The gluco-regulatory effects of 3,4-Methylenedioxymethamphetamine (MDMA): A controlled n-of-2 self-experimentation

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This study was not conducted in affiliation with any institution

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Key words: blood sugar; copeptin; ecstasy; hydration; MDMA; psychedelic drugs; thirst

Updated December 2021: *I have received some helpful comments back from peers, as well as having cortisol measured; as such this version is edited to reflect those changes. For full transparency, reviewer comments are in the Supplementary Material (at the end of this document), and significant changes in text are in red.*

Named peer-reviewer: Dr Ilsa Jerome (Multidisciplinary Association for Psychedelic Studies)

Abstract

Background: Psychedelic drugs are increasingly being researched for their therapeutic properties in terms of treatment resistant mental illnesses. However, little is known about how they impact metabolism, which has implications for those with metabolic diseases such as diabetes. Hypohydration has been argued by some to cause glucose dysregulation, particularly due to elevations in arginine vasopressin (AVP). 3,4-Methylenedioxymethamphetamine (MDMA) is known to elevate AVP, resulting in body water retention. Thus MDMA offers a paradoxical state of elevated AVP and hyperhydration, allowing us to uncouple the effects of hydration state and hydration physiology on metabolism.

Aims: This study aimed to test the impact of MDMA on gluco-regulation in a tightly controlled setting.

Methods: Using a non-blinded AB study design, this self-experimentation involved two female scientists who underwent one day pre-trial within-person control of diet and activity. After the pretrial control period, the experimenters did the control (CON; no-intervention) arm first, followed by the treatment (MDMA) arm. Testing involved fasting measures pre- and post-MDMA (or equivalent timepoints during CON), then a two hour 75 g oral glucose tolerance test, and a four hour follow up period. Blood samples, urine volume and specific gravity, body mass, and visual analogue scales were collected at set times throughout the testing period.

Results: Relative to CON, MDMA resulted in gluco-dysregulation in both experimenters, particularly in relation to causing hyperinsulinaemia. However, plasma copeptin concentration (as a marker of AVP) only increased in one experimenter. Both experimenters had elevated cortisol during MDMA compared to CON. For one experimenter, urine volume was greater during MDMA, with no distinct differences in specific gravity; for the other experimenter (with elevated copeptin concentrations), urine volume was lower with higher specific gravity. Visual analogue scales showed only the experimenter with high copeptin had increased thirst and xerostomia.

Conclusion: Overall, MDMA may be implicated in gluco-dysregulation, though caution needs to be taken when generalising from such a small study. Accordingly, these findings need to be replicated in a larger sample, as well as in conditions that are similar to a therapeutic setting. Since gluco-dysregulation occurred in both experimenters, but copeptin only increased in one experimenter, it is unlikely that AVP is the key mechanism of action. Cortisol is likely a key driver of the gluco-dysregulation reported. Preliminary recommendations are provided to help ensure the safety of MDMA therapy patients and recreational users.

Introduction

Psychedelic drugs are increasingly being evaluated for their efficacy in treating multiple mental illnesses, particularly in those that are resistant to current treatments, such as addiction, chronic depression, and post-traumatic stress disorder (e.g. Mitchell *et al.*, 2021; Multidisciplinary Association for Psychedelic Studies [MAPS], n.d.). 3,4-Methylenedioxymethamphetamine (MDMA), commonly known as the party drug 'ecstasy' is a key drug of interest in mental illness therapeutics due to its ability to induce a positive experience in a more predictable manner than other psychedelics such as lysergic acid diethylamide or psilocybin. This is likely due to MDMA not inducing as strong experiences of altered consciousness and mystical experiences which can sometimes lead to the 'bad trips' more commonly associated with classical psychedelic drugs (Holze *et al.*, 2020).

Current exclusion criteria in clinical studies using MDMA therapy primarily focus on ensuring volunteers are normo-tensive and have no obvious heart conditions, have a healthy liver, and do not suffer with conditions affecting water balance (e.g. MAPS, 2019; Mithoefer *et al.*, 2019). These criteria make sense according to our current understanding of the effects of MDMA, such as elevated heart rate and hyponatraemia. However, these physiological effects of MDMA that are only known because they have caused obvious harm and are relatively easy to identify and measure. In a therapeutic setting though, it is of utmost importance to understand the full range of potential side effects of any administered drug. Considering the current obesity and type 2 diabetes (T2D) epidemics (as well as the prevalence of other disorders like type 1 diabetes, and insulin resistance), understanding gluco-regulation in relation to MDMA administration may offer insights into safety measures patients and psychotherapists might need to take before undergoing therapy.

The reason hyponatraemia is common with MDMA use is due to elevations in the hormone arginine vasopressin (AVP). In the field of hydration and health, investigations regarding the role of AVP (which is elevated with body water losses) in blood sugar regulation are ongoing (Carroll & James, 2019). If one of the most accepted hypotheses is correct, then elevated AVP is implicated in gluco-dysregulation (Carroll *et al.,* 2016). As AVP is part of the hypothalamic-pituitary-adrenal (HPA) axis, and has a role in modulating corticotropin-releasing factor secretion, the hypothesis goes that increased AVP leads to a cascade whereby cortisol is secreted resulting in elevated hepatic glucose output (Melander, 2016). Further, elevated AVP can act on hepatic V1 receptors resulting in glycogenolysis (Koshimizu *et al*, 2021; Spruce *et al*., 1985). Accordingly, blood sugar concentrations increase, and gluco-regulation is disrupted (Carroll *et al.*, 2016; Carroll & James, 2019; Melander, 2016).

Furthermore, cell volume may be implicated in gluco-regulation; unlike hypohydration, MDMA is more likely to result in cell swelling via AVP-mediated water retention. *In vitro* studies demonstrate that hepatocyte cell swelling can inhibit glycogenolysis (Haussinger, 1996; Graf *et al.*, 1988) and stimulate glycogen synthesis (Baquet *et al*., 1990; Meijer *et al*., 1992; Peak *et al*., 1992). Thus from this perspective, MDMA may be implicated in improved gluco-regulation, resulting net null effect.

Whilst in healthy adults, hypohydration (with elevated serum osmolality and copeptin, as markers of AVP) does not appear to impact gluco-regulation (Carroll *et al*., 2019a), research has shown that medication-withdrawn participants with type 1 (Burge *et al.*, 2001) and type 2 diabetes (Johnson *et al.*, 2017) can experience gluco-dysregulation with hypohydration, perhaps due to changes in hydration physiology, or perhaps due to glucosuria when euhydrated (Carroll & James, 2019). However, unlike these studies, MDMA typically results in hyperhydration with reduced urine frequency, and co-occurring elevations in AVP, which is a physiologically unusual state to be in and may disrupt homeostatic processes even in healthy patients. Infusion of AVP to supraphysiological levels (> 100 pmol·L^{-1}) does marginally increase glycaemia (by ~0.8 mmol·L^{-1}) under fasted conditions, perhaps due to an increase in glucagon (Spruce *et al.*, 1985).

Research on the gluco-regulatory effects of MDMA is scarce due to obvious logistical reasons. One study in rats found hypoglycaemia (Soto-Montenegro *et al.*, 2007) with another finding the opposite (Banks *et al.*, 2009), whereas in a study in humans in an uncontrolled setting (*ad libitum* food and fluid intake), no clear effect on blood glucose concentrations was found (Downing, 1986). Since a hypothesised mechanism of AVP is the resultant increase in cortisol, it is worth noting that MDMA typically increases cortisol (Dolder *et al.*, 2018; Harris *et al.*, 2002; Hysek *et al.,* 2012; Hysek *et al.*, 2014), though a minority of studies have failed to see this (e.g. Henry *et al.,* 1978, which was conducted in a controlled setting).

Accordingly, the aim of this self-experiment was to test the gluco-regulatory impact of MDMA in healthy self-experimenters. We hypothesised that MDMA would result in an elevation in plasma copeptin concentrations, but this would not result in gluco-dysregulation due to counterregulatory effects of cell volume maintenance or expansion.

Methods

Participants

Two scientists self-experimented in this study, thus no recruitment, inclusion/exclusion criteria, power estimation, trial registration, nor ethical approval were required/implemented, in line with COPE guidance (COPE, 2015). Considering the nature of the experiment, the self-experimenters may or may not be included in the author list. Additionally, data regarding the location and dates of the experiment have been deliberately omitted, as have some of the analytic equipment by request of collaborators. Self-experimenters had a joint curiosity and/or academic interest in the research and collectively planned, implemented, and financially contributed to the project, along with the help of collaborators.

Study design

This was an unblinded (for logistical reasons) non-randomised controlled study (AB design) with both experimenters conducting the control (CON) arm first, followed by the treatment (MDMA) arm. The non-randomised nature of the study was chosen for logistical purposes. Initially, during the planning phase of the study, experimenters agreed a two consecutive day testing protocol was most feasible; thus in order to remove any negative metabolic, appetitive and/or psychological impacts of the after effects of MDMA administration (i.e. a 'comedown'), the CON arm was conducted first. In *Experimenter-b*, after successfully completing the CON arm, their cannula blocked overnight and the attempts at re-cannulation for the MDMA trial arm failed; therefore, their MDMA trial was conducted one month later in order to control for the menstrual cycle.

Prior to starting the study, experimenters had a standardisation day, whereby they minimised their activity and recorded their food and fluid intake (within-person), ready to be replicated on the two experimental days of the trial (**Figure 1**). Thus, the planned experimental protocol consisted of three days, which *Experimenter-a* underwent:
Day 1: Standardisation
Day 2: Control trial arm
Day 3: MDMA trial arm

Considering the failed cannulation for *Experimenter-b*, their trial schedule went as follows:
Day 1: Standardisation
Day 2: Control trial arm
****One month washout****
Day 3: Replicated standardisation from day 1
Day 4: MDMA trial arm

To ensure diet was accurately replicated before both CON and MDMA, 75 g maltodextrin was also consumed on the standardisation day at roughly the same time as the two trial arms. Thus on all three days (standardisation, CON, MDMA), experimenters woke up at ~0900 h, consumed 100 mL of water, waited one hour, consumed the 75 g oral glucose solution, then waited two hours before consuming any other food or fluid; the food and fluid consumed 2 h after the 75 g maltodextrin on day 1 (standardisation) was replicated on CON and MDMA (**Figure 1**). In the MDMA arm, the 100 mL of water included the MDMA dose (explained below).

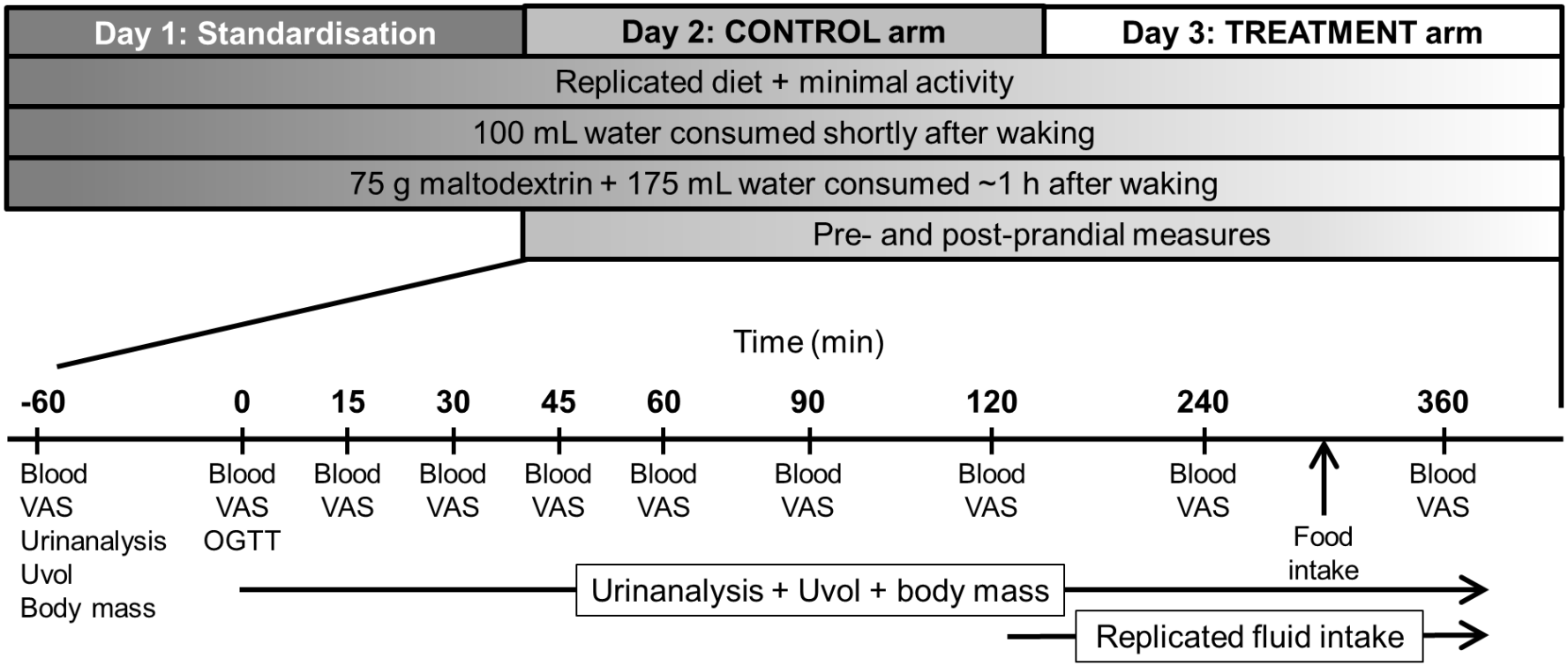

Figure 1. Protocol schematic. Food and fluid intake replicated within-person from the standardisation day. For *Experimenter-b*, CONTROL and TREATMENT trial arms were one month apart, and the Standardisation Day was repeated before the TREATMENT arm. During the TREATMENT arm, MDMA was consumed with the 100 mL water consumed after taking the -60 min measures
Abbreviations: OGTT, 75 g oral glucose tolerance test; MDMA, 3,4-Methylenedioxymethamphetamine; Uvol, urine volume; VAS, visual analogue scales

Experimental days

Experimenters started at 0900 h, and completed visual analogue scales (VAS) before brushing their teeth or voiding. Scales were 100 mm vertical lines which were marked by the experimenters with a horizontal line and scored from '0' (not at all/equivalent) to '100' (extremely/equivalent), and asked: "how hungry/full are you?", "how much do you think you can eat?", "how empty does your stomach feel?", "how thirsty are you?", "how dry does your mouth feel?", "how strong is your desire to consume salty/savoury/sweet/fatty foods?", "how awake/tired/happy/sad do you feel?", and "how much do you want to clench your jaw?".

Following this, experimenters provided a urine sample and took a post-void measure of nude body mass (Salter Glass Analyser Scales). Once nude body mass was obtained, experimenters re-dressed and re-weighed to obtain the weight of their clothes so subsequent measures did not require undressing each time. Urine was expelled into a glass which was pre-labelled in 100 mL increments in order to estimate urine volume. A dipstick (Medisave Urinalysis Reagent Strip, UK) was used to estimate urine specific gravity; if other parameters on the dipstick were not the baseline value on the score chart, these were also recorded; these are in the published dataset but not discussed herein. This protocol (urine volume, urine dipstick, and post-void body mass) was repeated throughout the testing period whenever the experimenters needed to void (**Figure 1**). There was no set schedule for voiding to minimise experimenter discomfort and to understand time trends in urination. Times of voids were rounded to the nearest half an hour for the purposes of figures. Urine and body mass measures continued until 1900 h on each testing day, approximately 180 minutes longer than blood and VAS measures were taken. Food and fluid remained standardised during these measures.

A cannula was then fitted in an antecubital vein and a baseline blood sample (6 mL) was drawn into a K2-ethylenediaminetetraacetic acid coated tube (BD, Oxford), then pipetted into three x 2 mL Eppendorfs for centrifugation. Whole blood glucose was measured using glucose strips (GlucoRx, Nexus, UK) to determine whole blood glucose concentrations before centrifugation. The plasma supernatant was aliquoted, and samples frozen at approximately -18 ºC.

After this baseline blood sample, experimenters consumed 100 mL water (CON), or MDMA (100 mg) + 100 mL water before waiting for one hour. The MDMA was tested with a reagent prior to experimentation using semi-quantitative methods (EZ Test amphetamine 2C-B, Netherlands). Such a method is accurate enough to detect MDMA, but can fail to detect other illicit substances (Camilleri & Caldicott, 2005). Whilst imperfect, these tests confirmed the presence of MDMA with relative purity (for the impurities tested, such as piperazines and paracetamol). The MDMA was provided as white crystalline rocks and powder from two batches that were crushed and mixed. Experimenters dissolved the powder in 100 mL water to aid ingestion, with ample water remaining to discard any remaining unpleasant taste.

Experimenters waited one hour to account for MDMA absorption, then completed VAS and had a pre-oral glucose tolerance test (OGTT) blood sample taken. Subsequently, experimenters consumed 75 g maltodextrin mixed with 175 g water within five minutes. 175 g water was determined prior to the experiment as the minimum water required to dissolve the maltodextrin powder; this was done to reduce the confounding influence of water ingestion on circulating AVP concentrations. Blood samples were taken following the

above protocol at 15, 30, 45, 60, 90, 120, 240, and 360 min post-glucose ingestion, and VAS were completed at 30, 60, 90, 120, 240, and 360 min. Approximately 5 mL of saline was reinfused to maintain the cannulae after each sample was drawn.

Diet (food and fluid) from the standardisation day was replicated throughout the experimental days, with no food/fluid consumed during the OGTT. Diet was controlled for within-person; thus, after the 120-min time point, blood glucose responses differ between experimenters due to the differences in their nutrient intake, rather than between-person differences in nutrient absorption and metabolism. For ease, both experimenters ate at the same time, at approximately 300 minutes post-glucose ingestion (i.e. 60 minutes before the final blood sample). Both experimenters also matched their post-OGTT fluid within-person.

Biochemical analyses

Whole blood glucose was measured immediately using glucose strips (GlucoRx, Nexus, UK). Plasma glucose, copeptin, and cortisol concentrations were measured (in singular) using autoanalyzers (anonymous). Urine specific gravity was estimated to the nearest 0.005 using a dipstick (Medisave Urinalysis Reagent Strip, UK). Plasma insulin concentrations were measured (in duplicate, with the average taken) using ELISA (Mercodia, Sweden). Plasma sodium was measured by indirect ion-selective electrode, and plasma urea and creatinine were measured by enzymatic assays using urease and creatininase, respectively. Plasma osmolality was estimated from plasma sodium, urea, potassium, and glucose, using the following formula (Bhagat *et al.*, 1984):

$$\text{Calculated osmolality} = 1.89\,[Na] + 1.38\,[K] + 1.03\,[\text{urea}] + 1.08\,[\text{glucose}] + 7.45$$

Due to the use of potassium EDTA as an anti-coagulant (which prevented the measurement of osmolality directly), K was imputed as 4.4 $mmol \cdot L^{-1}$ for all timepoints. As such calculated osmolality measures should be interpreted cautiously and are presented for the purpose of identifying potential trends rather than providing exact measures or inferences.

Statistical analyses

Due to the small sample size, no inferential statistics have been performed. Individual data have been shown for all variables. Area under the curve (AUC) was calculated as per Wolever (2004) (using Excel, Microsoft, USA), and incremental AUC (iAUC) was estimated fitting a cubic spline using the -integ- function on StataMP 16 (StataCorp, USA). Fasting insulin resistance was calculated using Homeostasis Model Assessment 2 (HOMA2; University of Oxford, UK). To minimize the chance of any experimenters being identifiable, an age range has been provided. Change (Δ) scores were calculated by subtracting the MDMA value from the CON value. All data are published and fully available (https://osf.io/sf4nq), or by emailing the author.

Results

Table 1 shows baseline experimenter characteristics. Both were tested during their estimated luteal phase of the menstrual cycle, though both suffer some level of oligo- and/or polymenorrhea making this estimation very approximate (estimates were based on the date of last onset of menses). A summary of the entire study results can be found in **Table 2**.

Table 1. Experimenter characteristics

	Experimenter-a	*Experimenter-b*
Sex	Female	Female
Age range (y)	25-35	25-35
Body mass (kg)	44.5	49.1
Body mass index (kg/m^2)	19.1	19.9
Regular medication	Selective serotonin reuptake inhibitors (20 mg/d fluoxetine)	None

Table 2. Overall differences and trends after MDMA administration (relative to CON)

	Experimenter-a	*Experimenter-b*	Comments
Body mass	↑	↑↑	
Urine volume	↑	↓	
Urine specific gravity	~?	↑	
Copeptin	~	↑	
Calculated osmolality	↑	↓	
Sodium	↑	↓	No indication of hyponatraemia
Urea	~	↑	Remained within norm ranges
Chloride	↑	↓	Remained within norm ranges
Creatinine	~?	↓	Remained within norm ranges
Glucose AUC	↑↑	↑	
Glucose iAUC	↑	↓	
Insulin AUC	↑	↑↑	
Insulin iAUC	↑	↑↑	
HOMA2	↑	↑↑	
Cortisol	↑	↑↑	
Thirst	↓	↑	
Xerostomia	↓	↑	
Hunger	↓	↑	
How much could they eat	↓	↑?	
How empty stomachs felt	↓	↑?	
Fullness	↑?	↓?	
Salt desire	↓	~	
Savoury desire	↓	↑	
Sweet desire	~	~	
Fatty desire	↓	~	
Wakefulness	↑	?	Experimenter-b had higher wakefulness during CON for the first 60 min, then higher wakefulness for MDMA for the remainder of testing
Tiredness	↓?	↑?	*Experimenter-a* was more tired during CON for the first 90 min, thereafter tiredness ratings were similar. *Experimenter-b* was more tired during MDMA for the first 90 min, then more tired during CON thereafter
Happiness	↑	↑↑	
Sadness	~?	↓	
Desire to clench jaw	~	↑	

↑ increased (red); ↑ ↑ increased more in this experimenter (red); ↓ decreased (green); ↓ ↓ decreased more in this experimenter (green); ~ no discernible difference (orange); ? indicates notable variability (orange, or pale ↑ red/↓ green if there is a potential trend)

Abbreviations: AUC, area under the curve; CON, control trial arm; iAUC, incremental area under the curve; MDMA, 3,4-Methylenedioxymethamphetamine

Hydration biomarkers

Body mass

Body mass can be seen in **Figure 2**. Most notably, after MDMA, body mass increased in both experimenters. In *Experimenter-a*, the rise in body mass was rapid and returned to baseline within ~90 min. In *Experimenter-b*, the rise in body mass was steady across the OGTT and post-OGTT period, declining around the approximate MDMA half-life (~7-8 h; Kalent, 2001) post-ingestion.

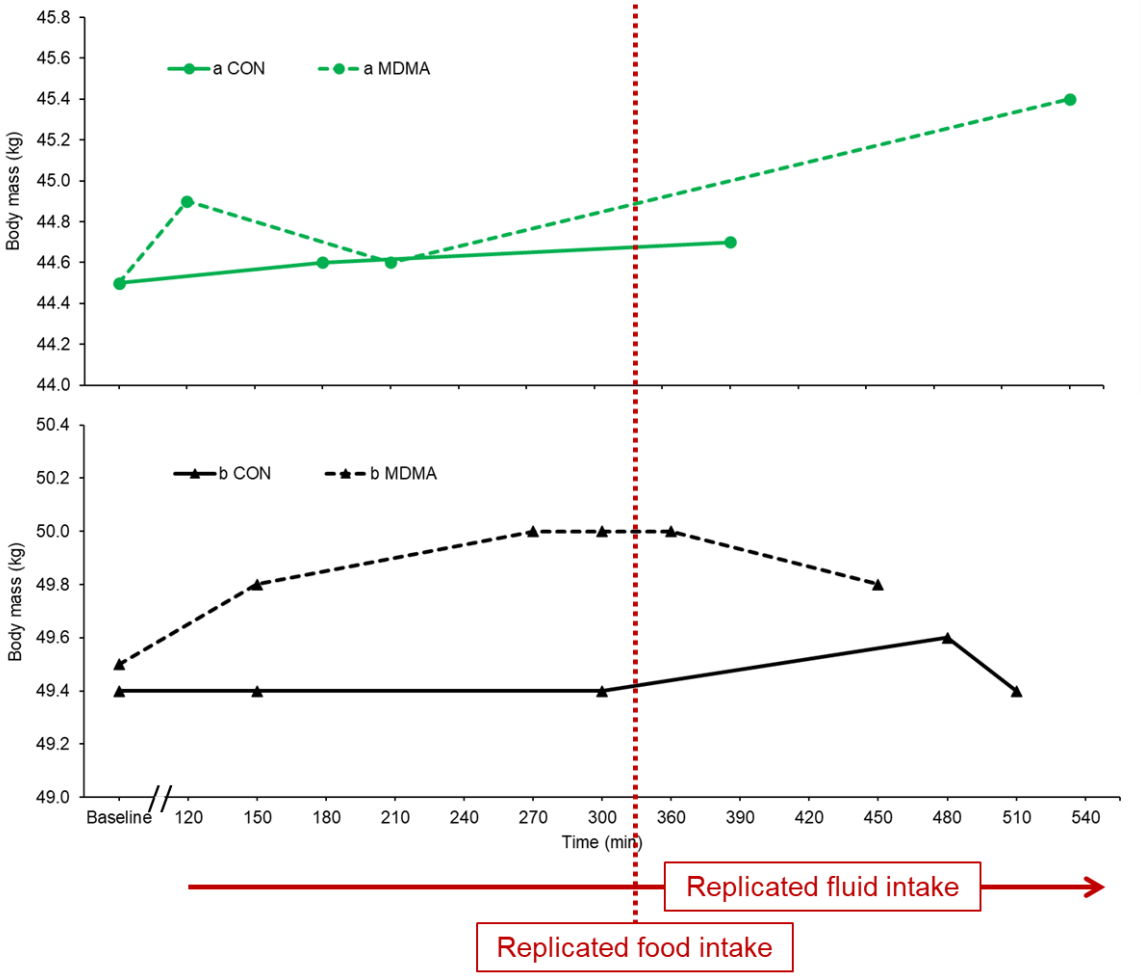

Figure 2. Body mass across the experimental period for *Experimenter-a* and *b*. Abbreviations: CON, control trial arm; MDMA, 3,4-Methylenedioxymethamphetamine trial arm

Urinary markers

The trends in body mass corroborate with trends in urine volume (**Figures 3** and **4**). Both experimenters urinated more frequently on MDMA over the testing period (*Experimenter-a* n = 3 CON, n = 4 MDMA; *Experimenter-b* n = 5 CON, n = 6 MDMA); however, the urine volume of each void was lower, with only one exception for *Experimenter-a*. Broadly speaking, as urine volume decreased, urine specific gravity increased, indicating more concentrated urine during MDMA (**Figure 5**); such an effect was minimal in *Experimenter-a*, whereas *Experimenter-b* showed a rapid increased in urine specific gravity during MDMA relative to CON during the first half of testing, followed by slightly lower specific gravity than CON during the latter hours of testing.

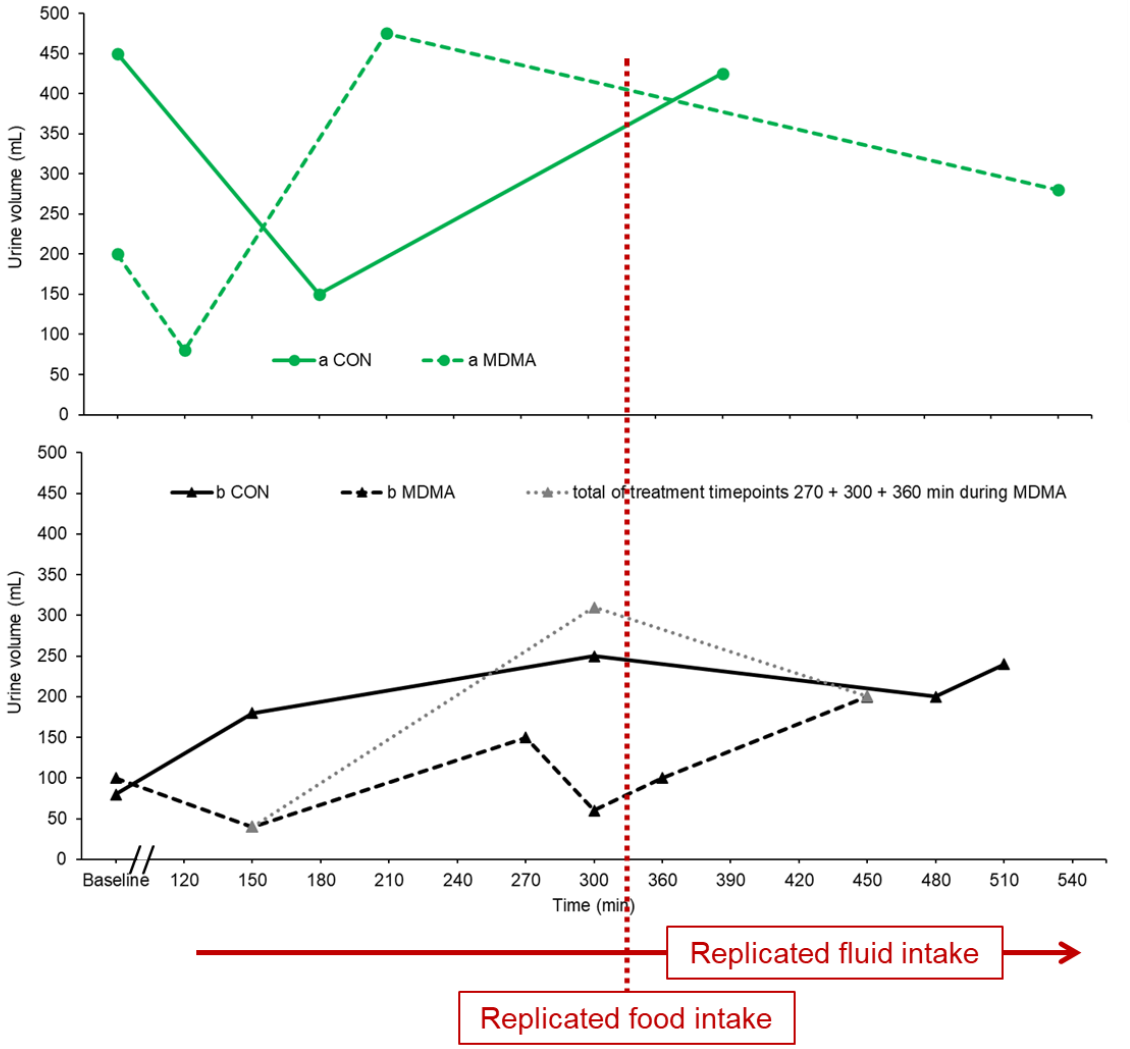

Figure 3. Urine volume across the experimental period for *Experimenter-a* and *b*. Abbreviations: CON, control trial arm; MDMA, 3,4-Methylenedioxymethamphetamine trial arm

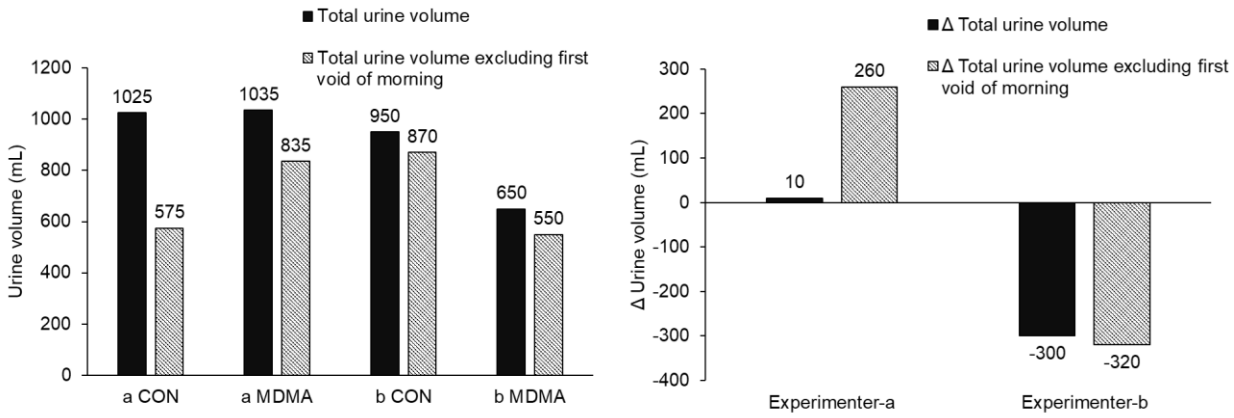

Figure 4. Urine volume across the testing period; change in urine volume between trial arms. Abbreviations: CON, control trial arm; MDMA, 3,4-Methylenedioxymethamphetamine trial arm

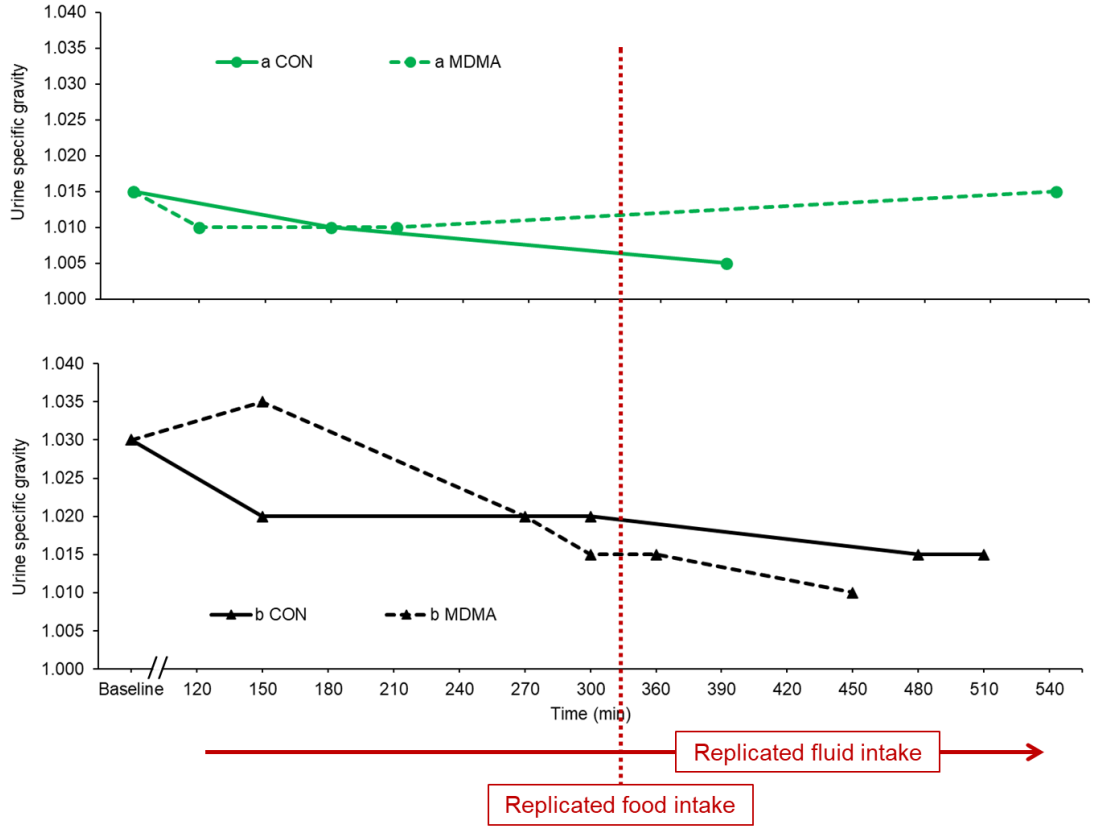

Figure 5. Urine specific gravity across the experimental period for *Experimenter-a* and *b*. Abbreviations: CON, control trial arm; MDMA, 3,4-Methylenedioxymethamphetamine trial arm

Plasma copeptin concentration

Plasma copeptin concentration increased after MDMA administration (but not during CON) for *Experimenter-b* only; *Experimenter-a* had similar plasma copeptin concentrations between both trial arms (**Figure 6**). For *Experimenter-b*, this elevation occurred prior to glucose ingestion (timepoint 0, i.e. within 60 minutes of MDMA administration), and peaked at 15 minutes post-glucose ingestion reaching 13.07 pmol·L^{-1} (Δ 9.99 pmol·L^{-1} relative to CON). The peak was followed by a gradual decline, with slightly lower than CON levels by 240 minutes post-glucose ingestion. During CON, copeptin concentrations remained stable across the testing period (within ~3 pmol·L^{-1}). For both experimenters, the almost immediate post-OGTT fluid (tea) and later food ingestion did not appear to impact copeptin concentration/dynamics in either trial arm.

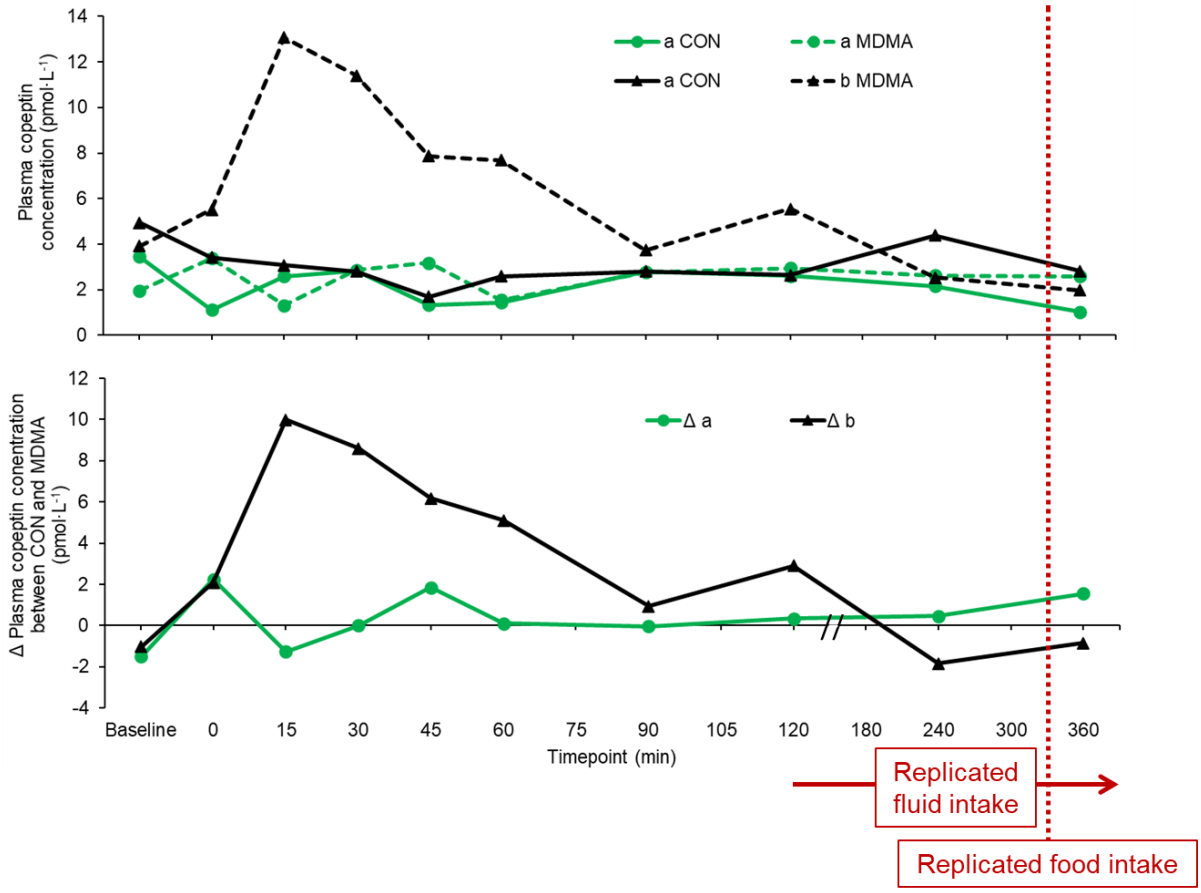

Figure 6. Plasma copeptin responses across the experimental period for *Experimenter-a* and *b*. Abbreviations: CON, control trial arm; MDMA, 3,4-Methylenedioxymethamphetamine trial arm

Plasma electrolytes and metabolites

Calculated plasma osmolality is shown in **Figure 7**. Due to the estimated nature of this measure, data should be considered cautiously, particularly as the range of change seems outwith typical values (i.e. > 10 mOsm·kg^{-1}). These data infer that during MDMA (relative to CON) plasma osmolality either increased or remained unchanged for *Experimenter-a*, but remained lower or was unchanged during MDMA relative to CON for *Experimenter-b*.

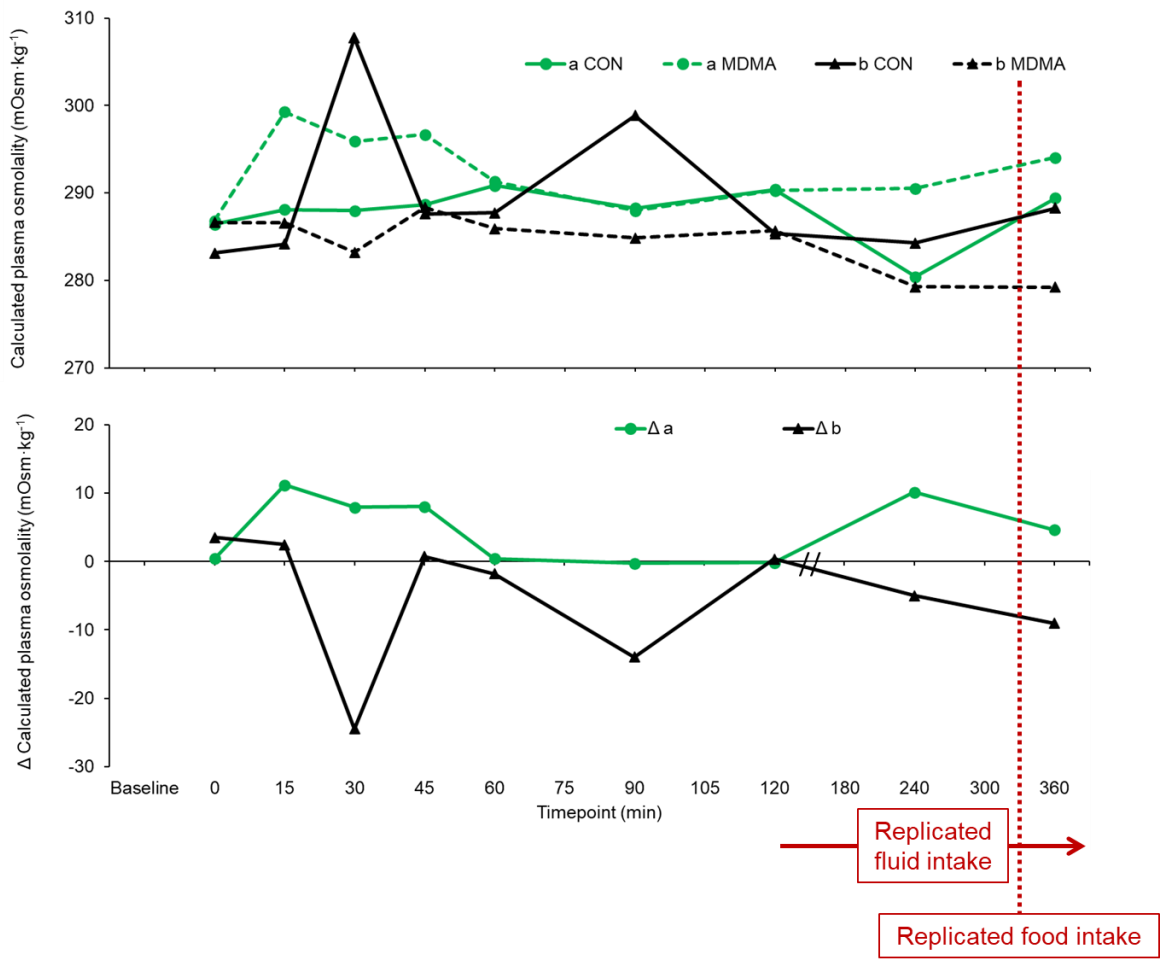

Figure 7. Calculated plasma osmolality across the experimental period for *Experimenter-a* and *b*. Abbreviations: CON, control trial arm; MDMA, 3,4-Methylenedioxymethamphetamine trial arm

The overall trend for plasma sodium (Na) levels was similar to calculated osmolality: *Experimenter-a* had similar or slightly higher Na during MDMA relative to CON (Δ range from -1 to 5 mEq·L^{-1}), whilst *Experimenter-b* had slightly lower Na during MDMA relative to CON (Δ range from -1 to -6 mEq·L^{-1}, with one outlier of Δ -14 mEq·L^{-1}). However, none of these absolute values were < 135 mEq·L^{-1}; thus there was no indication of clinically meaningful hyponatraemia. Interestingly, at the final timepoint, which was postprandial (after standardised within-experimenter food intake), plasma Na increased during CON, but decreased for both experimenters during MDMA.

In terms of other electrolyte/metabolite trends, overall: urea was similar during CON and MDMA for *Experimenter-a*, but higher during MDMA for *Experimenter-b*; chloride was higher during MDMA for *Experimenter-a*, but lower for *Experimenter-b* compared to CON; and creatinine was broadly similar (i.e. with fluctuations) for *Experimenter-a* during both trial arms, but lower during MDMA for *Experimenter-b* compared to CON (both experimenters' creatinine in both trial arms trended downwards throughout the testing period). All values were within expected norm ranges, or if anything slightly (but not concerningly) lower, with the exception that one measure for chloride at timepoint 30 min was slightly high for *Experimenter-b* during CON.

Gluco-regulation

Glucose regulation

Whole blood glucose concentrations were taken on the testing day and provided vastly different results to plasma glucose concentrations, perhaps due to contamination with EDTA which glucose sticks are not validated for. Considering the superior reliability of laboratory autoanalysers compared to glucose sticks, whole blood glucose data are shown in the supplementary material at the end of this document, and only plasma glucose data are discussed herein.

Plasma glucose concentration can be seen in **Figure 8**. Due to resource restraints, the baseline sample was unable to be measured for plasma glucose. One hour after consuming the MDMA (in a fasted state), plasma glucose was slightly higher in both experimenters relative to the same timepoint in CON (*Experimenter-a* +0.5 mmol·L^{-1}; *Experimenter-b* +1.3 mmol·L^{-1}). Postprandially, *Experimenter-a* had a peak during MDMA at 30 min post-glucose ingestion (glucose concentration at 30 min 10.9 mmol·L^{-1} MDMA *versus* 7.0 mmol·L^{-1} CON). Their glucose peak occurred later, at 45 min post-glucose ingestion, during CON and was lower (7.6 mmol·L^{-1}). *Experimenter-a* also had a second, smaller, peak of glucose at 60 min during CON. Other than these key differences, *Experimenter-a* had roughly similar plasma glucose concentrations throughout the rest of the testing phase.

Conversely, *Experimenter-b* had a clear postprandial glucose curve during CON, peaking at 60 min (6.5 mmol·L^{-1}), whereas their plasma glucose was more erratic during MDMA. During MDMA, their plasma glucose initially peaked earlier at 15 min (8.4 mmol·L^{-1}), dropping at 30 min (7.0 mmol·L^{-1}), and rising again at 45 min (8.3 mmol·L^{-1}). After this, *Experimenter-b* experienced hypoglycaemia during MDMA relative to CON at 90 min post-glucose ingestion (Δ -2.6 mmol·L^{-1}), returning to roughly pre-glucose ingestion levels by 120 min, followed by no distinct differences between MDMA and CON for the remaining timepoints.

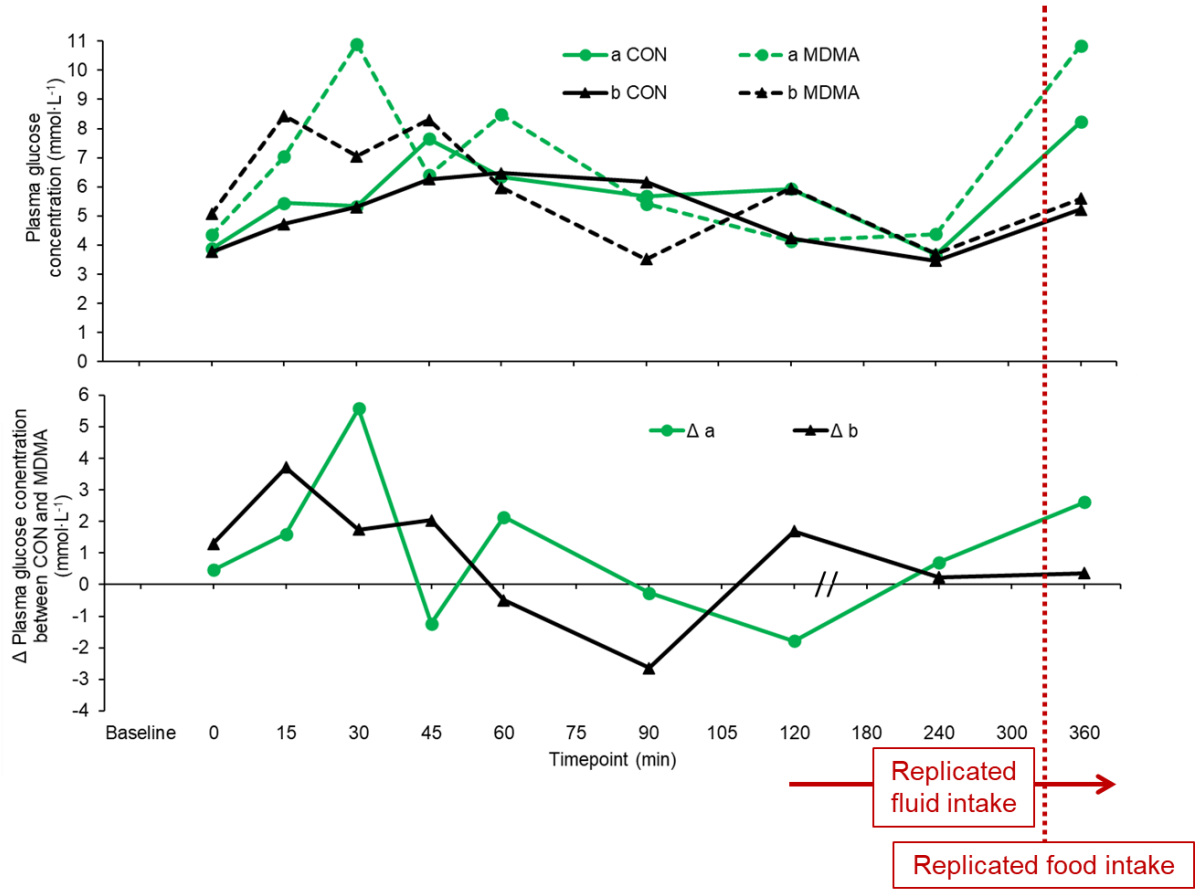

Figure 8. Plasma glucose responses across the experimental period for *Experimenter-a* and *b*. Abbreviations: CON, control trial arm; MDMA, 3,4-Methylenedioxymethamphetamine trial arm

Area under the curve for plasma glucose concentrations are presented in **Table 3**, showing that during the OGTT (timepoints 0-120 min) both experimenters had a trend for overall higher plasma glucose concentrations during MDMA relative to CON, with this effect being stronger for *Experimenter-a*. Incremental AUC showed concordant findings to AUC for *Experimenter-a*, with evidence of hyperglycaemia during MDMA compared to CON, but discordant findings for *Experimenter-b*, with more periods of hypoglycaemia than hyperglycaemia during MDMA compared to CON, relative to baseline (fasting) values.

Table 3. Plasma blood glucose area under the curve (mmol·120 min·L^{-1})

	Control arm	MDMA arm	Difference
	Area under the curve		
Experimenter-a	707.69	813.47	105.78
Experimenter-b	666.99	724.13	57.14
	Incremental area under the curve		
Experimenter-a	236.60	303.88	67.27
Experimenter-b	222.64	100.32	-122.32

Abbreviations: MDMA, 3,4-Methylenedioxymethamphetamine

Insulin regulation

Plasma insulin trends can be seen in **Figure 9**. At baseline, insulin concentrations were similar for both experimenters in both trial arms. One hour after consuming the MDMA (in a fasted state) plasma insulin was slightly higher in both experimenters relative to the same timepoint in CON (*Experimenter-a* +2.42 pmol·L^{-1}; *Experimenter-b* +6.47 pmol·L^{-1}). Postprandially, *Experimenter-a* had a similar insulin curve, but this was consistently higher during MDMA *versus* CON; as such peak insulin concentration took longer to reach with MDMA (45 min at 58.94 pmol·L^{-1} MDMA *versus* 30 min at 22.34 pmol·L^{-1} CON). After the OGTT, there were no discernible differences in plasma insulin concentrations for *Experimenter-a*.

Experimenter-b followed a similar trend of higher plasma insulin during MDMA relative to CON, however this response was vastly exaggerated compared to *Experimenter-a*. Peak insulin concentration occurred at 30 min (782.28 pmol·L^{-1}) for MDMA, compared to 45 min (58.15 pmol·L^{-1}) for CON. Additionally, *Experimenter-b* experienced a slight rebound of hyperinsulinaemia at 120 min. As with *Experiment-a*, *Experimenter-b* had similar post-OGTT insulin concentrations during MDMA and CON.

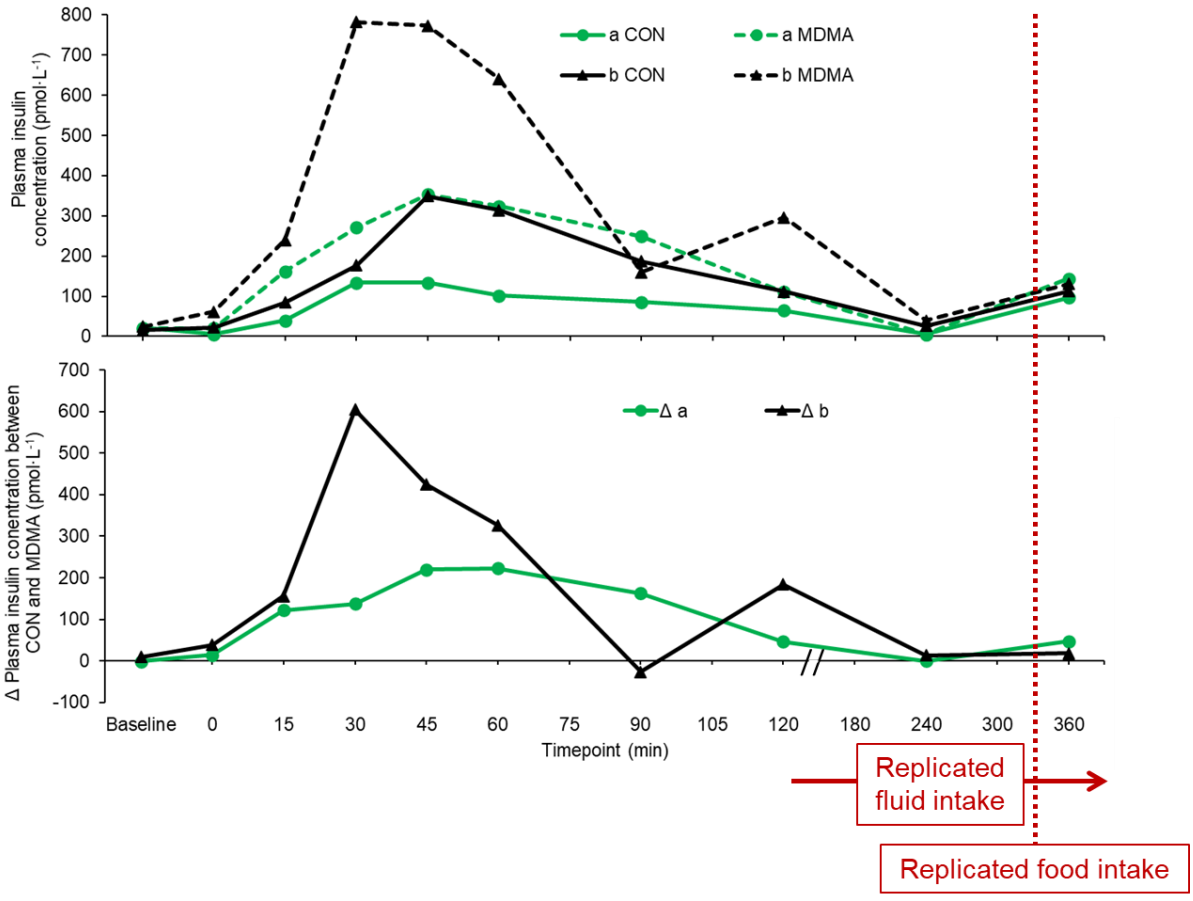

Figure 9. Plasma insulin responses across the experimental period for *Experimenter-a* and *b*. Abbreviations: CON, control trial arm; MDMA, 3,4-Methylenedioxymethamphetamine trial arm

Area under the curve for insulin is presented in **Table 4**, showing that during the OGTT (timepoints 0-120 min) both experimenters had notably higher plasma insulin concentrations during MDMA relative to CON, with this effect being stronger for *Experimenter-b*. Incremental AUC showed concordant findings to AUC for both experimenters. Insulin resistance (estimated from HOMA2) appeared to increase in both experimenters, with a larger effect in *Experimenter-b* (**Table 4**).

Table 4. Plasma insulin area under the curve (pmol·120 min·L^{-1}) and HOMA2

	Control arm	MDMA arm	Difference
	Area under the curve		
Experimenter-a	10488.92	28387.27	17898.36
Experimenter-b	23661.21	51038.01	27376.80
	Incremental area under the curve		
Experimenter-a	9750.08	26371.28	16621.2
Experimenter-b	20808.07	41870.95	21062.88
	HOMA2*		
Experimenter-a	-	0.37	-
Experimenter-b	0.39	1.15	0.76

*HOMA2 was unable to be calculated when insulin concentrations were < 20 pmol·L^{-1}
Abbreviations: HOMA2, Homeostasis Model Assessment 2; MDMA, 3,4-Methylenedioxymethamphetamine

Cortisol response

The plasma cortisol response can be seen in **Figure 10**. Overall, both experimenters had a drop in cortisol during CON and an increase (*Experimenter-b*) or no notable change from baseline (*Experimenter-a*) in cortisol during MDMA. In the fasted state, during MDMA, *Experimenter-a* had a decrease in cortisol pre- to post-MDMA ingestion (-76 nmol·L^{1}), which increased in the postprandial state. *Experimenter-a* had an earlier (30 min) and lower (604 nmol·L^{1}) peak than *Experimenter-b* (60 min, 741 nmol·L^{1}, respectively). *Experimenter-a* reached baseline or lower levels earlier than *Experimenter-b* (45 min *versus* 120 min, respectively). By the final blood sample, *Experimenter-b* ended on roughly the same cortisol levels during MDMA as CON, whereas *Experimenter-a* remained elevated (+179 nmol·L^{1}).

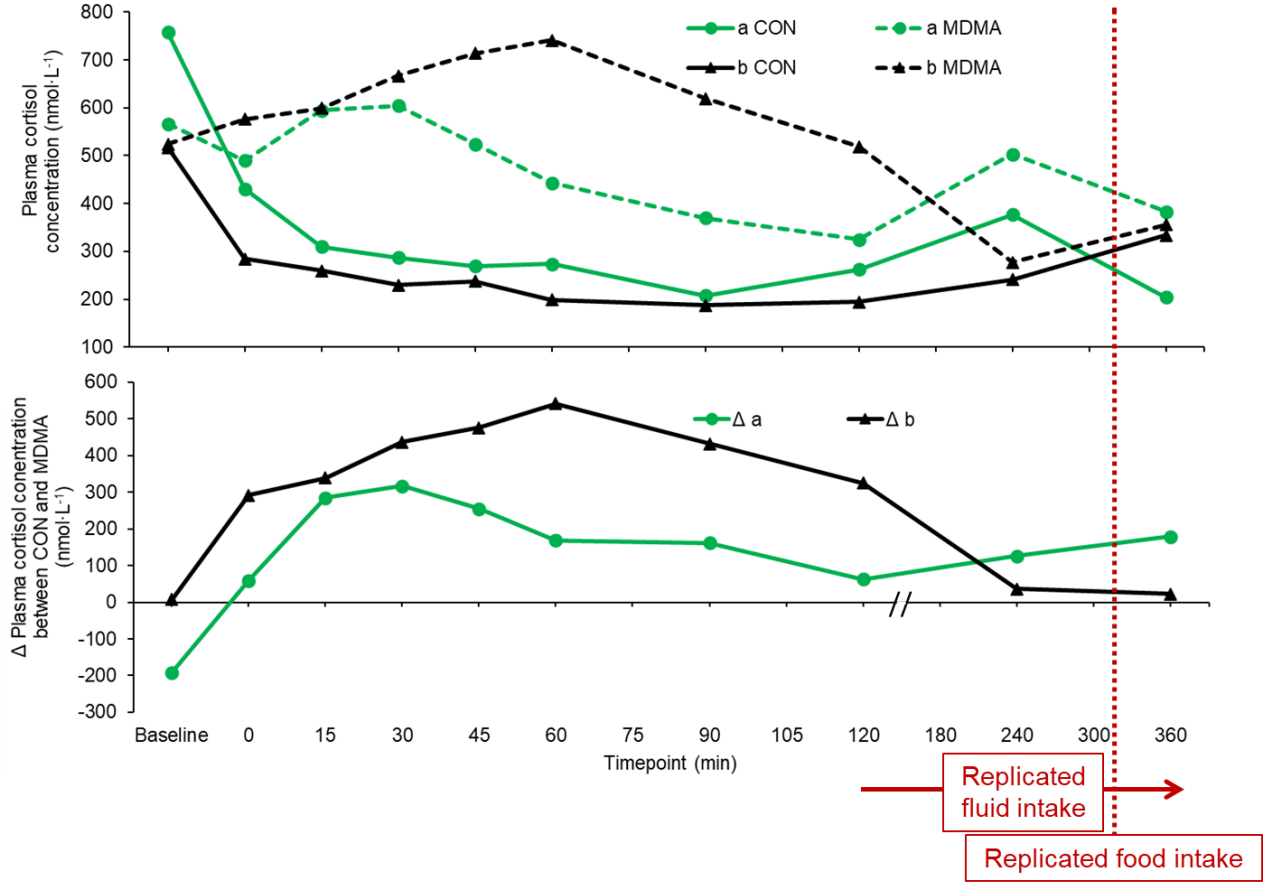

Figure 10. Plasma cortisol responses across the experimental period for *Experimenter-a* and *b*. Abbreviations: CON, control trial arm; MDMA, 3,4-Methylenedioxymethamphetamine trial arm

Appetite (visual analogue scales)

Due to the variability in some of these measures, along with *Experimenter-b* recalling some confusion with the scales during the MDMA trial arm (specifically understanding the meaning of the questions in relation to the appetite scales), these results should probably be interpreted with caution. As such, these are only discussed briefly.

Thirst appetite

Thirst ratings can be seen in **Figure 11**. Overall, thirst was notably higher during MDMA for *Experimenter-b* compared to CON, with this effect wearing off towards the end of the testing period (360 min). Comparatively, *Experimenter-a* had similar thirst ratings in both trial arms; if anything, their thirst was slightly lower during MDMA. Experimenters also rated how dry their mouth felt, and these ratings nearly exactly matched those of thirst (data not shown).

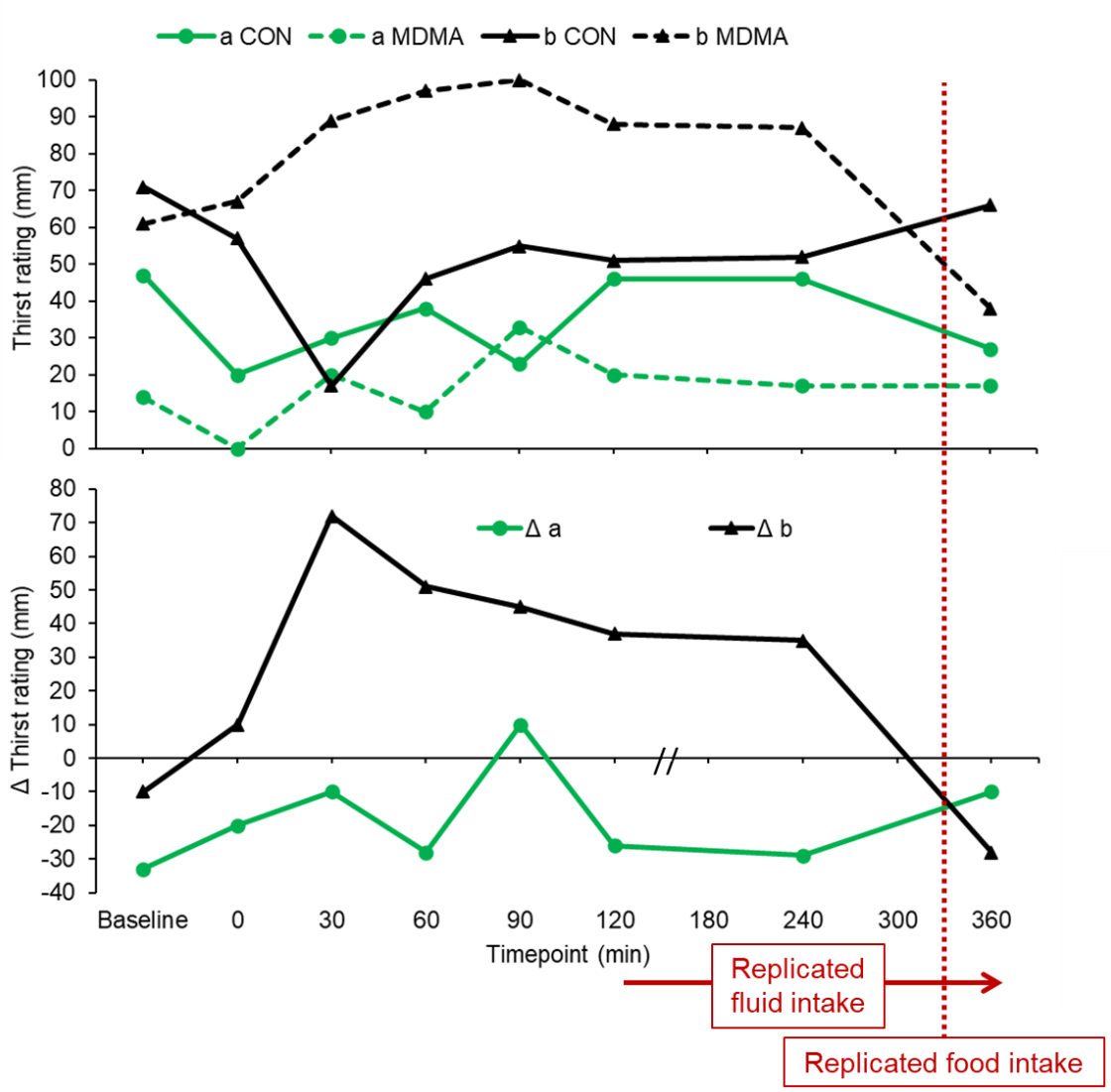

Figure 11. Thirst ratings across the experimental period for *Experimenter-a* and *b*. Abbreviations: CON, control trial arm; MDMA, 3,4-Methylenedioxymethamphetamine trial arm

Food appetites

Experimenter-b rated hunger as higher if anything during MDMA, whereas *Experimenter-a* rated hunger as lower during MDMA compared to CON. Whilst the magnitude of difference was larger between MDMA and CON, how much experimenters felt they could eat and how empty their stomachs felt roughly matched hunger ratings, and fullness ratings were roughly the inverse of hunger; these measures were somewhat erratic and hard to interpret however.

In terms of desires, *Experimenter-a* desired salt more strongly (ranging from 7-30 mm higher) during CON relative to MDMA; *Experimenter-b* had no difference in salt desires between trial arms (all rated 0 mm). For savoury desire, MDMA resulted in slightly lower desire in *Experimenter-a* with no discernible difference between trials for *Experimenter-b*. Differences emerged after the OGTT, however, whereby savoury desire increased rapidly for *Experimenter-b* on MDMA relative to CON, whereas *Experimenter-a* had higher post-OGTT savoury desire in CON, relative to MDMA. There were no differences in sweet desire for

either trial (nearly all points scored 0 mm for both experimenters). *Experimenter-a* had a strong desire for fatty 30 minutes into the OGTT in CON, but not MDMA, with no other notable differences between trials for either experimenter.

Wakefulness

Overall, *Experimenter-a* was more alert during MDMA compared to CON, and this was consistent across the full 360 min testing period. *Experimenter-b* reported higher alertness during CON during the first 60 min of testing, and higher alertness during MDMA thereafter. Tiredness ratings were more erratic for both experimenters making them harder to interpret. Broadly, *Experimenter-a* was more tired during CON for the first 90 min, thereafter tiredness ratings were similar. Conversely, *Experimenter-b* was more tired during MDMA for the first 90 min, then more tired during CON for the remaining testing period.

Mood

Both experimenters rated higher happiness during MDMA, though *Experimenter-b* had a much larger magnitude of difference with the greatest difference at 90 min (45 mm CON *versus* 95 mm MDMA); *Experimenter-a* had similar ratings for many of the timepoints with the greatest difference between trial arms also at 90 min (23 mm CON *versus* 51 mm MDMA). Sadness ratings were comparable between trial arms for *Experimenter-a*, whereas *Experimenter-b* rated sadness consistently lower during MDMA.

Experience

Experimenter-a had no desire to clench their jaw in either trial arm; *Experimenter-b* rated this desire at or near 100 mm (on a 100 mm scale) for the 120 min OGTT period during MDMA, after which this desire gradually decreased to 29 mm by 360 min (comparatively, these ratings were 0 mm during CON).

Whilst both experimenters were female, of a similar age and BMI, at the same estimated phase of their menstrual cycle, and took the same dosage of MDMA, their reported subjective experiences of the study were vastly different. *Experimenter-a* reported feeling the effects of MDMA, notably a ‘buzz’; however, they were still able to function effectively as an experimenter. On the contrary, *Experimenter-b* felt all the expected effects of MDMA overwhelmingly, including the desire for human contact (resulting in cuddling *Experimenter-a* under a blanket unhelpfully between blood samples), stroking soft materials, excessive talking, mild tremor/shaking, and feelings of euphoria (“ecstasy”). Accordingly, *Experimenter-b* was unable to be of use in terms of processing bloods and helping run the study until towards the end of the OGTT as the MDMA effects wore off. After the OGTT, both experimenters felt some fatigue, though their MDMA hangover (‘comedown’) felt less intense and barely noticeable than previous MDMA experiences, perhaps due to the (relative) calm context compared to usual settings of MDMA use, and the time of day the drug was administered, mitigating chrono-disruption.

Discussion

This non-randomised controlled self-experiment conducted by two female scientists overall found MDMA to cause fasting and postprandial gluco-dysregulation (variable and somewhat erratic plasma glucose and elevated plasma insulin). This finding did not support our hypothesis of no effect of MDMA on gluco-regulation. A key mechanism previous literature had proposed gluco-dysregulation to occur from was an elevation in plasma copeptin concentrations (as a marker of AVP) which would trigger the HPA-axis (cortisol) and/or activate V1 receptor (leading to glycogenolysis) (Carroll *et al.*, 2016; Carroll & James, 2019; Koshimizu *et al*, 2021; Melander, 2016; Spruce *et al*., 1985). However, only one experimenter demonstrated copeptin elevations with MDMA, whilst both experimenters experienced elevated cortisol and gluco-dysregulation. Thus, AVP may not be a necessary factor in MDMA-mediated gluco-dysregulation. However, AVP may be a contributory rather than a causal factor, explaining the greater hyperinsulinaemia in the experimenter who had elevated copeptin after MDMA compared to the experimenter whose copeptin did not increase after MDMA.

Individual responses

Both experimenters had vastly different subjective experiences, presenting the possibility for responders and non-responders. Previous research has suggested ~10 % of users are 'non-responders' to the subjective effects of MDMA (Peroutka *et al*., 1988); many factors might contribute to disparities in experiences, including MDMA metabolism via cytochrome P450 isozyme CYP2D6 activity (Studerus *et al.*, 2021). In the present study, the subjective differences in responses seemingly correlated with the different copeptin responses to MDMA in each experimenter.

Whilst legitimate non-response in *Experimenter-a* cannot be ruled out, one explanation for the differences in both the subjective effects and the copeptin response between the experimenters might be the use of selective serotonin reuptake inhibitors (SSRIs) in *Experimenter-a*. The SSRI used by *Experimenter-a* was 20 mg/d fluoxetine (which was taken as normal prior to the study). Previous research has shown that intravenous administration of the SSRI citalopram reduced the subjective (Leichti *et al.*, 2000) and cardiovascular (Leichti & Vollenweider, 2000) effects of MDMA in humans. Further, in men, 3 days of oral paroxetine reduced both subjective and physiological effects, despite a 30 % increase in plasma MDMA concentration (Farré *et al.*, 2007). Another study using 5 days of 20 mg fluoxetine found an attenuation of most of the positive subjective effects of MDMA (Tancer & Johanson, 2007). In these studies, AVP/copeptin, cortisol, nor gluco-regulation were measured. However, these findings are in accordance with the experience of *Experimenter-a*.

Serotonin can act to inhibit AVP (Ferris, 2000; Ishizuka *et al.*, 2010); however, the effect of SSRIs directly on AVP in humans is unclear (Golyszny & Obuchowicz, 2019; Kirchner *et al.,*1998), particularly considering rare instances (typically in older adults) of SSRI-induced hyponatraemia caused by the syndrome of inappropriate antidiuretic syndrome (Inaguma *et al.*, 2000), counter to our n-of-1 findings. Although generalisable inferences cannot be made with n = 1, it is noteworthy that *Experimenter-a* did not have a copeptin response to MDMA. Whether this was causally due to their chronic SSRI usage, a symptom of the illness the SSRIs were being used for, or they were legitimately a non-responder is unclear.

Nonetheless, such a finding offers the hypothesis that (in some people) chronic use of SSRIs mitigate the AVP response to MDMA, and this in part may explain the reduced subjective effects of MDMA in SSRI users. Further causal human research is certainly warranted.

MDMA exerts some of its subjective effects via oxytocin release (Kirkpatrick *et al.*, 2014). Oxytocin is molecularly nearly identical to AVP, with evidence suggesting some cross-talk between these molecules and their receptors (Song & Albers, 2018). Thus, the different responses in copeptin may represent differences in oxytocin and AVP/oxytocin receptor binding and could help explain the two experimenters' unique experiences.

If this is the case, AVP may have a significant role to play in MDMA-induced gluco-dysregulation, but only in 'responders'. This idea may be supported by the nearly identical trends during MDMA in copeptin and insulin responses in *Experimenter-b* (the 'responder'). Further, the gluco-regulatory responses between the two experimenters were opposing: *Experimenter-a* (MDMA 'non-responder') had higher glucose AUC, but lower insulin AUC during MDMA compared to *Experimenter-b*; accordingly it seems *Experimenter-a*'s (the 'non-responder') insulin was less responsive at mitigating hyperglycaemia, particularly during the first 90 min of the OGTT, and after food ingestion prior to the final measure at 360 min. Conversely, *Experimenter-b* had nearly similar glucose AUC during MDMA and CON, with much greater insulin release throughout nearly the entire testing period (up to 240 min). This may indicate insulin resistance as hyperinsulinaemia was required to maintain glucose homeostasis (though this did also appear to cause significant hypoglycaemia, as confirmed by differences in AUC and iAUC). Such a finding perhaps suggests that MDMA may result in poorer homeostatic regulation of glucose, particularly in 'responders'.

This conclusion is supported by the increase in (fasting) insulin resistance, as measured by HOMA2, in both experimenters after ingestion of MDMA. In both experimenters though, the primary gluco-dysregulation was seen in their insulin response, which warrants further investigation as it suggests MDMA-mediated hyperinsulinaemia in the context of 75 g sugar ingestion occurs via other mechanisms, not just (or at all) AVP. Previous research has yielded unclear results regarding the gluco-regulatory impacts of MDMA ingestion. In rats, MDMA has caused hypoglycaemia (Soto-Montenegro *et al.*, 2007), and hyperglycaemia (in obese Zucker rats) and hyperinsulinaemia (Banks *et al.*, 2009), whereas in an uncontrolled (i.e. *ad libitum* food and fluid intake) natural setting in humans, no obvious effect on blood sugar was found (Downing, 1986). Thus, our present work in a highly controlled setting using standardised metabolic testing (OGTT) partially supports some previous findings, with disparities in findings likely due to differences in methodologies.

Since publishing version 1 of this manuscript (June 2021), we have measured cortisol; these results offer insight into the gluco-regulatory response during MDMA. In both experimenters, MDMA resulted in an elevation in cortisol relative to CON. In *Experimenter-b*, this elevation was above baseline values for most of the OGTT. Comparatively, *Experimenter-a*'s cortisol remained at or below baseline values during MDMA, relative to a much greater reduction compared to baseline during CON. The elevation (relative to CON) in cortisol is overall in line with previous research (e.g. Dolder *et al.*, 2018; Harris *et al.*, 2002; Hysek *et al.,* 2012; Hysek *et al.*, 2014; Wolff *et al.*, 2012). Cortisol is known to increase hepatic gluconeogenesis and blood glucose concentrations (Khani & Tayek, 2001). Thus, it appears there may be

competing effects causing glucose dysregulation during MDMA in the present study: 75 g maltodextrin was orally ingested resulting in an increase of (exogenously-sourced) blood glucose; simultaneously MDMA triggered cortisol secretion, increasing (endogenously produced) blood glucose concentrations; and resultantly insulin responded to both stimuli. This insulin response was therefore exaggerated, perhaps explaining the erratic gluco-regulation seen in both experimenters (Figure 12).

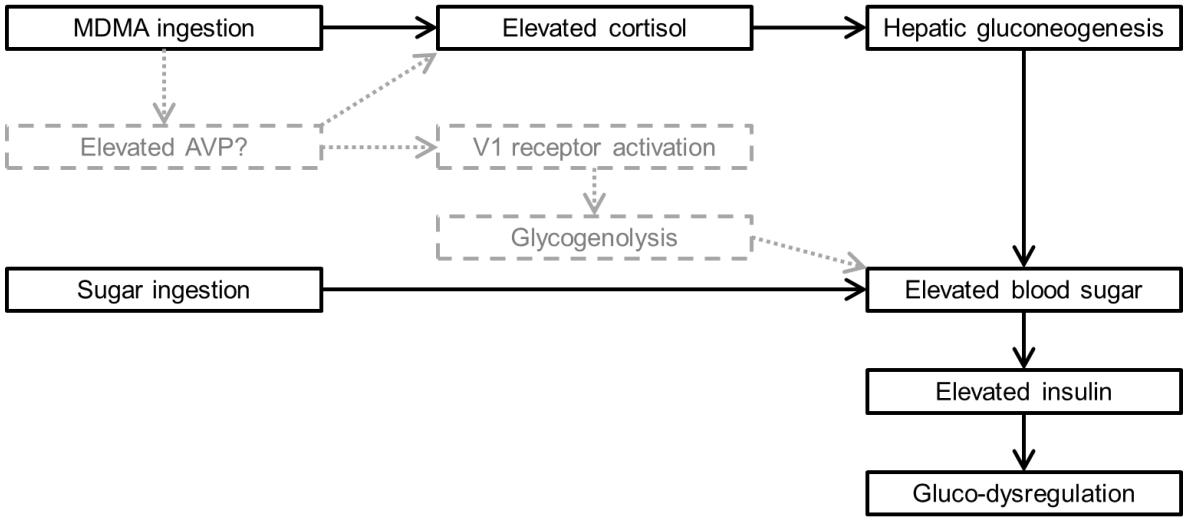

Figure 12. Hypothetic pathway potentially explaining the gluco-dysregulation seen in this experiment. Grey dashed relationships and mechanisms represent a second pathway only seen in one of the two experimenters. Abbreviations: AVP, arginine vasopressin; MDMA, 3,4- Methylenedioxymethamphetamine

From our present data, it appears that AVP is not the primary factor in the MDMA-induced cortisol response. As *Experimenter-b* had higher cortisol and higher copeptin, it may be that AVP had a contributory effect. However, this seems unlikely considering the time-trend responses of copeptin and cortisol were highly discordant. Thus it seems under MDMA, AVP is acting outwith the HPA axis, and likely acting via hypothalamic magnocellular neurons. This pathway also delivers oxytocin to peripheral circulation (Goncharova, 2013), and supports the hypothesis above regarding AVP-oxytocin cross-talk under MDMA. Nonetheless, Dolder *et al.* (2018) found improved subjective affect, despite a lack of increase in AVP, suggesting AVP is not needed for the subjective experience of MDMA. In all, these findings may imply that MDMA-induced cortisol acts partially or fully independently of AVP. We have recently demonstrated that in humans AVP (copeptin) can act seemingly independently of the HPA axis with acute physical stress (Carroll & Melander, 2021), adding credence to this notion, and (at least partially) discounting AVP actions on the HPA axis from gluco-dysregulation.

Hydration and gluco-regulation

The present study is difficult to interpret in relation to previous studies investigating hydration/AVP and gluco-regulation. In terms of cortisol, hypohydration-induced elevations appear to be specific to exercise contexts (Zaplatosch & Adams, 2020). Exercise contexts may translate to popular MDMA use (considering clubbing is a form of exercise in a typically

hyperthermic environment), but seem less relevant to the present study and therapeutic use. In the one study from the review by Zaplatosch & Adams that did not use an exercise dehydration protocol, cortisol did not differentially respond according to hydration status (i.e. high *versus* low copeptin) (Carroll *et al.*, 2019a), in line with the present findings.

In terms of direct gluco-regulation, medication-withdrawn patients with type 1 (Burge *et al.*, 2001) and 2 diabetes (Johnson *et al.*, 2017), hypohydration induced hyperglycaemia. We have previously hypothesised that this was likely due to increased glucosuria when patients were euhydrated, rather than hydration physiology *per se* (Carroll & James, 2019). However, it is interesting to observe that the postprandial cortisol response was similar between hypohydrated participants with (uncontrolled) type 2 diabetes (Johnson *et al.*, 2017) and the present study (particularly *Experimenter-b*). Though it should be noted that Johnson *et al.* did not measure AVP or copeptin.

In healthy participants, acute hypohydration does not impact glycaemia or insulinaemia, despite copeptin concentrations increasing approximately five-fold (i.e. more than that seen herein with *Experimenter-b*) (Carroll *et al.*, 2019a). Intravenous infusion of AVP to supraphysiological levels has resulted in fasting hyperglycaemia and elevated glucagon, but no change in insulin (Spruce *et al.*, 1985); thus the present results cannot be likened with the gluco-regulatory effects of exogenous AVP either since copeptin remained within an expected (albeit high in *Experimenter-b*) physiological range. Enhörning *et al.* (2019a) found copeptin 'responders' and 'non-responders' to a one-week increased water intervention; in 'responders', glucose and insulin did not change, but glucagon decreased. Extending this intervention to six weeks showed a small, statistically significant reduction in fasted plasma glucose (-0.2 $mmol \cdot L^{-1}$) (Enhörning *et al.*, 2019b); it is unclear if this small glucose reduction has clinical relevance though. Importantly, these findings were in those with elevated copeptin and urine concentration at baseline, unlike the present study.

Such differences between hydration-glycaemia work and the present study cast doubt upon the AVP-HPA-glycaemia hypothesis. However, the work of Enhörning *et al.* offers insights into 'responders' and 'non-responders' and the AVP-lowering effects of water intake. The present work extends these findings to the AVP response to MDMA, supporting previous MDMA research. MDMA ingestion does not always/reliably elevate circulating copeptin concentrations (Baggott *et al.*, 2016), and typically copeptin is elevated more in women than men after MDMA (Simmler *et al.*, 2011). Unlike a water intervention, it is currently unclear who might be a copeptin 'responder' to MDMA. Understanding this has implications for MDMA therapeutics, as many diseases co-occur with elevated copeptin which is associated with increased risk of adverse cardiovascular and renal events (Enhörning *et al.,* 2011; Enhörning *et al.*, 2015; Velho *et al.*, 2013; Velho *et al.*, 2018).

It is currently unclear whether an acute rapid increase in AVP (i.e. similar to that induced by MDMA) may pose an avoidable health risk, particularly in those with already elevated AVP, such as people with type 2 diabetes. This offers a difficult paradox: elevations of AVP (inferred from copeptin) are associated with adverse cardiovascular and renal events, and water is typically a reliable way to reduce circulating AVP levels. However, in the context of MDMA, consumption of water may result in hyponatraemia. Future research should understand the interaction between MDMA-induced AVP elevations, whether and how this can be mitigated by fluid ingestion, and work on identifying high-risk patients (e.g. a pre-

MDMA water intake prescription may be warranted). Further, based on the current data, it is unclear whether there was a true MDMA-AVP-feeding interaction, or whether the rapid elevation of copeptin in *Experimenter-b* would have occurred if the experimenter remained in a fasted state. Previous work has shown no interaction between copeptin responses and feeding under normal conditions (Carroll *et al.*, 2019a,b), though it is unclear if these findings can be extrapolated to an MDMA setting. Thus consumption of food shortly before/after MDMA ingestion may pose an additional risk to patients who may not be excluded from MDMA therapy based on current exclusion criteria. Equally, whether gluco-dysregulation would occur in the fasted state during MDMA in humans should be investigated.

The difficult-to-interpret results in relation to previous hydration-based research could be due to the conflicting state of hyperhydration and high AVP found in the present study (for *Experimenter-b* at least). This dynamic offers a unique insight by uncoupling the physiology of hydration (e.g. endogenous AVP) from the state of hydration (i.e. total body water) (Carroll & James, 2019). In previous research copeptin has been elevated via loss of body water, whereas the present research induced an increase in copeptin with a concomitant increase (or no change) in body mass, which is assumed to be primarily from water (i.e. the water co-consumed with the MDMA, the OGTT, and the within-experimenter standardised fluid intake post-OGTT). Since the present study found some form of gluco-dysregulation in both experimenters, but only elevated copeptin in one experimenter, this study appears to support a lack of clear relationship between AVP and gluco-dysregulation and such a finding appears independent of body water status. Additionally, it appears any hypothetical beneficial gluco-regulatory effects of (hepatocyte) cell swelling from water retention (Baquet *et al*., 1990; Haussinger, 1996; Graf *et al.*, 1988; Meijer *et al*., 1992; Peak *et al*., 1992) did not outweigh the detrimental gluco-regulatory effects of MDMA. Fluid intake pre- and during the OGTT was minimised and standardised to try and mitigate any confounding effects of excess water intake. It is, however, unclear whether the addition of fluid would have mitigated MDMA-induced glycaemic/insulinaemic dysregulation via cell swelling.

Hydration markers

In the present study, we found that copeptin concentrations increased in *Experimenter-b* (‘responder’) during MDMA most rapidly in the 15 minutes post-glucose ingestion (i.e. ~75 min post-MDMA ingestion). This elevation was in accordance with previous research, which reached peak AVP over 60-120 min (Henry *et al.*, 1998; Simmler *et al.*, 2011), with a gradual reduction over 4-6 hours (Henry *et al.*, 1998). Interestingly, the effect of MDMA on AVP may not occur in men (Simmler *et al.*, 2011), thus presenting a limitation regarding the already limited generalisability of the current study. *Experimenter-b* (‘responder’) showed a clear time trend of elevated copeptin preceding elevated body mass, with a time delay in copeptin lowering and body mass returning during MDMA. *Experimenter-a* however, showed an increase in body mass after the OGTT during MDMA without an increase in copeptin, suggesting MDMA exerted some effects of body mass retention outwith the effects of copeptin (since body mass was measured after urination). Notably, and notwithstanding the limitations with the estimation of plasma osmolality, the overall trend in calculated osmolality appears to be the inverse trend of plasma copeptin, particularly for *Experimenter-b* (as copeptin increased, osmolality decreased). Whilst this was a small change, it suggests some fluid could have been shunted into the extracellular space, thus diluting blood, since fluid ingestion was minimal during the OGTT part of the experiment.

Previous research on the effects of MDMA on osmolality have yielded mixed results. In placebo-controlled studies, no clinically meaningful effect has been found, broadly in line with the present findings with plasma Na staying in the expected normal range (indicating eunatraemia) (Henry *et al.*, 1998; Simmler *et al.*, 2011). The study by Simmler *et al.* allowed *ad libitum* fluid intake, suggesting some preservation of plasma osmolality even with elevated AVP and fluid ingestion. In a natural (pre-post clubbing) setting, osmolality reduced in some participants indicative of hyponatraemia (Wolff *et al.*, 2005). Evidence of hyponatraemia was also found in another placebo-controlled study which additionally tested the effects of water loading, finding AVP did not become elevated after MDMA (Baggott *et al.*, 2016). This provides some evidence that a pre-MDMA treatment water intervention with *ad libitum* fluid may be safe to mitigate any potential negative metabolic effects of MDMA in a therapeutic setting. As such, it is likely that MDMA exerts its effects on AVP unmediated by osmolality, and cases of hyponatraemia are from the excessive drinking and perhaps an interaction with other environmental factors, rather than directly from MDMA. The interaction between MDMA administration and fluid ingestion should be further investigated, as well as whether and how this might impact gluco-regulation. Whether salt intake can be manipulated to reduce risks of hyponatraemia should also be investigated, though this may also lead to a potentiation of AVP release.

A limitation of the study was that we did not standardise urination times. This was a methodological decision in order to reduce discomfort during the trials. In this instance, *Experimenter-a* did not void towards the end of the testing period during CON and *Experimenter-b* did not void towards the end for MDMA; these final voids may have influenced total urine volume trends across the testing period. Nevertheless, this study limitation offers interesting insight into void dynamics which are often missed when investigating hydration interventions (which more often test urine volume and/or concentration rather than frequency/pattern). The most notable trend is from *Experimenter-b* during MDMA, whereby several hours post-MDMA administration there was a cluster of three low-volume voids. Since the experimenters did not void unless they had the urge to, this may be indicative of MDMA exerting effects on bladder sensations independent of bladder fullness. As copeptin did not have a rebound effect of going below baseline concentrations, these bladder sensations could be due to other MDMA-mediated effects such as dysregulated acetylcholine (Carroll, 2020). *Experimenter-b* remarked that they often experience this urge to void as MDMA wears off during recreational use.

Trends in urine specific gravity further confirmed the differences in copeptin between the experimenters; *Experimenter-a* had broadly similar specific gravity between trials, whereas *Experimenter-b* had higher specific gravity during the peak phase of MDMA (indicating more concentrated urine). Of interest, urine specific gravity decreased to levels similar/slightly lower than CON by the time of the void cluster several hours post-MDMA. Typically, urine osmolality remains unchanged or increases after MDMA administration, in both controlled and natural settings (Simmler *et al.*, 2011; Wolff *et al.*, 2005), broadly in line with the present findings.

Thirst

During the latter phase of testing, *Experimenter-b* had high thirst, a dry mouth, low urine volume, high urge to void, and low urine concentration with similar/lower (compared to CON) copeptin concentrations. This bears some relation to the hydration physiology dysregulation

often reported in ageing (Carroll, 2020); accordingly MDMA may be useful model to help understand the physiological changes that occur as we age. One idea has recently been posited that cholinergic dysregulation occurs in both ageing and MDMA use and this may explain some of the current findings (Carroll, 2020). Typically thirst is framed as being controlled primarily by plasma osmolality and AVP. However, we can see some discordance with this in the present study (and MDMA use in general). Firstly, *Experimenter-a* reported slightly higher thirst during CON than MDMA, despite similar copeptin concentrations and slightly higher calculated plasma osmolality and Na concentrations during MDMA.

Secondly, *Experimenter-b* had elevated copeptin, and slightly lower plasma Na during MDMA relative to CON, yet experienced excessive thirst and xerostomia. This may provide evidence for AVP being a contributor of thirst, independent of plasma osmolality. However, the time trends for thirst and copeptin do not match, with MDMA-induced elevated copeptin returning to baseline by ~90 min, but excessive (above baseline) thirst continuing to ~240 min. Nonetheless, it was noteworthy that the trends in cortisol and thirst were similar for *Experimenter-b* during MDMA; whether this is causal is questionable, but stress and elevated cortisol are known to induce xerostomia (Gholami *et al.*, 2017). As such, the thirst experienced by *Experimenter-b* during MDMA may have been mediated by cortisol rather than hydration physiology *per se*. In terms of thirst regulation, it is also interesting that *Experimenter-b* started both arms with similar thirst, and during CON this thirst dropped quite rapidly in the first 30 min of the OGTT. It is unclear what drove this drop (the relatively small bolus of fluid from the OGTT may have contributed) particularly as there appeared to be a peak in calculated osmolality at 30 min which theoretically should have resulted in elevated thirst. Thus, neither AVP, nor plasma osmolality can be strongly attributed to the thirst ratings, in accordance with recent proposals (Carroll, 2020).

Further, since thirst ratings and dry mouth ratings were almost identical, it is unlikely that the thirst rating validly captured ‘true-thirst’ (as posited by Carroll, 2020), particularly as body mass was higher (or at least unchanged) during MDMA, indicating water retention (or at least lack of dehydration). This supports the idea that xerostomia is a central factor regulating thirst appetites, moving away from more classic osmo- (and volume-) regulatory ideas (Carroll, 2020).

Appetite

In terms of other appetites, the data were difficult to interpret. Typically amphetamines result in a loss of appetite (Bray, 1993); we did not clearly observe this. In terms of hydration, the most pertinent food appetite is salt. *Experimenter-b* who responded strongly to MDMA had no difference in salt desire (all rated 0 mm), however, *Experimenter-a* who did not respond much to MDMA had higher salt desire during CON (ranging from 7-30 mm higher than MDMA). Previous work shows euhydration to result in higher fasted salt desire than hypohydration (high copeptin and hyperosmolality) (Carroll *et al.*, 2019b), with no difference postprandially. The present findings may suggest previous findings were unrelated to copeptin or electrolyte levels since there was no agreement with salt desire according to copeptin status in either of the experimenters.

Whilst there were no clear differences in sweet desires, it is worth noting that *Experimenter-b* reported the glucose drink to taste notably and unbearably sweet with MDMA. To our knowledge, differences in taste perception have not previously been studied in humans

under the influence of MDMA. In rats, sweetness paired with MDMA administration can result in conditioned taste aversion and reduced intake of sweetness (saccharin) (Lin *et al.*, 1993). The present study may offer insight to such a phenomenon via increased taste perception of the sweet stimuli. This idea is speculative, and the mechanisms of action surrounding it and need to be elucidated and tested. This could offer insight into the appetite lowering effect of amphetamines, if both hedonic desires and hunger/satiety change.

Subjective effects

As with appetite, the measures of other subjective phenomenon were crude. Amphetamine-based drugs are normally associated with increased alertness, yet there was no clear trend for either experimenter. Previous research has suggested that amphetamines can have both stimulatory and depressant activity, particularly in the first hour of intake (Tecce & Cole, 1974); such an idea supports the trends seen in *Experimenter-b* ('responder').

Despite *Experimenter-a* perhaps being a 'non-responder', in accordance with the known effects of MDMA (van Wel *et al.,* 2012) both experimenters reported higher happiness during MDMA relative to CON, though this was stronger for *Experimenter-b*. More notably however, sadness ratings did not change for *Experimenter-a* ('non-responder') but were consistently lower for *Experimenter-b*. This is in line with the positive affect typically associated with MDMA use. It is unclear how SSRI usage in *Experimenter-a* may have influenced these ratings, though these findings of dulled affect are in accordance with previous research on SSRIs and subjective MDMA experiences (Farré *et al.*, 2007; Leichti *et al.,*2000; Tancer & Johanson, 2007). Corroborating the 'non-responder' hypothesis was the distinct lack of desire for *Experimenter-a* to clench their jaw ('gurning') during MDMA, which is a well-reported side effect of amphetamine-based drugs (Leneghan, 2013), and was experienced strongly by *Experimenter-b*.

Strengths and limitations

Of course, with n = 2, this experiment is extremely limited in terms of the inferences it can make (especially to men and postmenopausal women), particularly in light of the potential for a responder and non-responder. Such a study would be difficult to run in a larger sample particularly without a clear clinical endpoint. Accordingly, another key limitation is the purity of the MDMA taken. Whilst this was verified semi-quantitatively, it is unlikely that it was pure MDMA. Home testing kits are unable to provide accurate assessment of any impurities or additional compounds. The experience of *Experimenter-b* (the 'responder') fully accords with the known effects of MDMA. This adds credence that the MDMA administered was pure enough to offer insights into the physiological and metabolic effects of MDMA. We were also unable to measure glucagon which may have added mechanistic insight; we have plasma samples left if anyone would like to measure this.

The high level of control in the study is a key strength, with a fully diet- and activity-standardised pre-trial protocol, which even accounted for the OGTT glucose intake and wake times during the pre-trial standardisation day. Additionally, we measured multiple outcomes related to hydration physiology and health, using a standard laboratory protocol to test glucose metabolism (an OGTT), which to our knowledge has not previously been done. Some measures however, were less accurate, such as urine specific gravity being measured

using dipsticks with an accuracy of 0.005. Urine volume was also measured in a glass which had markings at 100 mL intervals, reducing the accuracy of this measure.

However, such high control also represents a limitation. Our study tested the gluco-regulatory effects using an OGTT, with 75 g of maltodextrin. Whilst this does not replicate a therapeutic session, it may offer insight into recommendations of what foods to avoid prior to MDMA therapy, which may be particularly pertinent for those with gluco-regulatory disorders. Based on this self-experiment in two healthy females, it is difficult to make evidence-based recommendations, but preliminarily, a higher fat, low glycaemic load, low protein (due to the insulinogenic effects of protein) pre-therapy snack or meal may be a safer recommendation to avoid hyperinsulinaemia and/or high glucose variability in a therapy session, at least until further research is conducted. Additionally, along with findings from other research, there is hints towards pre-MDMA water ingestion (+ 1 L/d for a week) to potentially reduce health risks from elevated AVP, particularly in those with high baseline AVP. Therapists should have appropriate training for dealing with hypoglycaemic episodes, and know the symptoms of potential cardiovascular or renal events (both during the MDMA sessions, and during follow-up). This study did not test the effects of water ingestion with MDMA; perhaps this would have influenced outcomes. Importantly, more metabolic research needs to be conducted in order that patients are fully informed of the risks. It is also near impossible to make recommendations for recreational users, other than if users have a gluco-regulatory disorder, they should probably be extra vigilant in monitoring their blood sugar.

Both experimenters were metabolically healthy; thus such findings may raise concerns for MDMA users (recreational, and those who use it therapeutically) who have gluco-regulatory disorders such as type 1 or 2 diabetes mellitus, though these populations certainly need independent testing. There was some evidence of hypoglycaemia with MDMA (relative to CON), and such an effect may be exaggerated in those with hyperinsulinaemia at baseline or those with clinical hypoglycaemia. Equally, higher insulin resistance was noted in both experimenters, again raising concerns for those with insulin resistance or a related disorder. The rise in copeptin in the ‘responder’ may pose health risks for those with elevated baseline copeptin or at high risk of cardiovascular events. Although a one-off administration is unlikely to cause harm, it is important for MDMA-therapeutic practitioners to understand basic gluco-regulation and have the necessary tools available to respond to any signs of adverse events.

Future research

To summarise key areas that warrant further investigation based off these extremely limited data, future research should investigate the following in the appropriate populations with larger sample sizes:

- The reliability of this study (i.e. replication), including a trial arm with fluid intake to induce greater water retention
- The role of SSRIs on the AVP and gluco-regulatory response to MDMA
- Markers (which may include SSRIs) of MDMA-induced AVP responders and non-responders
- The effects of acute and chronic pre-loading with water and/or salt prior to MDMA on AVP and gluco-regulation
- The gluco-regulatory and hydration regulatory effects of MDMA compared to similar psychostimulants (e.g. amphetamines)
- The gluco-regulatory effects in other populations, such as those with type 2 diabetes (with and without medication)

- The consistency of gluco-dysregulation whilst fasted and after intake of other energy sources (e.g. high fat pre-meal)
- The chronic implications of therapeutic use of MDMA, such as whether there is higher risk of myocardial infarction or continued insulin resistance
- The cardiometabolic effects of MDMA in natural settings, such as raves (i.e. with heat and/or physical activity)
- The appetitive effects of MDMA, including aspects such as sweet perception, desire, preference, and intake
- The relationship between AVP and subjective responses to MDMA
- The role of the cholinergic and other (non-osmoregulatory, such as cortisol) mechanisms in the MDMA thirst response
- The applicability of MDMA-induced thirst responses to other models of thirst dysregulation, such as ageing
- The need/appropriateness of electrolyte drinks to prevent hyponatraemia in a therapeutic setting, and whether such drinks further increase AVP and therefore, perhaps cardiometabolic health risk
- Sex differences in the above responses

Conclusion

Overall, this n = 2 self-experimentation showed MDMA induced higher plasma glucose variability and hyperinsulinaemia during fasting and after 75 g glucose ingestion, regardless of copeptin concentrations. The study raises questions regarding responders and non-responders of MDMA (particularly in light of chronic SSRI use), and whether copeptin could be used to determine or understand these differences and potential risks. Further research needs to investigate the metabolic and gluco-regulatory effect of MDMA in a range of participants in circumstances that mimic therapeutic and recreational settings. Additionally, the mechanisms underlying MDMA-induced insulin resistance should be elucidated. Whilst this small study does not give direct answers and cannot be generalised, we hope it gives a rationale for further investigation, by demonstrating glucose and insulin dysregulation under controlled physiological circumstances, and providing preliminary dietary recommendations to avoid ill-effects.

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References

Baggott, M. J., Garrison, K. J., Coyle, J. R., Galloway, G. P., Barnes, A. J., Huestis, M. A., & Mendelson, J. E., 2016. MDMA impairs response to water intake in healthy volunteers. *Advances in Pharmacological & Pharmaceutical Sciences,* 2175896, doi: 10.1155/2016/2175896.

Banks, M. L., Buzard, S. K., Gehret, C. M., Monroy, A. L., Kenaston, M. A., Mills, E. M., & Sprague, J. E., 2009. Pharmacodynamic characterization of insulin on MDMA-induced thermogenesis. *European Journal of Pharmacology*, 615(1-3), 257-61, doi: 10.1016/j.ejphar.2009.05.021.

Baquet, A., Hue, L., Meijer, A. J., van Woerkom, G.M. & Plomp, P. J., 1990. Swelling of rat hepatocytes stimulates glycogen synthesis. J*ournal of Biological Chemistry*, 265(2), 955-9.

Bhagat, C. I., Garcia-Webb, P., Fletcher, E., & Beilby, J. P., 1984. Calculated vs measured plasma osmolalities revisited. *Clinical Chemistry*, 30(10), 1703-5, doi: 10.1093/clinchem/30.10.1703.

Bray, G. A., 1993. Use and Abuse of Appetite-Suppressant Drugs in the Treatment of Obesity. *Annals of Internal Medicine,* 119(7 Pt 2), 707-713, doi:10.7326/0003-4819-119-7_part_2-199310011-00016.

Burge, M. R., Garcia, N., Qualls, C. R., Schade, D. S., 2001. Differential effects of fasting and dehydration in the pathogenesis of diabetic ketoacidosis. *Metabolism: Clinical & Experimental*, 50, 171-177, doi: 10.1053/meta.2001.20194.

Camilleri, A. M., & Caldicott, D., 2005. Underground pill testing, down under. *Forensic Science International*, 151, 53-8, doi: 10.1016/j.forsciint.2004.07.004.

Carroll, H. A., 2020. Redefining thirst: A conceptual four-compartment model characterising types of thirst, and their underlying mechanisms and interactions. *NutriXiv*, doi: 10.31232/osf.io/q7gvd.

Carroll, H. A., Betts, J. A., & Johnson, L., 2016. An investigation into the relationship between plain water intake and glycated Hb (HbA1c): a sex-stratified, cross-sectional analysis of the UK National Diet and Nutrition Survey (2008-2012). *British Journal of Nutrition*, 116(10), 1-11, doi: 10.1017/S0007114516003688.

Carroll, H. A., & James, L. J., 2019. Hydration, arginine vasopressin, and glucoregulatory health in humans: A critical perspective. *Nutrients*, 11(6), doi: 10.3390/nu11061201.

Carroll, H. A., & Melander, O., 2021. Copeptin response to acute physical stress during hypohydration: An exploratory secondary analysis. *BioRxiv*, doi: 10.1101/2021.09.19.460958.

Carroll, H. A., Templeman, I., Chen, Y-C., Edinburgh, R., Burch, E. K., Jewitt, J. T., Povey, G., Robinson, T. D., Dooley, W. L., Buckley, C., Rogers, P. J., Gallo, W., Melander, O., Thompson, D., James, L. J., Johnson, L., & Betts, J. A., 2019b. Hydration status affects thirst and salt preference but not energy intake or postprandial ghrelin in healthy adults: A randomised crossover trial. *Physiology & Behavior*, 212, 112725, doi: 10.1016/j.physbeh.2019.112725.

Carroll, H. A., Templeman, I. S., Chen, Y-C., Edinburgh, R. M., Burch, E. K., Jewitt, J. T., Povey, G., Robinson, T. D., Dooley, W. L., Jones, R., Tsintzas, K., Gallo, W., Melander, O., Thompson, D., James, L. J., Johnson, L., Betts, J. A., 2019a. The effect of hydration status on glycemia in healthy adults: A randomized crossover trial. *Journal of Applied Physiology,* 126(2), 422-430, doi:10.1152/japplphysiol.00771.2018.

COPE, 2015. The ethics of self-experimentation [online]. Available from: https://publicationethics.org/case/ethics-self-experimentation [accessed 31 May 2021].

Dolder, P. C., Müller, F., Schmid, Y., Borgwardt, S. J., & Liechti, M. E., 2018. Direct comparison of the acute subjective, emotional, autonomic, and endocrine effects of MDMA, methylphenidate, and modafinil in healthy subjects. *Psychopharmacology*, 235(2), 467-79, doi: 10.1007/s00213-017-4650-5.

Downing, J., 1986. The psychological and physiological effects of MDMA on normal volunteers. *Journal of Psychoactive Drugs,* 18(4), 335-40.

Enhörning, S., Brunkwall, L., Tasevska, I., Ericson, U., Persson Tholin, J., Persson, M., Lemetais, G., Vanhaecke, T., Dolci, A., Perrier, E. T., & Melander, O., 2019b. Water supplementation reduces copeptin and plasma glucose in adults with high copeptin: The H2O Metabolism Pilot Study. *Journal Clinical Endocrinology & Metabolism*, 104(6), 1917-25, doi: 10.1210/jc.2018-02195.

Enhörning, S., Hedblad, B., Nilsson, P. M., Engström, G., & Melander, O., 2015. Copeptin is an independent predictor of diabetic heart disease and death. *American Heart Journal,* 169(4), 549-556, doi: 10.1016/j.ahj.2014.11.020.

Enhörning, S., Struck, J., Wirfält, E., Hedblad, B., Morgenthaler, N., & Melander, O., 2011. Plasma copeptin, a unifying factor behind the metabolic syndrome. *Journal of Clinical Endocrinology and Metabolism*, 96(7), E1065-72, doi:

Enhörning, S., Tasevska, I., Roussel, R., Bouby, N., Persson, M., Burri, P., Bankir, L., & Melander, O., 2019a. Effects of hydration on plasma copeptin, glycemia and gluco-regulatory hormones: a water intervention in humans. *European Journal of Nutrition*, 58, 315-24, doi: 10.1007/s00394-017-1595-8.

Farré, M., Abanades, S., Roset, P. N., Perió, Torrens, M., O'Mathúna, B., Segura, M., de la Torre, R., 2007. Pharmacological interaction between 3,4-methylenedioxymethamphetamine (ecstasy) and paroxetine: Pharmacological effects and pharmacokinetics. *Journal of Pharmacology & Experimental Therapeutics*, 323(3), 954-62, doi: 10.1124/jpet.107.129056.

Ferris, C. F., 2000. Adolescent stress and neural plasticity in hamsters: a vasopressin-serotonin model of inappropriate aggressive behaviour. *Experimental Physiology*, 85(Suppl 1), 85–90, doi:10.1111/j.1469-445x.2000.tb00011.x.

Gholami, N., Sabzvari, B. H., Razzaghi, A., & Salah, S., 2017. Effect of stress, anxiety and depression on unstimulated salivary flow rate and xerostomia. *Journal of Dental Research, Dental Clinics, Dental Prospects*, 11(4), 247-52, doi: 10.15171/joddd.2017.043.

Gloyszny, M., & Obuchowicz, E., 2019. Are neuropeptides relevant for the mechanism of action of SSRIs? *Neuropeptides*, 75, 1-17, doi: 10.1016/j.npep.2019.02.002.

Goncharova, N.D., 2013. Stress Responsiveness of the Hypothalamic–Pituitary–Adrenal Axis: Age-Related Features of the Vasopressinergic Regulation. *Frontiers in Endocrinology*, vol. 4, pp. 26, doi: 10.3389/fendo.2013.00026.

Graf, J., Haddad, P., Haussinger, D., & Lang, F., 1988. Cell volume regulation in liver. *Renal Physiology and Biochemistry*, 11(3-5), 202-20.

Harris, D. S., Baggott, M., Mendelson, J. H., & Jones, R. T., 2002. Subjective and hormonal effects of 3,4-methylenedioxymethamphetamine (MDMA) in humans. *Psychopharmacology*, 162(4), 396-405, doi: 10.1007/s00213-002-1131-1.

Haussinger, D., 1996. The role of cellular hydration in the regulation of cell function. *Biochemical Journal*, 313, 697-710.

Henry, J. A., Fallon, J. K., Kicman, A. T., Hutt, A. J., Cowan, D. A., & Forsling, M., 1998. Low-dose MDMA ("ecstasy") induces vasopressin secretion. *Lancet*, 351(9118), 1784.

Holze, F., Vizeli, P., Müller, F., Ley, L., Duerig, R., Varghese, N., Eckart, A., Borgwardt, S., & Liechti, M. E., 2020. Distcinct acute effects of LSD, MDMA, and D-amphetamine in healthy subjects. *Neuropsychopharmacology*, 45, 462-71, doi: 10.1038/s41386-019-0569-3.

Hysek, C. M., Schmid, Y., Simmler, L. D., Domes, G., Heinrichs, M., Eisenegger, C., Preller, K. H., Quednow, B. B., & Liechti, M. E., 2014. MDMA enhances emotional empathy and prosocial behavior. *Social Cognitive & Affective Neuroscience*, 9(11), 1645-52, doi: 10.1093/scan/nst161.

Hysek, C. M., Domes, G., & Liechti, M. E., 2012. MDMA enhances “mind reading” of positive emotions and impairs “mind reading” of negative emotions. *Psychopharmacology*, 222(2), 293-302, doi: 10.1007/s00213-012-2645-9.

Inaguma, D., Kitagawa, W., Hayashi, H., Kanoh, T., & Kumon, S., 2000. Three cases of severe hyponatremia under taking selective serotonin reuptake inhibitor (SSRI) [Abstract only]. *Nihon Jinzo Gakki shi*, 42(8), 644-8.

Ishizuka, Y., Abe, H., Tanoue, A., Kannan, H., & Ishida, Y., 2010. Involvement of vasopressin V1b receptor in anti-anxiety action of SSRI and SNRI in mice. *Neuroscience Research*, 66(3), 233-7, doi: 10.1016/j.neures.2009.11.004.

Johnson, E. C., Bardis, C. N., Jansen, L. T., Adams, J. D., Kirkland, T. W., Kavouras, S. A., 2017. Reduced water intake deteriorates glucose regulation in patients with type 2 diabetes. *Nutrition Research*, 43, 25-32, doi: 10.1016/j.nutres.2017.05.004.

Kalent, H, 2001. The pharmacology and toxicology of “ecstasy” (MDMA) and related drugs. *Canadian Medical Association Journal*, 165(7), 917-28.

Khani, S., & Tayek, J. A., 2001. Cortisol increases gluconeogenesis in humans: Its role in the metabolic syndrome. *Clinical Science*, 101(6), 739-47, doi: 10.1042/cs1010739.

Kirkpatrick, M. G., Francis, S. M., Lee, R., de Wit, H., & Jacob, S., 2014. Plasma oxytocin concentrations following MDMA or intranasal oxytocin in humans. *Psychoneuroendocrinology*, 46, 23-31, doi: 10.1016/j.psyneuen.2014.04.006.

Kirchner, V., Silver, L. E., & Kelly, C. A., 1998. Selective serotonin reuptake inhibitors and hyponatraemia: Review and proposed mechanisms in the elderly. *Journal of Psychopharmacology*, 12(4), 396-400, doi: 10.1177/026988119801200411.

Koshimizu, T., Nakamura, K., Egashira, N., Hiroyama, M., Nonoguchi, H. & Tanoue, A. 2012. Vasopressin V1a and V1b Receptors: From Molecules to Physiological Systems. *Physiological Reviews*, 92(4), 1813-64.

Leichti, M. E., Baumann, C., Gamma, A., & Vollenweider, F. X., 2000. Acute psychological effects of 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") are attenuated by the serotonin uptake inhibitor citalopram. *Neuropsychopharmacology*, 22(5), 513-21, doi: 10.1016/S0893-133X(99)00148-7.

Leichti, M. E., & Vollenweider, F. X., 2000. The serotonin uptake inhibitor citalopram reduces acute cardiovascular and vegetative effects of 3,4-methylenedioxymethamphetamine ('Ecstasy') in healthy volunteers. *Journal of Psychopharmacology*, 14(3), 269-74, doi: 10.1177/026988110001400313.

Leneghan, S., 2013. The Varieties of Ecstasy Experience: A Phenomenological Ethnography. *Journal of Psychoactive Drugs*, 45(4), 347-354, doi:10.1080/02791072.2013.826561.

Lin, H. Q., Atrens, D. M., Christie, M. J., Jackson, D. M., & McGregor, I. S., 1993. Comparison of conditioned taste aversions produced by MDMA and d-amphetamine. *Pharmacology Biochemistry & Behavior*, 46, 153−156, doi:10.1016/0091-3057(93)90333-o.

MAPS, n.d. Participate in research [online]. Available from: https://maps.org/participate/participate-in-research [accessed 09 March 2021].

MAPS, 2019. Trial registration: A Multi-Site Phase 3 Study of MDMA-Assisted Psychotherapy for PTSD [online]. Clinical trial identifier number: NCT04077437. Available from: https://clinicaltrials.gov/ct2/show/NCT04077437 [accessed 09 March 2021].

Meijer, A. J., Baquet, A., Gustafson, L., van Woerkom, G.M., & Hue, L., 1992. Mechanism of activation of liver glycogen synthase by swelling. *Journal of Biological Chemistry*, 267(9), 5823-8.

Melander, O., 2016. Vasopressin, from Regulator to Disease Predictor for Diabetes and Cardiometabolic Risk. *Annals of Nutrition & Metabolism*, 68(Suppl 2), 24-28, doi: 10.1159/000446201.

Mitchell, J. M., Bogenschutz, M., Lilienstein, A., Harrison, C., Kleiman, S., Parker-Guilbert, K., Ot'alora G., M., Garas, W., Paleos, C., Gorman, I., Nicholas, C., Mithoefer, M., Carlin, S., Poulter, B., Mithoefer, A., Quevedo, S., Wells, G., Klaire, S. S., van der Kolk, B., Tzarfaty, K., Amiaz, R., Worthy, R., Shannon, S., Woolley, J. D., Marta, C., Gelfand, Y., Hapke, E., Amar, S., Wallach, Y., Brown, R., Hamilton, S., Wang, J. B., Coker, A., Matthews, R., de Boer, A., Yazar-Klosinski, B., Emerson, A., & Doblin, R., 2021. MDMA-assisted therapy for severed PTSD: A randomized, double-blind, placebo-controlled phase 3 study. *Nature Medicine*, 27, 1025-33, doi: 10.1038/s41591-021-01336-3.

Mithoefer, M. C., Feduccia, A. A., Jerome, L., Mithoefer, A., Wagner, M., Walsh, Z., Hamilton, S., Yazar-Klosinski, B., Ermeron, A., & Doblin, R., 2019. MDMA-assisted psychotherapy for treatment of PTSD: Study design and rationale for phase 3 trials based on pooled analysis of six phase 2 randomized controlled trials. *Psychopharmacology*, 236(9), 2735-45, doi: 10.1007/s00213-019-05249-5.

Peak, M., al-Habori, M., & Agius, L., 1992. Regulation of Glycogen-Synthesis and Glycolysis by Insulin, pH and Cell-Volume - Interactions between Swelling and Alkalinization in Mediating the Effects of Insulin. *Biochemical Journal*, 282(3), 797-805.

Peroutka, S. J., Newman, H., & Harris, H., 1988. Subjective effects of 3,4-methylenedioxymethamphetamine in recreational users. *Neuropsychopharmacology*, 1(4), 273-7.

Simmler, L. D., Hysek, C. M., & Liechti, M. E., 2011. sex differences in the effects of MDMA (ecstasy) on plasma copeptin in healthy subjects. *Journal of Clinical Endocrinology & Metabolism*, 96(9), 2844-2850, doi: 10.1210/jc.2011-1143.

Song, Z., & Albers, H. E., 2018. Cross-talk among oxytocin and arginine-vasopressin receptors: Relevance for basic and clinical studies of the brain and periphery. *Frontiers in Neuroendocrinology*, 51, 14–24, doi: 10.1016/j.yfrne.2017.10.004.

Soto-Montenegro, M. L., Vaquero, J. J., Arango, C., Ricaurte, G., Garcia-Barreno, P., Desco, M., 2007. Effects of MDMA on blood glucose levels and brain glucose metabolism. *European Journal of Nuclear Medicine & Molecular Imaging,* 34, 916-925, doi: 10.1007/s00259-006-0262-8.

Spruce, B. A., McCulloch, A. J., Burd, J., Ørskov, H., Heaton, A., Baylis, P. H., & Alberti, K. G. M. M. (1985). The effect of vasopressin infusion on glucose metabolism in man. *Clinical Endocrinology*, 22(4), 463-8, doi:10.1111/j.1365-2265.1985.tb00145.x.

Studerus, E., Vizeli, P., Harder, S., Ley, L., & Liechti, M. E., 2021. Prediction of MDMA response in healthy humans: A pooled analysis of placebo-controlled studies. *Journal of Psychopharmacology*, doi: 10.1177/0269881121998322.

Tancer, M., & Johanson, C.-E., 2007. The effects of fluoxetine on the subjective and physiological effects of 3,4-methylenedoixymethamphetamine (MDMA) in humans. *Psychopharmacology*, 189(4), 565-73, doi: 10.1007/s00213-006-0576-z.

Tecce, J. J., & Cole, J. O., 1974. Amphetamine Effects in Man: Paradoxical Drowsiness and Lowered Electrical Brain Activity (CNV). *Science*, 185(4149), 451-453, doi:10.1126/science.185.4149.451.

Van Wel, J. H. P., Kuypers, K. P. C., Theunissen, E. L., Bosker, W. M., Bakker, K., Ramaekers, J. G., 2012. Effects of Acute MDMA Intoxication on Mood and Impulsivity: Role of the 5-HT2 and 5-HT1 Receptors. *PLoS ONE*, 7(7), e40187, doi: 10.1371/journal.pone.0040187.

Velho, G., Bouby, N., Hadjadj, S., Matallah, N., Mohammedi, K., Fumeron, F., Potier, L., Bellili-Munoz, N., Taveau, C., Alhenc-Gelas, F., Bankir, L., Moarre, M., & Roussel, R., 2013. Plasma copeptin and renal outcomes in patients with type 2 diabetes and albuminuria. *Diabetes Care*, 36(11), 3639-45, doi: 10.2337/dc13-0683.

Velho, G., Ragot, S., El Boustany, R., Saulnier, P.-J., Fraty, M., Mohammedi, K., Fumeron, F., Potier, L., Marre, M., Hadjadj, S., & Roussel, R., 2018. Plasma copeptin, kidney disease, and risk for cardiovascular morbidity and mortality in two cohorts of type 2 diabetes. *Cardiovascular Diabetology*, 17, 110, doi: 10.1186/s12933-018-0753-5.

Wolever, T. M., 2004. Effect of blood sampling schedule and method of calculating the area under the curve on validity and precision of glycaemic index values. *British Journal of Nutrition*, 91, 295-301, doi:10.1079/BJN20031054.

Wolff, K., Tsapakis, E. M., Pariante, C. M., Kerwin, R. W., Forsling, M. L., Aitchison, K. J., 2012. Pharmacogenetic studies of change in cortisol on ecstasy (MDMA) consumption. *Journal of Psychopharmacology,* 26, 419-428, doi: 10.1177/0269881111415737.

Wolff, K., Tsapakis, E. M., Winstock, A. R., Hartley, D., Holt, D., Forsling, M. L., & Aitchison, K. J., 2005. Vasopressin and oxytocin secretion in response to the consumption of ecstasy in a clubbing population. *Journal of Psychopharmacology*, 20(3), 400-10. doi:10.1177/0269881106061514.

Zaplatosch, M. E., & Adams, W. M., 2020. The Effect of Acute Hypohydration on Indicators of Glycemic Regulation, Appetite, Metabolism and Stress: A Systematic Review and Meta-Analysis. *Nutrients*, 12(9), 2526, doi: 10.3390/nu12092526.

Supplementary material

Supplementary material 1: Whole blood glucose concentration and dynamics

We believe these data to be less reliable and valid than the plasma glucose presented in the main paper; these data are presented for transparency but should be interpreted cautiously. Whole blood glucose concentrations of the two experimenters are shown in **Figure S1.** Compared to CON, during MDMA, *Experimenters-a* and *b* had relatively similar fasting whole blood glucose concentration at baseline (Δ MDMA minus CON *Experimenter-a* -0.6 mmol·L^{-1}; *Experimenter-b* -0.4 mmol·L^{-1}) and one-hour post-MDMA ingestion prior to glucose ingestion (Δ MDMA minus CON *Experimenter-a* -0.6 mmol·L^{-1}; *Experimenter-b* 0.0 mmol·L^{-1}). As with plasma glucose concentrations, whole blood glucose concentrations to estimate insulin resistance via HOMA2 also demonstrated increased insulin resistance after MDMA ingestion (**Table S1**).

The postprandial whole blood glucose response during MDMA in *Experimenter-a* perhaps had an earlier peak (30 min MDMA *versus* 45 min CON). Comparatively, the peak for *Experimenter-b* was at 45 min for both trial arms. Whilst *Experimenter-a* had a similar post-prandial glucose response, *Experimenter-b* had a much higher whole blood glucose response during MDMA relative to CON. Area under the curve are presented in **Table S1**, corroborating a similar whole blood glucose response during the OGTT period (timepoints 0-120) for *Experimenter-a*, and relative gluco-dysregulation during MDMA *versus* control for *Experimenter-b*. Incremental AUC showed *Experimenter-a* experienced some hypoglycaemia during the OGTT, but the time above baseline during MDMA was higher than during CON.

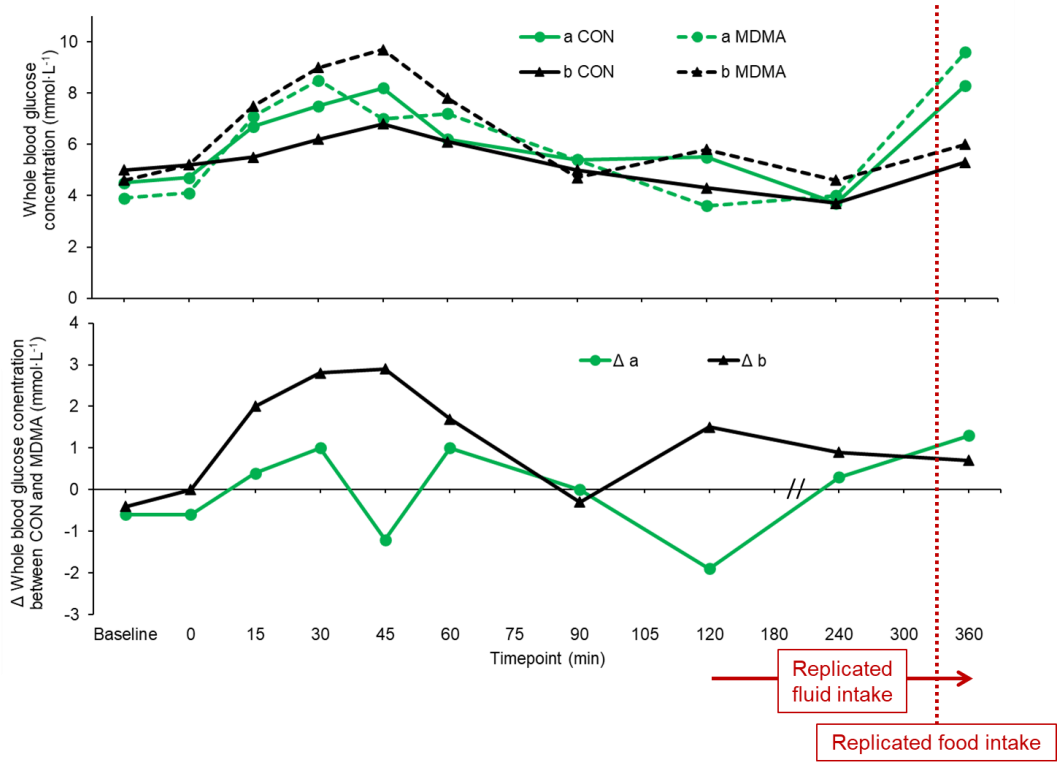

Figure S1. Whole blood glucose responses across the experimental period for *Experimenter-a* and *b*. Abbreviations: CON, control trial arm; MDMA, 3,4-Methylenedioxymethamphetamine trial arm

Table S1. Whole blood glucose area under the curve (mmol·120 min·L^{-1})

	Control arm	MDMA arm	Difference
	Area under the curve		
Experimenter-a	755.25	747.75	-7.5
Experimenter-b	668.25	835.50	167.25
	Incremental area under the curve		
Experimenter-a	185.69	265.96	80.27
Experimenter-b	42.30	198.00	155.70
	HOMA2*		
Experimenter-a	-	0.37	-
Experimenter-b	0.43	1.16	0.73

*HOMA2 was unable to be calculated when insulin concentrations were < 20 pmol·L^{-1}

Abbreviations: HOMA2, Homeostasis Model Assessment 2; MDMA, 3,4-Methylenedioxymethamphetamine

Supplementary material 2: Reviewer comments (from version 1)
HC changes:

1. Some errors have been amended:

(i) Peak insulin p. 18 for *experimenter-b* was accidentally misreported and is now amended to read 782.28 pmol·L^{-1}

(ii) Time to AVP peak was reported as quicker than previous research on p. 24; this has been amended to read "in accordance with" previous research, since this was approximately 75 min post-MDMA ingestion, and most research shows peak at 60-120 min post-administration

(iii) Some clarifications in-text have been made and typographical errors corrected which have not been highlighted as there were minor changes that did not impact the meaning of the text

2. Subheadings have been added to the discussion for improved clarity

3. Cortisol data have been added, including appropriate amendments to the methods, results, and discussion. The data for this study are published (https://osf.io/sf4nq). Since this version (December 2021, version 2) now includes cortisol data, the raw data are as follows:

Table S2. Raw cortisol data (nmol·L^{-1}) across the testing period (minutes from consuming the 75 g glucose drink)

	Baseline	0	15	30	45	60	90	120	240	360
E-a CON	758	431	310	287	269	274	208	262	377	204
E-a MDMA	566	490	595	604	524	443	370	325	503	383
E-b CON	517	284	260	230	238	199	187	194	241	334
E-b MDMA	524	576	599	667	714	741	619	519	277	356

Baseline represents pre-MDMA, timepoint 0 min represents 1 hour post-MDMA ingestion, or just 1 hour post-baseline measure for CON. Abbreviations: CON, control trial arm; *E-a*, *Experimenter-a*; *E-b*, *Experimenter-b*; MDMA, 3,4-methylenedioxymethamphetamine trial arm

Reviewer 1 (anon):
Remarked on the use of SSRIs in *Experimenter-a*, and how this might interact with AVP (copeptin).
HC: see response below to reviewer 2.

Reviewer 2 (Dr Ilsa Jerome, MAPS):
The biggest issue relates to MDMA and cortisol - studies in healthy controls consistently find elevated cortisol. Hence this seems like a promising and possibly additive explanation for an increase in insulin or reduction in glucose. Here are some examples.
https://pubmed.ncbi.nlm.nih.gov/28551715/
https://pubmed.ncbi.nlm.nih.gov/24097374/
https://pubmed.ncbi.nlm.nih.gov/22277989/
https://pubmed.ncbi.nlm.nih.gov/12172693/
HC: These references were appreciated and clearly missed during my initial wrote up. Since publishing version 1, we have analysed cortisol and can confirm an increase in cortisol during MDMA relative to CON in both *Experimenter-a* and *Experimenter-b* (i.e. regardless of copeptin changes). These results have been added in, as have the references above as appropriate in the introduction and discussion.

Typo noted in introduction: psylocibin. And amend lysergic acid to lysergic acid diethylamide.
HC: these have been amended.

Include https://www.ncbi.nlm.nih.gov/labs/pmc/articles/PMC6695343/ as reference for the current health exclusion criteria typically used in MDMA studies.
HC: this has been included.

A second point relates to the explanation or ideas for mechanism of action for MDMA-assisted therapy. You wrote "3,4-Methylenedioxymethamphetamine (MDMA), commonly known as the party drug 'ecstasy' is a key drug of interest in mental illness therapeutics due to its ability to induce ego dissolution with a positive experience in a more predictable manner than other psychedelics such as lysergic acid or psylocibin." That was likely what Shulgin and Leo Zeff and others started out with, but findings drawn from studies in healthy participants and patient populations suggest a more specific pathway or pathway that includes an increased ability to face emotionally upsetting information and a greater ability to tolerate unpleasant memories. There are likely more than one route for producing these effects - oxytocin is likely part of the mix, and 5HT2A receptor activity that is tempered by actions on other monoamines may be involved.
HC: These are all fair points. The comment in the introduction regarding ego dissolution has been removed, and Dr Jerome's comments can be seen here for further context/information.

On p. 9, the demographic information about the two experimenters, there is information that regular medications for Experimenter-A included SSRIs. This is an important detail and might be a strong alternative explanation for the difference in subjective effects. It is doubly interesting if despite these differences in subjective effects, both experimenters had changes in glucose regulation. Studies using pretreatment with various serotonin uptake inhibitors report that the pretreatment attenuates MDMA effects. This is one of the reasons that our studies require people on SSRIs or SNRIs to taper their medication prior to enrollment. There are no studies looking at the effects of longer maintenance periods on SSRIs and MDMA effects.
https://pubmed.ncbi.nlm.nih.gov/10731626/
https://pubmed.ncbi.nlm.nih.gov/11106307/
https://pubmed.ncbi.nlm.nih.gov/17047932/
https://pubmed.ncbi.nlm.nih.gov/17890444/
HC: I would like to thank Dr Jerome for this insight and providing study links which have been included in-text. I think this is a good example of how epistemic trespassing can overlook basic things. As a non-psychopharmacologist, I had assumed the SSRIs if anything would exacerbate the effects of MDMA. The text has been amended to incorporate these comments.

Dr Jerome also highlighted that this is a n = 2 study and all findings need to be taken within these limitations.
HC: I agree, and have added further comments to emphasise that this is a hypothesis generating paper, rather than a paper to establish causal and generalisable effects.

I wondered about relationships in terms of time as well as between the two participant/observers. For instance, did changes in gluco-regulation precede or follow changes in subjective effects? I'm not sure whether more charts and tables are needed so much as selecting measures or variables that might seem to be related and to look at any changes in time.
HC: I tried to make a figure to put this information together but it was not intuitive and did not really help understand the time trends. I welcome suggestions from readers on how to do this.

The biggest suggestion I would make at this point is to consider what future research you would like to see developed as a result of these observations. A controlled trial of the effects of MDMA on gluco-regjulation? Relationship between oxytocin, cortisol and blood sugar levels on self-reported thirst? A 2 x 2 study confirming whether SSRI+MDMA changes

effects on gluco-regulaton and, if so, whether they are related or unrelated to changes subjective effects? Just a paragraph describing whatever questions arose after you examined your data - even if it's something so boring as a fully blinded, placebo-controlled crossover study with confirmed MDMA following the procedures you used. That would be a good 'wrap up'.
HC: I have added a “future research” section to the discussion. There are a lot of unknowns so I just bullet pointed key areas I think should be investigated

Clinical trials in healthy controls continue to find paradoxical information about alertness/stimulation – I was hoping to pull up a source right away, the Kirkpatrick pooled study comes close, there’s also Bershad ‘MDMA verss stimulants’ but none note this directly. It is sort of visible in Vizeli et al. 2017 (pooled data, safety).
HC: I was not really sure what to do with this information so have left it here for readers to use if interested. I think this is the Bershad paper Dr Jerome has cited: https://pubmed.ncbi.nlm.nih.gov/27562198/

Dr Jerome and I also had discussions around this topic; a key discussion point that may be of interest to readers is below:

IJ: The circumstances under which hyponatremia occurs after MDMA may differ from laboratory or clinical settings in a number of ways, including greater physical activity and unrestricted fluid consumption; I am unconvinced that we need to require electrolyte containing drinks, but - at least until now - the thinking is likely that providing them can't do any harm. You are suggesting that this might not be the case?
HC: I think it is a good idea to offer isotonic drinks instead of water, in theory at least. The problem is that no one has really tested these things properly. I am hoping this study can spur on some enthusiasm for understanding the non-psychopharmacology related physiology of MDMA (and other drugs). For example, if I am right that an acute elevation of AVP does pose a health risk, electrolyte drinks might raise that risk even more (as salt = elevated AVP). This is why I think MDMA offers such a useful model—it poses such a complex question regarding hydration science that most people think is settled.

In saying that, I think you are right with regard to hyponatraemia risk (or lack of); water is probably safe (especially in a therapeutic setting) but there is no harm (or reduced potential for harm) with electrolyte drinks. However, I also think this should be studied, rather than speculated on. If people want to drink during MDMA therapy, then from a hyponatraemia risk point of view, isotonic drinks are probably safest. Better yet *might* be chewing gum/sweets/mints (assuming there's no choking hazard).

But yes, the flipside *could* be that by maintaining or potentially elevating osmolality, it might encourage AVP secretion (even more), which could increase cardiovascular event risks, if this is a true risk. it's difficult to tell, since the AVP response is clearly independent of osmolality under MDMA, but whether osmolality can still alter the AVP response isn't exactly clear in this case (that I'm aware of). So it's all kind of "maybe potentially".

Nonetheless, in a clinical setting, the risk of most adverse events is likely extremely low (though you probably know more than me about this). I guess I’d rather just know than speculate. I also suspect that different people will have different baseline risk characteristics which might dictate the best course of action, so finding out what these could be might help make broadly generalisable guidelines.