing identical glucose courses after glucagon stimulation with a simultaneous effect on plasma copeptin and GH [96]. Similar to our study, Lewandowski et al. observed an increase of copeptin levels in healthy volunteers, but no or an only mild increase in patients with cDI. Our study differs from Lewandowski et al. by a few points. First, we investigated the glucagon stimulation with the aim to assess the diagnostic potential of glucagon stimulation in differentiating cDI from PP. Lewandowski et al. included nine patients with cDI and no patient with PP, and therefore, a comparison between the copeptin response in these patients and investigation of the diagnostic accuracy of the glucagon test was not possible. Second, in our study all patients under desmopressin therapy discontinued their medication for 24h prior to the

test to avoid biased copeptin levels in medicated patients. To our knowledge, Lewandowski et al. did not mention discontinuation of desmopressin therapy in their test protocol. Third, and importantly, glucagon stimulation is known to induce nausea, a strong stimulus of arginine vasopressin/copeptin release. This is why in clinical routine; an antiemetic drug is usually given with the glucagon test. Lewandowski et al. performed the test without additional antiemetic medication, and therefore the stimulatory effect might be additionally triggered by nausea. Nausea is therefore a likely explanation for the higher copeptin response in their study compared to ours, as we administered ondansetron to all study participants upon glucagon and placebo stimulation to prevent confounded copeptin levels. Our data show only a minimal increase of nausea and likely minor influence on the copeptin levels.

In addition, it is known that an increase in glucose levels inhibits GH release, potentially explained by glucose-mediated hypothalamic somatostatin release [106]. On the other hand, the observed decrease of glucose levels during the glucagon stimulation could lead to inhibited hypothalamic somatostatin release, consequently leading to increasing GH levels. In animal studies, this mechanism was also suggested for the vasopressin system after intracerebroventricular infusion of a somatostatin analogue leading to inhibition of elevated plasma AVP levels [107]. Since a marked increase of glucose levels can be observed at 30 minutes, one further hypothesis might be an acute increase in osmolality, however, the delayed copeptin response (120/150 minutes after the glucose peak) is unlikely to be explained by this.

Our data suggest that the glucagon stimulation test has promising diagnostic potential. Importantly, glucagon had only a minimal effect on copeptin in patients with cDI, but increased copeptin levels considerably in patients with PP to almost fourfold from baseline. Using a copeptin cutoff of 4.6pmol/l at timepoint 150 minutes glucagon-stimulated copeptin discriminated between cDI and PP with high diagnostic accuracy. Further, as routinely used in children for diagnosis of GH deficiency since decades, the glucagon test could easily be applied in the paediatric age group after confirmation of our results in a paediatric study.

Today, the most reliable diagnostic test for the differential diagnosis of cDI and PP is copeptin measurement after hypertonic saline infusion test [57]. However, inducing hypernatremia requires close

monitoring with continuous medical supervision, making it challenging to carry out in the context of daily clinical practice. The arginine test has been proposed as an alternative non-osmotic stimulus with a short test duration and no need for continuous sodium monitoring [62]. Arginine is a less potent stimulus than hypertonic saline, and whether the test can be considered non-inferior to the hypertonic saline test is currently being investigated (ClinicalTrials.gov NCT03572166). Compared to arginine, glucagon seems to be more potent in increasing copeptin levels, but with a slightly longer test duration [62,97].

The tolerability and safety profile of the glucagon test was high. The observed adverse events after glucagon injection might best be explained by the rapid decrease of glucose causing headache, dizziness, cold feeling, and hot flashes, which, however, were all mild and resolved within minutes. Importantly, non-osmotic stimuli of the vasopressin system such as nausea and vomiting are known to confound copeptin levels and to influence the test results [108,109]. We, therefore, administered Ondansetron before the injection of glucagon. As a result, nausea experienced by participants was mild and unlikely to affect copeptin levels in this study. During glucagon and placebo stimulation, osmolality and plasma sodium levels showed no relevant change from baseline to the end of the test procedure, indicating no osmotic effect of glucagon on copeptin.

Our study had several limitations and strengths. As a proof of concept study, the sample size for deriving a cut-off to discriminate between cDI and PP was small. A larger study has to validate the proposed cutoffs in the future. In addition, we only included patients with a clear diagnosis of either cDI or PP and mainly patients with complete cDI. Diagnostic accuracy is likely to be overestimated and may be lower when including patients with an unclear diagnosis of polyuria polydipsia syndrome and when generalising our results to the broader population with more partial cDI patients. The given cut-offs should be interpreted with caution for patients with partial cDI since most of the patients had a complete cDI. Strengths are the novel and simple diagnostic approach in the differential diagnosis of diabetes insipidus and the randomized, placebo-controlled double-blind study design.

In conclusion, we show that glucagon-stimulated copeptin measurement is a safe and promising novel diagnostic test in the evaluation of diabetes insipidus.

**Declaration of interest**

We declare no competing interests.

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**Contributors**

CA edited the protocol; contributed to data collection, data analysis, and data interpretation; did the literature search; and wrote the manuscript. OG wrote the protocol, contributed to data collection did the literature search; and wrote the manuscript. DRV and LW covered all statistical aspects of the study, planned and performed data analysis, and edited the manuscript. GS edited the protocol, contributed to data collection, and edited the manuscript. MC-C edited the protocol, contributed to data analysis and data interpretation, edited the manuscript, and supervised all steps of the conduct of the study.

**SUPPLEMENTAL MATERIAL FOR MANUSCRIPT 1 (M1)**

**DETAILED ELIGIBILITY CRITERIA**

**Inclusion criteria for healthy participants:**

- Age 18-60 years
- Healthy participant with no medication except hormonal contraception

**Exclusion criteria for healthy participants:**

- BMI > 25kg/$m^2$ or < 18.5 kg/$m^2$
- Participation in a trial with investigational drugs within 30 days
- Vigorous physical exercise within 24 hours before the study participation
- Alcohol intake within 24 hours before study participation
- Pregnancy and breastfeeding
- Evidence of disordered drinking habits and diuresis defined as polyuria >50ml/kg body weight/24h and polydipsia >3l /24h
- Intention to become pregnant during the study
- Known allergy towards glucagon
- Evidence of an acute illness
- Long QT syndrome (i.e., QT time >450ms)
- Haemoglobin level below 120 g/l

**Inclusion criteria for patients:**

- Age 18-60 years
- Documented primary polydipsia or diabetes insipidus based on a water deprivation test or hypertonic saline infusion. Accordingly, patients must have evidence of disordered drinking habits and diuresis defined as polyuria >50ml/kg body weight/24h and polydipsia >3l /24h, or must be on regular daily Desmopressin medication.

**Exclusion criteria for patients:**

- BMI > 25kg/$m^2$ or < 18.5 kg/$m^2$
- Participation in a trial with investigational drugs within 30 days
- Vigorous physical exercise within 24 hours before the study participation

- Alcohol intake within 24 hours before study participation
- Pregnancy and breastfeeding
- Evidence of an acute illness
- Long QT syndrome (i.e., QT time >450ms)
- Haemoglobin level below 120 g/l

**Sample size for healthy volunteers**

For the primary analysis we performed a two-sided hypothesis test. The null hypothesis was that, in healthy volunteers, there was no difference in the primary endpoint between glucagon and placebo injection. I.e. under the null hypothesis, the expected difference was zero. The alternative hypothesis was that the difference in the primary endpoint between glucagon and placebo injection was not zero.

Sample size was estimated to be able to show a difference between glucagon and placebo injection in the maximal increase in copeptin within three hours in healthy volunteers. The calculation was based on data from Lewandowski et al. (2016) and Szinnai et al. (2007).

Assumptions glucagon arm: For the glucagon arm, we followed a multi-step resampling approach:

• Data from Lewandowski et al. (2016) from healthy controls and subjects without cDI with baseline and stimulated copeptin values available were used. Patients with very high copeptin values at baseline (≥ 20 pmol/l) were excluded.

• We derived the maximal changes for the remaining 23 subjects. Based on these data, we estimated the probability density function (pdf) and derived the bandwidth *bw* of a Gaussian smoothing kernel (3.62).

• In the first resampling step we sampled s = 10’000 times from the 23 data points with replacement. These samples were used as expected values $\mu_1$, ...,$\mu_{10'000}$ for the next step.

• In the second resampling step we simulated 10’000 times one random draw each from normal distributions with expected value $\mu = \mu_s$ and standard deviation $\sigma$ equivalent to $_{bw} = 3.62$. This resulted in a pool of 10’000 simulated data points following the density distribution from the original data.

Assumptions placebo arm: For the placebo arm we assumed the primary endpoint to follow a normal distribution with expectation zero. We based our assumptions on the results of Szinnai et al. (2007) who reported mean (sd) changes in copeptin in two control groups (subjects were allowed to consume non-alcoholic beverages except coffee ad libitum throughout the study period of 28h) of -0.8 (1.5) pmol/l and -0.7 (2.6) pmol/l. We assumed a slightly larger standard deviation of 3.0 pmol/l. The sensitivity of the sample size was examined by varying the expected mean value from -1 to 2 pmol/l.

Sample size calculation: Each sample size (ni=1,...,16 = 10, ..., 40) was evaluated by sampling R = 1000 times $n_i$ values from the simulated data pool for the glucagon arm and from the assumed distributions

for the placebo arm. For simplicity, we assumed the samples to be uncorrelated within subjects. The sampled values were tested for a difference between glucagon and placebo using the Wilcoxon rank sum test. We set the type I error probability, α, to 0.05 and the type II error probability, β, to 0.10 (90% power). The null hypothesis was rejected if the p-value of the statistical test was lower than α.

**Figure 6** shows the sensitivity of the sample size for the healthy volunteers assuming either a decrease, no change or an increase under placebo injection. Assuming a drop-out rate of 10 %, a total of 22 healthy volunteers should be recruited, in order to have 19 evaluable subjects. The respective sample size allows in at least 90 % of hypothetical repetitions of the study (i.e. with a power of 1 -β = 90%) to reject the null hypothesis.

**Sample size for patients with central diabetes insipidus**

The rationale for also including patients with central diabetes insipidus is to collect data on their reaction to glucagon since no data are available yet. This part is of pilot character and no hypothesis test is planned. We aim to estimate the maximal increase in copeptin under glucagon injection, which we expect to be very small at most.

Under stimulation with arginine infusion (Winzeler et.al. 2019, the Lancet) we have observed an average maximal copeptin increase of 0.69 pmol/l with a standard deviation of 1.1 pmol/l. The data-derived 95th percentile was an increase of 2.09 pmol/l. Assuming a similar reaction to glucagon, 10 patients with diabetes insipidus would provide 90% power to estimate the maximal change in copeptin with a precision such that the upper level of the 95% confidence interval would be lower than 2.0 pmol/l.

**Per protocol and sensitivity analyses**

Per protocol analyses: The primary finding was supported in both per protocol analyses: Median [IQR] difference between the increase in copeptin under glucagon and placebo were 7.04 [1.53, 15.23] pmol/l (p-value <0.001) for PPS-1 and 6.69 [1.51, 13.41] pmol/l (p-value <0.001) for PPS-2.

To examine the robustness and sensitivity of the treatment effect, supplementary per-protocol analyses were performed, excluding one participant without baseline copeptin measurement in the glucagon arm (n = 21), and another participant who showed signs of a vasovagal reaction before start of treatment (n = 20). In the latter we further performed two sensitivity analyses based on modified versions of the primary outcome, using the maximum of I) those measurements not exceeding ten times the baseline value ($Copeptin_{max} < 10 * Copeptin_{bsl}$) and II) those measurements not exceeding 100 pmol/l ($Copeptin_{max} < 100$ pmol/l).

Sensitivity analyses: In 4 subjects, extreme increases in copeptin levels larger than ten times the baseline value were observed (757.8, 461, 74.18, 98.99 pmol/l). In 2 of these subjects, the maximal observed copeptin level was also larger than 100 pmol/l. Replacing these extreme values by the respective maximal values smaller than ten times the baseline value (3.48, 6.1, 14.56, 10.14 pmol/l; sensitivity analysis 1) or by the respective maximal values ≤ 100 pmol/l (3.48, 6.1 pmol/l; sensitivity analysis 2) resulted in a smaller but still relevant difference between the increase in copeptin under glucagon and placebo: 4.36 [1.4, 8.33] pmol/l (p-value <0.001) for sensitivity analysis 1 and 4.36 [1.4, 8.45] pmol/l (p-value <0.001) for sensitivity analysis 2.

**Figure S4 a/b (M1) Receiver-operating-characteristic area under the curve (ROC) and best copeptin cutoffs (A) and individual copeptin levels (B) at timepoint 180 minutes.**

(A) ROC curve for copeptin measured after glucagon injection. The red point and text indicate the optimal cutoff (specificity, sensitivity). The red error bars indicate 95% confidence intervals. (B) Individual copeptin level for each patient. The black horizontal line indicates the proposed best thresholds to differentiate between the patient groups.

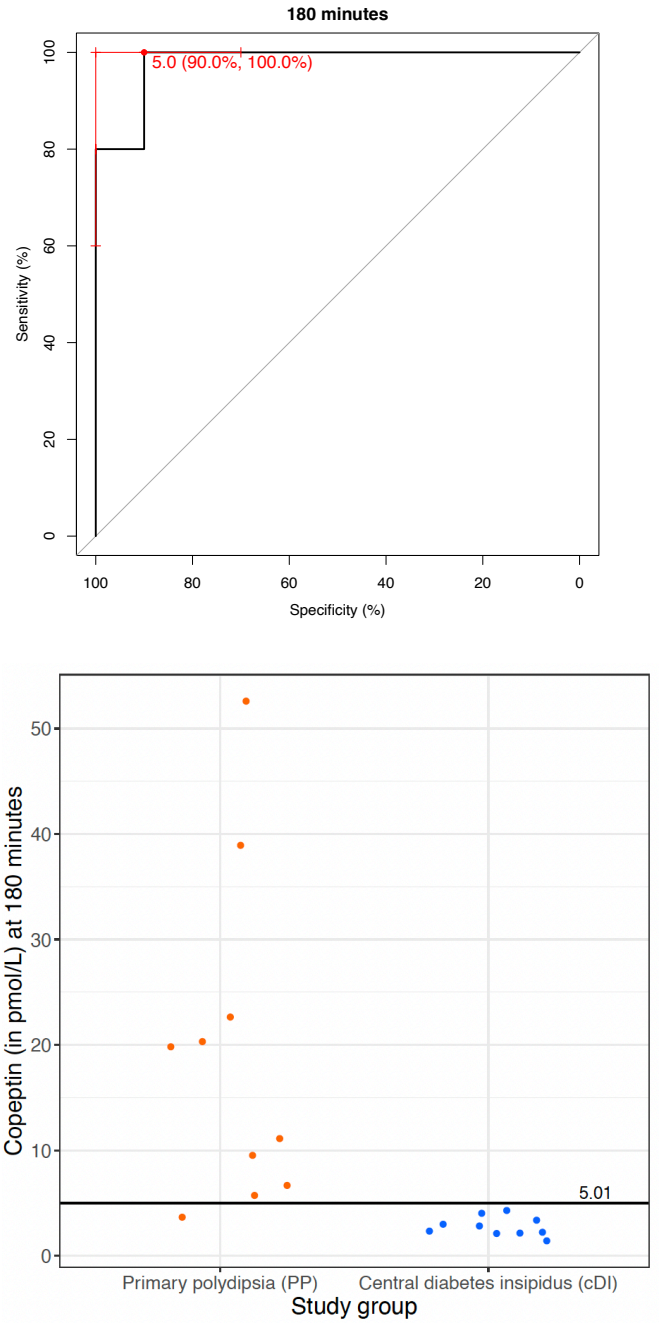
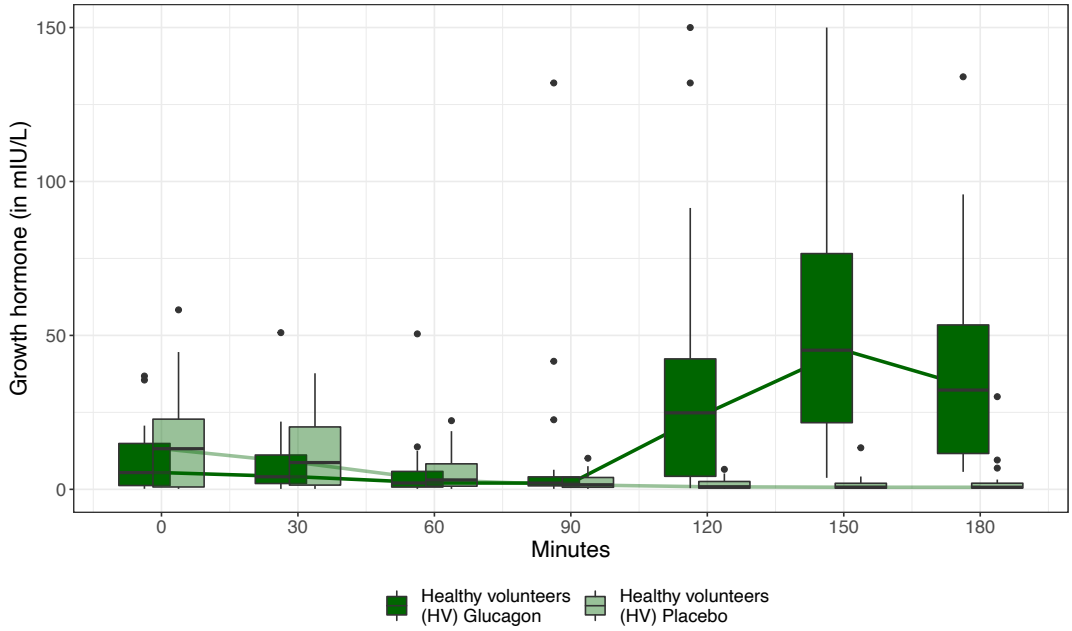


**Figure S5 a/b (M1) Course of growth hormone levels after glucagon stimulation in healthy participants (A), patients with central diabetes insipidus and primary polydipsia (B).**

Boxes span the interquartile range (IQR); the thick horizontal line is the median. Whiskers are the most extreme values lying within the box edge and 1.5 times the IQR. All other values are considered to be outliers and plotted as individual points.



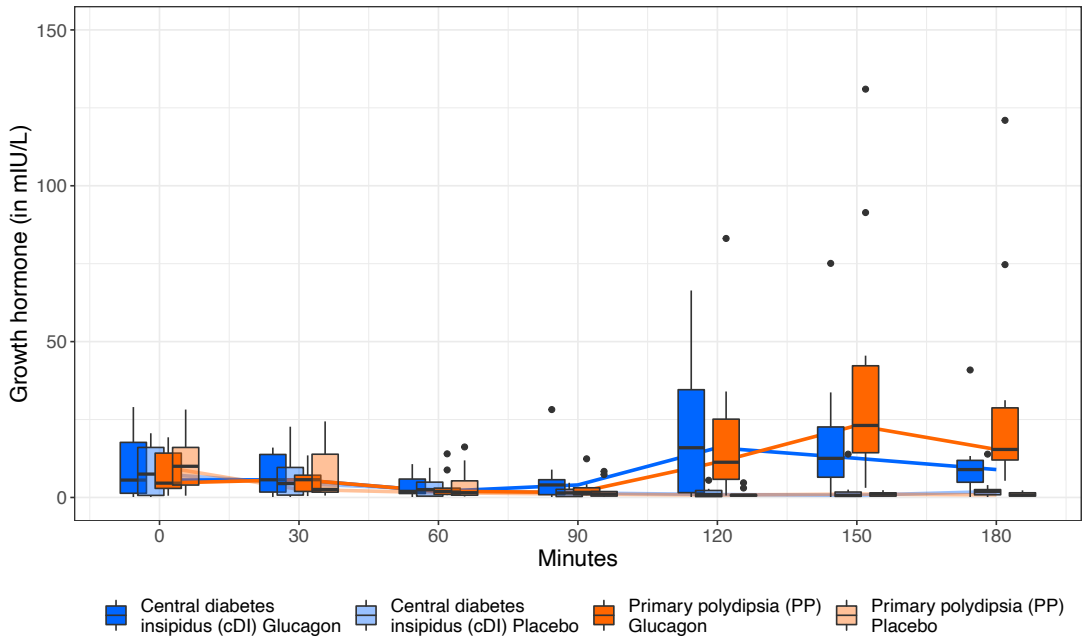



**Figure S6 (M1)**

Sensitivity of the sample size with respect to the expected change in the primary endpoint under placebo (θ). Curves are smoothed and are shown for illustration only.

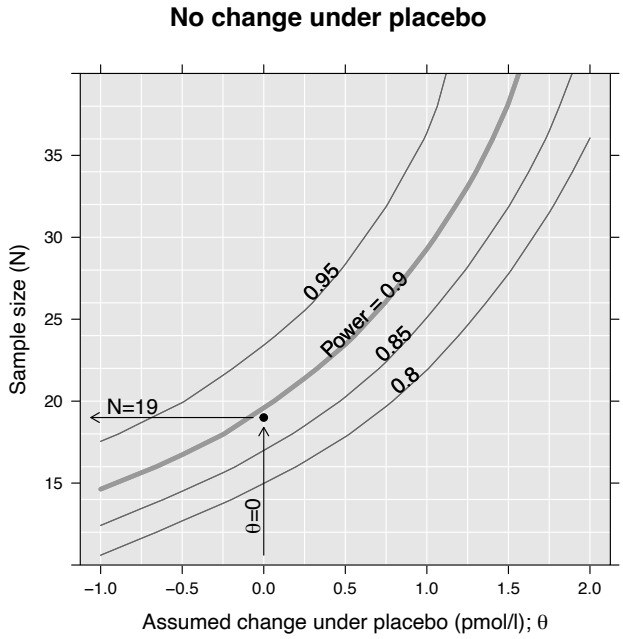


**Table S3 (M1) Best copeptin cutoffs at different timepoints**

| Timepoint | AUC [95% CI] | Optimal cut-off | Sensitivity [95% CI] | Specificity [95% CI] | Accuracy [95% CI] |
|---|---|---|---|---|---|
| 30 minutes | 0.88 [0.73, 1.00] | 2.3 | 0.80 [0.50, 1.00] | 0.90 [0.70, 1.00] | 0.85 [0.63, 0.96] |
| 60 minutes* | 0.81 [0.62, 1.00] | 3.3 | 0.90 [0.70, 1.00] | 0.60 [0.30, 0.90] | 0.75 [0.53, 0.90] |
| | | 2.4 | 0.60 [0.30, 0.90] | 0.90 [0.70, 1.00] | 0.75 [0.53, 0.90] |
| 90 minutes | 0.86 [0.70, 1.00] | 2.7 | 0.80 [0.50, 1.00] | 0.80 [0.50, 1.00] | 0.80 [0.58, 0.93] |
| 120 minutes | 0.93 [0.82, 1.00] | 3.9 | 0.90 [0.70, 1.00] | 0.90 [0.70, 1.00] | 0.90 [0.68, 0.98] |
| 150 minutes* | 0.99 [0.96, 1.00] | 4.6 | 1.00 [1.00, 1.00] | 0.90 [0.70, 1.00] | 0.95 [0.76, 1.00] |
| | | 3.7 | 0.90 [0.70, 1.00] | 1.00 [1.00, 1.00] | 0.95 [0.76, 1.00] |
| 180 minutes | 0.98 [0.93, 1.00] | 5.0 | 1.00 [1.00, 1.00] | 0.90 [0.70, 1.00] | 0.95 [0.76, 1.00] |

Diagnostic performance of copeptin measurements 30 to 180 minutes after glucagon injection in discriminating patients diagnosed with cDI from patients diagnosed with PP. Area under the receiver–operator characteristics curve (AUC), and the best copeptin cut-off with corresponding sensitivity, specificity, and accuracy with 95% confidence intervals (CI) are given for each timepoint (minutes). * For the timepoints 60 and 150 minutes there were two equally good candidates for the best copeptin cutoff.

## Table S5 (M1) Adverse events

| | Healthy participants | Diabetes insipidus | Primary polydipsia |
|---|---|---|---|
| **Cold feeling and cold sweats** | 3 | 1 | 1 |
| **Dry eyes** | 0 | 0 | 1 |
| **Headache** | 0 | 0 | 1 |
| **Hot flashes** | 2 | 0 | 0 |
| **Symptomatic drop in blood glucose levels** | 3 | 0 | 0 |
| **Presyncope (after venous cannula insertion)** | 1 | 0 | 0 |
| **Tremor** | 1 | 0 | 0 |

The number and type of adverse events by study group after glucagon stimulation is demonstrated. There were 10 adverse events in the healthy participants, 1 adverse event in patients with central diabetes insipidus, and 3 adverse events in patients with primary polydipsia. The most reported adverse events were cold feeling and cold sweats. Note: no adverse event under placebo was observed.

**MANUSCRIPT 2 (M2)**

# Central diabetes insipidus from a patients' perspective: management, psychological co-morbidities, and renaming of the condition: results from an international web-based survey

the DImond study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**ABSTRACT**

**Background** Central diabetes insipidus is a rare neuroendocrine condition. Data on treatment-associated side-effects, psychological comorbidities, and incorrect management are scarce. The aim of this study was to investigate patients' perspectives on their disease.

**Methods** This study used a cross-sectional, web-based, anonymous survey, developed by endocrinologists and patient representatives, to collect the opinions of patients with central diabetes insipidus on management and complications of their disease, psychological comorbidities, degree of knowledge and awareness of the condition among health-care professionals, and renaming the disease to avoid confusion with diabetes mellitus.

**Findings** Between Aug 23, 2021, and Feb 7, 2022, 1034 patients with central diabetes insipidus participated in the survey. 91 (9%) participants were children and adolescents (37 [41%] girls and 54 [59%] boys; median age 10 years [IQR 6–15]) and 943 (91%) were adults (757 [80%] women and 186 [20%] men]; median age 44 years [34–54]). 488 (47%) participants had isolated posterior pituitary dysfunction and 546 (53%) had combined anterior and posterior pituitary dysfunction. Main aetiologies were idiopathic (315 [30%] of 1034 participants) and tumours and cysts (pre-surgical: 217 [21%] of 1034 participants; post-surgical; 254 [25%] of 1034 participants). 260 (26%; 95% CI [0·23–0·29]) of 994 patients on desmopressin therapy had hyponatraemia leading to hospitalisation. Patients who routinely omitted or delayed desmopressin to allow intermittent aquaresis had a significantly lower prevalence of hyponatraemia compared with those not aware of this approach (odds ratio 0·55 [95% CI 0·39–0·77]; p=0·0006). Of patients who had to be hospitalised for any medical reason, 71 (13%; 95% CI 0·10–0·16) of 535 did not receive desmopressin while in a fasting state ('nil by mouth'/'nil per os' state) without intravenous fluid replacement and reported symptoms of dehydration. 660 (64%; 0·61–0·67) participants reported lower quality of life, and 369 (36%; 0·33–0·39) had psychological changes subjectively associated with their central diabetes insipidus. 823 (80%; 0·77–0·82) participants encountered a situation where central diabetes insipidus was confused with diabetes mellitus by health-care professionals. 884 (85%; 0·83–0·88) participants supported renaming the disease; the most favoured alternative names were vasopressin deficiency and arginine vasopressin deficiency.

**Interpretation** This is the largest survey of patients with central diabetes insipidus, reporting a high prevalence of treatment-associated side-effects, mismanagement during hospitalisation, psychological comorbidities, and a clear support for renaming the disease. Our data are the first to indicate the value of routinely omitting or delaying desmopressin.

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## INTRODUCTION

Central diabetes insipidus, a rare neuroendocrine condition with a prevalence of one in 25,000 people, is caused by arginine vasopressin deficiency.[26] The condition is characterised by the production of large volumes of unconcentrated urine, which are compensated for by excessive fluid intake.[22] Once diagnosed, desmopressin, a selective vasopressin V2 receptor agonist, is usually prescribed to overcome the symptoms of polyuria, polydipsia, and nocturia.[71]

Data about desmopressin-associated side-effects, insufficient awareness among medical professionals, and the prevalence of incorrect management of central diabetes insipidus are scarce and restricted to small studies or case series. Occasional published case reports show the tragic and fatal consequences of treatment neglect with omission of desmopressin during hospitalisation, which is partly explained by confusion among health-care professionals between central diabetes insipidus and diabetes mellitus.[110] These examples of mismanagement and confusion have given rise to increasing interest in the potential need for renaming central diabetes insipidus to avoid confusion with diabetes mellitus.

An enormous amount of research has been devoted to quality of life (QoL) in patients with anterior pituitary dysfunction; however, research covering QoL and psychological comorbidities in patients with central diabetes insipidus is scarce. A few small studies have shown that even if patients were asymptomatic in terms of polyuria and polydipsia, psychological comorbidities occur, with adverse effects on QoL, compared with individuals without diabetes insipidus.[59,111] However, important questions regarding psychopathological characteristics remain unanswered.

To address these issues, we aimed to assess patients' perspectives regarding their disease management, psychological comorbidities, knowledge and awareness of the disease among healthcare professionals, and renaming central diabetes insipidus.

## METHODS

### Study design and participants

This study used an anonymous, cross-sectional, web-based survey (DImond survey [Assessment of the characteristics of patients with central diabetes insipidus – from the diagnosis to the management of the condition]) done via the website of the Department of Clinical Research, University Hospital Basel, Basel, Switzerland. Patients with central diabetes insipidus were invited to participate in this voluntary 10 min survey. Patients younger than 18 years were defined as children and adolescents; those aged 18 years and older were defined as adults. The questions were developed by a multinational team of endocrinologists from Switzerland, the UK, and Ireland, together with patient representatives from the USA. The survey consisted of eight sections with 35 main questions, and it was implemented as a custom web application supporting smartphones, tablets, and computers. Data were stored in a secured database of the University of Basel, Basel, Switzerland. Participant anonymity was ensured by hosting the application on internal servers, not using any external service providers, or collecting identifying data (eg, IP addresses or user-agent strings). Additionally, only strictly necessary client-side cookies were used. A random token was generated when the user navigated to the first question. This token was valid for a short period, but it lost its validity after submitting the survey, thus allowing users to complete the questionnaire even after the loss of internet connection or with temporary interruptions.

Recruitment was done in three ways using different strategies and contact channels to gain a large sample that reflected the full spectrum of patients with central diabetes insipidus. (1) Physicians involved in this project informed patients with central diabetes insipidus by telephone and directly during routine visits or hospitalisations about this voluntary anonymous survey and shared the link to the homepage. Patients were contacted without any prespecified eligibility criteria. (2) Announcements were shared on websites of the UK Pituitary Foundation (on Oct 28, 2021) and Pituitary Worlds News (on Dec 17, 2021) with a description and direct link to the survey. (3) A description and link to the survey were shared on social media: a post was shared on the Got diabetes insipidus? Facebook group on Oct 20, 2021, and a post was shared on the Twitter account of the Pituitary Society on Dec 13, 2021. Before the start of the survey, patients or their legal representative were informed about the anonymity of the data collection, and that by consenting to participate, the data would be processed, analysed, and

published for research purposes (supplementary **(M2)**). The proposal of this survey was submitted to the local ethics committee, Ethical Committee Northwest and Central Switzerland, which confirmed that a research project with anonymous health-related personal data does not fall within the scope of the Swiss Human Research Act and study conduct permission was granted. We used the Checklist for Reporting Results of Internet E-Surveys and the Consensus-Based Checklist for Reporting of Survey Studies for reporting (supplementary **(M2)**).

**Outcomes**

The objectives were to investigate patients' perspectives on management and complications as in-patients and out-patients, psychological comorbidities, degree of knowledge and awareness among health-care professionals, and views for renaming central diabetes insipidus to avoid confusion with diabetes.

Disease management and complications were assessed through questions focused on occurrence and total number of hyponatraemic and hypernatraemic episodes since diagnosis, current and previous types and doses of desmopressin preparations, practice of intentionally delaying and omitting desmopressin dose to reduce the risk of hyponatraemia, occurrence of desmopressin access problems, and episodes of withdrawal from desmopressin treatment while in a fasting state ('nil by mouth'/nil per os' state) during hospitalisation. Occurrence of psychological problems after diagnosis subjectively associated with central diabetes insipidus (depressed mood, sleep disturbance, heightened anxiety, stress management disturbance, change in eating habits, and change in personality), change in QoL subjectively associated with central diabetes insipidus (social activities, recreation, and fun; physical wellbeing; and mental wellbeing), level of QoL, ability to trust others, social interaction, sexual arousal, and anxiety level in general life were assessed with a 10-point scale. Confusion of central diabetes insipidus with diabetes by health-care professionals and level of knowledge of physicians on central diabetes insipidus from a patients´ perspective was assessed on a 10-point scale.

**Statistical analysis**

No formal sample size calculation was made; a target sample size of more than 800 participants (with no allowance for multiplicity) was considered adequate. All statistical analyses used R (version 4·1·2). Discrete variables are expressed as frequencies and continuous variables reported as median and

interquartile range. Prevalence estimates are reported with 95% CI, calculated with the Wald Interval method. Data regarding the practice of delaying or omitting desmopressin dose until breakthrough symptoms (increased urinary frequency and strong thirst) occur to allow aquaresis, referred to by some as desmopressin escape or water off-loading, was collected. We refer to this method as desmopressin escape. A univariate logistic regression model was done to describe the association of desmopressin escape performance with the prevalence of hyponatraemia: patients aware of and used desmopressin escape were compared with patients who were aware of but did not follow the approach and with patients who were not aware and did not use the approach. We report odds ratios with 95% CI. Qualitative measures (i.e., diagnostic test burden, knowledge about central diabetes insipidus among physicians, and different psychological characteristics) were indicated on a visual analogue scale ranging from 0 (no, none, or minimum) to 10 (extreme or maximum). All analyses are exploratory and were assessed in the entire population, patients with isolated posterior pituitary dysfunction, and patients with combined anterior or posterior pituitary dysfunction separately.

**Role of funding source**

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

## RESULTS

### Baseline characteristics

Between Aug 23, 2021, and Feb 7, 2022, 1034 patients with central diabetes insipidus participated in the survey. 91 (9%) participants were children and adolescents (37 [41%] girls and 54 [59%] boys; median age 10 years [IQR 6–15]) and 943 (91%) were adults (757 [80%] women and 186 [20%] men]; median age 44 years [34–54]). 488 (47%) participants had isolated posterior pituitary dysfunction and 546 (53%) had combined anterior and posterior pituitary dysfunction. Median duration of central diabetes insipidus was 9 years (3–19) and the initial symptoms (e.g., polyuria, reported by 930 [90%] participants, polydipsia reported by 905 [88%] participants, and nocturia reported by 810 [78%] participants), began a median of 0·3 years (0·1–1·0) before diagnosis. An initial diagnostic provocation test was done in 602 (58%; 95% CI 0·55–0·61) participants (supplementary (M2)). The causes of central diabetes insipidus are reported in Figure 1 (M2) and Table 1(M2). The number of additional anterior pituitary hormone dysfunctions are reported in Figure 2 (M2) and the supplementary (M2).

### Management as in- and out-patients

994 (96%) participants were receiving desmopressin therapy (Figure 3 (M2); supplementary (M2)). Prevalence of the types of preparation and availability of desmopressin in the local pharmacy are reported in the supplementary (M2). 985 (95%) participants indicated that they saw a medical doctor twice (IQR one to two) a year for reviews and check-ups of their central diabetes insipidus: 857 (83%) saw an endocrinologist, 99 (10%) saw a general practitioner, 16 (2%) saw another specialist (e.g., oncologist), and 13 (1%) saw a nephrologist.

Desmopressin escape was used by 667 (67%; 95% CI 0·64–0·70) participants. 386 (39%; 0·36–0·42) of 994 participants used this approach daily, 160 (16%; 0·14–0·19) participants used this approach several times a week, and 121 (12%; 0·10–0·14) participants used the approach once a week. 205 (21%; 0·18–0·23) of the participants who were receiving desmopressin were not aware of desmopressin escape; 122 (12%; 0·10-0·14) were aware of desmopressin escape but did not use it.

In the out-patient setting, 230 (22%; 95% CI 0·20–0·25) participants reported episodes of hyponatraemia (median two episodes [IQR two to four]), with a similar incidence in adults and children and adolescents (supplementary (M2)). 211 (21 %; 0·22–0·27) participants treated in an out-patient setting who were receiving desmopressin medication reported episodes of hyponatraemia. Hyponatraemia was reported by 114 (17%; 0·17–0·20) of 667 patients who used desmopressin escape, 65 (32%; 0·26–0·28) of 205 participants who were not aware of desmopressin escape, and 32 (26%; 0·19–0·35) of 122 participants who were aware of desmopressin escape but did not use the method. Patients who used desmopressin escape had a significantly lower prevalence of hyponatraemia compared with those not aware of this method (OR 0·44; 95% CI 0·31–0·64; p<0·0001) and compared with those aware of desmopressin escape but who did not use this method (0·58; 0·37–0·92; p=0·018). There was no association between type of desmopressin preparation and prevalence of hyponatraemia (for oral versus nasal spray, supplementary (M2)).

364 (35%; 95% CI 0·32–0·38) participants reported an episode of dysnatraemia leading to hospital admission on at least one occasion. 273 (26%; 0·24–0·29) participants (259 [27%; 0·25–0·30] of 943 adults and 14 [15%; 0·08–0·23] of 91 children and adolescents) had a median of two (IQR one to three) hyponatraemia episodes. 260 (26%; 0·23–0·29) participants under desmopressin treatment reported episodes of hyponatraemia leading to hospitalisation. 145 (22%; 0·18–0·25) of 667 participants who used desmopressin escape, 69 (34%;0·27–0·40) of 205 participants not aware of desmopressin escape, and 46 (38%; 0·29–0·46) of 122 participants who were aware of but did not use desmopressin escape had hyponatraemia. Similar to the findings in the out-patient setting, participants who used desmopressin escape had a significantly lower hyponatraemia prevalence leading to hospitalisation compared with those not aware of this method (OR 0·55; 95% CI 0·39–0·77; p=0·0006) and with those aware of but who did not use this method (0·46; 0·31–0·69; p=0·0002; supplementary (M2)).

150 (15%; 95% CI 0·12–0·17) participants (128 [14%; 0·11–0·16] of 943 adults and 22 [24%; 0·15–0·33] of 91 children and adolescents) had hypernatraemia leading to hospital admission; a median of

one episode (IQR one to three) was reported per patient. 59 (6%; 0·04–0·07) patients had episodes of both hyponatraemia and hypernatraemia.

247 (24%; 95% CI 0·21–0·26) participants had problems accessing desmopressin during hospitalisation (e.g., for acute illness and elective surgery). Multiple factors could restrict access to medication; the most common reasons were non-availability of desmopressin (139 [56%; 0·50–0.62] participants), other reasons (e.g., desmopressin provided only on a scheduled time; 102 [41%; 0·35–0·47] participants), prescription of wrong dose (47 [19%; 0·14–0·24] participants), or complete absence of prescription (32 [13%; 0·09–0·17] participants).

535 (52%; 95% CI 0·49–0·55) participants had to avoid eating and drinking for a medical reason ('nil by mouth'/'nil per os' state) during hospitalisations (for any reason) in elective (475 [46%; 0·43–0·49] participants) or emergency (150 [15%; 0·12–0·17] participants) situations. During their hospitalisation, 290 participants (54%; 0·50–0·58) received no intravenous fluids; from these 209 (39%; 0·35–0·43) patients used their own desmopressin, desmopressin was given to ten (2%; 0·01–0·03) participants by the medical team, and 71 (13%; 0·10–0·16) participants received no desmopressin—these patients described classical symptoms of dehydration (e.g., extreme thirst, dry eyes and mouth, and nausea and shivering).

**Psychological co-morbidities**

369 (36%; 95% CI 0·33–0·39; equal proportion of participants with isolated posterior dysfunction and those with combined pituitary dysfunction) had psychological problems or recognised psychological changes associated with their central diabetes insipidus (Table 2 (M2)). 660 (64%; 0·61–0·67 participants; equal proportion of participants with isolated posterior dysfunction and those with combined pituitary dysfunction) reported lower QoL (six [IQR four to seven] out of ten on the visual analogue scale [VAS]). 538 (52%; 0·49–0·55) participants reported effects to social activities, 493 (48%; 0·44–0·51) to recreation and fun, 476 (46%; 0·43–0·49) to physical wellbeing, and 414 (40%; 0·37–0·43) to mental wellbeing. Rates on a VAS regarding anxiety levels in general life, ability to build trust with others, ability in social interaction, and sexual arousal are reported in Table 2 (M2). The

median rates were equal in participants with isolated posterior pituitary dysfunction and those with combined pituitary dysfunction; no major sex and age category specific differences were reported (supplementary (M2)).

**Knowledge and awareness of healthcare professionals and re-naming of the condition**

823 (80%; 95% CI 0·77–0·82) participants indicated that health-care professionals had confused their condition with diabetes mellitus on at least one occasion. 869 (84%; 0·82–0·86) participants thought that physicians in general (e.g., during routine or emergency hospital admissions) have insufficient understanding of central diabetes insipidus and rated the general knowledge of physicians (not involved in the regular treatment of their central diabetes insipidus) as a two (IQR one to four) out of ten on the VAS. 753 (87%; 0·84–0·89) of the 869 participants thought that this poor knowledge affected the management of their condition (e.g., repeated blood sugar measurements due to confusion).

884 (85%; 95% CI 0·83–0·88) preferred a renaming of the condition. The most common suggestions were vasopressin deficiency and arginine vasopressin deficiency. The one clear wish from all of the comments was not to use the term diabetes in the name of the disease.

**Figure 1 (M2) Causes of central diabetes insipidus**

The proportion of participants with isolated central diabetes insipidus cases and proportion with combined central diabetes insipidus and anterior pituitary dysfunction due to each clinical cause.

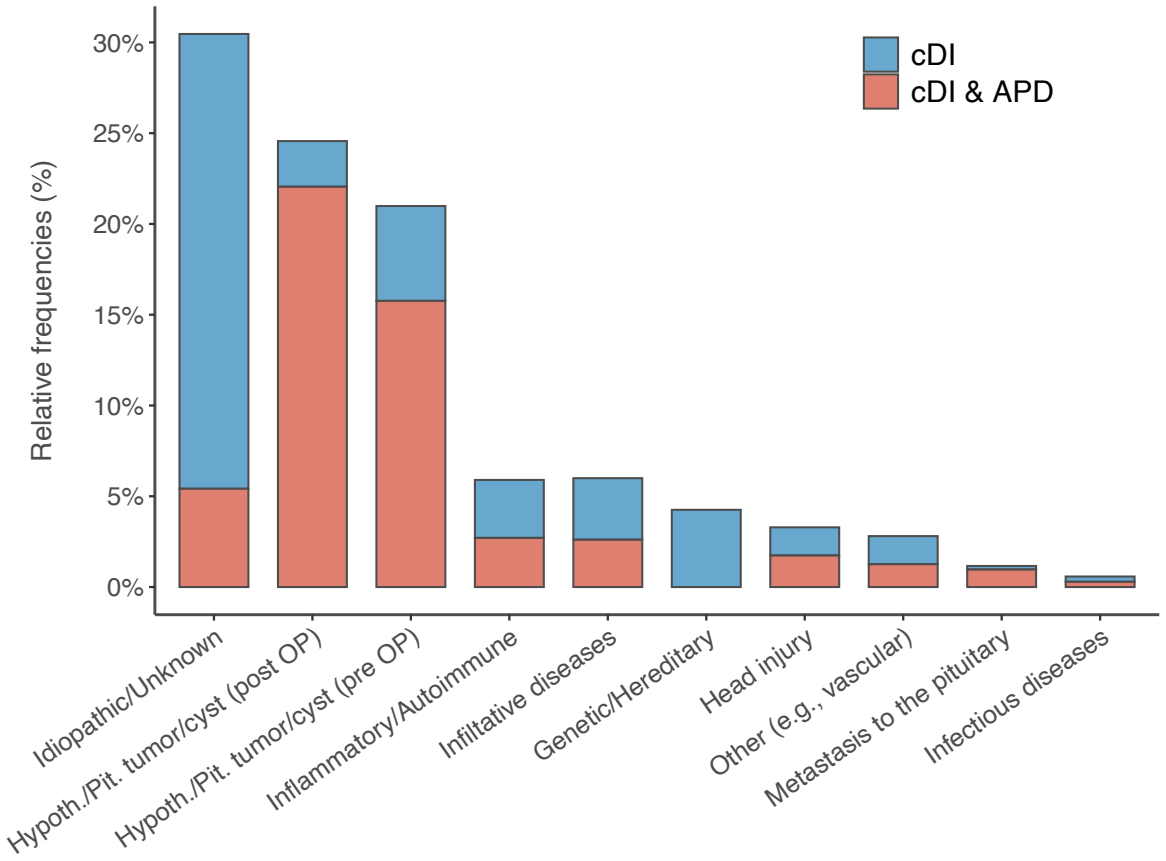

**Figure 2 (M2) Anterior pituitary dysfunction**

The numbers of patients with combined central diabetes insipidus and anterior pituitary dysfunction in each category grouped according to the hormones. ACTH=adrenocorticotropic hormone. FSH=follicle-stimulating hormone. LH=luteinising hormone. TSH=thyroid-stimulating hormone.

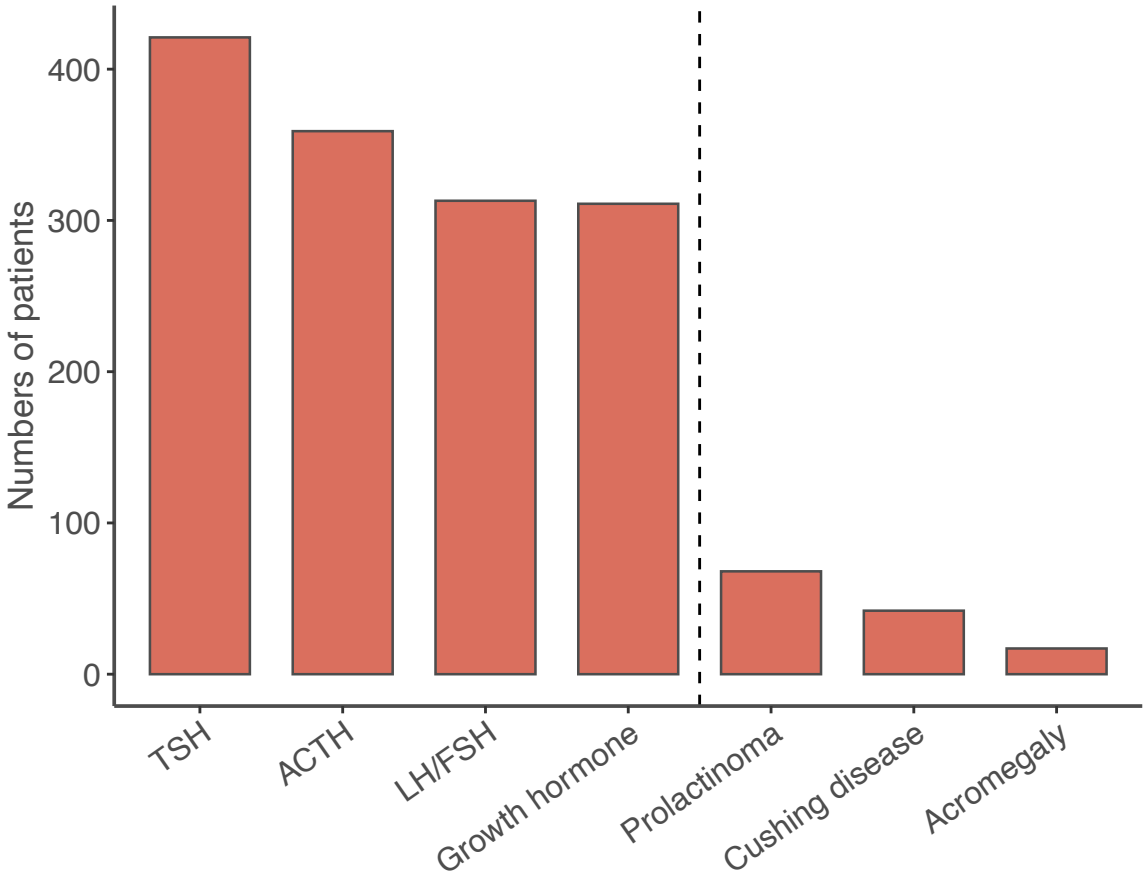

**Figure 3 (M2) Type of desmopressin preparation**

Bar plots represent the proportion of each desmopressin preparation.

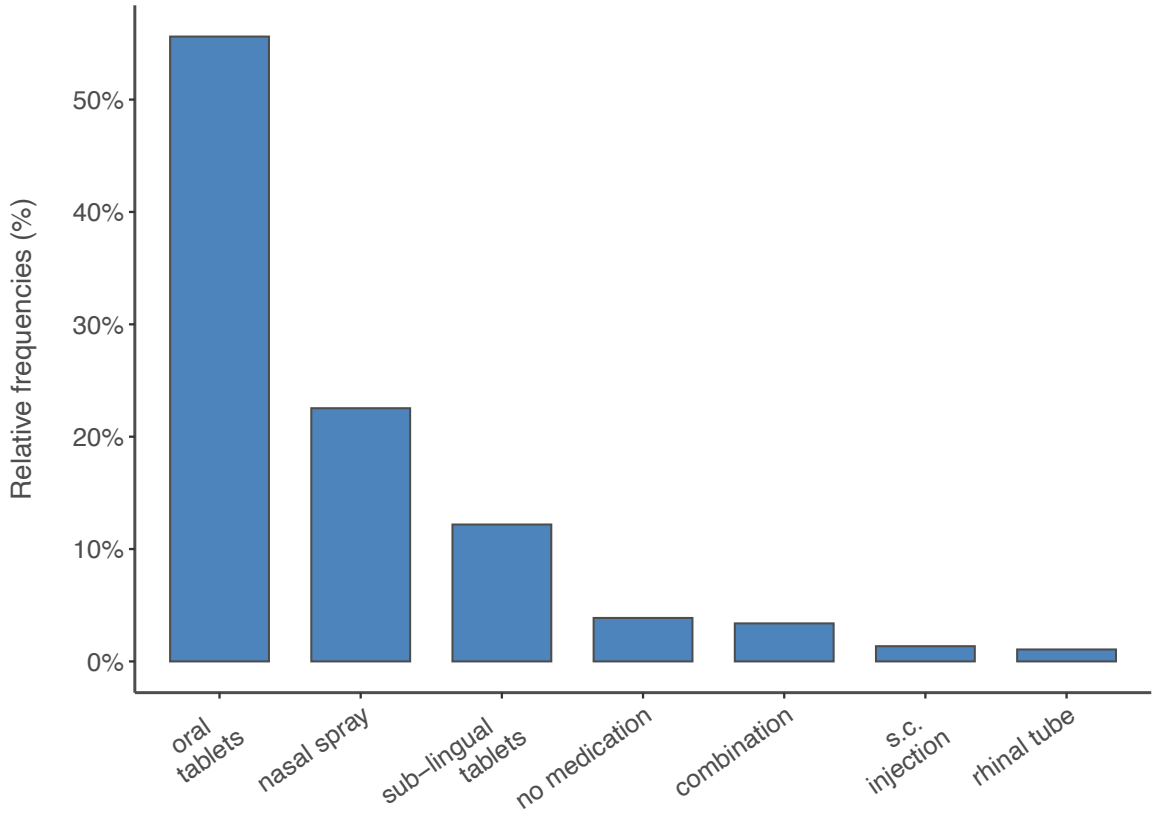

**Table 1 (M2) Baseline characteristics**

| | Full dataset (n=1034) | Participants with isolated posterior pituitary dysfunction | | Participants with anterior and posterior pituitary dysfunction | |
|---|---|---|---|---|---|
| | | Adults (n=444) | Children and adolescent (n=44) | Adults (n=499) | Children and adolescent (n=47) |
| Age, years | 42 (32–53) | 44 (35–53) | 7 (5–12) | 44 (34–54) | 11 (7–15) |
| Sex | | | | | |
| Female | 794 (77%) | 368 (83%) | 20 (45%) | 389 (78%) | 17 (36%) |
| Male | 240 (23%) | 76 (17%) | 24 (55%) | 110 (22%) | 30 (64%) |
| Weight, kg | 77·1 (63·0–95·0) | 77·1 (65·0–93·9) | 32 20–43) | 82·0 (68·0–98·0) | 44·9 (35·0–61·2) |
| Height, cm | 165 (158–173) | 167 (160–173) | 136 (122–155) | 168 (160–174) | 141 (114–157) |
| BMI, kg/m² | 27·6 (23·3–32·7) | 27·4 (23·4–32·5) | 16·8 (12·2–19·2) | 29·1 (24·9–33·4) | 21·7 (19·0–27·5) |
| Duration of central diabetes insipidus, years | 9·0 (3·0–19·0) | 9·0 (3·0–23·0) | 3·0 (1·0–5·0) | 10·0 (3·0–19·0) | 4·0 (2·5–7·0) |
| Duration of symptoms before diagnosis, years | 0·3 (0·1–1·0) | 0·5 ( 0·2–2·0) | 0·2 (0·1–0·7) | 0·2 (0·1–1·0) | 0·1 (0·0–0·3) |
| Symptoms at the time of diagnosis | | | | | |
| Polyuria* | 930 (90%) | 412 (93%) | 42 (95%) | 433 (87%) | 43 (91%) |
| Polydipsia* | 905 (88%) | 410 (92%) | 39 (89%) | 420 (84%) | 36 (77%) |
| Nocturia* | 810 (78%) | 386 (87%) | 35 (80%) | 365 (73%) | 24 (51%) |
| Cause of central diabetes insipidus | | | | | |
| Idiopathic or unknown | 315 (30%) | 240 (54%) | 19 (43%) | 51 (10%) | 5 (11%) |
| Hypothalamic or pituitary tumour or cyst (post-surgery) | 254 (25%) | 26 (6%) | 0 | 211 (42%) | 17 (36%) |
| Hypothalamic or pituitary tumour or cyst (pre-surgery) | 217 (21%) | 48 (11%) | 6 (14%) | 157 (31%) | 6 (13%) |
| Infiltrative disease (e.g., sarcoidosis or Langerhans cell histiocytosis) | 62 (6%) | 25 (6%) | 10 (23%) | 16 (3%) | 11 (23%) |
| Inflammatory or autoimmune (e.g., hypophysitis) | 61 (6%) | 30 (7%) | 3 (7%) | 28 (6%) | 0 |
| Genetic or hereditary | 44 (4%) | 40 (9%) | 4 (9%) | 0 | 0 |
| Head injury | 34 (3%) | 16 (4%) | 0 | 17 (3%) | 1 (2%) |
| Other causes (e.g., vascular or congenital) | 29 (3%) | 15 (3%) | 1 (2%) | 7 (1%) | 6 (13%) |
| Metastasis to the pituitary (e.g., lymphoma, breast cancer, or lung cancer) | 12 (1%) | 1 (<1%) | 1 (2%) | 10 (2%) | 0 |
| Infectious diseases (e.g., meningitis, encephalitis, or tuberculosis) | 6 (1%) | 3 (1%) | 0 | 2 (<1%)† | 1 (2%) |

Data are median (IQR) and n (%). *Remaining patients could not recall the symptoms (e.g., diagnosed in childhood). †This column will not add up to 100% because of rounding.

**Table 2 (M2) Psychological comorbidities**

| | Full dataset (n=1034) | Participants with isolated posterior pituitary dysfunction (n=488) | Participants with anterior and posterior pituitary dysfunction (n=546) |
|---|---|---|---|
| Psychological problems or changes since diagnosis | 369 (36%; [33–39]) | 173 (35%; [31–40]) | 196 (36%; [32–40]) |
| Heightened anxiety | 258 (25%; [22–28]) | 115 (24%; [20–27]) | 143 (26%; [23–30]) |
| Sleep disturbance | 263 (25%; [23–28]) | 113 (23%; [19–27]) | 150 (27%; [24–31]) |
| Depressed mood | 239 (23%; [21–26]) | 99 (20%; [17–24]) | 140 (26%; [22–29]) |
| Stress management disturbance | 181 (18%; [15–20]) | 86 (18%; [14–21]) | 95 (17%; [14–21]) |
| Change in eating habits | 168 (16%; [14–18]) | 82 (17%; [13–20]) | 86 (16%; [13–19]) |
| Change in personality | 124 (12%; [10–14]) | 51 (10%; [8–13]) | 73 (13%; [11–16]) |
| Documented psychological condition after the diagnosis | 111 (11%; [9–13]) | 41 (8%; [6–11]) | 70 (13%; [10–16]) |
| Reduced quality of life after the diagnosis | 660 (64%; [61–67]) | 308 (63%; [59–67]) | 352 (64%; [60–68]) |
| Social activities | 538 (52%; [49–55]) | 249 (51%; [47–55]) | 289 (53%; [49–57]) |
| Recreation and fun | 493 (48%; [44–51]) | 234 (48%; [44–52]) | 259 (47%; [43–52]) |
| Physical wellbeing | 476 (46%; [43–49]) | 218 (45%; [40–49]) | 258 (47%; [43–51]) |
| Mental wellbeing | 414 (40%; [37–43]) | 192 (39%; [35–44]) | 222 (41%; [37–45]) |
| Subjective rates on a visual analogue scale, median [IQR] | | | |
| QoL*† | 6 (4–7) | 6 (4–8) | 6 (4–7) |
| Ability to trust*† | 7 (4–8) | 7 (4–8) | 7 (4–8) |
| Social interaction*† | 7 (5–8) | 7 (6–8) | 7 (4–8) |
| Sexual arousal*†‡ | 3 (2–7) | 4 (2–8) | 3 (1–6) |
| Anxiety level in general life*§ | 6 (3–8) | 6 (3–8) | 6 (3–7) |

Data presented in median [IQR] and n (%; [95%-CI]). *Rated on a visual analogue scale from 0 (minimum, no, or none) to 10 (maximum or extreme). †Low score on this parameter reflects more adversely affected. ‡Answered by 819 patients. §High score on this parameter reflects more adversely affected. QoL=quality of life.

## DISCUSSION

The data from our survey, the largest of its kind in patients with central diabetes insipidus, indicate a high prevalence of treatment-associated side-effects leading to hospitalisation—particularly in patients unaware of the desmopressin escape approach—and psychological comorbidities, poor knowledge and awareness of central diabetes insipidus among health-care professionals, and strong support for renaming the condition.

It is often not sufficiently recognised that many patients with rare illnesses, such as central diabetes insipidus, are experts on their conditions. The experiential knowledge that patients acquire after years of treatment is hard-won and unique, and deserves to be considered, both clinically and in research studies. On the basis of this consideration, this survey was developed by a team of expert endocrinologists together with patient representatives using a novel web-based method.

Most commonly, central diabetes insipidus results from acquired disruptions of the hypothalamic–pituitary axis, and less than 10% of the cases are hereditary.[67,112] The spectrum of causes of central diabetes insipidus indicated by our data are consistent with available literature, with hypothalamo–pituitary tumours or cysts the most common cause of central diabetes insipidus (46%); however, there was a large proportion of idiopathic cases (30–50%), especially in isolated central diabetes insipidus.[67,112,113]

In anterior pituitary dysfunction, gonadotropins and growth hormone are usually more likely to be affected than adrenocorticotropic hormone and thyroid-stimulating hormone. Our data appear to show contradictory results, with thyroid-stimulating hormone as the most common concomitant hormone deficiency and growth hormone as the least common. We speculate that the discrepant high incidence of hypothyroidism might reflect, in part, the high prevalence of primary hypothyroidism in idiopathic central diabetes insipidus,[114] and is probably not distinguished from secondary hypothyroidism by patients. Additionally, not all adult patients with growth hormone deficiency are tested for or receive growth hormone replacement therapy, and they might be unaware of their deficiency.

Desmopressin is the current standard of care for central diabetes insipidus. Our data show a clear preference for the oral route of desmopressin in those switching the type of preparation. A possible

explanation is that alternative nasal preparations show great variability in effectiveness and switching to the oral route has been shown to improve overall control.[115,116] Compared with the results of post-marketing safety data, which indicate a lower risk of hyponatraemia with oral than with nasal desmopressin,[114,116] our data showed a similar prevalence of patient-reported hyponatremia in patients with both preparations. Nonetheless, the use of oral preparations should be preferred, and well-designed studies are needed to investigate this advantage. The antidiuretic effect of desmopressin can be affected by several factors, such as solute intake and excretion, and fluctuating bioavailability (eg, by nasal congestion for nasal sprays or concomitant food ingestion for oral route).[73] Despite this, patients often take a fixed dose at scheduled times. If not instructed on use of desmopressin escape, even normal daily fluid intake can result in water retention and development of hyponatraemia in the presence of sustained antidiuresis from rigid dose schedules. In the out-patient setting, a long-term follow-up study revealed a 27% prevalence of mild hyponatraemia and a 15% prevalence of profound hyponatraemia.[75] In our study, patient-reported hyponatraemia was less frequent, suggesting that laboratory-confirmed hyponatraemia might even be higher. Our data also suggest a larger proportion of patients with hyponatraemia leading to hospitalisation. Desmopressin escape, a method to delay or omit desmopressin to allow aquaresis, has long been advised by some physicians to counteract this risk.[1] Our data are the first to show the value of this clinical approach, with lower prevalence of hyponatraemia in patients practising desmopressin escape. Patients who were instructed to delay or omit the dose one or more times a week at initiation of desmopressin treatment had a lower prevalence of hyponatraemia. Hyponatraemia is particularly common in the out-patient setting,[75] and in the absence of patient education on hyponatraemia symptoms and desmopressin escape, it can quickly become life-threatening. In our opinion, desmopressin escape should be instructed at every initiation of desmopressin therapy because it is an approach resulting in immediate cost-free health-care improvements (ie, reduced prevalence of life-threatening hyponatraemia and hospitalisation). Future prospective studies should investigate whether this method does lead to lower risk for hyponatraemia. Furthermore, in paediatric patients a more careful regimen is needed, and parents must be educated about hyponatraemia as a result of inappropriate management of desmopressin and fluid intake.[117]

Our data also show that a large number of patients were unable to source desmopressin during hospitalisation when they were in a fasting state ('nil by mouth'/'nil per os') and without intravenous fluid replacement. Many of these patients reported symptoms of dehydration. Previously, Behan and colleagues[75] reported concerningly high rates of hypernatraemia, particularly in in-hospital settings, probably as a result of inappropriate management. However, once admitted with hyponatraemia, physicians intuitively tend to discontinue desmopressin treatment.[74] This can lead to rapid overcorrection of serum sodium and result in severe neurological injury, if not appropriately monitored.[74] These findings suggest that the in-hospital management of patients should be led, or at least accompanied by, a specialist because patients with central diabetes insipidus are known to be highly vulnerable to rapid volume depletion in the context of severe illness if not adequately managed.[110,118] Concern about mismanagement and delay of appropriate treatment led to a recent call for a campaign to increase awareness and education of medical personnel, and the request to include desmopressin as a high-alert medication with 24 h access in hospitals.[119] Consequently, the Society for Endocrinology UK published a clinical guidance covering the in-hospital management.[120]

Our data indicate a high prevalence of psychological comorbidities in central diabetes insipidus, particularly heightened anxiety, depressed mood, sleeping difficulties, and lower sexual drives, consistent with previously published studies.[59,85,86] The patients also reported a reduced QoL, despite reduction of polyuria with desmopressin therapy. Impaired QoL and psychological changes in patients with anterior pituitary dysfunction are well recognised, and replacement therapy improves symptoms.[78,79] By contrast, few attempts have been made to evaluate psychological comorbidities in isolated central diabetes insipidus. The available data suggest that reduced QoL is partly explained by fluctuations in desmopressin efficacy, leading to changes in symptom control, or by concomitant pituitary hormone deficiencies.[111,121,122] Our data show that the reduction in QoL is equally common in patients with isolated central diabetes insipidus and those with combined pituitary dysfunction, which challenges the assumption that concomitant pituitary hormone deficiencies are largely responsible.

Oxytocin, the second neuropeptide released from the posterior pituitary, is known to mediate neuropsychiatric effects, including antidepressant, anxiolytic, and socioemotional functioning

properties, suggesting a potential role for oxytocin deficiency in the increased psychopathology. This is supported by the results of the study by Aulinas and colleagues.[59] Of note, one study reported that a single dose of intranasal oxytocin improved emotion recognition in ten patients with craniopharyngioma and concomitant central diabetes insipidus.[123] Conversely, in neuropsychiatric conditions intranasal oxytocin has shown inconclusive results.[27] Future studies to investigate whether oxytocin deficiency occurs in central diabetes insipidus and whether treatment improves psychological symptoms would be of interest.

According to NHS England and the National Reporting and Learning System, 471 adverse incidents were reported from 2009 to 2015 involving desmopressin treatment.[110] Of these, prescription of the incorrect dose (n=56) and dose omission (n=76), were the most common errors.[110] Four of these dose omissions resulted in death due to severe dehydration.[124,125] Consequently, the NHS sent an alert to all doctors informing them of the risk of omitting this life-sustaining medication.[110] In line with this and a study by Dilrukshi and colleagues, 24% of hospitalised patients in our survey reported problems accessing desmopressin during routine or emergency hospitalisations, most commonly due to non-availability.[69] Owing to its rarity, central diabetes insipidus is a neglected condition among health-care professionals, and increased awareness of this disease is urgently needed. Additionally, central diabetes insipidus is often confused with diabetes mellitus. Patients in our survey indicated high rates of confusion with diabetes mellitus and insufficient understanding of central diabetes insipidus among health-care professionals. Together, confusion and poor knowledge about central diabetes insipidus, can significantly increase the risk of mismanagement during hospitalisation, as indicated in this survey. Future survey studies could explore the knowledge of health-care professionals on central diabetes insipidus and confirm the validity of the patient's perspective. Renaming of central diabetes insipidus and avoiding the word diabetes could help health-care professionals understand that central diabetes insipidus requires specialist life-sustaining therapy, which is distinct from diabetes mellitus. Several patient representative associations and foundations strongly support this approach. Most of the participants in our study suggest arginine vasopressin deficiency or vasopressin deficiency as alternative disease names; participants highlighted the need to not use diabetes in the name of the disease. For the

nephrogenic form, arginine vasopressin resistance or vasopressin resistance could be suggested.[69] A working group was set up in 2022 by the main endocrine societies worldwide (European Society of Endocrinology, Society for Endocrinology (UK), Endocrine Society, Endocrine Society of Australia, Brazilian Society of Endocrinology, Japanese Endocrine Society, Pituitary Society, European Society for Paediatric Endocrinology, American Society of Nephrology) to discuss and propose alternative names for central diabetes insipidus.

The main limitation of our study is that, due to the survey design, we cannot make causal inferences. We have no control group from the general population or patients with isolated anterior pituitary dysfunction for comparison and no standardised longitudinal assessment of outcomes for patients with and without central diabetes insipidus. Because our intent was not to assess causal effects, we did not use causal inference methods or adjust for confounding, and no weighting was done. Although a substantial amount of data shows higher psychological comorbidities in patients with anterior pituitary dysfunction compared with the general population, psychological burden could be associated with the underlying disease. Because our data indicate lower QoL and psychological comorbidities are similar in patients with isolated central diabetes insipidus and combined anterior pituitary dysfunction, it is reasonable to interpret our results as showing a higher psychological burden for patients with central diabetes insipidus than for members of the general population. The second main limitation is that due to the anonymous survey design, we have no empirical information on the representativeness of our very large sample. Our approach allowed us to include a very large sample and we assume that it reflects the views of patients, but we cannot rule out that selection bias affected our findings; however, we used a broad recruitment strategy, through social media, online dissemination, and personal contacts, and all actively recruited patients were contacted without any prespecified eligibility criteria. In our survey, 77% of the participants were females and the effect of gender on psychological outcomes is unclear; however, sex-stratification showed no major differences in our results. Third, also due to anonymity, response rates or differences according to each health-care system could not be analysed. Finally, the reliability of self-reported data, especially for unawareness of hyponatraemia not leading to hospitalisation, makes interpreting potential anterior pituitary dysfunction, such as hypothyroidism and

growth hormone deficiency, difficult; the absence of information about other comorbidities should be considered as a limitation. However, the outcomes of our survey are significant, objective events which patients most likely remember very well, limiting the potential effect of recall bias. In summary, our data underline the need to provide health-care professionals with more information about central diabetes insipidus and its management, and to better educate patients about the strategy of desmopressin escape. More research is needed on the prevalence of psychological comorbidities and possible treatment options in central diabetes insipidus. Furthermore, the renaming of central diabetes insipidus should be actively considered by members of the international endocrinology societies.

**Contributors**

CA designed the questionnaire; contributed to data collection, analysis, and interpretation; did the literature search; and wrote the manuscript. All authors modified and refined the questionnaire. MC-C edited the questionnaire, contributed to data analysis and data interpretation, edited the manuscript, and supervised all steps of the study. LGH contributed to the data interpretation and edited the manuscript. All other co-authors contributed to data collection, contributed to data interpretation, and revised the manuscript. All authors had access to all the data and had final responsibility for the decision to submit for publication.

**Declaration of interest**

We declare no competing interests.

**Data sharing**

We can share deidentified, individual participant-level data that underlie the results reported in this Article and related documents, including the study protocol and the statistical analysis plan. Data will be available with the publication of the manuscript and issued after receipt of a request detailing the study hypothesis and statistical analysis plan. All requests should be sent to the corresponding author. The steering committee of this study will discuss all requests and decide, based on the scientific rigor of the proposal, whether data sharing is appropriate. All applicants are asked to sign a data access agreement.

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**SUPPLEMENTAL MATERIAL FOR MANUSCRIPT 2 (M2)**

## Survey methodology

### Development and testing

The questions for this survey were developed together with all collaborators and the patient representative in an easy-to-understand language and given answer options were clearly formulated. Medical terms have been explained or replaced by non-medical alternative terms. The questions were developed as multiple-choice and checkbox questions, score ratings, and only a few as open-ended questions with free-text options. For some questions, the patients had the opportunity to state additional remarks in a comment field. This approach aimed to guarantee high data quality and low variability due to factors such as age and education. The involved patient representative proofed all questions.

There were three test runs with all collaborators and the patient representative to assess usability, simplicity, understandability, and technical functionality before the survey went online. The survey was an 'open' survey i.e., open for each visitor and available via the website of the Department of Clinical Research, University Hospital Basel, Switzerland. This website is mainly visited by researcher, students, and other affiliates of the University Hospital Basel. Patients were actively invited to the website or could access the link through announcements on the websites of *Pituitary World News* and *UK Pituitary Foundation*, Twitter account of the *Pituitary Society*, and Facebook Group 'Got Diabetes Insipidus?'. These websites are mainly visited by patients with pituitary conditions such as central diabetes insipidus. The questions of the survey were prespecified and remained unchanged through the conduct of the study. No detailed study protocol was used. All outcomes were described in the study proposal as listed in the CRF.

### Selection of patients (*Active* recruitment)

Patients with central diabetes insipidus were contacted as unselectively as possible, regardless of any pre-specified eligibility criteria. Physicians contacted patients on a regular base without any specific date restriction within the recruitment period (23.08.2021 to 07.02.2022). The aim of this approach was to include a heterogeneous cohort so that the data can be generalised for the population of patients with

central diabetes insipidus. Only patients with the central form of diabetes insipidus were contacted and invited.

All collaborators are experts in specialised centres who have access to large cohorts of patients with central diabetes insipidus that they either see regularly in their clinics or have included in previous studies. Patients were contacted via telephone or e-mail, direct contact at the clinic, and in addition, QR-codes (with direct link to the survey) were provided for patients sitting in the waiting-room. No incentives were offered (e.g., monetary or prizes).

**Selection of patients (*Passive* recruitment)**

In addition, patient associations (e.g., Pituitary World News, UK Pituitary Foundation, Pituitary Society) were also involved in the recruitment process, which have a considerable network of a heterogeneous group of patients with central diabetes insipidus. Announcements via their homepages were made with a short description and the link to the survey.

Patients with rare diseases tend to exchange experiences in specialised forums; therefore, the recruitment also involved patient representatives with direct access to a global network of patients, e.g., via the Facebook group 'Got diabetes insipidus?'). No incentives were offered (e.g., monetary or prizes). Patients were informed prior to the start of the survey, that this survey was only conducted for the central form of diabetes insipidus.

**Protection of anonymity**

No personal information such as ethnicity, country and region of participation were collected. No follow-up contact was made after the initial contact to ask for participation response. Data collected in this survey were stored in the secured database of the University Hospital Basel. Participant anonymity was ensured by hosting the application on internal servers, not using any external service providers or collecting identifying data (e.g., IP addresses or user-agent strings). Additionally, only strictly necessary client-side cookies were used.

Prior to the start of the survey, a notification was presented, that the survey is conducted in patients with the central form of diabetes insipidus, informed about the anonymity of the data collection with with no

allowance for multiplicity, and that by consenting to participate, the data would be processed, analysed, and published for research purposes - only by active confirmation using the *START* button the survey started.

**Prevention of missing survey data**

The survey consisted of eight sections. After staring the survey (through a *START* button), patients could see the percentage of questions that had been answered (e.g., 50% of 100% for half of the questions that had been answered) along with the survey questions. The survey was designed in a way that patients could only continue the survey if they had completely answered the previous question - each question was presented on one page, and only by answering the question could the next question be opened. Adaptive questioning was used i.e., certain questions were only conditionally displayed based on responses to other items to reduce number and complexity of the questions. In total, 35 main questions were presented each on one page. Patients were able to review and change their answers through a *BACK* button. The questions have not been randomised or alternated. By applying this technical method only complete surveys were saved, and no missing data was generated, and no completeness check was necessary.

No participation/response rate (ratio of unique visitors who agreed to participate/unique first survey page visitors) and completion rate (ratio of users who finished the survey/users who agreed to participate) can be calculated due to anonymity of the survey. Due to technical problems four responses in the system could not be analysed.

The data management was supported and provided by the IT team of the Department of Clinical Research, University Hospital Basel, Switzerland. The survey was hosted from one server and the data were saved on the MySQL-database; for the investigators the backend view Directus (directus.io) was used. As mentioned, multiple-choice and checkboxes, score rating (0=no/minimum to 10=extreme/maximum), and (only few) open-ended questions were computed. By selecting the predefined answers or entering the free-text and continuing to the next question, data were directly saved in the server of the University Hospital Basel. This technical method was chosen to avoid transfer errors between the survey platform and database.

**Representativeness of the study population**

Our approach allowed us to include a very large sample and we assume that it reflects the views of patients, but we cannot rule out that selection bias affected our findings; however, we used a broad recruitment strategy (active and passive recruitment), through social media, online dissemination, and personal contacts, and all actively recruited patients were contacted without any pre-specified eligibility criteria to minimise the main bias of selection.

## Results

### Diagnostic procedure of central diabetes insipidus

An initial diagnostic provocation test was performed in 58%(95%-CI [0·55-0·61]; n=602); of those, most underwent the water deprivation test (n=578) with a test burden of eight [6-10] points out of ten on a visual analogue scale (VAS), followed by the hypertonic saline infusion test (n=45) with a burden of six [3-9] VAS points, and the arginine infusion test (n=35) with a burden of five [3-9] VAS points. Reasons for discomfort during the test were the long thirst phase (n=489), long test duration (n=327), side effects (e.g., nausea) (n=197), repeated blood sampling (n=192), and other reasons (e.g., inexperienced medical team) (n=110). The remaining patients (e.g., patients after pituitary surgery) without a provocation test indicated that their diagnosis was made according to physical examination, blood and urine laboratory assessments, and imaging of the brain and pituitary gland.

### Anterior pituitary hormone dysfunctions

Patients with combined pituitary dysfunction (n=546) indicated additional deficiencies i.e., thyroid-stimulating hormone (TSH) (n=421), adrenocorticotropic hormone (ACTH) (n=359), luteinizing hormone/follicle-stimulating hormone (LH/FSH) (n=313), growth hormone (n=311), or excesses i.e., prolactinoma (n=68), Cushing's disease (n=42), and acromegaly (n=17) (Figure 2).

### Switch of desmopressin preparation and availability in the local pharmacy

Overall, 38% (95%-CI [0·35-0·41];n=389) switched at least once the type of preparation after diagnosis. Types of desmopressin preparation were switched **from**: 'nasal sprays' (n=209), 'rhinal tube' (n=103), 'oral tablets' (n=100), 's.c. injection' (n=19), and 'sub-lingual tablets' (n=7); **to**: 'oral tablets' (n=208), 'nasal sprays' (n=114), 'sub-lingual tablets' (n=85), 's.c. injection' (n=7), and 'rhinal tube' (n=5).

Reasons for switching the type were 'due to availability' (n=147), 'personal preferences' (n=134), 'doctors' recommendation' (n=99), and 'side effects (n=73)' (Figure 1S).

Half of the patients (46%; 95%-CI [0·43-0·49]; n=478) had encountered at least one occasion where they were unable to source their desmopressin in the local pharmacy.

**Hyponatraemia in the outpatient setting – adults & children/adolescents**

The hyponatraemia prevalence in adults was 22% (95%-CI [0·20-0·25]; n=212/943) and was 20% (95%-CI [0·12-0·28]; n=18/91) in children/adolescents. In patients under medication, the hyponatraemia prevalence in adults was 21% (95%-CI [0·19-0·24]; n=194/904) and was 19% (95%-CI [0·11-0·27]; n=17/90) in children/adolescents.

**Hospitalisation due to hyponatraemia – adults & children/adolescents**

**Patients at the age of ≥18**

The hyponatraemia prevalence in adults was 23% (95%-CI [0·19-0·26]; n=138/607) in patients performing desmopressin escape, 34% (95%-CI [0·27-0·40]; n=63/188) in those not aware of desmopressin escape, and 41% (95%-CI [0·32-0·51]; n=45/109) in patients aware of desmopressin escape but not using this method. Patients performing desmopressin escape had a significantly lower prevalence of hyponatraemia compared to those not being aware of this method (OR 0·58; 95%-CI [0·41-0·84]; p=0·0032) and to those aware of desmopressin escape but not using this method (OR 0·42; 95%-CI [0·27-0·64]; p<0·0001).

**Patients at the age of <18**

The hyponatraemia prevalence in children/adolescents was 12% (95%-CI [0·04-0·20]; n=7/60) in patients performing desmopressin escape, 35% (95%-CI [0·13-0·58]; n=6/17) in those not aware of desmopressin escape, and 8% (95%-CI [0·00-0·22]; n=1/13) in patients aware of desmopressin escape but not using this method. Patients performing desmopressin escape had a significantly lower prevalence of hyponatraemia compared to those not being aware of this method (OR 0·24; 95%-CI [0·07-0·88]; p=0·0285); however, not to those aware of desmopressin escape but not using this method (OR 1·58; 95%-CI [0·25-31·07]; p=0·6800).

**Figure S1 (M2) Desmopressin therapy – switch of preparation**

(A, B) Bar plots representing the frequency of each type of desmopressin changed from one preparation to another. (C) Bar plots represent the number of patients in each reason for a change in type of desmopressin.

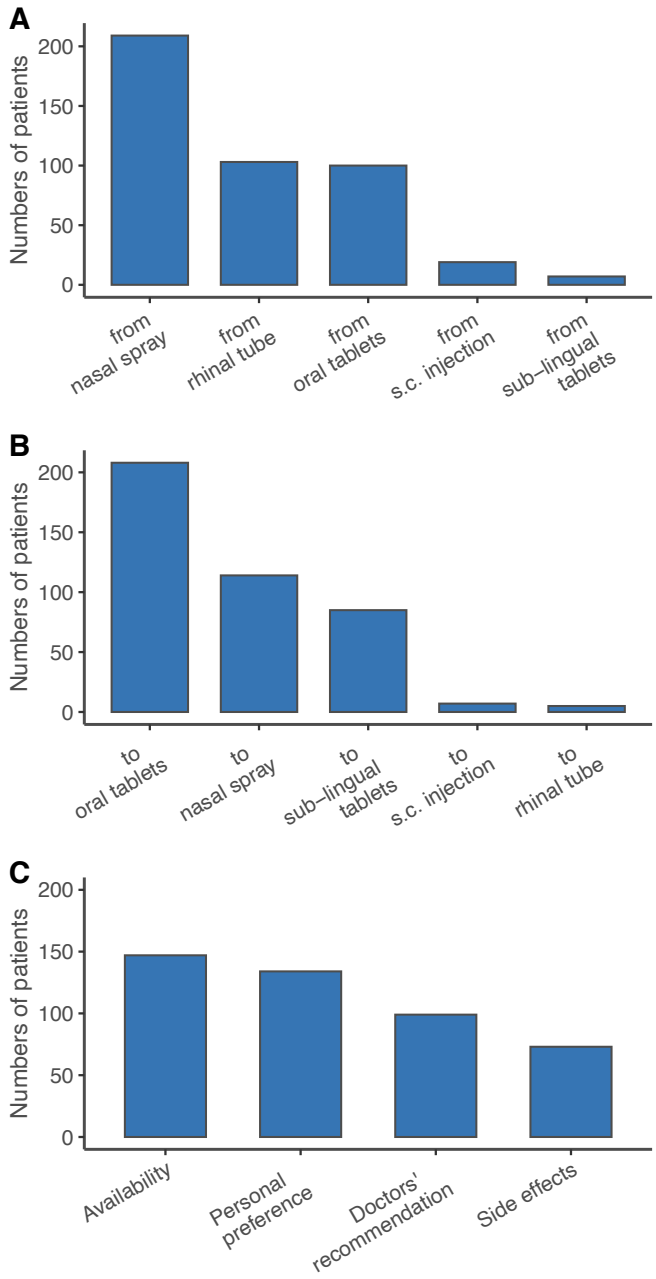

**Figure S2 (M2) Subjective rates on a visual analogue scale**

The median rate is presented as points with lines indicating the 25th to 75th percentile for the pooled data (grey), patients with isolated central diabetes insipidus (cDI, in blue), and patients with combined central diabetes insipidus and anterior pituitary dysfunction (cDI & APD, in red).

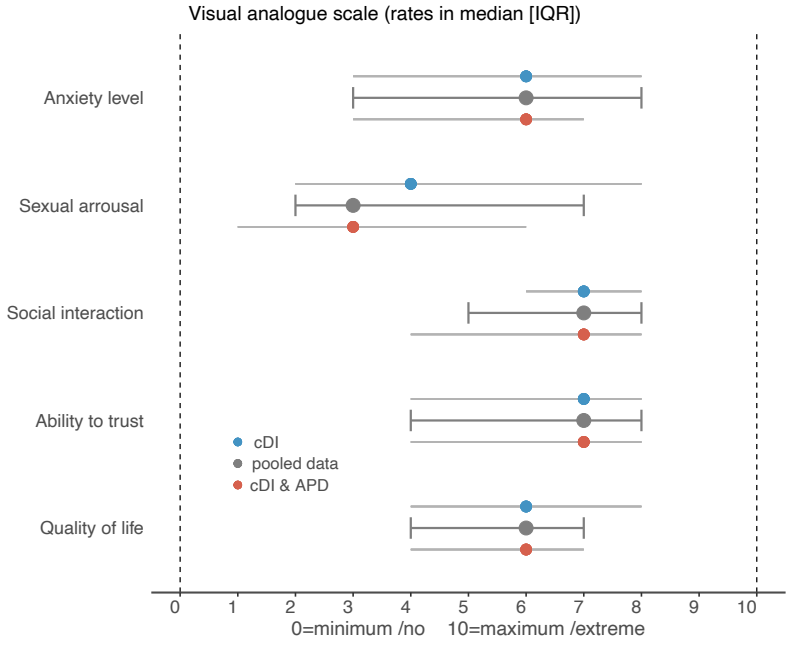

**Figure S3 (M2) Reasons for problems accessing desmopressin during hospitalisation**

Bar plots represent the number of patients in each reason for problems accessing desmopressin during hospitalisation**.**

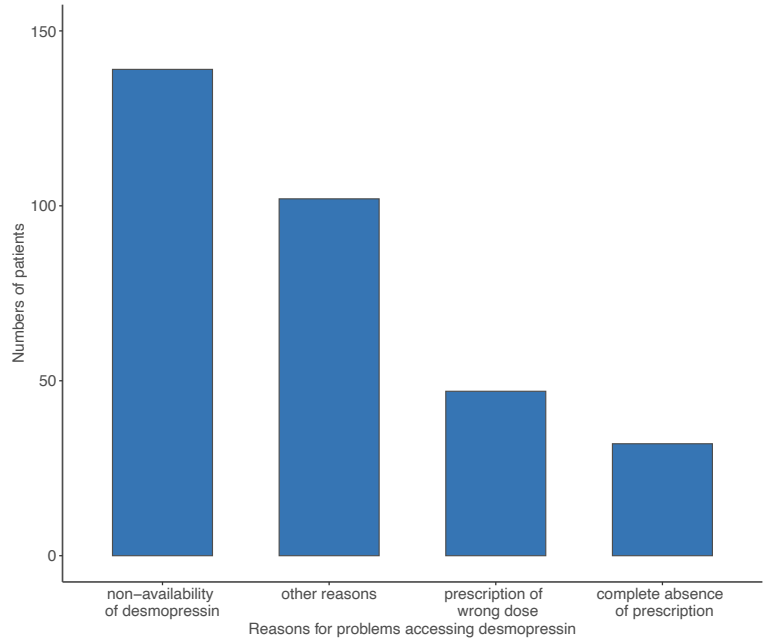

**Table S1 (M2) Aetiologies of central diabetes insipidus**

| | **Full dataset n=1034** | **Isolated posterior pituitary dysfunction** | **Anterior and posterior pituitary dysfunction** |
|---|---|---|---|
| **Idiopathic/unknown** | 315 (30) | 259 (25)* | 56 (5)* |
| **Hypothalamic/ Pituitary tumour/cyst** (post-surgery) | 254 (25) | 26 (3)* | 228 (22)* |
| **Hypothalamic/ Pituitary tumour/cyst** (pre-surgery) | 217 (21) | 54 (5)* | 163 (16)* |
| **Inflammatory/autoimmune** (e.g., hypophysitis) | 62 (6) | 33 (3)* | 28 (3)* |
| **Infiltrative disease** (e.g., sarcoidosis, Langerhans cell histiocytosis) | 61 (6) | 35 (3)* | 27 (3)* |
| **Genetic/familial** | 44 (4) | 44 (4)* | 0 (0)* |
| **Head injury** | 34 (3) | 16 (2)* | 18 (2)* |
| **Other causes** (e.g., vascular, congenital) | 29 (3) | 16 (2)* | 13 (1)* |
| **Metastasis to the pituitary** (e.g., lymphoma, breast cancer, lung cancer) | 12 (1) | 2 (0)* | 10 (1)* |
| **Infectious diseases** (e.g., meningitis, encephalitis, tuberculosis) | 6 (1) | 3 (0)* | 3 (0)* |
| Data are presented in numbers (%). * Calculated as proportions of the full dataset. n= numbers | | | |

## Table S2 (M2) Type of desmopressin preparation and the median total daily dose

| | Full dataset (n=1034) | |
|---|---|---|
| | **n (%; [95%-CI])** | **the median total daily dose in mcg [IQR]** |
| **oral tablets** | 575 (56; [53-59]) | 200 [150-400] |
| **nasal spray** | 233 (23; [20-25]) | 20 [20-40] |
| **sub-lingual tablets** | 126 (12; [10-14]) | 120 [120-240] |
| **subcutaneous injection** | 14 (1; [1-2]) | 2 [2-4] |
| **rhinal tube** | 11 (1; [1-2]) | 20 [15-45] |
| **combination of preparations** | 35 (3; [2-5]) | NA |
| **no medication** | 40 (4; [2-5]) | NA |
| NA= not available, n= numbers, mcg= microgram. Data presented in median [IQR] or n (%, [95%-CI]). | | |

**Table S3 (M2) Psychological co-morbidities – stratified by age category and sex**

| **Age category (years)** | **<18** | | **18-40** | | **41-60** | | **>61** | |
|---|---|---|---|---|---|---|---|---|
| | **female (n= 37)** | **male (n= 54)** | **female (n= 311)** | **male (n= 86)** | **female (n= 345)** | **male (n= 74)** | **female (n= 101)** | **male (n= 26)** |
| **Psychological problems or changes since diagnosis** | 14 (38; [24-54]) | 15 (28; [18-41]) | 121 (39; [33-44]) | 29 (34; [24-44]) | 124 (36; [31-41]) | 34 (46; [35-57]) | 26 (26; [18-35]) | 6 (23; [11-42]) |
| depressed mood | 7 (19, [9-33]) | 10 (19; [10-31]) | 82 (26; [21-31]) | 23 (27; [17-36]) | 78 (23; [19-27]) | 21 (28; [19-40]) | 16 (16; [10-24]) | 2 (8; [2-24]) |
| sleep disturbance | 7 (19, [9-33]) | 10 (19; [10-31]) | 91 (29; [24-34]) | 18 (21; [12-30]) | 91 (26; [22-31]) | 24 (31; [23-44]) | 18 (18; [12-26]) | 4 (15; [6-34]) |
| heightened anxiety | 6 (16; [8-31]) | 11 (20; [12-33]) | 91 (29; [24-34]) | 17 (20; [11-28]) | 90 (26; [22-31]) | 22 (30; [21-41]) | 17 (17; [11-25]) | 4 (15; [6-34]) |
| stress management disturbance | 3 (8; [3-21]) | 8 (15; [8-27]) | 73 (23; [19-28]) | 14 (16; [8-24]) | 58 (17; [13-21]) | 14 (19; [12-29]) | 10 (10; [5-17]) | 1 (4; [0-19]) |
| change in eating habits | 7 (19, [9-33]) | 8 (15; [8-27]) | 66 (21; [17-26]) | 19 (22; [13-31]) | 49 (14; [11-18]) | 10 (14; [8-23]) | 9 (9; [5-16]) | 0 (0; [0-13]) |
| change in personality | 4 (11; [4-25]) | 8 (15; [8-27]) | 30 (10; [6-13]) | 13 (15; [8-23]) | 47 (14; [10-18]) | 13 (18; [11-28]) | 8 (8; [4-15]) | 1 (4; [0-19]) |
| **Documented psychological condition after the diagnosis** | 3 (8; [3-21]) | 5 (9; [4-20]) | 44 (14; [10-18]) | 11 (13; [6-20]) | 26 (8; [5-11]) | 14 (19; [12-29]) | 7 (7; [3-14]) | 1 (4; [0-19]) |
| **Reduced *QoL* after the diagnosis** | 27 (73; [57-85]) | 34 (63; [50-75]) | 208 (67; [62-72]) | 64 (74; [65-84]) | 205 (59; [54-64]) | 52 (70; [59-79]) | 56 (55; [46-65]) | 14 (54; [35-71]) |
| social activities | 24 (65; [49-78]) | 29 (54; [41-66]) | 169 (54; [49-60]) | 51 (59; [49-70]) | 169 (49; [44-54]) | 41 (55; [44-66]) | 46 (46; [36-55]) | 10 (38; [22-57]) |
| recreation and fun | 16 (43; [29-59]) | 24 (44; [31-58]) | 156 (50; [45-56]) | 52 (60; [50-71]) | 156 (45; [40-50]) | 38 (51; [40-62]) | 47 (47; [37-56]) | 8 (31; [17-50]) |
| physical wellbeing | 15 (41; [26-57]) | 24 (44; [31-58]) | 145 (47; [41-52]) | 52 (60; [50-71]) | 145 (42; [37-47]) | 34 (46; [35-57]) | 39 (39; [30-48]) | 6 (23; [11-42]) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| mental wellbeing | 15 (41; [26-57]) | 19 (35; [24-49]) | 120 (39; [33-44]) | 43 (50; [39-61]) | 120 (35; [35-40]) | 36 (49; [38-60]) | 27 (27; [19-36]) | 2 (8; [2-24]) |
| **Subjective rates on a VAS, median [IQR]** | | | | | | | | |
| QoL*, B | 7 [6, 7] | 8 [6, 8] | 6 [4, 7] | 6 [4, 7] | 6 [4, 7] | 6 [3, 7] | 6 [4, 7] | 7 [6, 8] |
| Ability to trust*, B | 7 [5, 8] | 8 [6, 9] | 6 [3, 8] | 6 [3, 7] | 7 [4, 8] | 7 [4, 8] | 8 [6, 8] | 7 [7, 8] |
| Social interaction*, B | 8 [6, 8] | 7 [7, 9] | 7 [4, 8] | 6 [4, 8] | 7 [5, 8] | 6 [3, 8] | 8 [7, 8] | 8 [7, 9] |
| Sexual arousal*, A, B | NA | NA | 3 [1, 7] | 7 [3, 9] | 3 [2, 7] | 3 [1, 7] | 3 [1, 4] | 5 [3, 8] |
| Anxiety level in general life*, C | 4 [2, 7] | 6 [2, 7] | 7 [4, 8] | 6 [3, 8] | 6 [3, 7] | 6 [3, 7] | 5 [3, 7] | 3 [2, 7] |

Data presented in median [IQR] or n (%; [95%-CI]).*Rated on a visual analogue scale (VAS) from 0 (=minimum/no) to 10 (=maximum/extreme). A = answered by 819 patients. B = low score on this parameter reflects more adversely affected. C = high score on this parameter reflects more adversely affected. n= numbers. QoL= quality of life

**Table S4 (M2) Association of 'desmopressin method' and hyponatraemia (odds ratios; 95%-CI)**

| Outcome | Effect: performance of the 'desmopressin escape' method | OR | 95%-CI | p-value |
|---|---|---|---|---|
| **Hyponatraemia** (in the outpatient setting) | "yes, performing this method" **vs.** "not aware of this method" | 0·44 | 0·31-0·64 | <0·0001 |
| | "yes, performing this method" **vs.** "yes, but not following this approach" | 0·58 | 0·37-0·92 | 0·0178 |
| **Hyponatraemia** (leading to hospitalisation) | "yes, performing this method" vs. "not aware of this method" | 0·55 | 0·39-0·77 | 0·0006 |
| | "yes, performing this method" vs. "yes, but not following this approach" | 0·46 | 0·31-0·69 | 0·0002 |
| *OR= odds ratio; 95%-CI= confidence interval;* vs.=versus | | | | |

**Table S5 (M2) Association of type of desmopressin preparation and hyponatraemia (odds ratios; 95%-CI)**

| Outcome | Effect: type of preparation | OR | 95%-CI | p-value |
|---|---|---|---|---|
| **Hyponatraemia** (in the outpatient setting) | oral **vs.** nasal spray | 1·22 | 0·74-2·07 | 0·4520 |
| **Hyponatraemia** (leading to hospitalisation) | oral **vs.** nasal spray | 0·77 | 0·49-1·21 | 0·2440 |
| *OR= odds ratio; 95%-CI= confidence interval;* vs.=versus. Only analysed for patients who did not switch the type of preparation since diagnosis. | | | | |

**Outpatient setting** (Only analysed for patients who did not switch the type of preparation since diagnosis)

The hyponatraemia rate was 20% (95% CI 0·16-0·24; n=79/398) for the oral preparation, 17% (95% CI 0·11-0·23; n=23/136) for the nasal spray, 15% (95% CI 0·05-0·25; n=7/47) for the sub-lingual preparation, 33% (95% CI 0·03-0·64; n=3/9) for the sub-cutaneous preparation, 0% (95% CI 0·00-0·00; n=0/4) for the rhinal tubes, and 15% (95% CI 0·00-0·31; n=3/20) for those with a combined preparation.

**Leading to hospitalisation** (Only analysed for patients who did not switch the type of preparation since diagnosis)

The hyponatraemia rate was 22% (95% CI 0·18-0·26; n=86/398) for the oral preparation, 26% (95% CI 0·19-0·34; n=36/136) for the nasal spray, 28% (95% CI 0·15-0·40; n=13/47) for the sub-lingual preparation, 22% (95% CI 0·00-0·49; n=2/9) for the sub-cutaneous preparation, 50% (95% CI 0·01-0·99; n=2/4) for the rhinal tubes, and 25% (95% CI 0·06-0·44; n=5/20) for those with a combined preparation.

**MANUSCRIPT 3 (M3)**

# Oxytocin in response to MDMA provocation test in patients with arginine vasopressin deficiency (central diabetes insipidus)

## - a single-centre case-control trial with nested placebo-controlled cross-over

the OxyMA study


**Authors**

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**ABSTRACT**

**BACKGROUND** Disruptions of the hypothalamic-pituitary axis can cause an arginine vasopressin deficiency (AVP-D), also known as central diabetes insipidus (cDI). Due to the close anatomical proximity, these patients are also at high risk of additional oxytocin deficiency. However, an oxytocin deficiency has never been proven. Here, we used 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy'), known for strong activation of the central oxytocinergic system, as a biochemical and psychoactive provocation test to reveal an oxytocin deficiency in patients with AVP-D (cDI).

**METHODS** This single-centre case-control trial with nested placebo-controlled, cross-over included 15 patients with AVP-D (cDI) and 15 matched healthy controls (by age, sex, BMI) between February 2021 and April 2022 at the University Hospital Basel, Switzerland. Participants were assigned to a single oral dose of MDMA (100 mg) or placebo first, using block randomisation, with a wash-out period of at least two weeks between both experimental sessions. Participants and investigators assessing the outcomes were blinded to the assignment. Oxytocin was measured at 0, 90, 120, 150, 180, and 300 minutes after MDMA or placebo. The primary outcome was the area under the plasma oxytocin concentration curve (AUC) after drug intake. The AUC was compared between groups and conditions using a linear mixed effects model. Subjective drug effects were assessed throughout the experiment using 10-point visual analogue scales. Acute adverse effects were assessed before and 360 minutes after drug intake using a 66-item List-of-Complaints.

**FINDINGS** In response to MDMA, in healthy controls, median plasma oxytocin at baseline was 77 pg/ml [59-94] and increased by 658 pg/ml [355-914], resulting in an AUC of 102,095 pg/ml [41,781, 129,565]; while in patients, oxytocin at baseline was 60 pg/ml [51-74] and only slightly increased by 66 pg/ml [16-94], resulting in an AUC of 6,446 pg/ml [1,291-11,577]. The effect of MDMA on oxytocin was statistically significantly different between both groups showing an 82 % (95%-CI [70, 186]) higher AUC for oxytocin in healthy controls compared with patients (difference: 85,678 pg/ml (95%-CI [63,356 to 108,000], $p<0{\cdot}0001$)). The increase in oxytocin in healthy controls was associated with typical strong subjective pro-social, empathic, and anxiolytic effects, while in patients, only minimal subjective effects were observed in agreement with the lack of oxytocin increase.

In healthy controls and patients, the most reported complaints were ‘fatigue’ (7 (47 %) and 8 (53 %)), ‘lack of appetite’ (8 (53 %) and 10 (67 %)), ‘lack of concentration’ (7 (47 %) and 8 (53 %)), and ‘dry mouth’ (8 (53 %) and 8 (53 %)). In addition, 2 (13 %) healthy controls and 4 (27 %) patients developed transient mild hypokalaemia.

**INTERPRETATION** These results provide evidence for an oxytocin deficiency in patients with AVP-D (cDI), laying the groundwork for a new hypothalamic-pituitary disease entity.

**FUNDING** Swiss National Science Foundation, Swiss Academy of Medical Sciences, and G&J Bangerter-Rhyner-Foundation.

**REGISTRATION** ClinicalTrials.gov NCT04648137

## INTRODUCTION

Arginine vasopressin (AVP) and oxytocin (OXT) are both nine-amino acid neuropeptides produced in the hypothalamic supraoptic and paraventricular nuclei (SON/PVN) and released into circulation from axon terminals projecting to the posterior pituitary.[12] A disrupted hypothalamic-pituitary axis caused by inflammation, tumours, or head trauma can cause AVP deficiency (AVP-D), also known as central diabetes insipidus (cDI), a condition characterised by polyuria and consecutive polydipsia.[23] Once diagnosed, desmopressin, a selective AVP receptor 2 agonist, is prescribed to treat AVP-D (cDI) symptoms.[23] Despite adequate treatment with desmopressin, patients often report residual psychological symptoms such as heightened anxiety levels, difficulties describing or expressing emotions, and depressed mood, leading to a reduced quality of life.[59,85,86,88,124,126]

Due to the anatomical proximity, a disrupted AVP system leading to AVP-D (cDI) could also disturb the OXT system leading to OXT deficiency. The central oxytocinergic system is key in regulating socio-emotional functioning, including attachment and pair bonding, fear extinction, emotion recognition, and empathy.[12] Therefore, increased psychopathological findings in patients with AVP-D (cDI) may be caused — at least partially — by an additional OXT deficiency. However, OXT deficiency has never been proven and established as a disease entity, as basal levels are unreliable and ideal sampling methods are controversial.[90,127,128] For other pituitary hormones, a provocation test to stimulate the respective hormone is often applied in case of a suspected deficiency. Still, no standard provocation test for OXT has been established, and testing attempts or physiological stimuli (e.g., exercise) have failed to reveal a consistently strong increase in OXT levels.[85,129]

3,4-Methylenedioxymethamphetamine (MDMA, 'ecstasy') is used recreationally for its effects on empathic feelings and sociability. Several studies have documented marked increases in circulating OXT levels in response to MDMA in healthy adults.[130-132] The prosocial effects of MDMA on emotion processing and social interaction, such as increased trust, closeness to others, identification of facial emotions, and fear extinction, are mediated partly by a strong OXT release.[133]

Given the residual psychopathological symptoms in patients with AVP-D (cDI) and that a potential OXT deficiency could be the underlying cause, we investigated MDMA as a biochemical and psychoactive provocation test to reveal an OXT deficiency in these patients. We hypothesised that OXT

would show a robust increase in response to MDMA in healthy controls and a blunted response in patients with AVP-D (cDI).

## METHODS

### Trial design

This was a randomised, double-blind, placebo-controlled, case-control with nested cross-over trial in 15 patients with AVP-D (cDI) and 15 healthy controls conducted between February 2021 and April 2022 at the University Hospital Basel, Switzerland. The study conformed to the Declaration of Helsinki and was approved by the Ethics Committee Northwest Switzerland (EKNZ, No 2020-02147). The use of MDMA was authorised by the Swiss Federal Office for Public Health, Bern, Switzerland (BAG, No 2020/013366). All participants provided written informed consent before participating in the study. The study was registered at ClinicalTrials.gov NCT04648137.

### Participants

Adult patients with a confirmed diagnosis of AVP-D (cDI) and healthy controls were included. Healthy controls were matched according to factors known to affect OXT levels, i.e., age (+/-3), sex, body mass index [BMI] (+/-2), and menopause/hormonal contraceptives. All patients were screened/examined for somatic and psychological comorbidities and only included if no other somatic and no psychological illnesses were present. The main exclusion criteria were regular consumption of alcoholic beverages, tobacco smoking (>10 cigarettes/day), documented cardiovascular disease or uncontrolled arterial hypertension, current or previous major psychiatric disorder or psychotic disorder in first-degree relatives (assessed by the Semi-structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Axis I disorders), lifetime prevalence of illicit substance use >10 times (except for tetrahydrocannabinol, THC) or any time within the previous two months and during the study period, and the use of medications that may interfere with the study medications (e.g., any psychiatric medication). Full in- and exclusion criteria are provided in the supplementary (M3).

### Study procedure

This study included a screening visit followed by a baseline evaluation on the same day, two 7-hour main visits, and phone calls three days after each visit conducted to inquire about subacute adverse events. The wash-out period between both main visits lasted ≥14 days. Enrolled participants were randomised to receive either MDMA or placebo first.

At baseline, participants underwent a detailed psychological evaluation, including the assessment of anxiety levels by using Spielberger's State-Trait Anxiety Inventory (STAI – Trait subscale), scoring the mood via the Beck's Depression Inventory (BDI II), the degree of alexithymia by using the Toronto Alexithymia Scale (TAS-20), and general health status by using the Short Form 36 Health Survey (SF-36). Detailed description and references of the questionnaires are provided in the supplementary (M3).

At the main visits, participants presented in the morning after an 8-hour food fasting state. The use of alcohol was prohibited 24 hours before each visit. Participants were also asked to refrain from other substance use during the study and were screened for drug abuse consumption in urine (opiates, cocaine, amphetamines, methamphetamines, and THC) at the beginning of each visit. The experimental visits were conducted in a quiet standard hospital patient room. Only one participant and one investigator were present during the experimental visits. An intravenous catheter was placed in an antecubital vein for blood sampling. Patients with additional secondary adrenal insufficiency on hydrocortisone (HCT) substitution were asked to take 50 mg HCT instead of the regular dose at the experimental visits (30 mg morning and 20 mg noon dose), and the patients were asked to take desmopressin as regular. Female participants were screened for pregnancy before each visit, and the visits were conducted during the follicular phase to account for cyclic changes. One main visit lasted for 360 minutes after drug administration, and participants were under continuous medical supervision until any subjective effects had completely subsided. A standardised breakfast was served before drug intake, and lunch was served after 3 hours.

**Autonomic and adverse effects of special interest, and adverse events**

Blood pressure, heart rate, and tympanic body temperature were repeatedly measured 60 minutes before, at drug intake, and every 30 minutes after drug intake. Adverse effects of special interest (AESI) were assessed 60 minutes before and 360 minutes after drug administration using a pre-defined 66-item List-of-Complaints (LC). Additional adverse events were assessed during and three days after each experimental session. All events were handled in accordance with the Swiss legal framework and reported if necessary. An annual safety report was provided to the local ethics committee and an independent monitoring was performed.

**Subjective drug effects**

Subjective effects were assessed repeatedly throughout the treatment visit (at 0, 30, 60, 90, 120, 150, 180, 240, 300, and 360 minutes) using visual analogue scales (VASs). The VASs were presented as 10 cm horizontal lines ranging from 0 to 10, with 'not at all' (i.e., 0) on the left and 'extremely' (i.e., 10) on the right, or were bidirectionally ranging from -5 to +5, with 0 being the neutral measure (i.e., 'no effect'). The following items known to be sensitive to the acute subjective effects induced by MDMA were used: 'any effect,' 'good effect,' 'bad effect,' 'liking effect,' 'feeling high,' 'stimulation,' 'fear,' 'satisfaction,' 'happiness,' 'trust,' 'talkative,' 'openness,' 'want to be close to others,' 'want to be embraced,' 'want to embrace someone,' 'want to be with others,' and 'feeling close to others'.[13,16-18] Acute anxiety was assessed using the STAI-State sub-scale (STAI-S) right before drug intake (0 minutes) and re-assessed 180 minutes after drug administration.

**Study drugs**

Oral MDMA was prepared as opaque gelatine capsules containing 25 mg of pharmaceutically pure MDMA hydrochloride (ReseaChem GmbH, Burgdorf, Switzerland) with mannitol filler and administered as a single dose of 100 mg (four capsules à 25 mg) orally. Oral placebo was prepared as identical opaque gelatine capsules filled with mannitol only. All products were prepared and quality-controlled according to GMP guidelines. Given prior pharmacokinetic studies, peak effects of MDMA were expected after 2.5 hours and expected psychoactive effects of 6 hours.[130]

**Blood samples**

Samples were collected to determine OXT and sodium levels at timepoint 0, 90, 120, 150, 180, and 300 minutes, and for plasma copeptin at 0 and 120 minutes. Samples were taken as aliquots (EDTA plasma, lithium-heparin, and serum) immediately centrifuged at 4 °C at 3000 rpm for 10 min, then stored at -80 °C until batch analysis. Sodium levels were measured by analysing venous blood gas. Copeptin levels were measured in serum using a commercial automated immunofluorescence assay (B.R.A.H.M.S Copeptin-proAVP KRYPTOR, Brahms, Thermo Scientific Biomarkers, Hennigsdorf, Germany). Extracted EDTA plasma OXT was created using Oasis® PRiME HLB 96-well plate, 30 mg sorbent (Waters Corporation, Milford, MA). Further, for OXT determination, the Oxytocin ELISA kit (ENZO Life Sciences, Ann Arbor, MI) (sensitivity 15pg/ml (range 15.6 - 1,000.0 pg/ml)) was used. Prolactin

was measured using an electrochemiluminescence assay. The intra-assay coefficient of variation for our OXT is 1.59 %, and the inter-assay coefficient of variation is 4.97 %. The antiserum displays cross-reactivity with mesotocin of 7%, arginine vasotocin of 7.5%, and <0.02% for other related molecules. Copeptin, OXT, and prolactin were analysed blinded at the end of the study in one batch.

MDMA and its primary metabolite 3,4-methylenedioxyamphetamine (MDA) were determined in human plasma using high-performance liquid chromatography-tandem mass spectrometry (HPLC–MS/MS). The lower limit of quantification of MDMA and MDA were 0.5 and 1 ng/ml, respectively. A validated bioanalytical method was used for the analysis.[134] Pharmacokinetic parameters were estimated using non-compartmental methods in Phoenix WinNonlin 8.3 (Certara, Princeton, NJ, United States).

**Emotion recognition and empathy tasks**

At the expected peak concentration of OXT (timepoint 150 minutes)[130], participants performed the Multifaceted Empathy Task (MET) and Face Emotion Recognition Task (FERT). The MET is a reliable and valid task to assess the cognitive and emotional aspects of empathy[135] that has been proven to be sensitive to the effects of OXT[16] and MDMA.[136] The FERT assesses the recognition of basic emotions, including ten neutral faces and 160 faces that express one of four basic emotions (i.e., happiness, sadness, anger, and fear), with pictures morphed between 0% (i.e., neutral) and 100% in 10% steps. A detailed description of the tasks is provided in the supplementary (M3).

**Sample size estimation**

Given the lack of data on oxytocin after MDMA in patients with AVP-D (cDI) prior to this study, assumptions for sample size calculation were discussed in a panel of expert neuroendocrinologists. According to available data from healthy adults assessing OXT levels after a single oral dose of MDMA[130], we assumed a mean maximum increase in plasma OXT levels after MDMA of 810 pg/mL (SD 330) in healthy controls. A 30% reduced response, i.e., plasma OXT levels after MDMA of 550 pg/mL (SD 100) in patients with AVP-D (cDI), was considered a minimum clinically meaningful difference. With a power of 80%, a two-sided significance level α of 0.05, and assuming a pooled SD of 240 pg/mL (according to Cohen's calculation), a total of n=15 was estimated for each group.

**Randomisation**

Participants were assigned to receive MDMA or placebo first, using block randomisation (blocks of four) to counterbalance the intervention order according to a predefined randomisation list. The GMP facility generated the allocation sequence, the study investigators performed the randomisation, and an intervention order was assigned to each participant number. Only the GMP facility and the principal investigator had access to the code (sealed opaque envelopes). The randomisation list was unknown to the participants, the investigators, and the study nurses involved in the trial.

**Objectives**

The primary objective was to evaluate the OXT response to MDMA compared to placebo in patients with AVP-D (cDI) and healthy controls. The secondary objective was to evaluate baseline psychological characteristics and physical and mental health between both groups using the questionnaires STAI-T, TAS-20, BDI-II, and SF-36. Other objectives were descriptive in nature. We aimed to evaluate the time course of 20 subjective effects, emotion recognition and empathy at 150 minutes, and acute anxiety levels at 180 minutes after drug intake.

**Statistical analysis**

Demographic information was described as mean (SD), median [interquartile range (IQR)], or absolute (relative) frequency, as appropriate. The plasma OXT level after MDMA administration was described by mean (standard deviation, SD) at each time point, and the time course was visualised. The primary endpoint, net incremental area under the curve (AUC) in both directions (positive and negative) was calculated to evaluate the change in OXT from 0 to 300 minutes.[137] The continuous primary endpoint AUC was analysed using a restricted maximum likelihood (REML)-based repeated measures approach (i.e. linear mixed-effects regression model) using the R-package "nlme" (version 3.1-160). We analysed treatment (MDMA vs placebo) and participant group (healthy control vs patient), and the interaction between both as fixed effects and OXT-level at timepoint 0 (i.e., right before the study drug) of each visit was included as a continuous covariate. A random intercept for study participants was added to the model to account for the correlation between measures within the same participant, and a general unstructured variance-covariance structure was used to model the within-patient errors. The degrees of freedom were approximated with the Newton-Raphson Algorithm. Significance tests were based on the

Wald-statistic using a two-sided alpha of 0.05. We calculated the percentage difference between group under the MDMA condition based on the estimates of the model using a bootstrapping method with 1000 bootstrap replicates and a percentile method to determine the 95% confidence interval from all obtained estimates.

The baseline psychological characteristics were compared using Wilcoxon rank-sum tests as a posthoc analysis. The p-values were corrected for multiple testing using the conservative Bonferroni method. Other secondary endpoints (i.e., FERT, MET, STAI-S, subjective effects) were described by median [IQR] and visualised using boxplots. The time course of the subjective effects was visualised using the mean (SD). For each subjective effect, we calculated the maximal change (either positive or negative) and the net incremental AUC as a measure of the total effect. Finally, we calculated the Pearson correlation coefficient for the maximal change in each subjective effect and the maximum level of OXT. The time course of each safety outcome (blood pressure, heart rate, and tympanic body temperature) was described, and the time to maximum increase was calculated. The total number of complaints reported on the LC was described by mean (SD) at the end of each study visit and during the phone interview three days after the study visit. No data imputation was foreseen for missing data. No dropouts or lost-to-follow-up occurred. Only the full analysis set was used for statistical analysis. All analyses were performed in R version 4.2.2.

**Role of funding source**

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

## RESULTS

### Baseline characteristics

Between February 01, 2021, and May 01, 2022, 15 patients with AVP-D (cDI) and 15 matched healthy controls participated in this study (Figure 1 (M3)). Median age was 34 years ([IQR 25-46], 53 % (n=8) females) in patients and 35 years ([26-48], 53 % (n=8) females) in healthy controls. Of the patients with AVP-D (cDI), 53 % (n=8) had an isolated posterior pituitary dysfunction, and 47 % (n=7) had a combined pituitary dysfunction. Baseline characteristics are summarised in Table 1 (M3).

### Psychological baseline evaluation

In patients with AVP-D (cDI) compared with healthy controls, we observed statistically significantly higher total median scores in trait anxiety (STAI-T: 41 points [34-48] vs. 28 points [24-31]; p=0·0143), alexithymia levels (TAS-20: 47 points [38-59] vs. 30 points [29-37; p=0·0365), and depression symptoms (BDI-II: 6 points [3-17] vs. 1 point [0-2]; p=0·0437) (Table S1 (M3), Figure S1 (M3)). In the SF-36, patients showed lower self-reported mental health scores than healthy controls (mental health sub-scale: 48·6 points [42·8-52·4] vs. 54·4 points [51·8-55·2]; p=0·0199) (Table S1 (M3)).

### OXT in response to MDMA and placebo stimulation

The time course of plasma OXT after MDMA administration is shown in Figure 2 (M3), Table 2 (M3). Maximum changes in OXT levels from baseline (i.e., right before study drug intake) and the time to peak level are demonstrated in Table 2 (M3).

In healthy controls, median OXT at baseline was 77 pg/ml [59-94] and peaked in response to MDMA stimulation at 624 pg/ml [235-959] after 180 minutes; the resulting maximum change in OXT was 658 pg/ml [355-914]. In patients with AVP-D (cDI), OXT at baseline was 60 pg/ml [51-74] and peaked in response to MDMA at 92 pg/ml [79-110] after 150 minutes; the resulting maximum change in OXT was 66 pg/ml [16-94]. Neither group revealed any notable changes in plasma OXT under placebo (Figure S3 (M3)).

The plasma OXT AUC is visualised in Figure S3 and described in Table 2 (M3). In response to MDMA administration, an eight-fold increase in OXT levels was observed in healthy controls with an AUC of 102,095 pg/ml [41,782-129,565], whereas no notable increase was measured in patients with AVP-D

(cDI) with an AUC of 6,446 pg/ml [1,291-11,577]. In response to placebo, the plasma OXT AUC was 2,175 pg/ml [-3,750, 3,754] in healthy controls and -1,343 pg/ml [-3,860, -580] in patients (Figure S3 (M3), Table S2 (M3)).

The effect of MDMA on OXT was statistically significantly different in healthy controls compared with patients, i.e., the net incremental AUC was 85,678 pg/ml (95%-CI [10,800 to 63,356], p<0·0001) higher in healthy controls than in patients (adjusted for baseline OXT). Given the estimates for the OXT for both groups after MDMA administration, healthy controls had an 82% (95%-CI [70, 186]) higher AUC compared to patients. Additional subgroup analyses are provided in the supplementary; Figures S4 (M3), S5 (M3), S6 (M3). Copeptin remained stable from 0 to 120 minutes in response to MDMA and placebo in both groups (Figures S7 (M3), S8 (M3)). Prolactin increased slightly and equally in both groups in response to MDMA (Figure S9 (M3)).

**Acute subjective emotional effects in response to MDMA**

At the beginning of the experimental session, a comparable increase in state anxiety was observed in healthy controls and patients (STAI-S: 30 points [27-33] vs. 31 points [28-34]). In response to MDMA stimulation, at the expected peak concentration of OXT (180 minutes), the anxiety score decreased to 24 points [22-27] in healthy controls, while no anxiolytic effect was observed in patients, and their anxiety levels remained stable at 30 points [29-37] (Figure 2 (M3)).

Overall, the MDMA-induced acute subjective effects assessed from VASs were lower or absent in patients compared to healthy controls (Table S3 (M3)). Specifically, patients exhibited lower effects, i.e., for ‘any effect,’ ‘good effect,’ ‘liking effect,’ ‘feeling high,’ ‘stimulation,’ ‘fear reduction,’ ‘satisfaction,’ ‘happiness,’ ‘trust,’ ‘talkative,’ and ‘openness,’ as shown in Figure 3. There were moderate correlations between maximum change in some subjective effects and maximum change in OXT levels (Table S3 (M3)). Additional assessed subjective effects are provided in the supplementary; Figure S10 (M3).

**Emotion recognition in the FERT and empathy rating in the MET**

In healthy controls, MDMA impaired the recognition of faces displaying negative emotions, such as “anger,” “sad,” and “fear,” with no alteration in the correct identification of “happy” or “neutral” faces

(Figure S11 (M3)). In contrast, in patients with AVP-D (cDI), MDMA differentially impaired the recognition of faces displaying “happy” and “sad” emotions without an effect on “anger,” “fear,” or “neutral” faces (Figure S11 (M3)). In healthy controls, MDMA increased explicit and implicit emotional empathy ratings for positive valence stimuli, whereas patients revealed a contra-directional effect with lower ratings. MDMA decreased explicit and implicit emotional empathy ratings for negative valence stimuli in healthy controls and patients with AVP-D (cDI), with a stronger decrease in healthy controls. MDMA showed no effect on cognitive empathy scores in both groups compared with placebo (Figure S12 (M3)).

**Safety and tolerability**

MDMA moderately increased blood pressure, heart rate, and body temperature showing no major differences between both groups (Table 3 (M3), Figure S13 (M3)). MDMA increased the acute (360 minutes after drug intake) and subacute (three days after the experimental session) number of complaints assessed with the LC similarly in both groups (acute number of complaints: patients 6·6 [SD 6·5] and healthy controls 6·3 [5·0]; subacute number of complaints: patients 3·6 [SD 2·5] and healthy controls 3·6 [2·7]) (Table 3 (M3), Tables S4 (M3), S5 (M3)). The most frequent acute adverse effects after MDMA administration included headache, tiredness, and lack of appetite (Table 3 (M3)). In addition, 2 (13 %) healthy controls and 4 (27 %) patients developed transient mild hypokalaemia; no episode of new onset hyponatremia was recorded in healthy controls and patients throughout the sessions (Table 3 (M3)). One patient presented with mild hyponatremia at the beginning of the experimental session due to a slight desmopressin overdose and remained stable throughout the session. No serious adverse events were reported.

**Figure 1 (M3) Trial profile**

Flow-chart showing screening, inclusion, randomisation, and participation throughout the study for patients with arginine vasopressin deficiency (central diabetes insipidus) (AVP-D (cDI)) and healthy controls (HC).

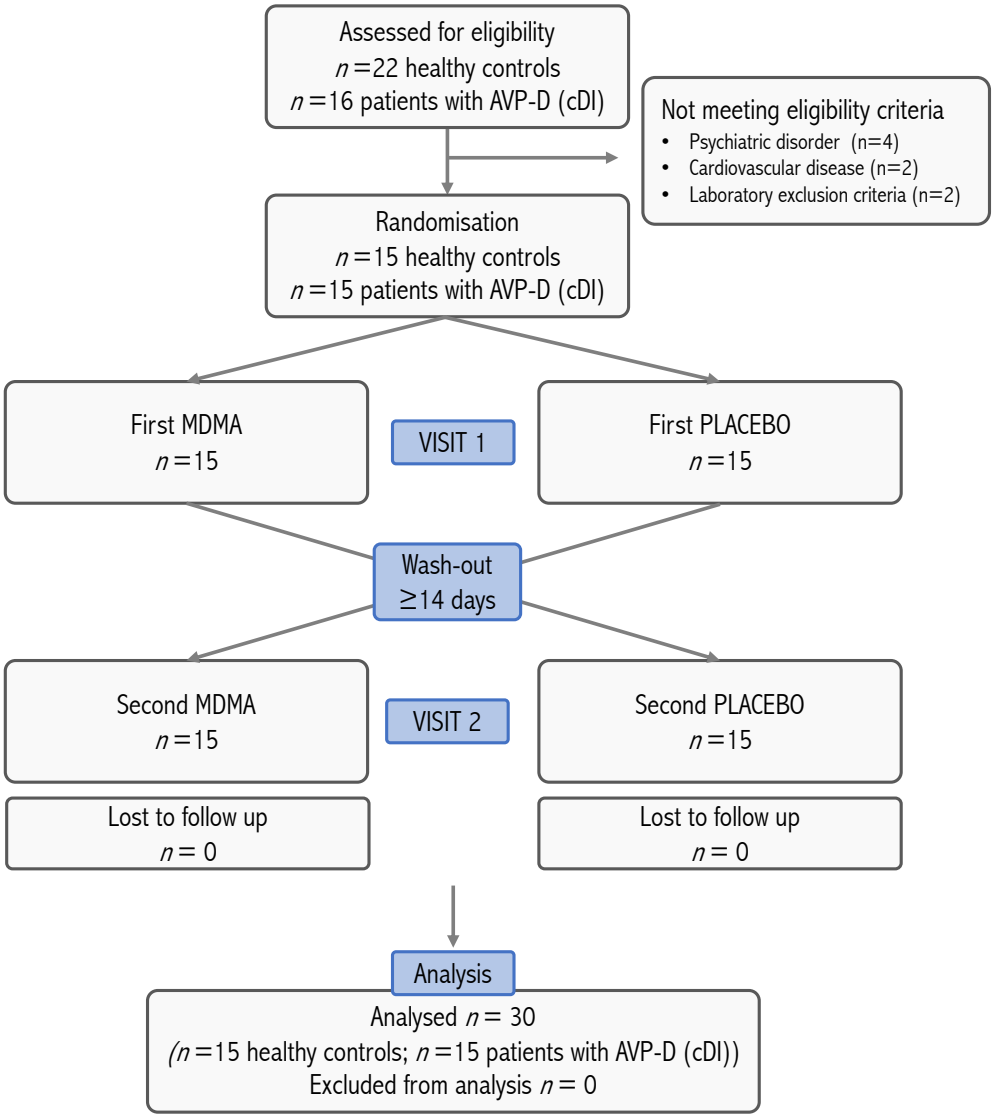

**Figure 2 (M3) Plasma oxytocin and anxiety symptoms in response to MDMA stimulation**

(A) OXT increased after MDMA stimulation in healthy controls, while no change was observed in patients with arginine vasopressin deficiency (central diabetes insipidus). Data are expressed as mean ± standard deviation for patients with arginine vasopressin deficiency (central diabetes insipidus (in red)) and healthy controls (in blue). (B) Increased state anxiety was observed in healthy controls and patients and decreased at 180 minutes in response to MDMA stimulation in healthy controls, while no such anxiolytic effect was observed in patients, and their anxiety levels remained stable. Boxes span the interquartile range (IQR); the thick horizontal line is the median. Whiskers are the most extreme values lying within the box edge and 1·5 times the IQR.

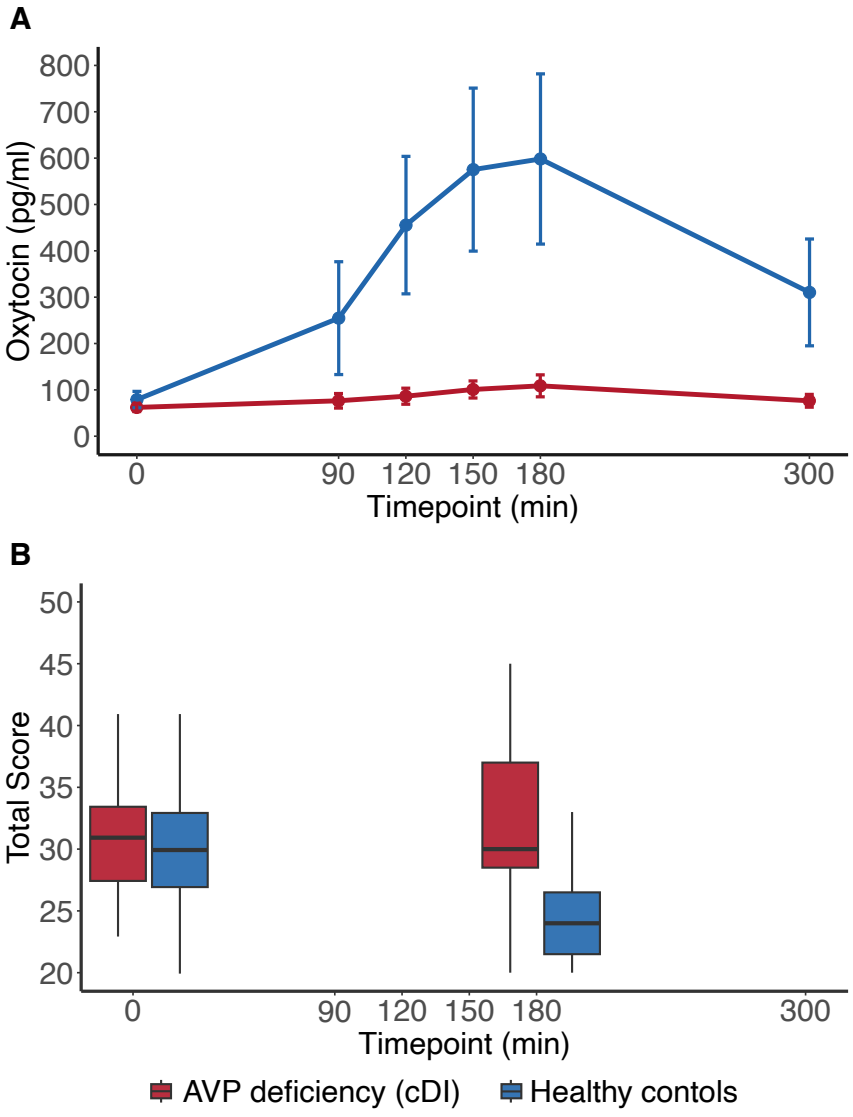

**Figure 3 (M3) Subjective effects in response to MDMA stimulation**

MDMA-induced acute subjective effects assessed on VAS scores were lower or absent in patients with arginine vasopressin deficiency (central diabetes insipidus) than in healthy controls. The data are presented as mean ± standard deviation for healthy controls (in blue) and patients with arginine vasopressin deficiency (central diabetes insipidus (in red)).

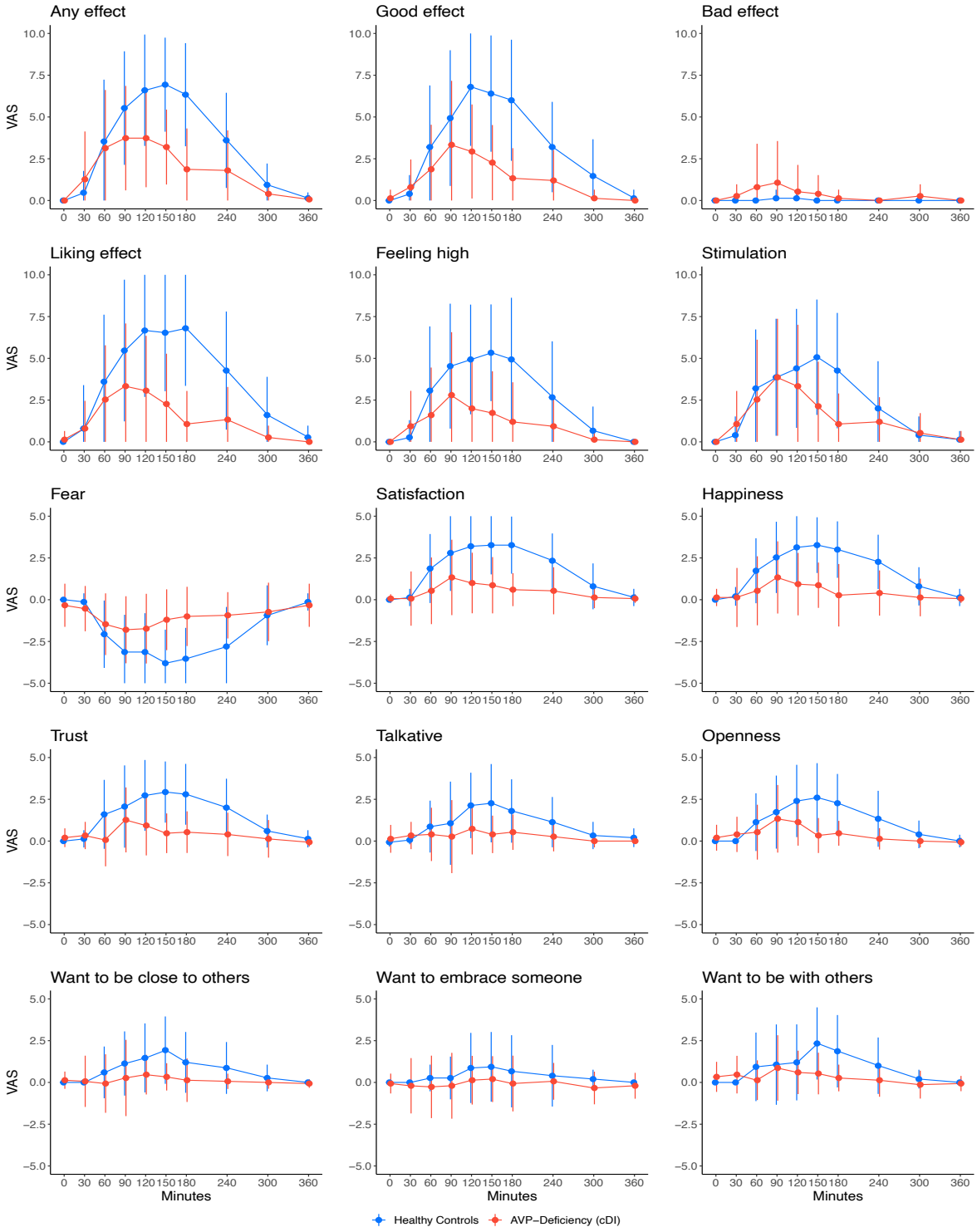

**Table 1 (M3) Baseline characteristics by group**

| | Patients with arginine vasopressin deficiency (central diabetes insipidus) (n=15) | | Healthy controls (n=15) | |
|---|---|---|---|---|
| | **Placebo** | **MDMA** | **Placebo** | **MDMA** |
| Age, years | 34 [25, 46] | 34 [25, 46] | 35 [26, 48] | 35 [26, 48] |
| Sex, female | 8 (53%) | 8 (53%) | 8 (53%) | 8 (53%) |
| Sex, male | 7 (47%) | 7 (47%) | 7 (47%) | 7 (47%) |
| Ethnicity, Caucasian | 14 (93%) | 14 (93%) | 15 (100%) | 15 (100%) |
| Ethnicity, Indian | 1 (7%) | 1 (7%) | 0 (0%) | 0 (0%) |
| Weight, kg | 74 (13) | 74 (13) | 70 (10) | 70 (10) |
| Height, cm | 174 (11) | 174 (11) | 173 (10) | 173 (10) |
| BMI, kg/m² | 24·4 (3·1) | 24·4 (3·1) | 23·2 (2·1) | 23·2 (2·1) |
| **Cause of central diabetes insipidus** | | | | |
| Idiopathic or unknown | 5 (33%) | 5 (33%) | NA | NA |
| Pituitary adenoma (post-surgery) | 3 (20%) | 3 (20%) | NA | NA |
| Hypothalamic or pituitary tumour or cyst (e.g., craniopharyngioma or Rathke cleft cyst) | 3 (20%) | 3 (20%) | NA | NA |
| Inflammatory or autoimmune (e.g., hypophysitis) | 3 (20%) | 3 (20%) | NA | NA |
| Genetic or hereditary | 1 (7%) | 1 (7%) | NA | NA |
| Anterior pituitary deficiency | 7 (47%) | 7 (47%) | NA | NA |
| Degree of AVP deficiency, partial | 3 (20%) | 3 (20%) | NA | NA |

Data presented as mean (SD), median [IQR], or frequency (%). BMI=body mass index; AVP= arginine vasopressin; NA = not applicable. BMI= body mass index; MDMA= methylenedioxymethamphetamine

**Table 2 (M3) The time course, net incremental AUC, maximum change, and time to peak of plasma oxytocin levels in each group in response to MDMA stimulation in the full analysis set.**

| | **Patients with arginine vasopressin deficiency (central diabetes insipidus) (n=15)** | | **Healthy controls (n=15)** | |
|---|---|---|---|---|
| OXT level, pg/ml | **Mean (SD)** | **Median [IQR]** | **Mean (SD)** | **Median [IQR]** |
| at 0 minutes | 62 (18) | 60 [51-74] | 79 (35) | 77 [59-94] |
| at 90 minutes | 76 (31) | 68 [52-96] | 255 (244) | 141 [121-341] |
| at 120 minutes | 86 (35) | 73 [62-110] | 456 (297) | 504 [192-589] |
| at 150 minutes | 101 (37) | 92 [79-110] | 575 (352) | 540 [273-844] |
| at 180 minutes | 109 (47) | 90 [72-134] | 598 (367) | 624 [235-959] |
| at 300 minutes | 76 (28) | 70 [62-89] | 310 (230) | 203 [181-391] |
| AUC | 7,093 (8,938) | 6,446 [1,291-11,577] | 89,532 (57,149) | 102,095 [41,782-129,565] |
| mean difference in AUC between groups | 85,678; 95%-CI [63,356 to 108,000] | | | |
| Maximum change | 60 (48) | 66 [16-94] | 609 (336) | 659 [355-914] |
| Time to peak, minutes | 140 (53) | 150 [105-180] | 166 (47) | 180 [150-180] |
| Data presented as mean (SD) and median [IQR]. AUC= Area under the OXT concentration curve; OXT = Oxytocin. | | | | |

**Table 3 (M3) Safety outcomes in each group in response to MDMA and placebo stimulation in the full analysis set**

| | Patients with arginine vasopressin deficiency (central diabetes insipidus) (n=15) | | Healthy controls (n=15) | |
|---|---|---|---|---|
| | **MDMA** | **Placebo** | **MDMA** | **Placebo** |
| **Clinical safety measures** | | | | |
| Maximum systolic blood pleasure, in mmHg | 143 (10) | 131 (7) | 146 (13) | 129 (9) |
| Maximum diastolic blood pleasure, in mmHg | 84 (7) | 78 (9) | 84 (9) | 78 (9) |
| Maximum heart rate, in bpm | 93 (12) | 83 (8) | 92 (14) | 78 (9) |
| Maximum tympanic temperature, in °C | 36·9 (0.2) | 36·9 (0.2) | 37·1 (0.3) | 37·0 (0.3) |
| **Adverse effects of special interest** | | | | |
| total number of acute reported complaints assessed with the 66-item List-of-Complaints [A] | 6·6 (2·5) | 1·9 (1·5) | 6·3 (3·5) | 2·0 (1·5) |
| Fatigue | 7 (47%) | 2 (13%) | 8 (53%) | 4 (27%) |
| Lack of appetite | 8 (53%) | 0 (0%) | 10 (67%) | 0 (0%) |
| Lack of concentration | 7 (47%) | 0 (0%) | 8 (53%) | 1 (7%) |
| Dry mouth | 8 (53%) | 0 (0%) | 8 (53%) | 0 (0%) |
| total number of subacute reported complaints assessed with the 66-item List-of-Complaints [B] | 3·6 (2.5) | 2·5 (2.9) | 3·6 (2.7) | 1·4 (0.8) |
| Headache | 7 (47%) | 2 (13%) | 5 (33%) | 1 (7%) |
| Fatigue | 7 (47%) | 3 (20%) | 6 (40%) | 1 (7%) |
| Lack of energy | 4 (27%) | 1 (7%) | 2 (13%) | 0 (0%) |
| Dullness | 1 (7%) | 4 (27%) | 5 (33%) | 1 (7%) |
| **Adverse events** | | | | |
| transient mild hypokalaemia, number | 4 (27%) | 0 (0%) | 2 (13%) | 0 (0%) |

Data presented as mean (SD), median [IQR], and frequency (%). A= change from the assessed complaints at 360 minutes after drug administration; see full list of complaints in the appendix. B= assessed three days after each visit via phone calls; see full list of complaints in the appendix.

## DISCUSSION

This study has three main findings. First, in response to MDMA stimulation, we demonstrated an expected eight-fold increase in plasma OXT levels in healthy controls, while in patients with AVP-D (cDI), no notable increase was observed. Second, this lack of OXT increase in patients was associated with lower MDMA-induced subjective pro-social, empathic, and anxiolytic effects. Third, already at baseline, patients exhibited significantly higher anxiety, alexithymia, and depression symptoms than healthy controls. Together, these findings indicate for the first time a clinically relevant OXT deficiency in patients with AVP-D (cDI).

The central oxytocinergic system and related limbic networks affect complex neural circuits of socio-emotional behaviour and promote pro-social effects such as in-group favouritism and protection against social threats, trust and attachment, empathy, and emotion recognition.[12] Over the years, only limited research has been devoted to a potential OXT deficiency in patients with hypothalamic-pituitary dysfunction. Few studies attempted to measure OXT in these patients and mainly focused on basal measurements delivering inconclusive results. Although some demonstrated slightly lower basal OXT levels in patients with AVP-D (cDI), others could not confirm these findings or even demonstrated contra-directional higher levels compared to controls.[85,86,88,126] Notably, similar to other pituitary hormones, single basal levels are unreliable in identifying a deficiency in this context.[128] According to a meta-analysis, peripheral OXT levels correlate with central levels only after stimulation but not at baseline.[138] Using an innovative provocation test, our results clearly indicate an OXT deficiency in patients with AVP-D (cDI), laying the groundwork for a new hypothalamic-pituitary entity. An important question, however, is whether this deficiency is related to alterations or dysfunctions in socio-emotional behaviour.

The few studies investigating psychological comorbidities in patients with AVP-D (cDI) have suggested heightened anxiety and alexithymia, reduced empathic abilities, higher levels of self-reported autistic traits, lower levels of joy when socialising, and lower scores in an emotion recognition task.[59,86,88,89] Studies in patients with craniopharyngioma (CP), a condition carrying a high risk of developing AVP-D (cDI) – either through direct tumour-induced or post-surgical damage to the SON/PVN - revealed personality changes (31%) and increased psycho-social comorbidities (47%), including anxiety,

depression, and social withdrawal.[80,81] Consistent with these observations, we demonstrate high anxiety and alexithymia levels in patients with AVP-D (cDI) already at baseline. Importantly, despite selection of patients without actively treated psychological co-morbidities, about half of the patients exhibited clinically relevant anxiety and possible to clinically manifest alexithymia symptoms. The cause for these impairments may be multi-factorial; it is, however, tempting to assume that some of these difficulties are attributable to undiagnosed OXT deficiencies. In line with this, in our study, the lack of increase in OXT levels in patients with AVP-D (cDI) was associated with increased anxiety.

In past decades, OXT knockout (OXT-KO) models have been used to identify dysfunctional aspects of social behaviour in an OXT-deficient state. Generally, an impairment in forming social memories and more anxiety-related behaviours were observed.[139,140] Indeed, mental conditions, such as autism spectrum disorder (ASD), anxiety and depression disorder, have been linked to lower endogenous OXT or impaired signalling.[141] Intranasal OXT has been experimentally administered to ameliorate symptoms, e.g., in ASD; however, with inconsistent results.[142] It is important to consider that in these conditions, in contrast to AVP-D (cDI), no OXT deficiency per se has been proven; the evidence is largely based on studies reporting individual variations in peripheral basal OXT levels or in genes involved in OXT signalling or OXT receptor expression.[143] The effects of OXT administration in AVP-D (cDI) have only been reported in one case report and a small study: In a six-year-old patient following pituitary surgery, the parents had recognised personality changes, such as increased social isolation and decreased interest in physical contact with family members. Following treatment with intranasal OXT, the patient re-engaged in play with family members and positive social interactions with peers.[144] A study of ten patients with childhood-onset CP (of whom nine had AVP-D (cDI)) demonstrated an improvement in the previously impaired ability to categorise negative emotions after a single dose of intranasal OXT.[123] Future studies in a larger patient population should investigate the potential therapeutic use of intranasal OXT.

In our study, we used MDMA as a stimulus for OXT, whose release is linked to the empathic and prosocial profile of MDMA, including closeness to others, openness, trust, happiness, and feelings of well-being.[130,133,134] By stimulating the oxytocinergic neurons, MDMA induces not only an increase in peripheral levels (via the posterior pituitary) but also central OXT-mediated behavioural effects. In rats,

AVP was not affected by MDMA, as also observed in our study, reflected by unchanged copeptin levels.[145] The anxiolytic effects observed upon MDMA stimulation might be due to the strong increase and consequent action of OXT within core regions for fear processing, such as the amygdala. Supporting this, we have confirmed the anxiolytic properties and furthermore demonstrate reduced recognition of negative emotions (i.e., "anger," "fear," and "sad") in the FERT in response to MDMA in healthy subjects. In contrast, anxiolytic effects and reduced recognition of "fearful" emotions were not observed in patients with AVP-D (cDI). There is research evidence of enhancing effects on emotional empathy for positive stimuli in the MET for both MDMA and intranasal OXT.[16,146] Although not powered for this task, we observed contra-directional effects in AVP-D (cDI) patients with no increase in 'positive empathy,' that is, the ability to share, celebrate, and enjoy others' positive emotions, a state which correlates with increased prosocial behaviour, social closeness, and well-being. This lack of 'positive empathy' in patients with AVP-D (cDI) might contribute to the psychological findings observed in our study. Taken together, our data show that almost all subjective effects of MDMA were reduced or even absent in patients, in contrast to the expected effects in healthy controls, which reflects the hormone's absence and, in agreement, consequent lack of function in central key regions important for socio-emotional processing. These findings contradict the previous paradigm that OXT stimulation is only attributed to a secondary role in MDMA effects. Our results, in contrast, suggest a paradigm shift and underline the importance of OXT as a key feature of MDMA's effects.

The present study has limitations. First, this was a single-centre trial, the sample size was limited and did not allow for appropriate sub-group analysis, and not blinded in respect to both groups. Second, MDMA at 100 mg produced pronounced acute mood and cardio stimulant effects, and a lower dose could likely be used if this approach is further developed into a routine clinical test. Third, there is an ongoing discussion on the currently available EIA and RIA oxytocin assays. However, all samples were measured in one batch, and an equally strong relative increase of OXT upon MDMA stimulation has been shown and reproduced with both assays throughout different laboratories. Lastly, possible selection bias of patients prone to psychological deficits cannot be excluded. However, due to our strict eligibility criteria, we only included patients without current active mental illness and no somatic illness other than

pituitary dysfunction. Therefore, the psychological assessment might even show higher values in the general population of patients.

In conclusion, this study provides evidence of a clinically relevant OXT deficiency in patients with AVP-D (cDI). These findings indicate a new hypothalamic-pituitary entity and contribute to deepening our understanding of OXT as a key hormone in centrally generated socio-emotional effects reflected by reduced pro-social, empathic, and anxiolytic effects in patients with an OXT deficiency. Future studies should evaluate if OXT replacement therapy can alleviate residual symptoms related to OXT deficiency in patients with AVP-D (cDI).

**Contributors**

CA wrote the protocol, contributed to data collection, analysis, and interpretation, did the literature search, and wrote the manuscript. MC-C edited the protocol, contributed to data analysis and interpretation, edited the manuscript, and supervised all steps of the conduct of the study. MEL edited the protocol, contributed to data analysis and interpretation, and edited the manuscript. NR covered all statistical aspects of the study, planned and performed data analysis, and edited the manuscript. FH contributed to the study design, data analysis, and data interpretation, and revised the manuscript. NV and AE contributed to data analysis and interpretation and revised the manuscript. RM and NH contributed to data collection and revised the manuscript. COS contributed to the study design, and data interpretation, edited the protocol and revised the manuscript. MH contributed to data analysis and interpretation and revised the manuscript. MCC, CA, FH, MEL, NR, and NH verified the data; MCC, CA, NR, and NH had access to all raw data, and all authors had final responsibility for the decision to submit for publication.

**Declaration of interest**

MEL has received consulting and license fees and funding from Mindmedicine for patents and know-how related to MDMA; the received fees and funding are not related to the present study. MEL owns stocks of Mindmedicine. All other authors declare no competing interests.

**Data sharing**

We may share de-identified, individual participant-level data that underlie the results reported in this Article and related documents, including the study protocol and the statistical analysis plan. Data will be available with the publication of our main manuscript on receipt of a request detailing the study hypothesis and statistical analysis plan. All requests should be sent to the corresponding author. The steering committee of this study will discuss all requests and decide, based on the scientific rigor of the proposal, whether data sharing is appropriate. All applicants are asked to sign a data access agreement.

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**SUPPLEMENTAL MATERIAL FOR MANUSCRIPT 3 (M3)**

**Full in- and exclusion criteria**

**Inclusion criteria diabetes insipidus**:

1. Confirmed diagnosis of central diabetes insipidus*
2. Age 18 – 65 years

**Inclusion criteria for healthy controls**:

1. Healthy controls
2. Matched for age, sex, body mass index, and oestrogen replacement/menopause/hormonal contraceptives to patients with central diabetes insipidus
3. No medication, except hormonal contraception

**Exclusion criteria**:

1. Participation in a trial with investigational drugs within 30 days
2. Illicit substance use (except for cannabis) more than 10 times in a lifetime or any time within the previous two months
3. Consumption of alcoholic beverages >15 drinks/week
4. Tobacco smoking >10 cigarettes/day
5. Cardiovascular disease, i.e., coronary artery disease, heart failure with a left ventricular ejection fraction <40%, stroke in the last three months, atrial fibrillation/flatter, Wolff-Parkinson-White-Syndrome
6. Uncontrolled arterial hypertension (>140/90 mmHg) or hypotension (systolic blood pressure <85mmHg)
7. Current or previous major psychiatric disorder, e.g., major depression, schizophrenia spectrum disorder
8. Psychotic disorder in first-degree relatives
9. Regular intake of selective serotonin reuptake inhibitors or monoamine oxidase inhibitors
10. Pregnancy and breastfeeding
11. Diagnosed chronic kidney disease > grade III (glomerular filtration rate < 30ml/min)
12. Diagnosed liver cirrhosis or alanine aminotransferase or aspartate aminotransferase levels 2.5 times above the normal range

* Initial diagnosis was made based on clinical presentation, laboratory results, radiological findings, and according to the water deprivation test and copeptin-based hypertonic saline test or arginine infusion test.

**Assessment of psychopathology at baseline**

The State-Trait Anxiety Inventory (STAI) is a validated and reliable questionnaire to assess anxiety symptoms.[147] We used the Trait-Scale of the STAI (STAI-T) to determine the general anxiety levels of a participant at the baseline evaluation and the State-Scale (STAI-S) for the experimental session. The total score is calculated based on responses to 20 items, with scores ranging from 1 ("almost never") to 4 ("almost always"). Total scores for each sub-scale range from 20 to 80, with higher scores indicating more pronounced levels of anxiety and scores ≥40 indicating clinically significant anxiety symptoms.

The Becks-Depression Inventory II (BDI-II) is a validated and reliable questionnaire to assess the severity of depressive symptoms.[148] Based on responses to 21 items, a total score is calculated. The total scores range from 0 to 63, with higher scores indicating greater severity of depressive symptoms (score≤16, mild mood disturbance; 17 to 20, borderline clinical depression, 21 to 30, moderate depression, and score ≥31, severe depression).

The Toronto-Alexithymia Scale 20 (TAS-20) is a validated tool to assess socioemotional functioning, measuring the ability to express and identify one's emotions.[149] Based on responses to 20 items ranging from 1 ("strongly disagree") to 5 ("strongly agree"), a global score is calculated. The total scores range from 20 to 100, with higher scores indicating worse alexithymia. A score of ≥61 indicates alexithymia (difficulty understanding one's own emotions), a score of 52 to 60 possible alexithymia, and a score of ≤51 non-alexithymia.

In addition, the SF-36, a tool to assess quality of life, was used to assess overall physical and mental health.[150] In the SF-36, eight multi-item scales are to calculate overall dimensions. The overall physical health dimension is calculated using the scores obtained from physical functioning, bodily pain, role limitations due to physical health problems, and general health perception scales. For each dimension, the scores range from 0 to 100, with higher scores indicating better health and less disability. The overall mental health dimension is calculated using the scores obtained from role limitations due to personal or emotional problems, general mental health, social functioning, and energy/fatigue scales.

**Emotion recognition and empathy tasks**

At the expected peak concentration of oxytocin (OXT) (timepoint 150 minutes)[130], participants performed the Multifaceted Empathy Task (MET) and Face Emotion Recognition Task (FERT). The MET is a reliable and valid task to assess the cognitive and emotional aspects of empathy [135] that has been proven to be sensitive to the effects of OXT[16] and MDMA.[136] The computer-assisted test consists of 40 photographs showing people in emotionally charged situations. To assess cognitive empathy, participants were required to infer the mental state of the subject in each scene and indicate their corresponding mental state from a list of four responses. Cognitive empathy is reflected in the percentage of correct responses among the total responses. To measure emotional empathy, participants are asked to rate how much they feel for the individual in each scene (i.e., explicit emotional empathy) and how much they are aroused by each scene (i.e., implicit emotional empathy) on a 1 to 9-point scale. The three aspects of empathy are each tested with 20 stimuli with positive valence and 20 with negative valence, resulting in a total of 120 trials. The FERT assesses the recognition of basic emotions. This task includes ten neutral faces and 160 faces that express one of four basic emotions (i.e., happiness, sadness, anger, and fear), with pictures morphed between 0% (i.e., neutral) and 100% in 10% steps. Two female and two male images were used for each of the four emotions. Stimuli were shown in random order for 500 ms, followed by the rating screen, where participants had to indicate the correct emotion. The facial images were derived from the Ekman and Friesen series.

**Figure S1 (M3) Psychological questionnaires at baseline**

Symptoms of (A) trait anxiety, (B) alexithymia, and (C) depression were worse in patients with AVP deficiency (cDI) (in red) in healthy controls (in blue). Boxes span the interquartile range (IQR); the thick horizontal line is the median. Whiskers are the most extreme values lying within the box edge and 1·5 times the IQR.

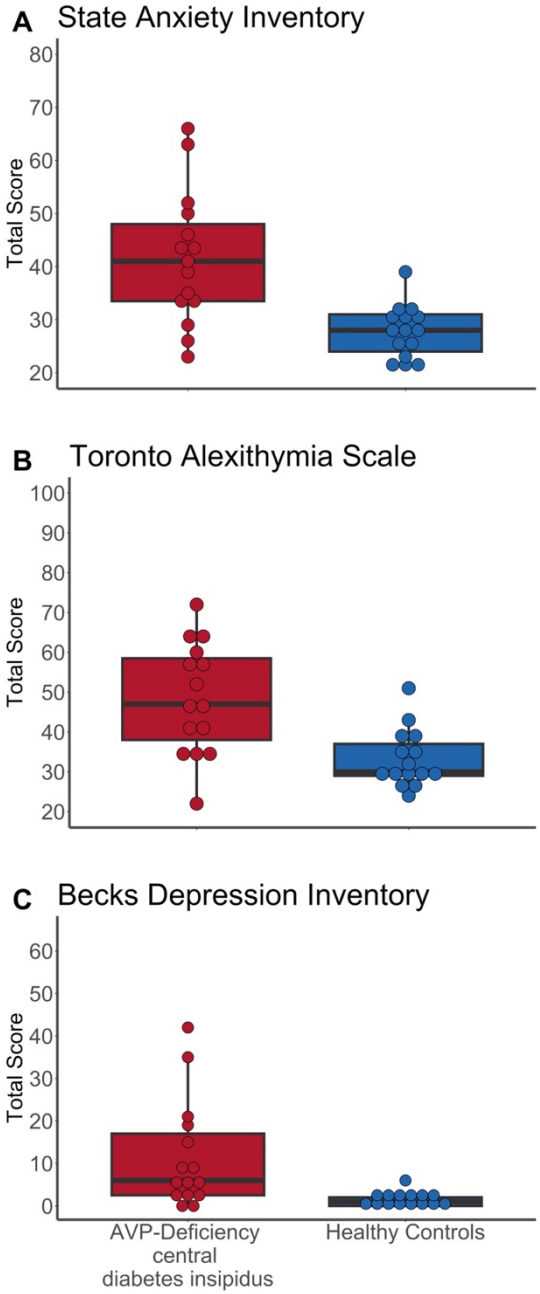

**Figure S2 (M3) Baseline plasma oxytocin levels**

The baseline plasma oxytocin for both experimental sessions before MDMA or placebo intake was pooled for healthy controls (in blue) and patients with AVP deficiency (cDI) (in red). No notable change between both groups was observed. Boxes span the interquartile range (IQR); the thick horizontal line is the median. Whiskers are the most extreme values lying within the box edge and 1.5 times the IQR.

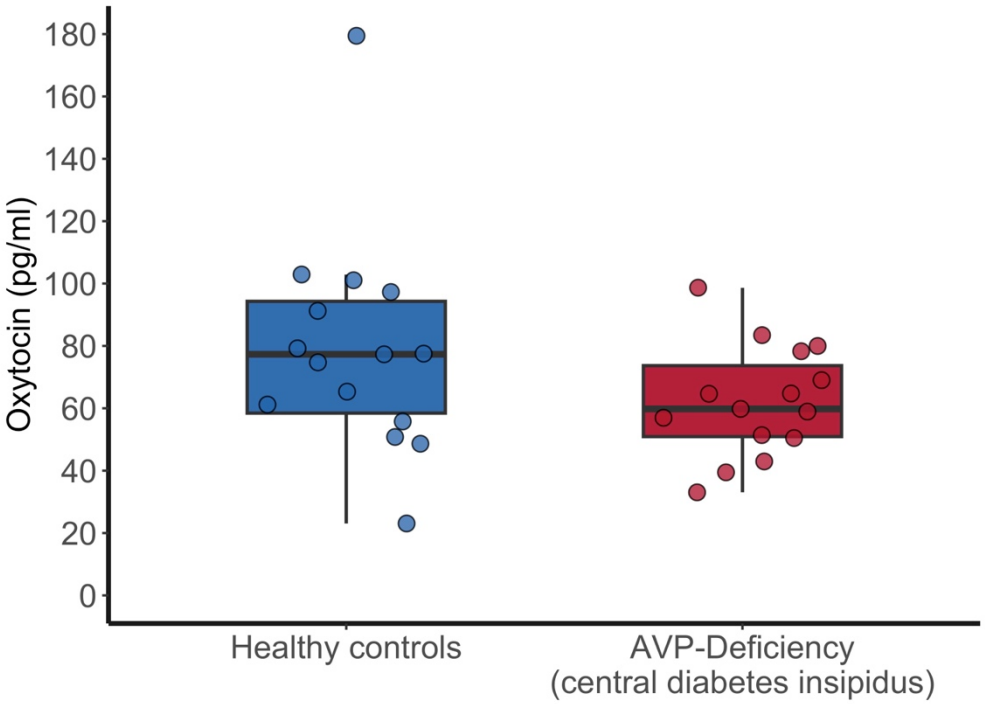

**Figure S3 (M3) Incremental area under the plasma oxytocin concentration curve**

For each participant, the net incremental area under the concentration curve (AUC) for plasma oxytocin was calculated from 0 to 300 minutes after drug intake. The net incremental AUC is visualised for both groups by treatments with boxplots. There is a clear positive AUC in healthy controls (in blue) in response to MDMA administration, while in patients with AVP deficiency (cDI) (in red), no notable change was observed. Boxes span the interquartile range (IQR); the thick horizontal line is the median. Whiskers are the most extreme values lying within the box edge and 1.5 times the IQR.

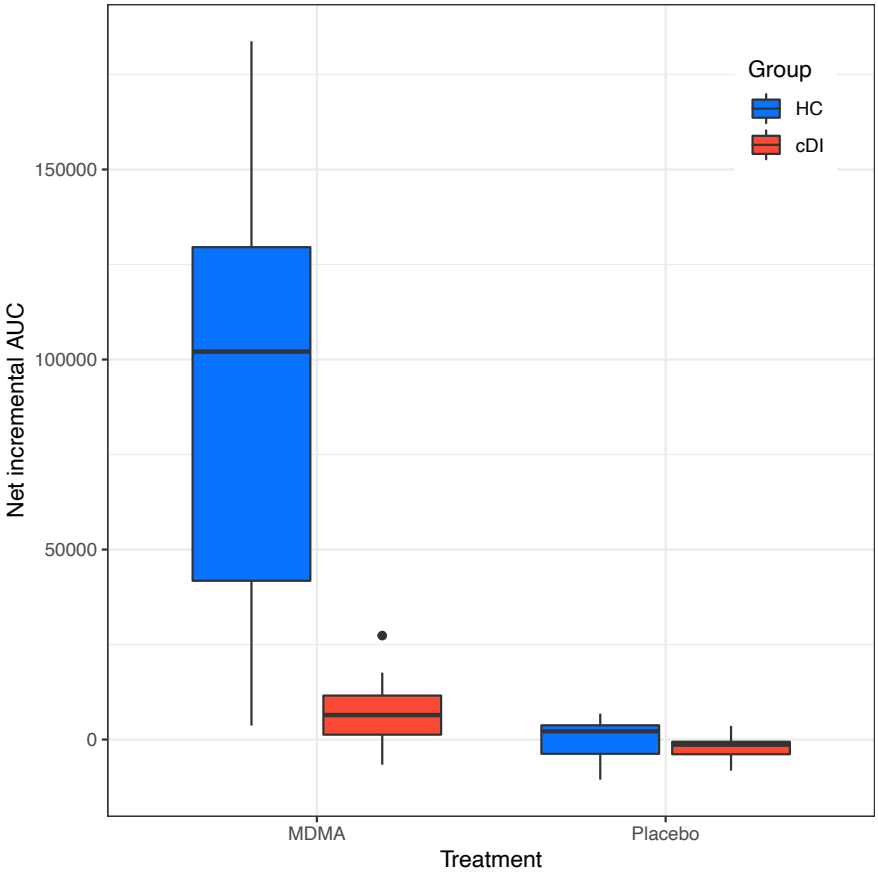

**Figure S4 (M3) Subgroup analyses**

The area under the oxytocin concentration curve (AUC) is visualised by sex ([A] females vs males in AVP deficiency (cDI) and HC), pituitary dysfunction ([B] isolated posterior dysfunction vs anterior & posterior dysfunction), degree of AVP deficiency ([C] complete vs partial), and duration of disease [D]. The degree of AVP deficiency was determined according to the diagnostic testing (*Christ-Crain M. et al, 2019, Nature Primer*). A higher net incremental AUC in response to MDMA administration in patients with partial compared to complete AVP deficiency (cDI). No major differences were observed for the other subgroups. Boxes span the interquartile range (IQR); the thick horizontal line is the median. Whiskers are the most extreme values lying within the box edge and 1.5 times the IQR.

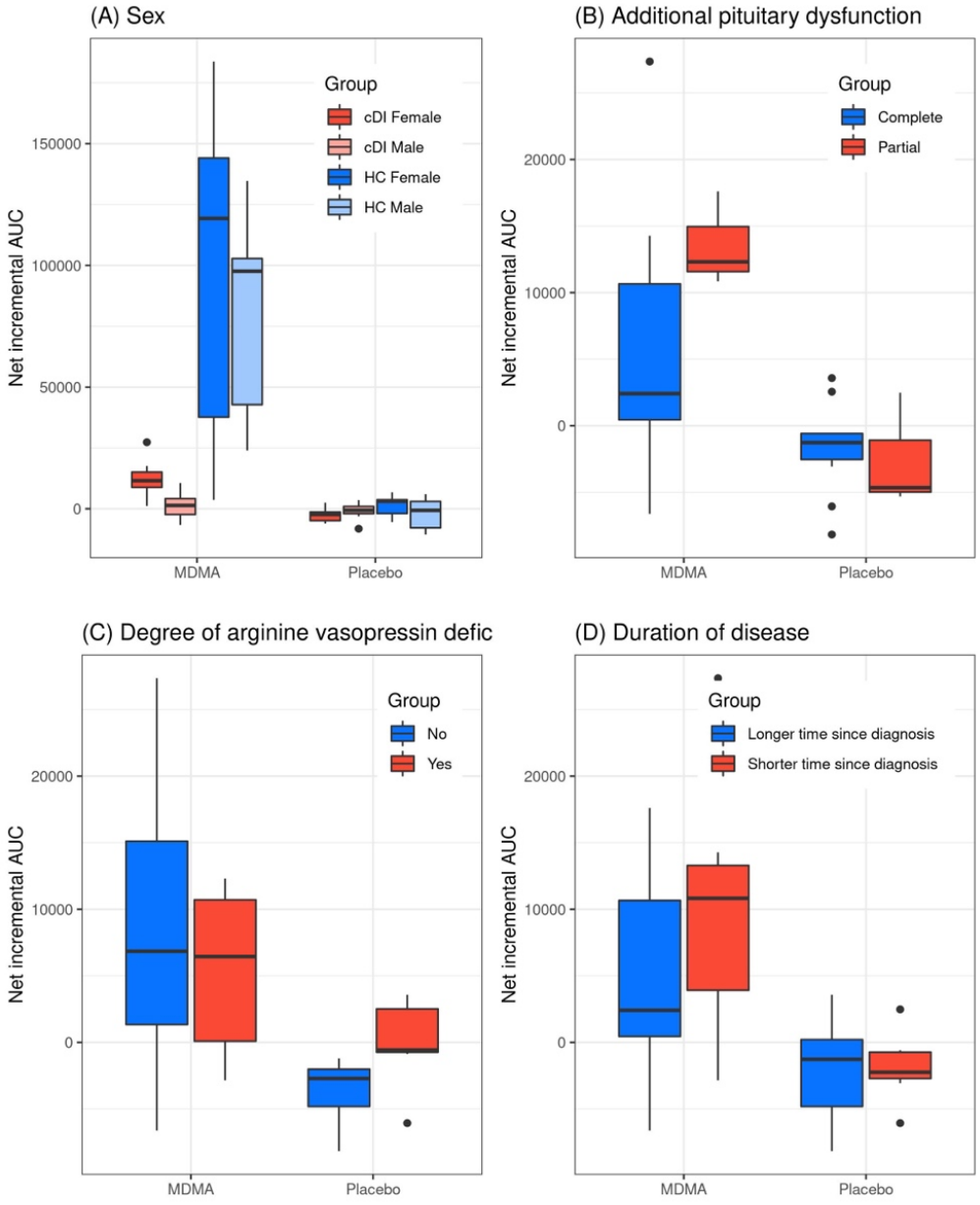

**Figure S5 (M3) Plasma oxytocin in response to MDMA stimulation – sex differences**

OXT increased after MDMA stimulation in healthy controls, while no change was observed in patients with AVP deficiency (cDI). Healthy female controls and female patients show a slightly stronger OXT response to MDMA compared to healthy male controls and male patients. Data are expressed as mean ± standard deviation for patients with AVP deficiency (cDI) (female in yellow; male in orange) and healthy controls (female in dark purple, male in light purple). Data presented as mean +/- standard deviation.

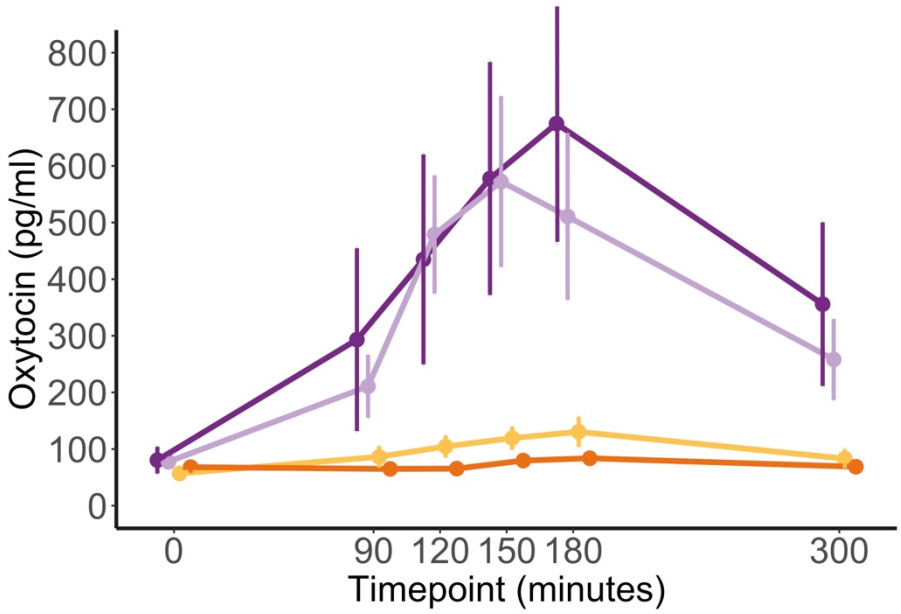

**Figure S6 (M3) Plasma oxytocin in response to MDMA stimulation – patient groups**

OXT increased after MDMA stimulation in healthy controls, while no change was observed in patients with AVP deficiency (cDI). Data are expressed as mean ± standard deviation for patients with AVP deficiency (cDI) (complete AVP deficiency in red; partial AVP deficiency in orange; genetic/hereditary cause in green) and healthy controls (in blue). Data presented as mean +/- standard deviation.

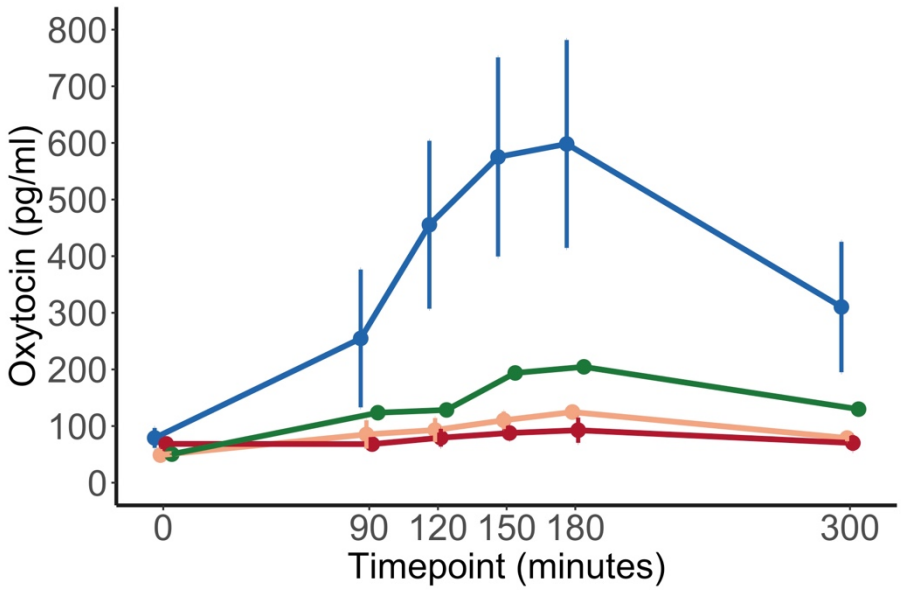

**Figure S7 (M3) Time course of copeptin levels for MDMA and placebo stimulation**

Copeptin, as a measure of the arginine vasopressin system, was at 0 minutes and 120 minutes. Copeptin levels remained stable in response to MDMA and placebo in both groups (healthy controls in blue, patients with AVP-deficiency (cDI) in red). Boxes span the interquartile range (IQR); the thick horizontal line is the median. Whiskers are the most extreme values lying within the box edge and 1.5 times the IQR.

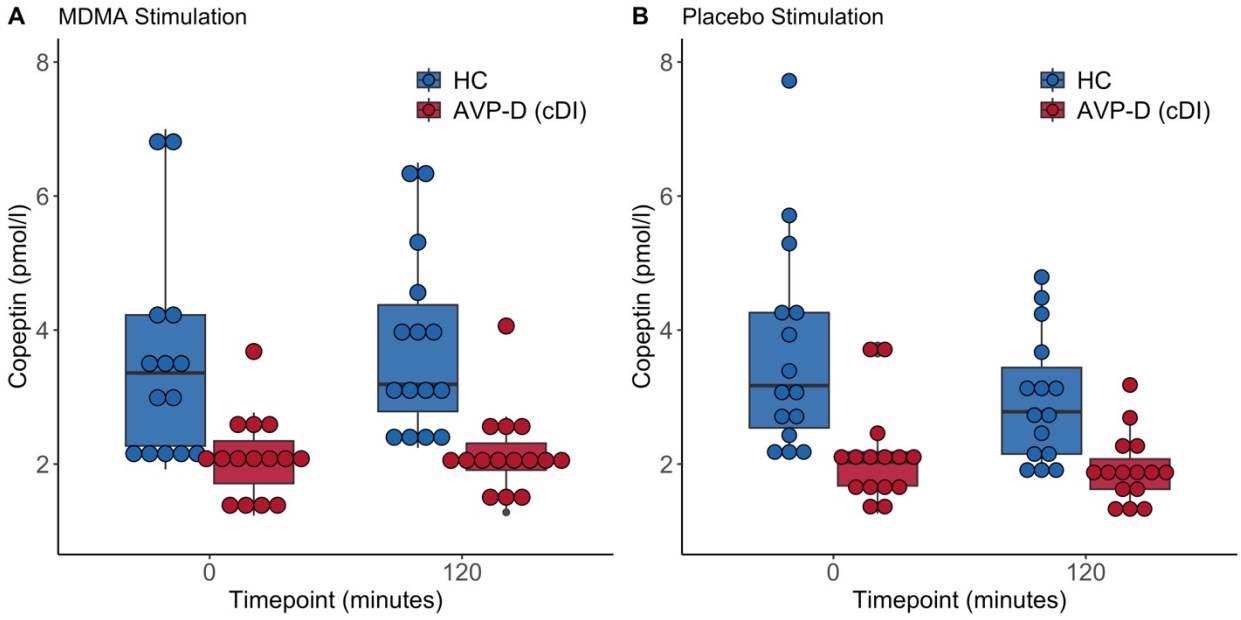

**Figure S8 (M3) Change of plasma copeptin levels in response to MDMA and placebo**

Copeptin, as a measure of the arginine vasopressin system, was assessed at baseline (0 minutes) and 120 minutes. Copeptin levels remained stable in response to MDMA and placebo in both groups, and no major change was observed (healthy controls in blue, patients with AVP-deficiency (cDI) in red). Boxes span the interquartile range (IQR); the thick horizontal line is the median. Whiskers are the most extreme values lying within the box edge and 1.5 times the IQR.

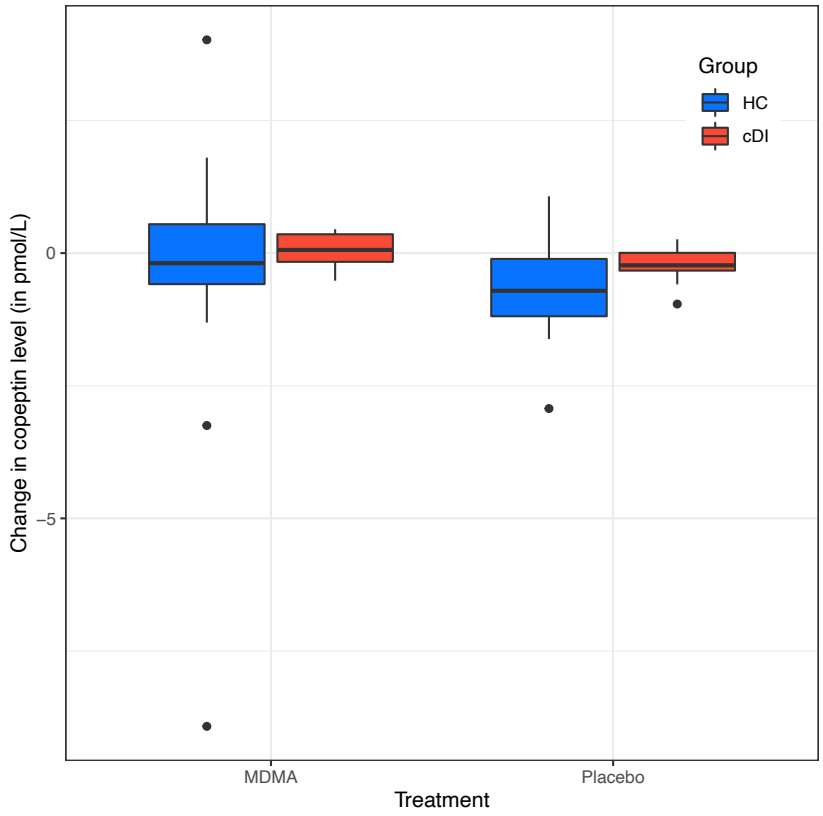

**Figure S9 (M3) Plasma prolactin in response to MDMA and placebo**

Prolactin was assessed at baseline (0 minutes) and 150 minutes. Prolactin increased slightly in response to MDMA equally in healthy controls and patients with AVP deficiency (cDI). Boxes span the interquartile range (IQR); the thick horizontal line is the median. Whiskers are the most extreme values lying within the box edge and 1.5 times the IQR.

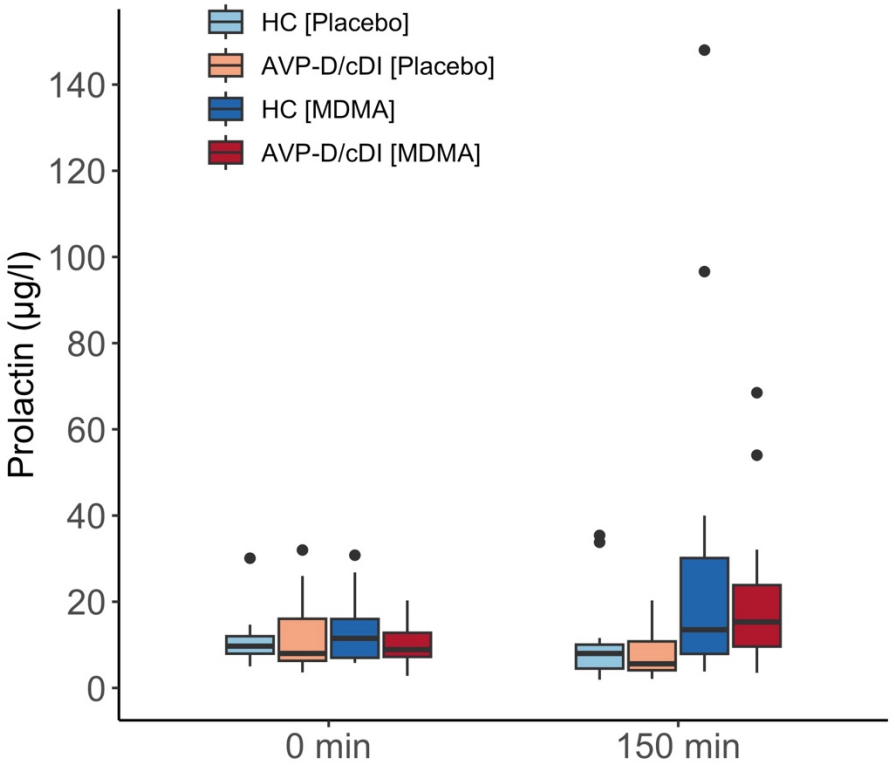

**Figure S10 (M3) Additional subjective drug effects response to MDMA**

Subjective effects were assessed repeatedly throughout the treatment visit (at 0, 30, 60, 90, 120, 150, 180, 240, 300, and 360 minutes) using visual analogue scales (VASs). The VASs were presented as 10 cm horizontal lines ranging from 0 to 10, with 'not at all' (i.e., 0) on the left and 'extremely' (i.e., 10) on the right, or were bidirectionally ranging from -5 to +5, with 0 being the neutral measure (i.e., 'no effect'). Data presented as mean +/- standard deviation for healthy controls in blue, patients with AVP-deficiency (cDI) in red.

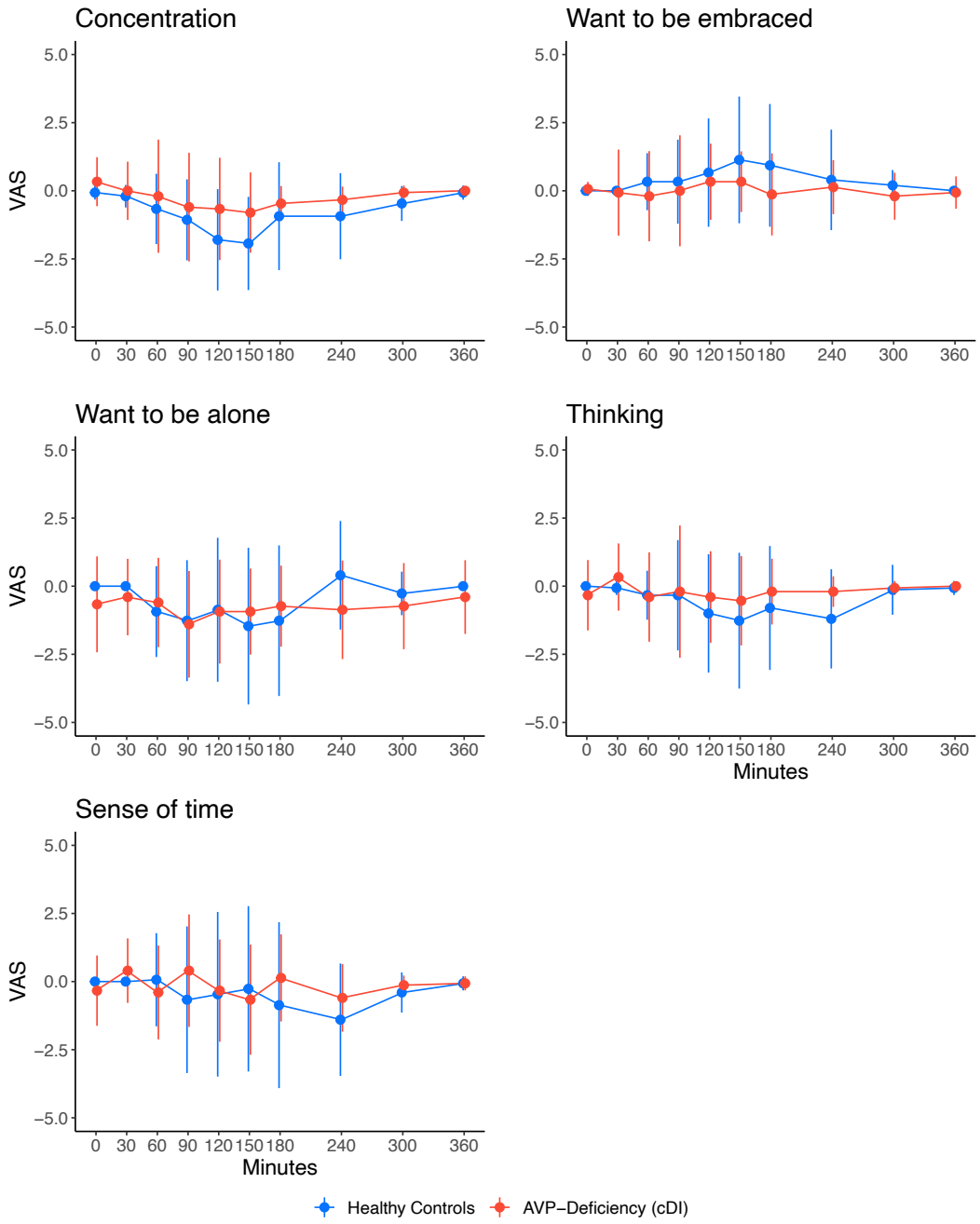

**Figure S11 (M3) Correct emotion recognition in the FERT**

In healthy controls, MDMA impaired the recognition accuracy of faces displaying “anger,” “sad,” and “fear,” with no alteration in correctly identifying “happy” or “neutral” faces. In patients, MDMA impaired the recognition of faces displaying “happy” and “sad” emotions with no effect on “anger,” “fear,” or “neutral” faces. Boxes span the interquartile range (IQR); the thick horizontal line is the median. Whiskers are the most extreme values lying within the box edge and 1.5 times the IQR.

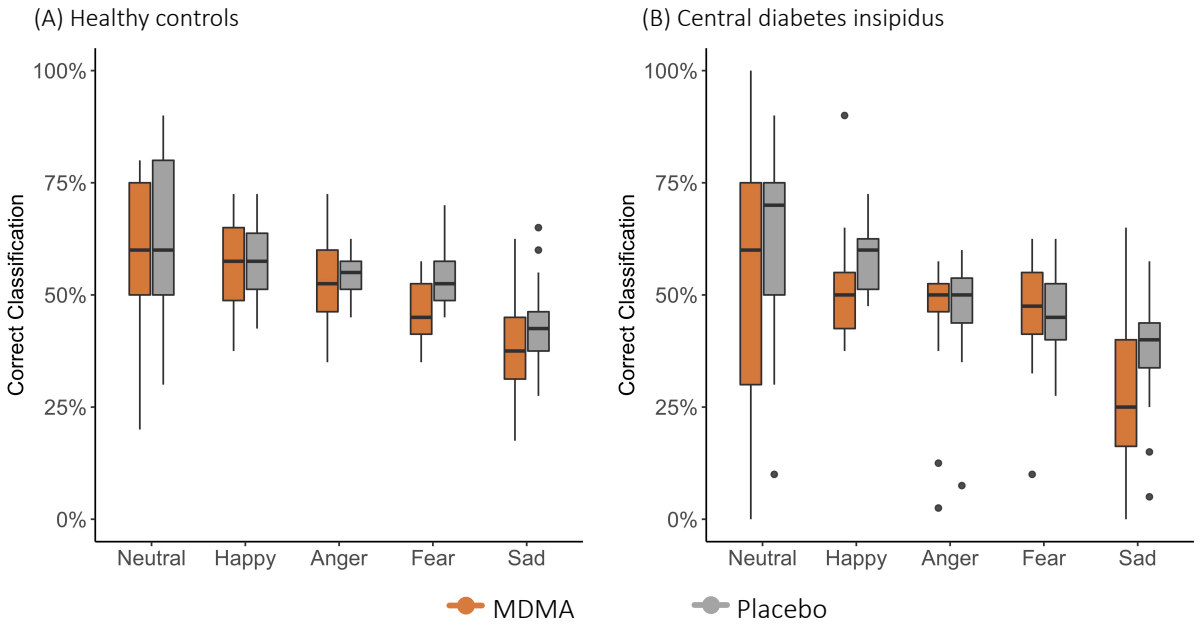

**Figure S12 (M3) Emotional empathy in the MET**

MDMA increased explicit and implicit emotional empathy ratings for positive valence stimuli in healthy controls (in blue), whereas a contra-directional effect was observed in patients with AVP deficiency (cDI) (in red). MDMA decreased explicit and implicit emotional empathy ratings for negative valence stimuli, with a stronger decrease in healthy controls. Boxes span the interquartile range (IQR); the thick horizontal line is the median. Whiskers are the most extreme values lying within the box edge and 1.5 times the IQR.

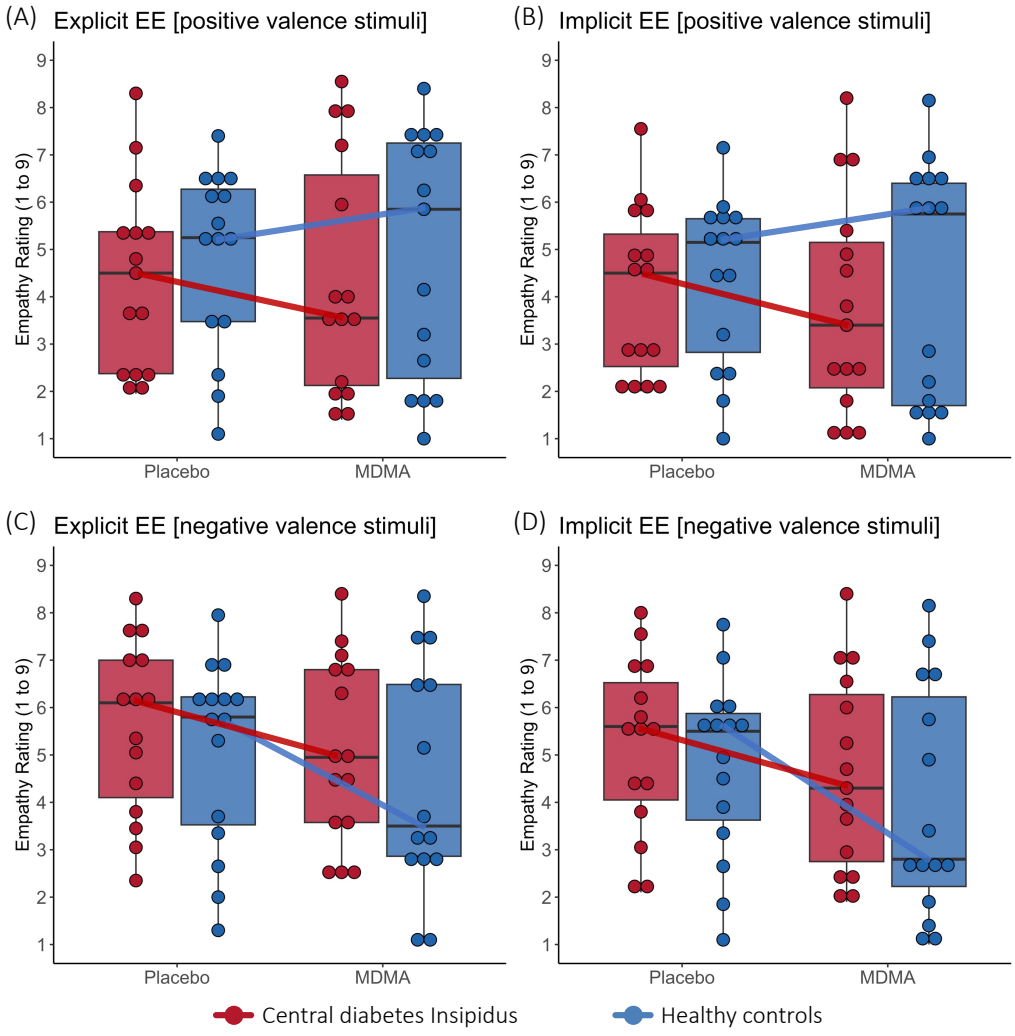

**Figure S13 (M3) Safety outcomes for the MDMA session**

As safety variables, vital signs were measured 60 minutes before and every 30 minutes after drug intake during both experiments. Blood pressure (systolic and diastolic), heart rate, temperature, and plasma sodium are presented as mean +/- standard error for patients with AVP deficiency (cDI) (in red) and healthy controls (in blue). Blood pressure increased moderately in response to MDMA stimulation in both groups. Heart rate and body temperature showed less variability.

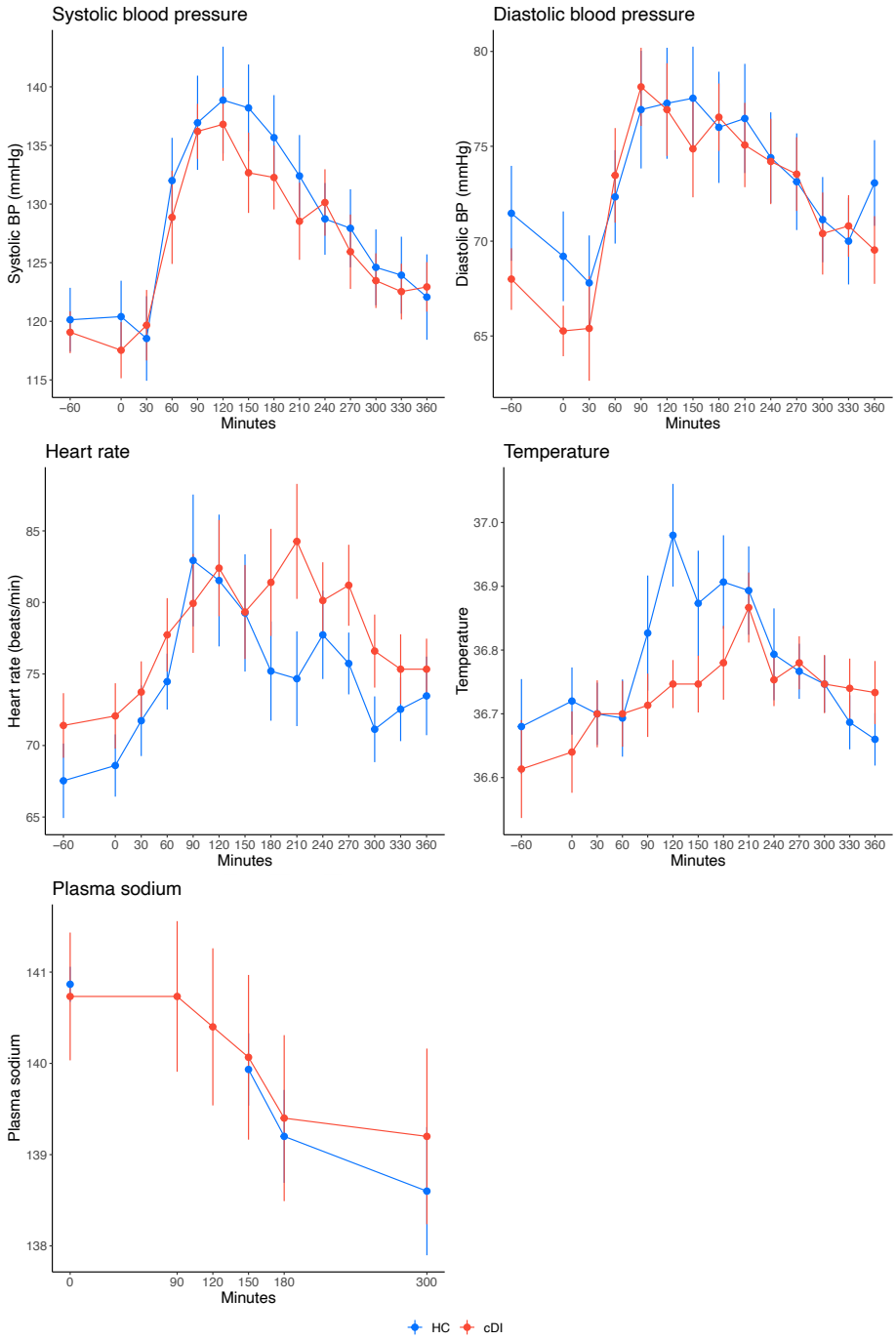

**Figure S14 (M3) Plasma MDMA and MDA concentrations**

Plasma MDMA and metabolite concentrations were in the expected range compared to prior publications.[130] No differences in MDMA or MDA were observed between groups. The $T_{max}$ values for MDMA in healthy controls and patients were 3.2 h (SD 1.2) and 3.5 h (1.1), and for MDA, 4.9 h (0.5) and 4.8 h (0.6), respectively. The $C_{max}$ values for MDMA were 222 ng/ml (58) and 192 ng/ml (34), and for MDA, 8.3 ng/ml (1.9) and 8.4 ng/ml (2.0), respectively. Data presented as mean +/- standard deviation for healthy controls in white, and patients with AVP-deficiency (cDI) in black.

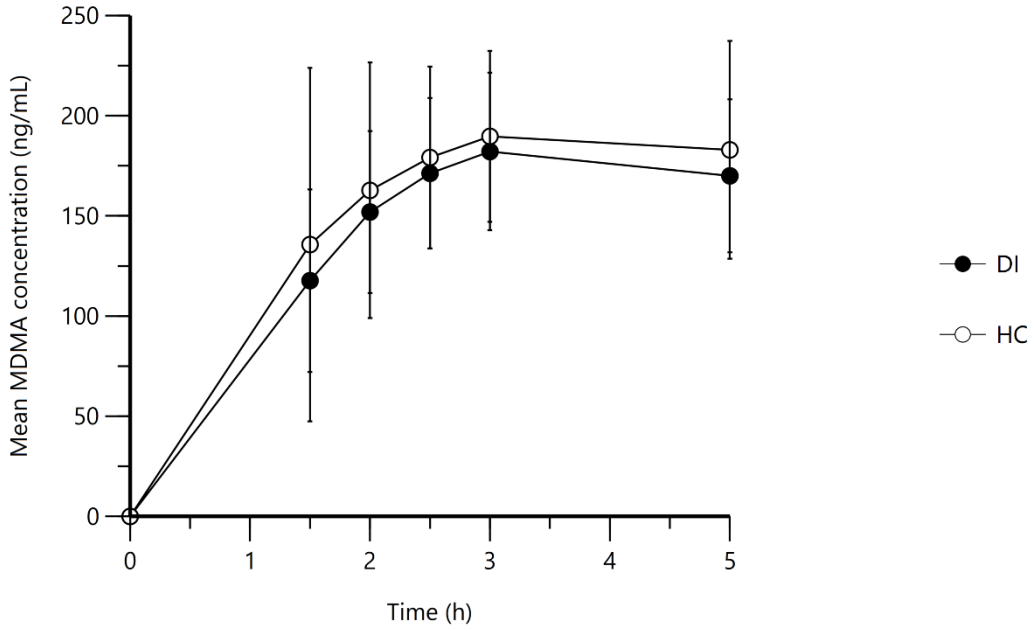

**Table S1 (M3) Baseline psychological and quality of life questionnaires**

| | **Patients with arginine vasopressin deficiency (central diabetes insipidus) (n=15)** | **Healthy controls (n=15)** | **Adjusted p-value** |
|---|---|---|---|
| **STAI Trait**, total score | 41 [34, 48] | 28 [24, 31] | 0·0143 |
| **TAS-20**, total score | 47 [38, 59] | 30 [29, 37] | 0·0365 |
| difficulty describing feelings | 13 [12, 17] | 8 [7, 9] | 0·0228 |
| difficulty identifying feeling | 16 [9, 20] | 9 [7, 11] | 0·2806 |
| externally oriented thinking | 20 [17, 22] | 15 [13, 18] | 0·5186 |
| **BDI-II**, total score | 6 [3, 17] | 1 [0, 2] | 0·0437 |
| cognitive-affective | 4 [1, 12] | 0 [0, 1] | 0·0308 |
| somatic | 3 [2, 5] | 0 [0, 2] | 0·0885 |
| cognitive | 1 [0, 6] | 0 [0, 1] | 0·2021 |
| somatic-affective | 5 [2, 11] | 1 [0, 2] | 0·0791 |
| **SF 36**, total score | | | |
| mental health | 49 [43, 52] | 54 [52, 55] | 0·0199 |
| physical health | 56 [54, 60] | 58 [57, 59] | 1·0000 |
| emotional well-being | 80 [56, 84] | 84 [76, 88] | 0·5647 |
| role emotional | 100 [67, 100] | 100 [100, 100] | 0·1637 |
| social functioning | 100 [75, 100] | 100 [100, 100] | 1·000 |
| energy/fatigue | 55 [40, 73] | 65 [60, 75] | 1·000 |
| general health | 77 [60, 87] | 90 [85, 97] | 0·1199 |
| pain | 100 [84, 100] | 100 [100, 100] | 0·7661 |
| role physical | 100 [100, 100] | 100 [100, 100] | 1·000 |
| physical functioning | 100 [95, 100] | 100 [100, 100] | 1·000 |

Data presented as median [inter-quartile range, IQR]. The scores between both groups were compared using the Wilcoxon rank-sum test and the p-values adjusted for multiple testing using the Bonferroni correction. STAI= State-Trait Anxiety Inventory; TAS-20= Toronto Alexithymia Scale; BDI II= Beck's Depression Inventory II; SF 36= Short Form 36 Health Survey

### Table S2 (M3) Results of the primary analysis

The estimates, 95% CIs and p-values of the mixed model comparing the groups are presented. The results table shows significant treatment-by-group interaction effects, meaning that the effect of MDMA administration compared to placebo was different in patients with AVP deficiency (cDI) compared to healthy controls (HC). The net incremental area under the curve (AUC) after MDMA in AVP deficiency (cDI) patients was, on average, 85678 lower than in HCs. In the placebo condition, the between-group difference was much smaller. There was no association between the baseline level of OXT and the net incremental AUC.

| | **Estimate** | **95%-CI** | **p-value** |
|---|---|---|---|
| **(Intercept)** | 104664.81 | [75264.39, 134065.23] | <0.0001 |
| Treatment (Placebo over MDMA) | -91572.93 | [-113480.42, -69665.43] | <0.0001 |
| Group (AVP-D (cDI) over HC) | -85677.68 | [-107999.75, -63355.62] | <0.0001 |
| Oxytocin level at 0 minutes (baseline) | -191.46 | [-509.47, 126.55] | 0.2274 |
| Treatment (Placebo over MDMA): Group (AVP-D (cDI) over HC) | 82962.68 | [52065.57, 113859.80] | <0.0001 |

### Summary of the net incremental AUC for plasma oxytocin levels in each group and treatment

| **Group, Treatment** | **Mean (SD)** | **Median [IQR]** |
|---|---|---|
| AVP-D (cDI) \| MDMA | 7 093 (8938) | 6 446 [1 291, 11 577] |
| AVP-D (cDI) \| Placebo | -1 856 (3295) | -1 343 [-3 860, -580] |
| HC \| MDMA | 89 532 (57149) | 102 095 [41 781, 129 565] |
| HC \| Placebo | -242 (5580) | 2175 [-3 750, 3 754] |

### Statical analysis of the primary outcome using a T-test

Parallel group t-test comparing the difference in AUC between the MDMA and placebo condition between the groups (i.e., HC and AVP-deficiency (cDI)). The results are consistent with our primary analysis with a p-value <0.0001.

t = 5.5688, df = 14.877, p-value = 5.534e-05

95% CI of the between-group difference: [49867.28, 111783.82]

## Table S3 (M3) Maximum change and AUC in VAS scores in response to MDMA stimulation

| | Patients with arginine vasopressin deficiency (central diabetes insipidus) (n=15) | | Healthy controls (n=15) | | Correlation to maximum OXT change[A] |
|---|---|---|---|---|---|
| **Visual analogue scale item** | **Maximum change** | **AUC** | **Maximum change** | **AUC** | |
| Any effect[B] | 4·0 [3·0, 7·5] | 600 [300, 930] | 9·0 [7·5, 9·0] | 1260 [1013, 1605] | 0·54 |
| Good effect[B] | 4·0 [2·0, 5·0] | 360 [135, 585] | 10·0 [7·0, 10·0] | 1350 [705, 1545] | 0·56 |
| Bad effect[B] | 0·0 [0·0, 1·0] | 0 [0, 30] | 0·0 [0·0, 0·0] | 0 [0, 0] | 0·01 |
| Liking effect[B] | 4·0 [4·0, 6·0] | 300 [120, 780] | 10·0 [8·0, 10·0] | 1530 [1035, 1740] | 0·57 |
| Feeling high[B] | 2·0 [2·0, 5·0] | 240 [120, 450] | 8·0 [6·0, 10·0] | 1020 [735, 1230] | 0·57 |
| Stimulation[B] | 4·0 [2·0, 7·0] | 330 [120, 990] | 8·0 [6·0, 9·0] | 840 [540, 1065] | 0·47 |
| Fear[C] | -2·0 [-4·5, 0·0] | -120 [-473, 0] | -5·0 [-5·0, -4·5] | -885 [-1020, -518] | -0·47 |
| Satisfaction[C] | 2·0 [1·0, 3·0] | 90 [0, 330] | 5·0 [4·0, 5·0] | 705 [578, 795] | 0·57 |
| Happiness[C] | 1·0 [0·5, 3·0] | 60 [0, 300] | 5·0 [3·5, 5·0] | 630 [548, 788] | 0·53 |
| Trust[C] | 1·0 [0·0, 3·0] | 30 [0, 195] | 4·0 [3·0, 5·0] | 600 [443, 795] | 0·53 |
| Talkative[C] | 1·0 [1·0, 2·5] | 60 [30, 248] | 4·0 [2·0, 4·5] | 375 [158, 630] | 0·54 |
| Openness[C] | 1·0 [0·0, 2·5] | 60 [0, 240] | 4·0 [2·0, 4·5] | 525 [83, 668] | 0·52 |
| Want to be embraced[C] | 0·0 [0·0, 1·0] | 0 [-30, 15] | 1·0 [0·0, 2·5] | 30 [0, 210] | 0·49 |
| Want to embrace someone[C] | 0·0 [0·0, 1·0] | 0 [-30, 45] | 1·0 [0·0, 2·0] | 30 [0, 173] | 0·51 |
| Want to be with others[C] | 0·0 [0·0, 2·0] | 0 [-30, 128] | 4·0 [0·0, 5·0] | 480 [0, 570] | 0·60 |
| Feeling close to others[C] | 1·0 [0·0, 1·0] | 0 [-15, 60] | 3·0 [1·0, 4·0] | 390 [30, 510] | 0·54 |
| Thinking[C] | 0·0 [0·0, 2·5] | -45 [-143, 0] | 0·0 [0·0, 1·5] | -210 [-338, 0] | 0·14 |
| Sense of time[C] | 2·0 [0·0, 2·5] | -90 [-188, 90] | 0·0 [0·0, 3·0] | -60 [-743, 165] | 0·53 |
| Concentration[C] | -2·0 [-3·0, -1·5] | -90 [-278, -53] | -3·0 [-3·5, -2·0] | -240 [-548, -45] | 0·49 |
| Want to be alone[C] | 0·0 [0·0, 1·0] | 0·0 [-225, 0] | 0·0 [0·0, 1·5] | -15 [-533, 98] | 0·82 |

Data presented as median [IQR]. AUC = Area under the VAS score curve. VAS= Visual analogue scale. (**A**)= Pearson correlation coefficients between the maximum change for the VAS-measures and the maximum change in oxytocin level. The VASs were presented as 10 cm horizontal lines ranging from (**B**) 0 to 10, with 'not at all' (i.e., 0) on the left and 'extremely' (i.e., 10) on the right, or were bidirectional ranging from (**C**) -5 to +5, with 'not at all' or 'decelerated' (i.e., -5) on the left and 'extremely' or 'racing' (i.e., +5) on the right and 0 being the neutral measure (i.e., 'no effect').

## Table S4 (M3) List of complaints (acute adverse events)

The number of complaints per participant was assessed at 0 and 360 minutes after drug intake using a predefined 66-item List-of-complaints. At 0 minutes, there were few complaints for the MDMA and placebo visit, and the number of complaints slightly increased after MDMA administration in both groups.

| | **Patients with arginine vasopressin deficiency (central diabetes insipidus) (n=15)** | | | | **Healthy controls (n=15)** | | | |
|---|---|---|---|---|---|---|---|---|
| | **MDMA** | **MDMA** | **Placebo** | **Placebo** | **MDMA** | **MDMA** | **Placebo** | **Placebo** |
| **Minutes** | **0** | **360** | **0** | **360** | **0** | **360** | **0** | **360** |
| Headache | 3 (20) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Fatigue | 3 (20) | 8 (53) | 3 (20) | 2 (13) | 3 (20) | 8 (53) | 2 (13) | 0 (0) |
| Tickling sensation in the throat | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (7) | 0 (0) |
| Imbalances | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 3 (20) | 0 (0) | 0 (0) | 0 (0) |
| Intermittent shortness of breath | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Feeling of suffocation | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Shortness of breath | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Crying tendency | 0 (0) | 0 (0) | 1 (7) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Coughing | 1 (7) | 1 (7) | 1 (7) | 1 (7) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Weakness | 0 (0) | 2 (13) | 0 (0) | 0 (0) | 0 (0) | 2 (13) | 0 (0) | 0 (0) |
| Lack of appetite | 0 (0) | 8 (53) | 1 (7) | 0 (0) | 0 (0) | 10 (67) | 0 (0) | 0 (0) |
| Hiccups | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Palpitations | 0 (0) | 3 (20) | 0 (0) | 0 (0) | 0 (0) | 3 (20) | 0 (0) | 0 (0) |
| Difficulty swallowing | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (7) | 1 (7) | 1 (7) |
| Stings, pain or pulling in the chest | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Rapid exhaustion | 0 (0) | 2 (13) | 1 (7) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Anxiety | 0 (0) | 1 (7) | 0 (0) | 0 (0) | 0 (0) | 1 (7) | 0 (0) | 0 (0) |
| Feeling of fullness | 0 (0) | 1 (7) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Abdominal pain | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Fainting | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Hypersensitivity to odours | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Dullness | 2 (13) | 4 (27) | 2 (13) | 0 (0) | 0 (0) | 4 (27) | 1 (7) | 0 (0) |
| Heartburn | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Nausea | 0 (0) | 3 (20) | 0 (0) | 0 (0) | 0 (0) | 2 (13) | 0 (0) | 0 (0) |
| Vomiting | 0 (0) | 1 (7) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Trepidation | 0 (0) | 1 (7) | 1 (7) | 0 (0) | 0 (0) | 1 (7) | 0 (0) | 2(13) |
| Diarrhoea | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Constipation | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Lack of energy | 0 (0) | 2 (13) | 1 (7) | 0 (0) | 0 (0) | 1(7) | 0 (0) | 0 (0) |
| Frequent urination | 0 (0) | 1 (7) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Irritability | 0 (0) | 0 (0) | 1 (7) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Musings | 0 (0) | 2 (30) | 1 (7) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Severe sweating | 0 (0) | 5 (27) | 0 (0) | 0 (0) | 0 (0) | 5 (33) | 0 (0) | 0 (0) |
| Back pain | 2 (13) | 1 (7) | 2 (13) | 0 (0) | 1 (7) | 1 (7) | 1 (7) | 2 (13) |
| Joint or limb pain | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Lack of concentration | 0 (0) | 7 (47) | 1 (7) | 0 (0) | 0 (0) | 8 (53) | 0 (0) | 0 (0) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Inner restlessness | 0 (0) | 3 (20) | 0 (0) | 0 (0) | 0 (0) | 1 (7) | 1 (7) | 0 (0) |
| Cold feet | 1 (7) | 4 (27) | 1 (7) | 0 (0) | 1 (7) | 3 (20) | 1 (7) | 0 (0) |
| Feeling of heaviness or tiredness in the legs | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Feeling of heaviness | 0 (0) | 1 (7) | 1 (7) | 0 (0) | 0 (0) | 3 (20) | 0 (0) | 0 (0) |
| Restlessness in the legs | 0 (0) | 0 (0) | 4 (27) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Lack of sexual arousal | 1 (7) | 1 (7) | 2 (13) | 1 (7) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Sexual arousal | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (7) | 0 (0) | 0 (0) | 0 (0) |
| Hypersensitivity to cold | 1 (7) | 2 (13) | 1 (7) | 0 (0) | 1 (7) | 1 (7) | 0 (0) | 0 (0) |
| Hypersensitivity to warm/hot | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Insomnia | 0 (0) | 0 (0) | 1 (7) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Excessive need for sleep | 1 (7) | 1 (7) | 1 (7) | 0 (0) | 1 (7) | 1 (7) | 0 (0) | 0 (0) |
| Bad dreams | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Hot flushes | 1 (7) | 2 (13) | 0 (0) | 1(7) | 0 (0) | 5 (33) | 0 (0) | 0 (0) |
| Occupational or private worries | 0 (0) | 0 (0) | 1 (7) | 1(7) | 1 (7) | 0 (0) | 1 (7) | 0 (0) |
| Itching | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Slight blushing | 1 (7) | 1(7) | 1 (7) | 1(7) | 0 (0) | 1(7) | 0 (0) | 0 (0) |
| Freeze | 1 (7) | 0 (0) | 1 (7) | 1 (7) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Increased appetite | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Dizziness | 0 (0) | 3 (20) | 0 (0) | 0 (0) | 0 (0) | 5 (33) | 0 (0) | 0 (0) |
| Fear of death | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Cloudy thoughts | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Dry mouth | 1 (7) | 8 (53) | 1 (7) | 0 (0) | 0 (0) | 8 (53) | 0 (0) | 0 (0) |
| Tremor | 0 (0) | 2 (13) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Numbness | 0 (0) | 1 (7) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Inner tension | 0 (0) | 2 (13) | 0 (0) | 0 (0) | 0 (0) | 1 (7) | 0 (0) | 0 (0) |
| Neck or shoulder pain | 2 (13) | 2 (13) | 2 (13) | 1 (7) | 2 (13) | 2 (13) | 1 (7) | 1 (7) |
| Sore or scratchy throat | 2 (13) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (7) | 1 (7) |
| Weight loss | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Weight gain | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Forgetfulness | 1 (7) | 2 (13) | 2 (13) | 0 (0) | 0 (0) | 3 (20 | 0 (0) | 0 (0) |
| Bruxism, jaw stiffness | 0 (0) | 1 (7) | 0 (0) | 0 (0) | 1 (7) | 4 (27) | 1 (7) | 0 (0) |
| Data presented as frequency (percentage, %). | | | | | | | | |

## Table S5 (M3) List of complaints (sub-acute adverse events)

Three days after each experimental session, the number of complaints per participant was assessed in a telephone interview using the predefined 66-item List of complaints.

| | Patients with arginine vasopressin deficiency (central diabetes insipidus) (n=15) | | Healthy controls (n=15) | |
|---|---|---|---|---|
| | **MDMA** | **Placebo** | **MDMA** | **Placebo** |
| Headache | 7 (47) | 2 (13) | 5 (33) | 1 (7) |
| Fatigue | 7 (47) | 3 (20) | 6 (40) | 1 (7) |
| Tickling sensation in the throat | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Imbalances | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Intermittent shortness of breath | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Feeling of suffocation | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Shortness of breath | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Crying tendency | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Coughing | 1 (7) | 1 (7) | 0 (0) | 1 (7) |
| Weakness | 1 (7) | 2 (13) | 2 (13) | 0 (0) |
| Lack of appetite | 2 (13) | 1 (7) | 5 (33) | 0 (0) |
| Hiccups | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Palpitations | 2 (13) | 0 (0) | 0 (0) | 0 (0) |
| Difficulty swallowing | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Stings, pain or pulling in the chest | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Rapid exhaustion | 1 (7) | 1 (7) | 3 (20) | 0 (0) |
| Anxiety | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Feeling of fullness | 0 (0) | 0 (0) | 1 (7) | 0 (0) |
| Abdominal pain | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Fainting | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Hypersensitivity to odours | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Dullness | 1 (7) | 4 (27) | 5 (33) | 1 (7) |
| Heartburn | 0 (0) | 0 (0) | 0 (0) | 1 (7) |
| Nausea | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Vomiting | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Trepidation | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Diarrhoea | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Constipation | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Lack of energy | 4 (27) | 1 (7) | 2 (13) | 0 (0) |
| Frequent urination | 1 (7) | 0 (0) | 2 (13) | 0 (0) |
| Irritability | 2 (13) | 0 (0) | 0 (0) | 0 (0) |
| Musings | 1 (7) | 1 (7) | 0 (0) | 0 (0) |
| Severe sweating | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Back pain | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Joint or limb pain | 0 (0) | 0 (0) | 1 (7) | 0 (0) |
| Lack of concentration | 0 (0) | 3 (20) | 0 (0) | 0 (0) |
| Inner restlessness | 1 (7) | 0 (0) | 0 (0) | 0 (0) |
| Cold feet | 1 (7) | 0 (0) | 1 (7) | 0 (0) |
| Feeling of heaviness or tiredness in the legs | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Feeling of heaviness | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Restlessness in the legs | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Lack of sexual arousal | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Sexual arousal | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Hypersensitivity to cold | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Hypersensitivity to warm/hot | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Insomnia | 3 (20) | 0 (0) | 2 (13) | 0 (0) |
| Excessive need for sleep | 2 (13) | 1 (7) | 1 (7) | 0 (0) |

| | | | | |
|---|---|---|---|---|
| Bad dreams | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Hot flushes | 0 (0) | 0 (0) | 1 (7) | 0 (0) |
| Occupational or private worries | 0 (0) | 1 (7) | 0 (0) | 0 (0) |
| Itching | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Slight blushing | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Freeze | 0 (0) | 0 (0) | 1 (7) | 0 (0) |
| Dizziness | 0 (0) | 0 (0) | 1 (7) | 0 (0) |
| Increased appetite | 0 (0) | 0 (0) | 1 (7) | 0 (0) |
| Fear of death | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Cloudy thoughts | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Dry mouth | 1 (7) | 1 (7) | 0 (0) | 1 (7) |
| Tremor | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Numbness | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Inner tension | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Neck or shoulder pain | 0 (0) | 1 (7) | 0 (0) | 0 (0) |
| Sore or scratchy throat | 1 (7) | 1 (7) | 0 (0) | 0 (0) |
| Weight loss | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Weight gain | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Forgetfulness | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Bruxism, jaw stiffness | 0 (0) | 0 (0) | 1 (7) | 1 (7) |
| Data presented as frequency (percentage, %). | | | | |

# 6. DISCUSSION OF THE FINDINGS OF THE PhD PROJECT AND DIRECTIONS FOR FUTURE RESEARCH

This PhD project has the following main findings: First, glucagon-stimulated copeptin has the potential to provide a safe and precise test in the differential diagnosis of AVP deficiency. Second, once diagnosed and treated with desmopressin, a high prevalence of hyponatremia can be observed in patients; importantly, performing the 'desmopressin escape' method, an approach to routinely omit or delay desmopressin to allow aquaresis, demonstrated a lower hyponatremia prevalence. Third, if hospitalised, high rates of mismanagement and treatment error occur, partly explained by confusion with diabetes mellitus. Fourth, despite adequate treatment with desmopressin, patients report psychological comorbidities and lower QoL. Finally, using a novel provocation test with MDMA, our results are the first to demonstrate a clinically relevant OXT deficiency with associated psychopathological findings.

Given the rarity of AVP deficiency, few clinicians outside specialised centres have developed expertise in diagnosing and treating patients with this deficiency, emphasising the need for simple diagnostic algorithms with high accuracy. Although recent laboratory advances have improved these algorithms, the final diagnosis is a composite of clinical, laboratory and radiographic findings.[23,70] The classic indirect WDT was for decades considered the gold standard in evaluating patients with PPS; however, the poor diagnostic accuracy[57] and resulting incorrect diagnosis and wrong treatment carries the risk for life-threatening consequences.[74,151,152] Copeptin-based tests with hypertonic saline infusion or arginine infusion demonstrate higher diagnostic accuracy.[60] However, these tests are associated with potential side effects and require a weight-adjusted infusion via an intravenous line, making close monitoring by medical personnel essential.[57,62] Although arginine infusion has been shown to provide an overall simplified diagnostic procedure, its accuracy is somewhat lower compared to the hypertonic saline infusion test, probably owing to its weaker potency in stimulating copeptin.[60,62] As part of this PhD project, we demonstrated the potential utility of glucagon as an alternative – presumably non-osmotic – diagnostic test in differentiating AVP deficiency from PP.[153] Glucagon provocation is a well-established test in evaluating the anterior pituitary function and has a stronger stimulative potency than arginine.[154] Our first data provide a high diagnostic accuracy of 95%, with only mild side effects and an overall safe

testing procedure.[153] Overall, glucagon provocation might be a valuable alternative with a stronger stimulative potency than arginine and a safer and more simplified test protocol than hypertonic saline. As the diagnostic workup in the ambulatory setting outside of specialised centres and the lack of a standardised paediatric approach represent a particular challenge, glucagon stimulation could be used, especially in these settings.[155] **Accordingly, the next step will be a prospective validation study in a larger cohort of adult and paediatric patients in an ambulatory setting and would be of great clinical value.**

The exact underlying mechanisms of how glucagon stimulates the posterior pituitary and its differences from arginine remain elusive. While arginine induces a copeptin peak at 60 minutes, glucagon is characterised by a more delayed peak at 150 minutes.[62,153] Hypoglycaemia is one of the most potent non-osmotic stimuli for the pituitary gland.[102,156] Therefore, copeptin after hypoglycaemia induced by an insulin tolerance test (ITT) provides strong stimulation to the posterior pituitary.[157] Since changes in glucose dynamics, as observed upon an ITT, seem to trigger copeptin secretion, we hypothesised that the changes in glucose dynamics upon glucagon might contribute to the copeptin release. Already in 1975, a relationship between the rapid drop in elevated glucose levels and the increase in growth hormone (GH) in response to glucagon was suggested.[104] In support of this hypothesis, the same group demonstrated a suppressed GH peak in response to glucagon if the participants received an additional continuous glucose infusion, preventing a rapid glucose drop.[104] Glucose course after glucagon might mimic a hypoglycaemic state, characterised by a rapid decrease in elevated glucose levels, without leading to absolute hypoglycaemia. We tested this hypothesis in a secondary analysis and demonstrated a clear positive correlation between the rapid drop in glucose levels and the subsequent increase in copeptin in response to glucagon.[158] However, causality cannot be concluded from these observations in our correlation study, therefore **further research is needed to investigate the effects of the stimulative mechanisms of glucagon on the posterior pituitary in more detail**.

Once a patient has been correctly diagnosed with an AVP deficiency, desmopressin treatment is usually initiated with effective long-term control of polyuria.[24] Physiologically, fluid intake suppresses AVP secretion, allowing an aquaresis to prevent water retention.[7] Under desmopressin, however, even normal modest fluid intake is retained as there is constant antidiuresis until the effects subside and therefore

dilutional hyponatremia is a common side effect.[159] A high prevalence of hyponatremia of up to 30% is mostly reported for the outpatient setting and might be explained by the lack of education on the correct use of desmopressin.[75] Our results confirm the high prevalence of hyponatremia leading to hospitalisation and are the first to demonstrate the value of the 'desmopressin escape' method to counteract this risk. Endocrinologists should educate patients about these strategies and apply an individualised approach at desmopressin initiation. Furthermore, **data from a prospective long-term study is needed to prove the efficacy of this method and investigate whether this approach lowers the risk of hyponatremia compared to a more rigid dose schedule.** In addition, some have suggested an association between the desmopressin formulation and hyponatremia risk, reporting a higher risk for nasal desmopressin. More precisely, one study reported a 60% risk reduction for hyponatremia in patients who switched from nasal to oral preparation.[77] Conversely, another study reported a 33% hyponatremia rate within a four-week dose titration period after switching from a nasal to an oral formulation.[116] Although our data do not support the hypothesis on differences between available formulations (e.g. nasal spray vs oral) and hyponatremia prevalence, one should emphasise the high patient-reported rates of switching from nasal to oral formulations in our cohort, mostly due to patient preference, pointing to overall better symptom control. **Future studies could investigate subjective symptom control and the rate of hyponatremia in, for example, a cross-over study between the nasal and oral formulations of desmopressin.**

Even without desmopressin, patients with a functioning osmoregulated thirst perception and free access to water can compensate for urinary water loss through increased fluid intake. Therefore, hypernatremia, an indicator of inadequate fluid intake, rarely occurs in patients with access to fluids.[75] Importantly, limited access to fluids, brought about, for example, by non-availability or restricted intake, unconsciousness, or acute concurrent illness, can lead to life-threatening hypernatraemic dehydration.[75] In support of this, hypernatremia is mostly reported in hospitalised patients, presumably owing to a lack of knowledge on correct fluid management and treatment failures by the medical team.[75] In our cohort, one in four hospitalised patients reported symptoms of dehydration and mismanagement as a result of an inability to use their desmopressin while in a fasting state without intravenous fluid replacement – a high-risk situation for hypernatraemic dehydration. Of note, such scenarios have been reported in several

cases with serious adverse outcomes, including death.[110,119] In line with our results, particularly during hospitalisation, the reported rate of hypernatremia is around 20%, most likely the result of inappropriate fluid management.[75] Importantly, evidence from our study and others reinforces the need to raise awareness and educate medical personnel about correct fluid management and demand the inclusion of desmopressin as a high-alert medication with 24-hour access in hospitals.[119,120] One possible additional explanation for these observed treatment errors might be the high confusion rate with diabetes mellitus, as reported by our cohort, presumably when they are under the care of non-endocrine specialists.[69] In our study, 87% of patients reported that the lack of knowledge and the resulting clinical confusion affected the management of their condition, for example repeated blood sugar measurements. Subsequent to these unfortunate but avoidable cases of treatment error and the results of our study showing strong patient support for renaming the condition, a working group of representatives from international endocrinology societies now propose changing the name of ‘diabetes insipidus’ to ‘arginine vasopressin deficiency’ for central aetiologies and ‘arginine vasopressin resistance’ for nephrogenic aetiologies.[160-162] In response to these recent movements, **we are currently planning a European AVP-deficiency Registry Study** to investigate, in a first step, retrospectively these incidences across European centres and, in a second step, prospectively (over 5 years) elucidate, for example, the impact of educating patients on the use of the ‘desmopressin escape’ approach on desmopressin-associated side effects and the renaming of ‘diabetes insipidus’ on treatment errors during hospitalisation.

Although hormone replacement therapy with desmopressin improves clinical outcomes in patients with AVP deficiency, evidence suggests that patients still do not reach population norms regarding QoL and psychological deficits.[111] In light of these deficits, recent studies have suggested a link between a possible additional OXT deficiency and accompanying psychological deficits.[87] However, the available data are purely based on correlations and associations, showing high variability in the findings, and should be interpreted with considerable limitations. Some of these studies have even shown questionable results. For example, contrary to expectations, in the study by Daubenbüchel et al. basal OXT levels were higher in patients with extensive hypothalamic involvement (anterior and posterior regions) than in those with only anterior hypothalamic involvement.[126] In addition, in the studies of Daughters et al.

and Gebert et al., to our surprise hypopituitarism was associated with reduced basal OXT levels, irrespective of the presence of an AVP deficiency, and higher basal OXT levels were associated with higher trait anxiety.[85,88] Even more surprising, Eisenberg et al. observed higher basal OXT levels in patients with AVP deficiency compared to healthy controls.[86] These questionable results demonstrate the importance of assessing OXT using a biochemical and psychoactive provocation test, since basal levels are unreliable in proving a deficiency and controversial in assessing the hormone's central effect on socio-emotional functioning.[90,127,128] In our study, we used MDMA as a stimulus for OXT. MDMA is a psychoactive drug whose distinct empathogenic and prosocial profile has been linked to the central stimulation of OXT.[130,133,163] For the first time, our results revealed an OXT deficiency using this unique provocation test, laying the groundwork for a new hypothalamic-pituitary entity. Importantly, we also answered the question of whether this deficiency in OXT is related to alterations or dysfunctions in socio-emotional behaviour in patients with AVP deficiency. In our study, healthy controls experienced acute prosocial, empathogenic and anxiolytic effects in response to MDMA stimulation, while patients with AVP deficiency showed markedly reduced acute subjective effects upon stimulation. These findings reflect an absence of the hormone and, in agreement, consequent dysfunction in central regions important for socio-emotional processing. It should nevertheless be mentioned that MDMA stimulation is not feasible in all patients (e.g. in those with cardiovascular conditions) and probably cannot be implemented in clinical routine as a standard provocation test. **Therefore, an alternative simplified approach must be developed for clinical routine**. Mimicking the effects of MDMA with substances that have already been approved might be a possibility.[164,165] Although the exact mechanisms of how MDMA stimulates OXT have not been fully elucidated, it has been suggested that MDMA directly activates oxytocinergic neurons in the SON/PVN – both regions with a high density of 5-$HT_{1A}$ receptors.[145] It is known that MDMA primarily increases synaptic 5-HT levels by inhibiting presynaptic 5-HT transporters (SERT) and interacting directly with various 5-HT receptors.[164] The activation of post-synaptic 5-$HT_{1A}$ receptors and consequent OXT release might be via direct partial agonistic action at these receptors and indirect MDMA-induced 5-HT release from pre-synaptic axon terminals.[164] In line with this, the administration of 5-$HT_{1A}$ agonists facilitates social behaviour and increases OXT levels in rats without affecting AVP.[145,165] In further support of this, MDMA-induced OXT release can

be blocked by pre-treatment with 5-$HT_{1A}$ antagonists.[145,166] In humans, pre-treatment with citalopram (a selective 5-HT uptake inhibitor, SSRI) or duloxetine (5-HT and norepinephrine uptake inhibitor) attenuated the acute effects (by interfering with SERTs) and inhibited the MDMA-induced OXT release. The neurochemical mechanisms of MDMA can be hypothetically simplified as a blockade of 5-HT reuptake (similar to SSRIs, e.g. fluoxetine), induction of serotonin and dopamine release (similar to serotonin-dopamine reuptake inhibitors, e.g. sertraline) and agonistic effect at $HT_{1A}$ receptors (e.g. buspirone). **Theoretically, a combined single administration of these substances** (e.g. SSRI and 5-$HT_{1A}$ receptor agonists) **could be investigated as an alternative simplified provocation test for OXT for clinical routine.**

To prove a causal relationship between the observed psychological deficits and an OXT deficiency, data from interventional studies with OXT replacement demonstrating an improvement in these impairments are needed. Up to now, the effects of OXT administration in patients with AVP deficiency have only been reported in one case, and a small study of ten patients investigating the ability to categorise emotions after a single dose of intranasal OXT has shown promising but still limited data.[123,144] However, data on the long-term effects of OXT in this patient population are not available, and so far, no interventional trial in patients with AVP deficiency has addressed this issue. We are currently planning an **interventional study in a larger patient population to investigate the potential therapeutic use of OXT replacement to ameliorate symptoms in these patients in whom we have now provided evidence for a high risk for OXT deficiency.**

Furthermore, our study focused on identifying an OXT deficiency and assessing the association with psychopathological characteristics. However, OXT also has other functions, including obstetric ones.[10,11] Complications during labour or difficulties with breastfeeding in patients with AVP deficiency have not been adequately investigated and available data are limited to some case series of patients with hypopituitarism.[167-171] Some reported successful spontaneous labour[167,168] without requiring OXT administration, suggesting that pituitary OXT may not be mandatory for spontaneous labour initiation or partially preserved. In contrast, more recently, in a case series of twelve patients, Aulinas et al. reported spontaneous labour only in those with isolated anterior pituitary dysfunction, but in the four patients with AVP deficiency, delivery took place through caesarean section or vaginal delivery that

required intravenous OXT administration.[171] These findings further align with the fact that only half of the patients were breastfeeding at the time of hospital discharge.[171-173] To address this lack of information and further characterise this new hypothalamic-pituitary condition, **an investigation of a large cohort is needed**.

## 7. CONCLUSION AND CLOSING REMARKS

This MD-PhD thesis focuses on patients with AVP deficiency and investigates a new diagnostic provocation test, treatment-associated side effects and possible methods to counteract these, complications and treatment errors during hospitalisation, patients' views on a possible renaming of the condition, socio-emotional changes and psychological comorbidities, and a possible additional OXT deficiency using a psychoactive and biochemical provocation test.

The results of the published articles will contribute significantly to an improvement in the diagnostic evaluation of patients with suspected AVP deficiency and the complications associated with desmopressin treatment, as well as providing a new method for lowering the risk of hyponatremia. The findings will also help to support the renaming of the disease to improve safety, increase awareness of the disease among healthcare professionals and provide the groundwork for the treatment of OXT deficiency as a new hypothalamic-pituitary condition.

Studies in future will provide data on the validity of the glucagon provocation test in patients with suspected AVP deficiency, investigate whether the ‘desmopressin escape’ method will lower the incidence of hyponatremia, assess whether the renaming leads to lower rates of confusion with diabetes mellitus and resulting treatment errors, as well as evaluating the potential treatment effects of OXT replacement on residual psychological symptoms in patients with AVP deficiency.

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