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Hydration status affects thirst and salt preference but not energy intake or postprandial ghrelin in healthy adults: A randomised crossover trial

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ABSTRACT

Background: Few studies have investigated the effect of hydration status on appetite for food in healthy adults. Prior work suggests hydration status does not alter appetite or energy intake, with mixed findings regarding appetite hormone secretion. However, an extensive investigation into both the psychological and physiological appetitive responses to hydration status has never been conducted.

Objective: To investigate the effect of hydration status on multiple facets of appetite.

Design: After 3 days pre-trial standardization, a range of appetite tasks were conducted when hypohydrated (HYPO) and euhydrated (EUHY) in 16 healthy participants (8 men). Hydration status was manipulated via dehydration in a heat tent for 60 min and subsequent fluid restriction (HYPO) or replacement (EUHY). The next day, a food reward computer task was completed followed by an *ad libitum* pasta meal. Pre- and post-prandial visual analogue scales assessing hunger, fullness, and flavour desires (sweet, salty, savoury and fatty) were additionally completed. Blood samples were taken the previous day before the hydration interventions in a euhydrated state, and in the fasted and post-prandial state during HYPO and EUHY.

Results: HYPO induced -1.9 ± 1.2% body mass change, compared to -0.2 ± 0.6% , with accompanying changes in markers of hypohydration which were not seen during EUHY. A higher desire for foods was associated with a higher water content but the association was weaker in EUHY compared to HYPO, (β = -0.33 mm/g of food water content, $p < 0.001$) in the food reward task. Visual analogue scales showed similar hunger and fullness between interventions, but during HYPO there was consistently higher thirst (average range in difference 27–32 mm across all time points) and lower fasted desire for salt (−23, 95% CI −10, −35 mm). *Ad libitum* energy intake (HYPO 1953 ± 742 kJ, EUHY 2027 ± 926 kJ; $p = 0.542$) and post-prandial ghrelin concentrations (HYPO 180 ± 65 pg mL^{-1}, EUHY 188 ± 71 pg mL^{-1}; $p = 0.736$) were similar by hydration status.

Conclusions: An acute manipulation to hydration status altered desire for salt and foods of differing water contents, but did not influence energy intake at an *ad libitum* pasta meal. Further research should investigate whether these appetites would alter food choice.

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1. Introduction

Raised urine osmolality (≥800 mOsm kg^{-1}), as a marker of inadequate hydration, has been positively associated with obesity [8]. However higher water intake (as a proxy for better hydration) also occurs with a cluster of other healthful lifestyle behaviours known to impact appetite control, such as higher physical activity and higher intake of dietary fibre [21]. Such confounding influences create difficulties when making causal inferences regarding the role of hydration status on appetite control. Ensuring adequate hydration via increased fluid consumption could offer an attractive avenue to help reduce energy intake due to it being a cost-effective and easily understood intervention, yet little research has investigated the acute and causal effects of hypohydration on appetite control, let alone the chronic effects. Furthermore, considering the current prevalence of obesity, much research is invested in understanding factors that alter energy intake in an attempt to try and mitigate positive energy balance, yet studies investigating appetite do not always control for hydration status, even if acute fluid intake is standardised. As such, there is a need to understand the causal effects of hydration status on both physiological and psychological appetite to aid health interventions, as well as improve the reliability of the research that underpins these interventions.

Hydration status may influence appetite via several mechanisms. Pre-meal water ingestion can reduce energy intake, both acutely (single meal tests in a research facility) and over two weeks, particularly in elderly populations [13,26,32]. However, mechanisms surrounding the acute effect of pre-meal water ingestion are likely to reflect gastric emptying (which is slower in older adults) and distention rather than changes in hydration status *per se* [13,26]. Research in rats has reported that cellular hypohydration induced via ingestion of hypertonic saline reduced food intake, due to upregulation of inhibitory neural networks which control appetite [4]. This phenomenon (dehydration-induced anorexia) has been hypothesised to prevent hyperosmolality caused by food ingestion and, whilst evidence is limited, has also been reported in humans [1]. Finally, in mice, higher intestinal osmolarity suppressed ghrelin more than lower intestinal osmolarities [24], which suggests hypohydration would suppress hunger (supporting the hypohydration-induced anorexia hypothesis). It remains unlikely, however whether whole-body hydration status would sufficiently alter intestinal osmolality (which is affected to a greater degree from food and fluid consumption), and therefore ghrelin secretion or energy intake in humans.

Previous research in humans has typically used exercise-induced hypohydration in order to ascertain the appetitive effects of hydration status [12,22]. Whilst both studies found increased thirst when participants were hypohydrated, Corney et al. [12] found decreased subjective fullness and no effect on acylated ghrelin whereas Kelly et al. [22] found no effect on subjective hunger ratings, but lower ghrelin concentrations. Such discordance may be attributed to methodological differences such as time of eating after exercise. For example, Corney et al. [12] tested energy intake at breakfast the next day (13 h post-exercise), compared to 30 min post-exercise in the study by Kelly et al. [22]. As exercise may induce differential acute *versus* chronic effects on appetite (reducing appetite acutely, but potentially increasing it chronically; [19]), difficulties arise when making comparisons or inferences regarding how hydration status impacts appetite when using exercise as a model for altering hydration status.

Research investigating the effect of non-exercise induced changes in hydration status and appetite is lacking. Studies altering hydration status via only modifying fluid intake (rather than via other means such as exercise) might have greater applicability to the general population who may typically be more prone to hypohydration due to low fluid intake. Evidence from one study was concordant with the exercise studies, reporting no changes in *ad libitum* energy intake after 24 h fluid restriction compared to euhydration, regardless of whether fluid was available with the meal [11].

Further, whilst physiological (*e.g.* serum osmolality) and psychological (*e.g.* visual analogue scales) measures are often employed in studies investigating the role of hydration status in appetite control, several gaps remain in the literature. Firstly, to our knowledge, no study has measured plasma copeptin concentrations (as a marker for arginine vasopressin; a hormone implicated in body water preservation during dehydration) to understand how this physiological response interacts with appetite. Secondly, the effect of hydration status on desires for specific foods or drinks with differing profiles of water content has never been investigated. This is important to understand as higher water content foods are typically lower in energy density so may aid in overall energy balance. This study will be the first to take these measures, adding substantially to the current literature base. Thirdly, there is currently a paucity of evidence in this topic; understanding the reliability of previous research is therefore important mechanistically before future work investigating chronic effects. The present study therefore aimed to acutely investigate the role of hydration status on multiple facets of appetite control in healthy adults, whilst understanding key underlying physiological and psychological mechanisms.

2. Subjects and methods

2.1. Participants

Full details of the experimental design and protocol have been described previously [7]. In brief, 16 healthy adults (n = 8 men) consented to take part in this research. The mean ± standard deviation (*SD*) age of the participants was 30 ± 9 y, with a body mass of 71.7 ± 9.6 kg, and body mass index of 24.0 ± 3.4 $kg{\cdot}m^{-2}$. All participants self-reported being healthy (no known cardiometabolic disease, drug dependency, taking essential medication or supplements, or weight loss >5 kg in last 6 mo), and women confirmed they were not pregnant or breastfeeding.

2.2. Experimental design

This was a randomized crossover trial, with trials separated by 5–35 days to account for the menstrual cycle where appropriate (women who were not on continuous hormonal contraceptives were tested during the estimated follicular phase of their menstrual cycle, 3–10 d after onset of menses). Each trial arm consisted of three monitoring days, a dehydration/rehydration intervention day, and a full trial day of testing in the laboratory (**Fig. 1**; see Supplementary Material Table S1 for any deviations to registered protocol and Subsection S1 for our approach to the methods) which have been detailed previously [7] and are given in brief below. Data were collected in South West England between June 2016 and January 2017, inclusive.

2.2.1. Pre-trial monitoring phase

For three days pre-intervention, participants recorded energy intake (weighed food diaries), and their physical activity energy expenditure was measured (ActiHeart™; CamNtech, Cambridge, England). Participants successfully replicated these patterns on the subsequent trial arm ([7]; nutrient profile of the pre-trial diet analyses are found in the Supplementary Material Table S2). Morning body mass (after voiding, but before the first eating or drinking occasion; Inner scan; body composition monitor, model BC-543, TANITA corp. Japan) was recorded during this three-day pre-trial period, along with urine specific gravity of the first morning void. On the third monitoring day, participants were additionally instructed to consume a minimum 40 $mL{\cdot}kg^{-1}$ lean body mass of non-alcoholic fluid to ensure euhydration before entering the experimental phase of the protocol. No fluid or food was allowed after 2200 h on this third day.

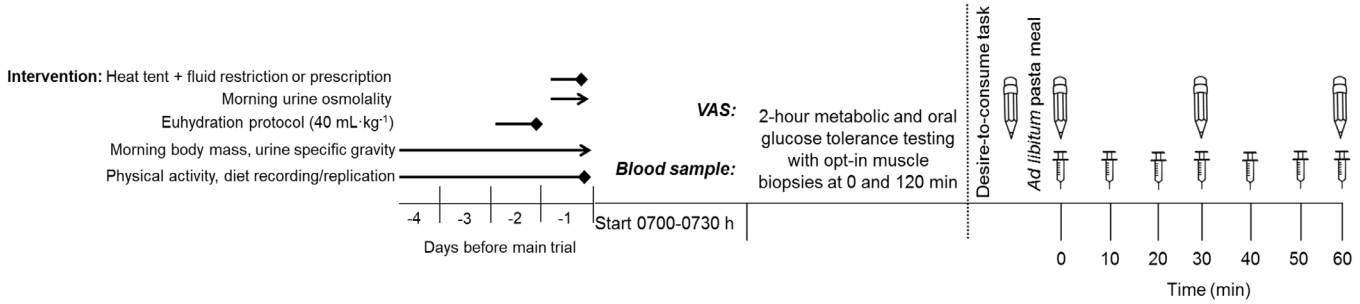

Fig. 1. Protocol schematic of the trial. Arrows represent that the measures were also taken on the main trial day; diamond arrows represent measures stopped on that day. Abbreviations: VAS, visual analogue scales.

2.3. Experimental protocol

2.3.1. Intervention day

On the intervention day, participants arrived at the laboratory between 0600-1000 h for a 10 mL baseline blood sample in a euhydrated state. Blood concentrations of various analytes obtained from this venepuncture further confirmed compliance to the pre-trial monitoring and control phase. This was demonstrated by similar levels of fasted plasma adrenocorticotropic hormone, copeptin and cortisol concentrations, fasted serum glucose and insulin concentrations, and serum osmolality (all $p \geq 0.152$; see [7] for full details).

Participants were then placed in a heat tent wearing a sweat suit for 60 min inducing similar body mass losses between trials (HYPO 0.6 ± 0.3 kg, EUHY 0.5 ± 0.3 kg; $p = 0.503$). Post-heat tent, participants were provided with either 3 mL kg^{-1} body mass (HYPO), or 40 Ml kg^{-1} lean body mass plus 150% water (sweat) losses of plain water only, metered evenly across the day until 2200 h (EUHY). All other fluids were prohibited and participants were only allowed to eat from a list of low-water-content foods (Supplementary Material Subsection S2), with food and fluid abstention again from 2200 h. Physical activity energy expenditure (HYPO 3294 ± 1654 kJ d^{-1}, EUHY 3222 ± 1723 kJ d^{-1}; $p = 0.641$) and energy intake (HYPO 9473 ± 3120 kJ d^{-1}, EUHY 9982 ± 4036 kJ·d^{-1}; $p = 0.410$) were similar between trials during the intervention day. The nutrient profile of the diet analyses during the intervention are shown in Supplementary Material Table S3.

2.3.2. Laboratory testing day

On the test day, participants arrived at the laboratory between 0700 and 0730 h in an overnight fasted and fluid restricted state from 2200 h the previous night, provided a urine sample and had their body mass recorded (as previously described), after which participants were asked to rest in a supine position for 10–15 min. As per the primary aim of the study, several metabolic tests were conducted (resting metabolic rate, opt-in muscle biopsies, and a two-hour oral glucose tolerance test consisting of ingestion of 75 g of glucose), in the 3–4 h prior to the appetite tasks.

The food reward task and *ad libitum* pasta meal (described below) were conducted in a private resting room in the laboratory with the doors closed over. The participant was lying semi-supine or sitting up in an adjustable medical bed, according to their comfort. Participants were allowed to use their phone or laptop, or watch the television fitted in the resting room (which had access to Netflix and BBC iPlayer) whilst eating. Participants were left alone with minimal external disturbances.

After completing the oral glucose tolerance test [7], a measure of food reward was taken, adapted from Rogers and Hardman [28]. This was administered to participants on a laptop computer (ASUS Transformer 550) and comprised of computerised visual analogue scales (VAS) anchored between 0 'not at all' and 100 'extremely'. Participants were presented with 20 images of 50 g portions of various foods and drinks and instructed to imagine consuming a single bite or sip of that food or beverage. Food pictures for this task were selected to represent high (mean water content 35.5 ± 4.5 g/50 g portion) and low water (mean water content 16.9 ± 12.2 g/50 g portion) content foods spanning a range of nutrient profiles. Food pictures were taken with standardised lighting, camera angle, plate (round, white), and background (black).

Participants were asked to rate the pleasantness of taste ('how much do you like this food'), and their desire to consume the entire portion ('how strong is your desire to consume this food right now') of each food by using the left and right arrows on the laptop to move the vertical rating line along a horizontal scale from a starting point of 50%. Here, pleasantness of taste is defined as "food liking" and desire to consume is defined as the momentary value of a food or beverage to the individual at the time of ingestion. A series of studies have demonstrated that this measure of food reward is comparable or even superior to traditional measures of food reward (*e.g.* willingness to pay) when predicting subsequent ad-libitum intake of the task food [28].

This task was implemented using software written using Matlab (v 2012a) with the psychophysics toolbox (v 3.0.13; [5]). Participants did not taste the foods in this adapted version of the task in order to reduce confounding for the *ad libitum* pasta meal and VAS (described below). Details of the nutrient breakdown (calculated from manufacturers' labels) of the foods presented are provided in the Supplementary Material Table S4.

After the food reward task, participants were given VAS to assess further aspects of appetite. Scales were 100 mm anchored between 0 and 100 representing the two extremes of each scale, with '0′ representing the least (*e.g.* 'not at all') and '100′ representing the most (*e.g.* 'extremely'). Participants were asked to make a vertical line on each scale for each question. Questions assessed hunger ("How hungry do you feel?"), fullness ("How full do you feel?"), perception of how much could be eaten ("How much food do you think you can eat?"), thirst ("How thirsty do you feel?"), and desire for sweet ("How strong is your desire to eat something sweet?"), savoury ("How strong is your desire to eat something savoury?"), salty ("How strong is your desire to eat something salty?"), and fatty ("How strong is your desire to eat something fatty?") foods. Scales were analysed by measuring the distance to the nearest millimetre from the far left-hand side of the scale ('0′) to the line marked by the participant, providing a score out of 100.

A large homogenous bowl of white pasta (Sainsbury's Penne) and tomato sauce (Morrisons Bolognese Sauce) was then presented (served weight [excluding Tupperware] HYPO 2088 ± 54 g, 10,393 ± 182 kJ; RE 2029 ± 133 g, 10,294 ± 348 kJ). Full details of the pasta meal preparation and serving method are given in Supplementary Material Subsection S3. Pasta and tomato sauce was chosen as it has been used in previous research to assess *ad libitum* energy intake (*e.g.* [9]) and is an easy to standardize, generically liked food. Participants had 30 min to eat, and were asked to do so until they were comfortably full. Bowls were topped up twice during this 30 min period to ensure finishing a bowl was not responsible for meal termination. No fluid was allowed before or during the test meal. After

30 min, 10 mL blood samples were drawn at 10 min intervals for a 60 min postprandial period. Postprandial VAS were repeated at 0, 30 and 60 min.

2.4. Blood handling

Six millilitres of whole blood was decanted into two ethylenediaminetetraacetic acid-coated (EDTA) tubes (BD, Oxford, UK), and spun for 10 min at 2500–3446 *g* at 4 °C. Four millilitres of whole blood was decanted into a serum tube (BD, Oxford, UK), left for at least 30 min at room temperature and then spun as per the plasma samples. The plasma and serum were then aliquoted into separate Eppendorfs and frozen at -20 °C before being moved to a -80 °C freezer for longer-term storage.

2.5. Blood analysis

All metabolites and hormones (except total ghrelin) were measured in a fasted state at baseline before the fluid intervention and before the oral glucose tolerance test commenced on the main trial day. Postprandial measures included: serum glucose and insulin concentrations to determine the glycemic and insulinemic response; plasma copeptin concentration as a marker of hydration status and AVP secretion; and total ghrelin concentration (at 60 min post-pasta meal only). Metabolites and hormones were measured using commercially available ELISAs (serum insulin, Mercodia; plasma total ghrelin, Bertin Pharma), automated immune analyzers (plasma copeptin, ThermoFisher Kryptor Compact Plus) and spectrophotometric assays (serum glucose, RX Datona, Randox Laboratories). Osmolality was measured using freezing-point depression (serum osmolality, Gonotec Osmomat auto; urine osmolality, Micro-Osmometer 3300). Coefficient of variations of these analyses can be found in Supplementary Material Table S5.

3. Statistical analysis

The primary aim of this study was to investigate whether the glycemic response is influenced by hydration status [7]. Therefore, the study was powered based on a pilot study assessing the effect of hydration status on the blood glucose response in five healthy participants [6], indicating we would need 16 participants to detect ($\beta = =0.95$ and $\alpha = =0.05$) the predicted difference in blood glucose at 45 min after consuming 75 g glucose ($D = 1.1$ mmol L^{-1} with *SD* in control group of 1.1 mmol L^{-1} resulting in an effect size of $dz = =1$) as part of the oral glucose tolerance test conducted immediately prior to the appetite portion of the study.

Data were analysed using paired samples *t*-test, 2-way repeated measures (trial, time, trial*time) analysis of variance (ANOVA), or non-parametric equivalents as appropriate (SPSS, version 22, IBM). The degree of asphericity was assessed using Greenhouse-Geisser epsilon; values <0.75 were corrected for using Greenhouse Geisser correction and values >0.75 used Huynh-Feldt correction.

For the food reward task, analyses were conducted using R software [31] using the lme4 add-on package [2], and figures were created using the ggplot2 add-on package [33]. Macronutrient composition of foods (g/100 g) were taken from nutrient labels on food packaging. Water content was estimated by subtracting the grams of macronutrients and salt from the total weight of the product; the remainder was assumed to be water, though it is acknowledged that a fraction of this will be other micronutrients not commonly listed on nutrition labels.

As each participant completed the food reward task twice for each of the 20 foods, the assumption of independence of errors was violated. Therefore a multi-level modelling approach was used to account for the intra-class correlation between individual participant responses and individual foods [18,27]. Cross-classified multilevel regressions were therefore used, with individual ratings nested within participants and foods to analyse associations between hydration status (person level), nutrient content (food level) and desire to consume (food level).

A multilevel regression model was specified for each hypothesis, and the desire-to-consume rating for each food from the food reward task was treated as the dependent variable in four separate models. For the first model, hydration status (HYPO, EUHY), energy density (kJ/g) and water content (g/100 g) were entered as predictors, with an interaction term between hydration status and water content and hydration status and energy density. Next, a model with hydration status, energy density and water content was specified and an interaction term between hydration status and salt content and water content was included. Finally, a model with sugar content and energy density as predictors of desire to consume was run. Thus the results will model the desire (y) for each nutrient tested (water, salt, sugar) (x) according to hydration status (HYPO *versus* EUHY). Data are reported as mean ± SD, or mean and 95% confidence intervals as appropriate. This research gained ethical approval from the NHS Health Research Authority Frenchay (ref: 16/SW/0057), and was registered at clinicaltrials.gov (ref: NCT02841449) and osf.io (ref: osf.io/ptq7m).

4. Results

During HYPO, participants achieved −1.3 ± 0.9 kg (−1.9 ± 1.3%) body mass change compared to −0.1 ± 0.4 kg (−0.2 ± 0.6%) during EUHY (HYPO *versus* EUHY $p < 0.001$). Accompanying changes confirming HYPO were also seen in other markers of hydration status and are reported elsewhere (see [7] for full details).

4.1. Food reward task

Participant liking of foods did not change according to hydration status (**Fig. 2**). During EUHY, the association between food desire and water content was 0.33 (95% CI -0.53, -0.13) mm/g lower compared to HYPO, independent of energy density ($p < 0.001$; **Table 1**, **Fig. 2**). Further, the association between food desire and salt content was 7.81 (95% CI 0.04, 15.59) mm/g higher during EUHY *versus* HYPO ($p = 0.049$; Table 1, Fig. 2). There was no difference in desire for sugar according to hydration status (hydration status*sugar content $\beta = =-0.03$, 95% CI −0.32, 0.26 mm, $p = 0.850$; Table 1, Fig. 2).

4.2. Visual analogue scales

All measures had a significant time effect ($p \leq 0.003$), except thirst (time $F = 0.445$, $p = 0.563$) and desire for sweet (time $F = 0.883$, $p = 0.399$). Hunger, fullness, how much participants felt they could eat, and desire for sweet and fatty foods had no trial (all $p \geq 0.254$) or trial*time (all $p \geq 0.062$) effects (Figs. 3 and 4).

HYPO induced consistently higher reporting of thirst (trial $F = 52.207$, $p < 0.001$; trial*time $F = 0.419$, $p = 0.646$; Fig. 3), and lower reporting of desire for savoury foods (trial $F = 6.871$, $p = 0.021$; time $F = 53.746$, $p < 0.001$; trial*time $F = 0.403$, $p = 0.574$; Fig. 4). Before eating, there was a higher reported desire for salty foods during EUHY, with this difference dissipating after consuming the pasta meal (trial $F = 4.815$, $p = 0.047$; time $F = 10.835$, $p = 0.003$; trial*time $F = 4.480$, $p = 0.022$; Fig. 4).

4.3. Ad libitum pasta meal

During HYPO, participants consumed 712 ± 280 g (1953 ± 742 kJ), compared to 757 ± 353 g (2027 ± 926 kJ) during EUHY ($p = 0.542$; Fig. 5). On average, 1.6 ± 0.6 g of salt was consumed within the meal during HYPO, and 1.7 ± 0.8 g during EUHY ($p = 0.539$).

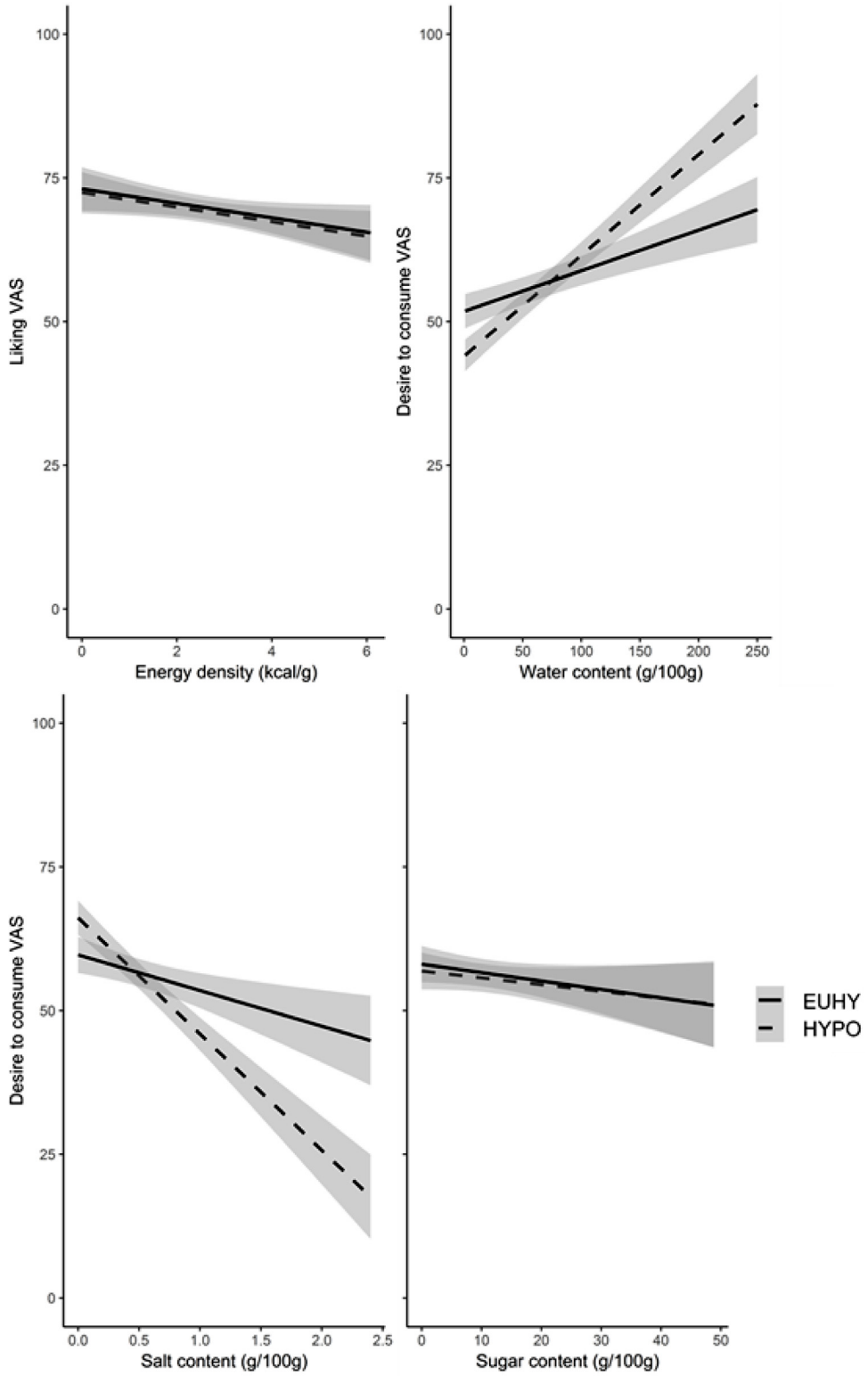

Fig. 2. Participant liking and participant desire to consume for foods of different water, salt and sugar content according to hydration status. Shaded error bands: 95% confidence intervals. Abbreviations: HYPO, hypohydrated trial arm; EUHY, euhydrated trial arm; VAS, 100 mm visual analogue scale.

4.4. Postprandial blood metabolite and hormone concentrations

Plasma copeptin concentrations remained consistently elevated in the fasted and postprandial state during HYPO compared to EUHY (trial $F = 10.064$, $p = 0.007$; time $F = 2.413$, $p = 0.166$; trial*time $F = 0.987$, $p = 0.344$; Fig. 6). Whilst there was a distinct postprandial serum glucose (time $F = 3.687$, $p = 0.030$) and insulin (time $F = 1.493$, $p = 0.029$) response in both trials, these did not differ by hydration status (glucose trial $F = 0.482$, $p = 0.500$; trial*time $F = 0.275$, $p = 0.772$; insulin trial $F = 3.289$, $p = 0.093$; trial*time $F = 0.078$, $p = 0.925$; Fig. 7). Total ghrelin was similar between HYPO (180 ± 65 pg mL^{-1}) and RE (188 ± 71 pg mL^{-1}) 60 min after eating

Table 1
Multi-level regressions investigating the relationship between hydration status and desire for foods with differing water, salt, and sugar content.

	Model 1			Model 2			Model 3		
	β	95% CI	*p*	β	95% CI	*p*	β	95% CI	*p*
Fixed Parts									
Intercept	31.89	2.14, 61.65	0.046	43.94	12.50, 75.38	0.013	69.46	56.51, 82.42	<0.001
Hydration status	22.54	2.32, 42.77	0.029	8.33	−4.04, 20.70	0.187	1.19	−3.80, 6.18	0.640
Water content (g/100 g)	0.44	0.16, 0.73	0.005	0.33	0.03, 0.63	0.048			
Energy density (kcal/g)	−0.87	−6.17, 4.43	0.751	−1.28	−6.31, 3.75	0.625	−5.48	−8.95, −2.02	0.006
Hydration status*water content	−0.33	−0.53, −0.13	0.001	−0.20	−0.35, −0.05	0.011			
Hydration status*energy density	−0.93	−4.63, 2.77	0.623						
Salt (g/100 g)				−8.17	−19.46, 3.11	0.171			
Hydration status*salt content (g/100 g)				7.81	0.04, 15.59	0.049			
Sugar (g/100 g)							0.02	−0.44, 0.48	0.941
Hydration status*sugar content (g/100 g)							−0.03	−0.32, 0.26	0.850
Random Parts									
σ^2	577.687			574.214			598.477		
$\tau_{00,\ Food}$	112.298			115.370			144.351		
$\tau_{00,\ Participant}$	184.804			184.891			184.284		
N_{Food}	20			20			20		
$N_{Participant}$	16			16			16		
ICC_{Food}	0.128			0.132			0.156		
$ICC_{Participant}$	0.211			0.211			0.199		
Observations	640			640			640		
r^2 / $\Omega_0{}^2$	0.439 / 0.437			0.442 / 0.441			0.418 / 0.416		

Abbreviations: β, unstandardised beta coefficient; CI, confidence interval; ICC, intraclass correlation.
Model 1: Hydration status (HYPO, EUHY), energy density (kcal/g), water content (g/100 g), hydration status * water content, hydration status * energy density.
Model 2: Model 1 (excluding hydration status * energy density) + salt content (g/100 g), hydration status * salt content.
Model 3: Model 1 (excluding hydration status * energy density) + sugar content (g/100 g), hydration status * sugar content.

($p = 0.736$; $n = 13$).

5. Discussion

In this randomized crossover trial, we found that acute hypohydration did not alter most facets of appetite in healthy adults, despite causing notable changes in markers of hydration status. Specifically, urine osmolality and urine specific gravity both crossed boundaries set to identify hypohydration, whilst plasma copeptin concentrations (as a marker of arginine vasopressin) increased to levels seen in those with cardiometabolic diseases [15,17]. Therefore the level of hypohydration achieved can be deemed physiologically meaningful. If confirmed by future research, the major implication of our findings may be that when measuring subjective hunger/fullness, *ad libitum* energy intake (using a homogenous high water content meal), or postprandial ghrelin concentration, hydration status does not necessarily have to be controlled

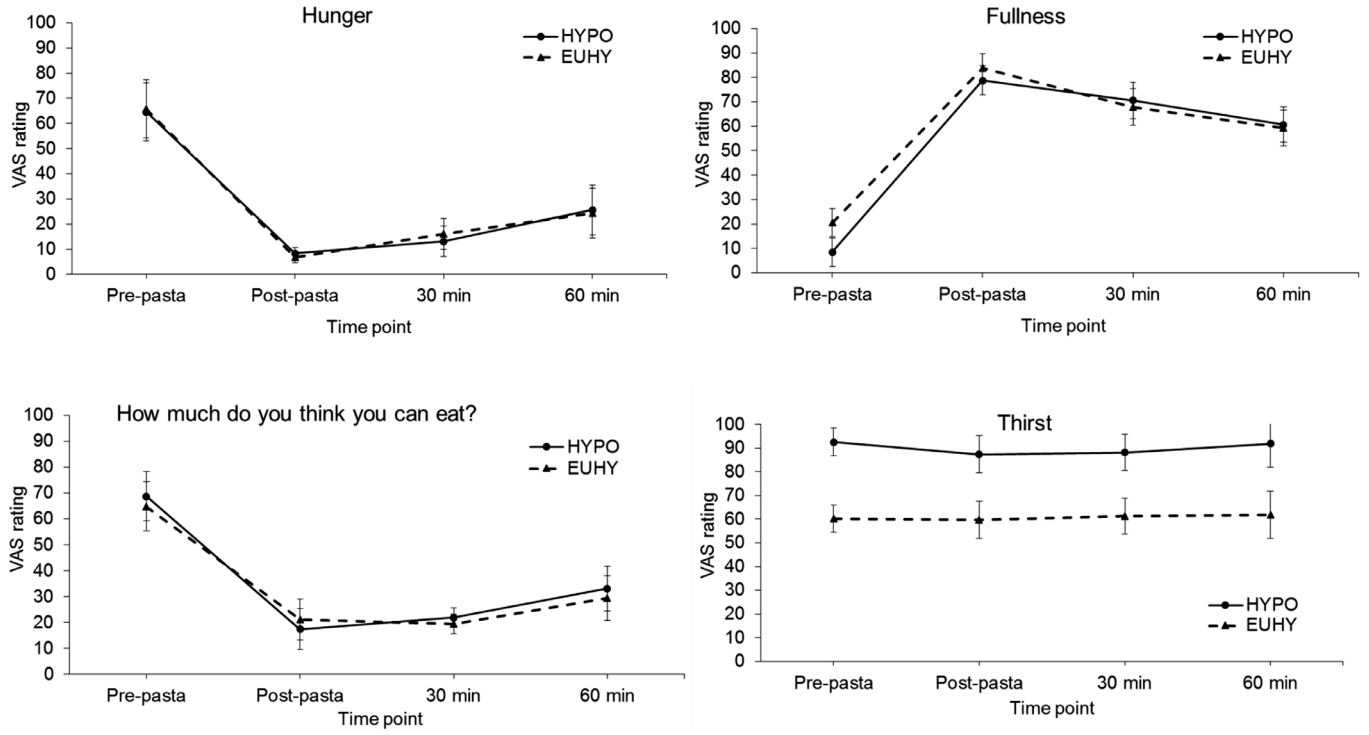

Fig. 3. Visual analogue scales assessing various aspects of appetite on a 0 (not at all) to 100 (very much) mm scale. Error bars: normalised confidence intervals. Abbreviations: HYPO, hypohydrated trial arm; EUHY, euhydrated trial arm; VAS, visual analogue scale.

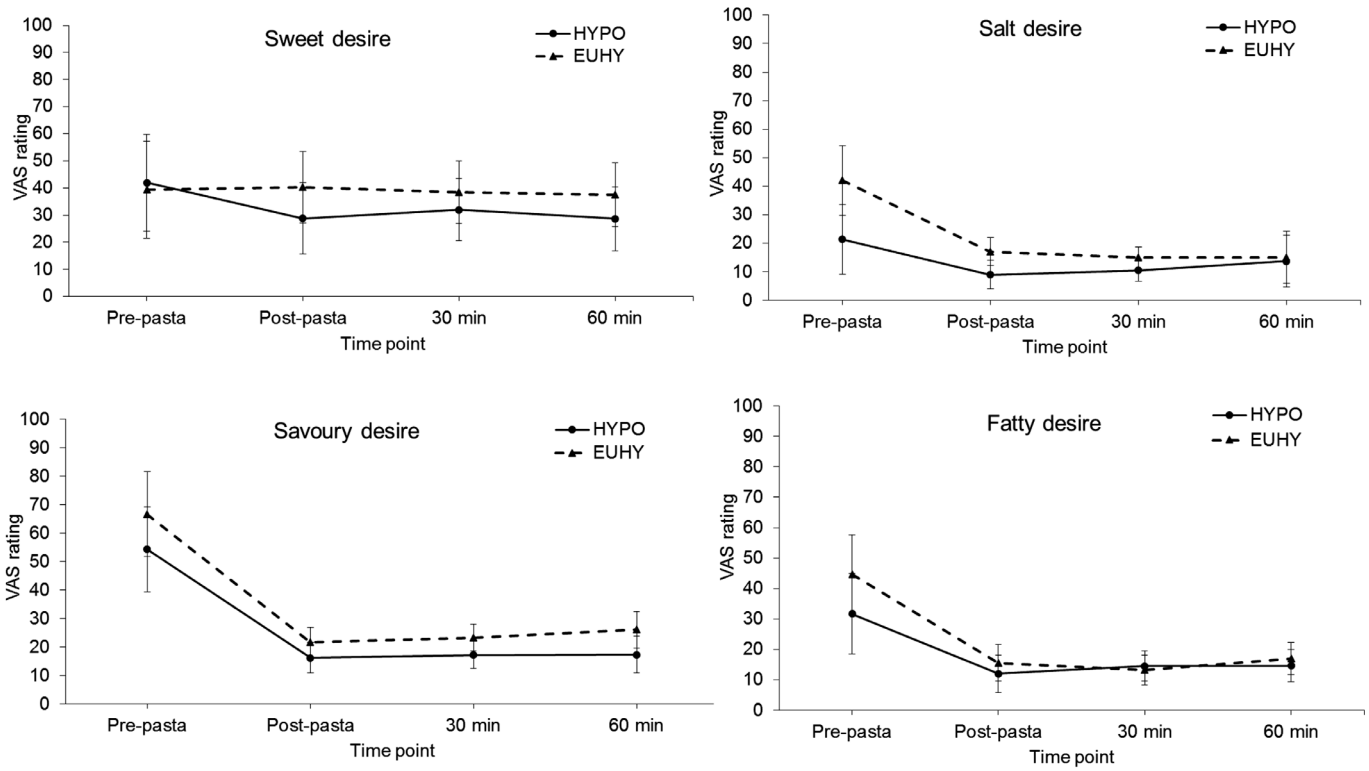

Fig. 4. Visual analogue scales assessing flavour desires on a 0 (no desire) to 100 (high desire) mm scale. Error bars: normalised confidence intervals. Abbreviations: HYPO, hypohydrated trial arm; EUHY, euhydrated trial arm; VAS, visual analogue scale.

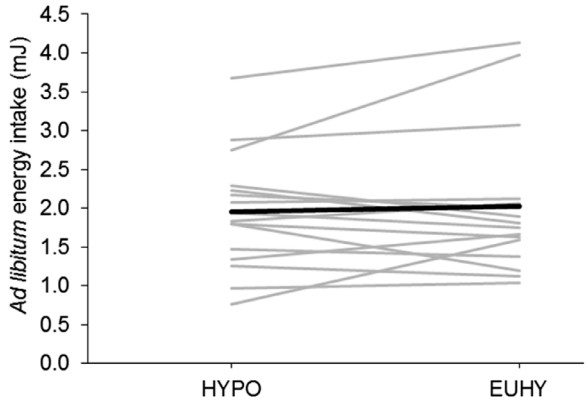

Fig. 5. Individual (grey lines) and overall (black line) energy intake (mJ) at an *ad libitum* pasta meal. Abbreviations: HYPO, hypohydrated trial arm; EUHY, euhydrated trial arm.

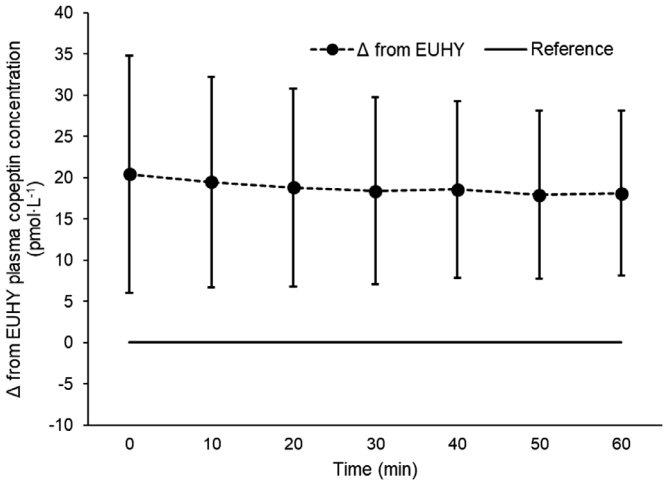

Fig. 6. Change HYPO compared to EUHY in plasma copeptin concentrations (pmol L^{-1}) after an *ad libitum* pasta meal (n = 14). Error bars: 95% confidence intervals. Abbreviations: HYPO, hypohydrated trial arm; EUHY, euhydrated trial arm.

for, assuming the population studied are healthy adults. However if tasks involve food choice then the water and salt content of the foods, and the hydration state of the participant may need to be considered.

Our research found no effect of hydration status on *ad libitum* energy intake during a homogenous pasta meal. This is consistent with research using exercise-induced dehydration protocols [12,22], and corroborates work demonstrating energy intake was not influenced by fluid restriction-induced hypohydration [11]. However, it cannot be ruled out that energy intake might have been affected indirectly had there been different availability of food choices (*e.g.* foods of differing salt or water content, or perceived palatability). Equally, energy intake may have differed had *ad libitim* fluid intake been allowed before and/or during the meal test, though previous research suggests this would not be the case [11].

Considering there were no differences in energy intake, it is unsurprising that postprandial total ghrelin concentrations were also similar between HYPO and EUHY. Despite similar energy intakes in previous work, the effect of hydration status on ghrelin secretion has been inconsistent. In the study by Kelly et al. [22], ghrelin concentrations were consistently higher when participants were euhydrated, both during and after exercise and eating 30 min post-exercise. However, in accordance with our findings, other research has shown no effect of hydration status on (acylated) ghrelin concentrations [11,12]. Plasma copeptin concentrations remained elevated during HYPO compared to EUHY; thus it is unlikely that copeptin (as a surrogate marker for arginine vasopressin) is implicated in energy intake during an *ad libitum* meal, nor does it appear to interact with total ghrelin (which did not differ 60 min post-meal).

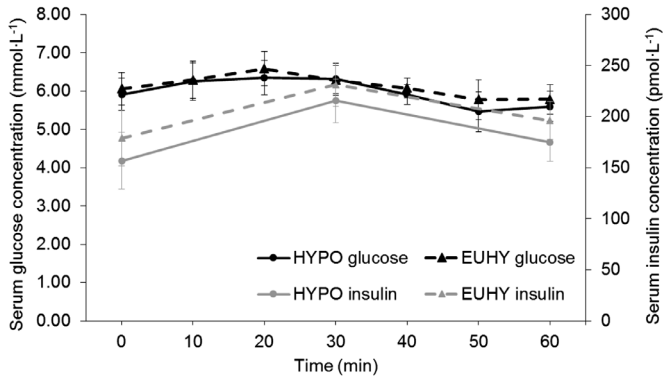

Fig. 7. Serum glucose (mmol L^{-1}) and insulin (pmol L^{-1}) concentrations after an *ad libitum* pasta meal. Error bars: normalised confidence intervals.Abbreviations: HYPO, hypohydrated trial arm; EUHY, euhydrated trial arm.

The similar energy intake between hydration states is in accordance with ratings of appetite which showed hunger and fullness to be similar between HYPO and EUHY. These findings are similar to some [11,22] but not all [12] previous work. It is reassuring that our findings are similar to Corney et al. [11] as their method of hypohydration was also fluid restriction, improving comparability between our studies. The discordance of our findings with Corney et al. [12] may be due to the use of exercise, though Kelly et al. [22] also used an exercise-dehydration model, perhaps suggesting there is an interaction between exercise hypohydration, interval before test meal, and appetite ratings.

Hypohydration induced significantly and consistently higher thirst ratings as per previous work [11,12,22] and is in accordance with higher serum osmolality and plasma copeptin concentrations. The higher thirst ratings in the VAS during HYPO were reflected in the food reward task by a weaker association between increasing food desire with higher food water content during EUHY compared to HYPO. These findings offer an interesting paradigm. There is growing evidence that elevated arginine vasopressin concentrations might have undesirable health consequences [15,17]. With 1–2% body mass loss, we achieved levels of arginine vasopressin (inferred from copeptin concentrations) correlated with poorer health. The level of hypohydration we achieved also strengthened the association of increased food desire with higher water content foods.

Higher water content foods typically have higher micronutrient density and lower energy density, and are therefore more likely to be representative of a healthier diet. Thus it is of interest to further investigate whether higher water content foods are chosen in a hypohydrated state, and whether these likely healthier choices can mitigate the potential harm caused by elevated arginine vasopressin concentrations. Whilst future research should investigate the impact of hydration status on food choice, we did not find a difference in *ad libitum* energy intake, despite the pasta meal provided being high water content. This may be due to the homogeneity of the pasta, lack of food choice, or differences in participant likings of the meal, but may also be indicative that desire for higher water content foods during HYPO does not translate to greater consumption. Alternatively, since hypohydration as been associated with poorer health outcomes (*e.g.* [8]), our findings may be an artefact of the acute study design which may not translate to chronic states of hypohydration. Further, chronically elevated AVP may mitigate the effects of acute food choices lower in energy density.

Additionally, there was a negative association between salt-content and desire-to-consume during HYPO. For comparability, estimated standardised beta coefficients suggested the association from the food reward task was slightly larger for water compared to salt desire (EUHY compared to HYPO beta water −0.14 *versus* beta salt 0.08 mm/1 *SD* change). Thus, whilst per gram salt desire was greater, this comparison suggests desire for water was a more prominent sensation. Such findings perhaps reflect the greater thirst during HYPO driving a stronger desire for water content than EUHY-induced salt desire. Concordant findings were found in the pre-meal VAS, though differences in the trial arms dissipated after eating. The higher desire for salt from the preprandial VAS during EUHY decreased after eating to levels of HYPO which remained relatively constant. This may suggest that euhydration is the driver of increased desire for salt, and food intake (which in this case contained approximately a quarter of the daily recommended maximum intake) being able to satisfy this desire.

Higher salt-preference during EUHY is discordant with previous work in exercise-induced salt loss and salt preference [23], potentially due to the methodological differences in inducing hypohydration (*i.e.* exercise *versus* fluid restriction). As current public health guidelines aim to reduce salt intake for general health [14], these results are somewhat paradoxical; maintaining euhydration may have health benefits (*e.g.* [10,25]), but the higher salt preference during EUHY may cause higher salt intake. With our data alone it is unclear whether the effect could be extrapolated to chronic behaviours and should be investigated longitudinally in future research. In saying this, energy intake, and therefore by proxy salt intake did not differ between hydration status. This could mean that salt desire does not lead to higher salt intake in this context, or that the homogenous pasta meal, which did not allow the addition of salt, was insufficient to determine the effect of this higher desire on intake.

Previous work has found that heat plus exercise-induced hypohydration followed by rehydration without Na^+ repletion led to increased Na palatability [30]. Our research adds to this by showing concordant palatability findings with Na repletion (from the pasta meal) after fluid restriction alone. Having a higher desire for salt may be due to the increased fractional excretion of Na associated with lower urine osmolality [1]; therefore higher preference for salt could be a method of preventing Na losses and maintaining osmoregulation during EUHY. However, this greater excretion of Na when euhydrated is not a consistent finding [20], perhaps suggesting higher serum osmolality and arginine vasopressin caused by hypohydration leads to a reduction in salt desire which might be mediated by the renin-angiotensin-aldosterone system [29]. Such findings potentially have important health implications which need to be investigated longitudinally as understanding how Na consumption is regulated may help population-based health recommendations to reduce overall salt intake [30].

There was consistently a slightly higher desire for savoury foods during EUHY, by 5–12 mm on the VAS. Whilst this reached statistical significance, the small difference in average ratings suggests these findings may not be meaningful. Such a small change in perception is unlikely to cause a change in behaviour, particularly as there is no known theory as to why savoury foods may be desired more strongly during EUHY. Alternatively, this finding may represent savoury foods being generally higher in salt (compared to sweet or fatty foods), slightly increasing participants' desire to consume.

Caution should be taken when interpreting our results as the study was not powered specifically for these appetite tasks, though our sample size is concordant with previous work. Accordingly, our findings need to be replicated in larger trials. Further work needs to investigate whether hydration status impacts actual food choices which we were unable to capture using a homogenous pasta meal, particularly in light of our findings suggesting lower salt and higher water content foods may be favoured during mild hypohydration. Whilst understanding the acute effects of hydration status is highly important, primarily because hydration status can fluctuate rapidly throughout the day, it is likely that distinct subsets of the population are chronic low water consumers, which may be indicative of chronic mild hypohydration [3,16]. Therefore future research should explore the causal effects of chronic hypohydration on energy intake, food choices, and energy balance.

Additionally, the food reward task has only been validated in parallel group study designs, reducing the validity of its use in our study. However, there was no association with the sugar content of foods,

improving the reliability of the findings as there is no basis in which sugar would be desired more under either hydration state. The robustness of these findings was also improved by liking for foods being consistent across conditions, suggesting we accurately captured trait liking and state desire.

Overall, despite inducing meaningful increases in plasma copeptin and serum osmolality, most facets of appetite were unaffected by an acute manipulation to hydration status, though increased desire for higher water-content, lower-salt foods were found during HYPO. We found that thirst ratings were notably higher during HYPO compared to EUHY, suggesting that there is no interaction between hunger and thirst, corroborated by similar postprandial plasma ghrelin concentrations despite significantly higher plasma copeptin concentrations during HYPO compared to EUHY. Energy expenditure, energy intake, blood analytes, and markers of hydration were all similar before the intervention, reducing the likelihood of confounding factors (such as pre-trial energy intake) influencing the results, thus improving the reliability of our findings. Although no inferences regarding food choice or chronic hypohydration can be made, the extent of physiological and psychological facets of appetite that we measured have never concomitantly been studied before, improving our understanding of these interactions. Our findings confirm previous work that maintaining euhydration may not be a suitable health intervention to mitigate excessive energy intake, at least acutely. Additionally, research may not need to control for hydration status when investigating *ad libitum* energy intake using a homogenous high-water meal or ratings of appetite, unless the study is pertaining to thirst or desire for salt.

Declaration of Competing Interest

HAC and LJJ have accepted conference fees from Danone. OM has received consultancy honoraria from Danone Research. LJJ has previously received funding for hydration-related research from PepsiCo Inc., the European Hydration Institute, and Volac International Ltd. and has performed consultancy work for Lucozade Ribena Suntory. LJ has received funding from Kellogg Europe and Danone Baby Nutrition. JAB has received funding from Lucozade Ribena Suntory, PepsiCo Inc., and Kenniscentrum Suiker. None of the other authors has any conflicts of interest, financial or otherwise, to disclose.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.physbeh.2019.112725.

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