Effect of hydration status and fluid availability on *ad-libitum* energy intake of a semi-solid breakfast.

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Key words

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Abstract

This study investigated the effects of hydration status and fluid availability on appetite and energy intake. Sixteen males completed four 24 h trials, visiting the laboratory overnight fasted on two consecutive days. Standardised foods were provided during the 24 h and on day two an *ad-libitum* semi-solid porridge breakfast was provided. Water intake during the 24 h (0 or 40 $mL \cdot kg^{-1}$) and fluid provision during the *ad-libitum* breakfast were manipulated so subjects were euhydrated with (EU-F) and without fluid (EU-NF) available at breakfast; and hypohydrated with (HYPO-F) and without fluid (HYPO-NF) available at breakfast. Blood samples (0 and 24 h), urine samples (0-24 h) and subjective responses (0, 24 and 24.5 h) were collected. HYPO trials decreased body mass by ~1.8%. Serum and urine osmolality increased and plasma volume decreased during HYPO trials (P<0.001). Total urine output was greater during EU than HYPO trials (P<0.001). *Ad-libitum* energy intake was not different between trials: 2658 (938) kJ (EU-F), 2353 (643) kJ (EU-NF), 2295 (529) kJ (HYPO-F), 2414 (954) kJ (HYPO-NF), (P=0.131). Fluid intake was ~200 mL greater during HYPO-F than EU-F (P<0.01). There was an interaction effect for thirst (P<0.001), but not hunger or fullness. These results demonstrate that mild hypohydration produced by inadequate fluid intake and fluid availability during eating does not influence *ad-libitum* energy intake of a semi-solid breakfast, at least in healthy young males.

Introduction

Deviations in energy balance (positive and negative) can have a profound effect on health (Kleiner, 1999), thus a better understanding of the physiological systems affecting energy balance is required. Alterations in appetite influence energy intake and consequently may potentially impact on energy balance. Whilst much research has focused on the effects of the energy containing macronutrients on appetite, (Rodin *et al.,* 1988; Metges and Barth, 2000; Anderson *et al.*, 2002; Clegg and Shaftat, 2010), relatively little is known about how deviations in water balance and water intake impact upon appetite and energy intake.

Currently, the effect of hydration status on appetite regulation and *ad-libitum* energy intake in humans is not fully understood, but there are a number of situations where hydration status might impact appetite regulation and thus health or performance of an individual. Hypohydration might develop rapidly due to an acute loss of body water due to either exercise or heat exposure (Corney *et al*., 2015) or more slowly due to a chronic inadequate fluid intake (James and Shirreffs, 2013). Whilst hypohydration appears to be more prevalent among athletes competing in certain sports, it is also common in children (Stookey *et al*., 2012), the elderly (Lavizzo-Mourey, 1987), as well as the general adult population (Mears and Shirreffs, 2014).

Research in animal models has consistently reported water intake being a major determinant of the amount of energy consumed (Lepkovsky *et al*., 1957; Silanikove, 1992; Senn *et al*., 1996; Watts, 1999). For example, Silanikove (1992) suggested that when water availability was reduced in ruminants there was a parallel reduction in *ad-libitum* feed intake. Similarly, Lepkovsky et al. (1957) reported that the restriction of fluid during feeding reduced energy intake in rats. Often (Senn *et al*., 1996; Watts *et al*.,

1999), these animal studies induce relatively large levels of hypohydration that are not consistent with the level of hypohydration commonly seen in humans.

In humans, only a limited number of studies have investigated the impact of water balance on appetite regulation or energy intake. Shirreffs *et al.* (2004) reported a reduction in energy intake with 37 h of complete fluid restriction compared to when fluids were provided *ad-libitum*. Similarly, Engell (1988) reported a reduction in energy intake during 6 meals over 48 h when fluid was restricted at meal times. In contrast, two recent studies (Kelly *et al.*, 2012; Corney *et al.*, 2015) observed no difference in *ad-libitum* energy intake between euhydrated and hypohydrated (2-3% body mass loss) conditions, with hypohydration induced using a combination of exercise and fluid restriction. In contrast to the studies of Shirreffs *et al*. (2004) and Engell (1988), Kelly *et al*. (2012) and Corney *et al*. (2015) provided fluid during feeding. Taken together with the animal literature, these studies suggest that whilst fluid restriction might result in hypohydration, it might be the fluid restriction during eating rather than the presence of hypohydration at the start of the meal that reduces energy intake in humans.

Therefore the purpose of the current study was to examine the effects of hydration status and/ or fluid availability during eating on *ad-libitum* energy intake. It was hypothesised that fluid restriction during feeding would reduce energy intake in both hypohydrated and euhydrated conditions, but that hypohydration would not affect energy intake when fluid was available.

Methods

Subjects

Sixteen healthy males (age: 25 (4) years; height: 1.78 (0.07) m; body mass: 72.6 (8.6) kg; body fat 15.1 (4.4)%; body mass index: 22.9 (1.7) $kg \cdot m^{-2}$) volunteered for the present study, which was approved by the University's Ethical Advisory Committee. Subjects were non-smokers, were not currently on a weight gain/weight loss diet, had not been on any such diet during the previous 6 months, and were habitual breakfast eaters. Subjects completed a health-screening questionnaire and provided written informed consent. Using G*Power 3.1.6 and the data of Engel (1988), an a priori power calculation with α of 0.05, statistical power of 0.8 and an estimated between groups correlation of 0.5 determined that 13 subjects would be required to reject the null hypothesis. Therefore, to ensure an adequate sample size and maintain counterbalancing 16 subjects were studied.

Experimental protocol

All subjects completed a familiarisation trial followed by 4 experimental trials, which were completed in a randomised, counterbalanced fashion and separated by at least 7 days. For each trial, subjects underwent a 24 h period of dietary manipulation and control and an *ad-libitum* breakfast was provided at 24 h. Water intake during day one and fluid availability during the breakfast were manipulated during each trial. This meant that the *ad*-libitum breakfast was served to subjects euhydrated with (EU-F) and without (EU-NF) fluid available during eating; and hypohydrated with (HYPO-F) and without (HYPO-NF) fluid available during eating.

During the familiarisation trial, subjects arrived at the laboratory overnight fasted (~10 h) and emptied their bladder and bowels before body mass was recorded to the nearest 10 g (Adam CFW 150 scale; Adam Equipment Co Ltd, Milton Keynes, UK) and height was measured to the nearest 1 mm (Stadiometer, Seca Ltd, Germany). Subcutaneous skinfold measurements were obtained (Tricep, Biceps, Subscapular and Suprailiac) and body fat

118 percentage was estimated using the Siri equation (Durnin and Wormersley, 1974).
119 Subjects were then provided with the *ad-libitum* breakfast (as described below).

120 For each experimental trial, subjects visited the laboratory on two consecutive mornings
121 in an overnight fasted state and at a time typical for them to consume breakfast (7-10 am).
122 On day one, subjects emptied their bladder and bowels and their nude body mass was
123 measured. Following 15 min seated rest, a baseline blood sample (15 mL) was collected
124 from an antecubital vein and a subjective feelings questionnaire (Flint *et al.*, 2000) was
125 completed. Questions asked were: "How thirsty do you feel?" "How hungry do you feel?"
126 and "How full do you feel?" with verbal anchors "not at all" and "extremely" at 0 mm
127 and 100 mm, respectively. Subjects were provided with food and drink for the next 24 h
128 and left the laboratory. On day two, subjects arrived again in an overnight fasted state
129 and all measurements previously made on day one were repeated. After blood sampling,
130 subjects consumed an *ad-libitum* porridge breakfast for a period of 30 min, after which
131 they completed a final subjective feelings questionnaire.

132 **Dietary intake and standardisation**

133 During the 48 h before the first experimental trial subjects completed a weighed record of
134 all food and drink consumed. They also recorded any light habitual physical activity.
135 These diet and activity patterns were then replicated in the 48 h preceding subsequent
136 experimental trials. Subjects refrained from any strenuous physical activity, alcohol
137 intake and dietary supplementation during the 48 h before trials. To help ensure
138 euhydration at the start of trials, subjects consumed an amount of water equivalent to 40
139 $mL \cdot kg^{-1}$ body mass in 6 aliquots over the 24 h pre-trial period. This water was consumed
140 in a manner identical to during euhydrated trials. During experimental trials subjects

consumed only food and drink provided to them and only performed light habitual physical activity.

For all trials, food was provided to subjects on day one in the form of dry foods (pizza, crisps, cereal bars, chocolate bars, sandwiches) to minimise water intake through foods. The appropriate amount of water was also provided to subjects during euhydrated trials (EU-F and EU-NF). Energy provided in foods was equal to the subjects estimated resting energy expenditure (Mifflin et al., 1990) multiplied by a physical activity level of 1.6. Nutritional intake (mean (SD)) for the 24 h was 10648 (859) kJ; 68 (11) g protein; 327 (35) g carbohydrate; 108 (12) g fat; 22 (7) g fibre. Total water provided during euhydrated trials was 40 $mL \cdot kg^{-1}$ body mass (2903 (332) mL) and provided in 6 equal aliquots consumed at set times during each trial, (i.e 0 h, 4 h, 6 h, 8 h 10 h and 13 h after the start of each trial). No water was consumed in the hypohydrated trials.

The *ad-libitum* breakfast consisted of porridge oats (Ready Brek, Weetabix, Kettering, UK) and semi-skimmed milk (Tesco Stores Ltd., Chestnut, UK) in a ratio of 100 g porridge oats to 400 mL milk. Each bowl of porridge received identical heating and cooling before being served. The *ad-libitum* breakfast was served in a custom built feeding booth inside an isolated feeding laboratory to minimise external distractions and to allow food to be provided with minimal interaction. Subjects were given standardised instructions to eat until they were 'comfortably full and satisfied' and to indicate satiation by leaving the booth and taking a seat in the adjoining laboratory. They had to remain in the laboratory for the whole 30 min eating period, and could return to the booth and continue eating if they desired, although no subject did. Subjects were initially provided with a single bowl of porridge and once approximately ½ to ¾ of the first bowl had been consumed a fresh bowl of porridge was supplied. This process continued until subjects indicated satiation by leaving the booth. Warm porridge was continually available for

subjects in the feeding booth. The time interval at which a new bowl of porridge was provided was determined during the familiarisation trial. This meant that finishing a bowl of porridge did not act as a satiety cue. During EU-F and HYPO-F, 500 mL of water and 500 mL of low sugar cordial were provided for subjects to drink *ad-libitum*, whilst during EU-NF and HYPO-NF no fluid was provided. During the EU-F and HYPO-F trials additional drink was available if required.

Sample handling and analysis

Venous blood samples (15 mL) were taken from an antecubital vein after 15 min rest in an upright seated position. Five mL blood was mixed with K_2EDTA (1.75 mg·mL^{-1}) and used for the determination of haemoglobin concentration using the cyanomethaemoglobin method and haematocrit by micro-centrifugation (Hawksley, Lancing, Sussex, UK). Haemoglobin and haematocrit values were used to estimate changes in plasma volume relative to 0 h (Dill and Costill, 1974). Five mL of blood was dispensed into a K_2 EDTA, (1.75 mg·mL^{-1}) tube (Sarstedt, Leicester, UK) containing a solution (10 µl·mL^{-1} blood) of potassium phosphate buffered saline (0.05 M), p-hydroxymercuribenzoic acid (0.05 M) and sodium hydroxide (NaOH), (0.06 M). The tube was then centrifuged at 3307 g for 10 min at 4^{o}C. Plasma was then transferred to a plain tube containing 1 M HCl (100 µl·mL^{-1} plasma) and centrifuged for a further 5 min before being stored at -20^{o}C for 24 h and then at -80^{o}C until analysis of acylated ghrelin concentration by enzyme-linked immunoassay (SPI BIO, Montigny le Bretonneux, France; intra-assay coefficient of variation 12%). The remaining blood (5 mL) was allowed to clot and the serum was separated by centrifugation at 3307 g for 10 min at 4^{o}C. Serum was refrigerated, before analysis for osmolality by freezing-point depression (Gonotec Osmomat 030 Cryoscopic Osmometer; Gonotec, Berlin, Germany). For urine samples at 0 h and 24 h subjects completely emptied their bladder and collected the entire

volume, whilst all urine produced between 0 h and 24 h was collected in a container provided. The volume and osmolality of all urine samples were determined.

Statistical Analysis

All data were analysed using statistical package SPSS 20.0 (Chicago, IL, USA) and initially checked for normality of distribution using a Shapiro-Wilk test. Data containing two factors were analysed using a two-way repeated-measures ANOVA. Significant differences were identified by Bonferroni-adjusted paired t-tests for normally distributed data or Bonferroni-adjusted Wilcoxon signed-rank tests for non-normally distributed data. Data containing one variable were analysed using one-way repeated measures ANOVA followed by Bonferroni-adjusted paired t-tests or Bonferroni-adjusted Wilcoxon signed-rank tests, as appropriate. Normally distributed data are presented as mean (SD). Non-normally distributed data are presented as median (range). All differences were accepted as being significant when $P<0.05$.

Results

Pre-trial measurements

Pre-trial body mass ($P=0.920$), urine osmolality ($P=0.260$) and serum osmolality ($P=0.243$), were not different between trials, indicating subjects started each trial in a similar hydration state.

Hydration variables

There was a main effect of trial ($P<0.05$) and time ($P<0.001$), as well as an interaction effect ($P<0.001$) for body mass. Body mass was similar at 0 h, but was lower at 24 h during HYPO-F and HYPO-NF compared with EU-F and EU-NF ($P<0.001$) and over the

trial body mass loss was greater during HYPO-F and HYPO-NF compared with EU-F and EU-NF (P<0.001) (Table 1). Total 24 h urine output was greater for EU-F and EU-NF than HYPO-F and HYPO-NF (P<0.001) (Table 1).

For both urine (Fig 1a) and serum (Fig 1b) osmolality, there were main effects of trial (P<0.001) and time (P<0.001), as well as interaction effects (P<0.001). Urine osmolality (P>0.163) and serum osmolality (P>0.492) did not change for EU trials over the 24 h, but both increased during HYPO trials (P<0.001). Furthermore, whilst there was no difference in urine or serum osmolality at 0 h, both were greater during HYPO trials compared to EU trials (P<0.001) at 24 h. The change in plasma volume over the trial meant that plasma volume at 24 h was greater during EU-F and EU-NF than HYPO-F and HYPO-NF (P<0.001) (Table 1).

A*d-libitum* energy intake and subjective responses

There was no difference between trials for *ad-libitum* energy intake (P=0.131) (Fig 2). Furthermore, there was no difference in energy intake when data were grouped according to hydration status, (EU trials 2491 (796) kJ; HYPO trials 2313 (737) kJ; P=0.120) or fluid availability (F trials (2460 (761) kJ; NF trials 2344 (780) kJ; P=0.410). More fluid was consumed during HYPO-F, (618 (251) mL) than during EU-F (400 (247) mL) (P<0.05).

For acylated ghrelin, there was a main effect of time (P<0.01), but no main effect of trial (P=0.089) or interaction effect (P=0.985). Mean values decreased over the 24 h for all trials, but this only reached significance during HYPO-F (P<0.05) and tended to decrease during EU-F (P=0.052) (Table 2).

There was a main effect of time (P<0.001) and trial (P<0.001), as well as an interaction effect (P<0.001) for subjective feelings of thirst (Table 3a). Compared to 0 h, thirst was increased at 24 h during HYPO-F (P<0.001) and HYPO-NF (P<0.05) and reduced at 24.5 h during EU-F (P<0.01) and HYPO-F (P<0.01). Thirst was greater at 24 h during HYPO trials compared to EU trials (P<0.001) as well as at 24.5 h during NF trials compared to during F trials (P<0.001). For both fullness (Table 3b) and hunger (Table 3c) there was a main effect of time (P<0.001), but no main effect of trial (P>0.294) or interaction effect (P>0.069).

Discussion

This study compared energy intake, acylated ghrelin and subjective appetite responses to alterations in hydration status and fluid availability. The main findings indicated that *ad-libitum* energy intake, acylated ghrelin and appetite sensations were not different between trials, although thirst was increased with hypohydration. These findings suggest that appetite and energy intake in humans are not affected by moderate levels of hypohydration or fluid restriction, which contrasts with previous research in animals (Lepkovsky *et al*., 1957; Silanikove, 1992; Senn *et al*., 1996; Watts, 1999) and humans (Engell, 1988; Shirreffs *et al*., 2004), as well as our main hypothesis.

Shirreffs *et al*. (2004) reported that complete fluid restriction over a 37 h period reduced *ad-libitum* energy intake by ~28% compared to a euhydrated control trial where subjects were free to consume fluids *ad-libitum*, despite no difference in subjective appetite responses. Shirreffs *et al*. (2004) limited subjects to the consumption of “dry foods” during the fluid restriction trial, but subjects could consume any foods during the control trial. It is possible that these changes in eating behaviour or the consumption of energy

containing fluids in the control trial might explain the difference in energy intake between the trials. Engell (1988) investigated energy intake during six consecutive meals over 48 h and found that when fluid was limited to 43% of *ad-libitum* fluid intake, *ad-libitum* energy intake was reduced by ~37% compared to a trial where fluids were available *ad-libitum*. Although fluid was restricted, hydration status was not measured and therefore the findings have been attributed to a close relationship between eating and drinking patterns (Engell, 1988). This is further described by McKiernan *et al.* (2008) who reported that drinking independently of eating is rare and approximately 75% of daily fluid intake is consumed at meal times.

Although it appears there is a strong behavioural link between food intake and drink intake, it has been suggested that there may be certain other physiological mechanisms that might explain changes in energy intake in response to fluid restriction and/ or hypohydration. Walsh *et al.* (2004) and Oliver *et al.* (2008) have reported that hypohydration decreases salivary flow rate. Others have reported that hypohydration decreases the rate of gastric emptying and reduces gastric secretions (Neufer *et al.*, 1989; Rehrer *et al.*, 1990). Symptoms of dry mouth, which are likely related to a reduced salivary flow rate have been reported to decrease energy intake in irradiated patients (Bäckström *et al.*, 1995) and the elderly (Lovat, 1996). This reduced energy intake may be due to peri-prandial feelings of satiety from reduced palatability of foods and/ or slower oral processing (i.e. increased chewing etc.) (Smit *et al.*, 2011). Silanikove (1992) linked reductions in salivary secretions and feed intake in ruminants during 72 h water restriction. These mechanisms might provide a plausible explanation as to why previous studies that restricted fluid ingestion during eating (Engell, 1988; Shirreffs *et al.*, 2004) or restricted foods to those with a low moisture content (Shirreffs *et al.*, 2004) observed reduced energy intake with hypohydration/ fluid restriction. Alterations in hydration

status might also influence appetite regulation and a previous study reported that acylated ghrelin was reduced after exercise-induced dehydration compared to when euhydration was maintained (Kelly *et al*., 2012), but that there was no change in peptide YY or pancreatic polypeptide. Neither the present study nor that of Corney *et al*. (2015) observed any differences in acylated ghrelin between hypohydration and euhydration. The divergent findings between studies might be accounted for by the different protocols used to induce hypohydration.

Both Kelly *et al.* (2012) and Corney *et al.* (2015) reported no difference in energy intake from a breakfast buffet meal when subjects were either euhydrated or hypohydrated at the start of the meal, with hydration status manipulated through a combination of exercise and fluid restriction. Both these studies provided fluids *ad-libitum* during eating and as such support the notion that hydration status does not affect *ad-libitum* energy intake when fluids are provided with a meal. In line with this, studies in rats report a rapid restoration of normal eating patterns when water is provided again after 5 days of dehydration induced anorexia produced by saline ingestion (Watts, 1999).

As discussed above, there are a number of studies in both humans (Engell, 1988; Shirreffs et al. 2004) and animals (Lepvoksky et al. 1957; Silanikove, 1992; Senn *et al.*, 1996; Watts, 1999) that suggest fluid restriction during eating decreases energy intake, but the results of the present study do not support this. Even comparison of just the EU-F and EU-NF trials with a t-test revealed no effect of fluid restriction (P=0.128). We speculate that the lack of agreement between this and previous studies might be caused by two possible explanations. Firstly, the choice of a semi-solid breakfast might have been enough to maintain energy intake during the meal. Indeed, whilst thirst compared to 0 h was increased immediately before the meal during HYPO-NF, the consumption of the meal (but no fluid) reduced thirst such that it was no longer different from 0 h. We chose

to use the single item porridge breakfast in the present study as previous studies have utilised a buffet style breakfast (i.e. Kelly *et al.,* 2012 and Corney *et al.,* 2015). Buffet style meals are known to encourage over feeding (Mirtch *et al.,* 2006) and might encourage learned eating behaviours between trials (e.g. I ate one slice of bread last week, so I'll do the same this week). Secondly, breakfast is perhaps the most habitual meal of the day and therefore expected satiety might have a greater impact on energy intake at breakfast than the effects of small manipulations of hydration status or fluid availability. Future studies should examine eating behaviour at meals other than breakfast, as well as over longer time periods, incorporating multiple meals.

Previous studies that have examined the influence of hydration status on appetite regulation (Kelly et al., 2012; Corney et al., 2015) have induced hypohydration of 2-3% body mass in comparison to the <2% induced in the present study. Therefore, at least in healthy young populations, small deviations in hydration status (<2-3% body mass) are unlikely to explain alterations in eating behaviour. Whether hypohydration of greater than 2-3% body mass influences appetite and eating behaviour is not known. The reduction in food intake caused by hypohydration in animal studies is often associated with much larger degrees of hypohydration (Senn *et al*., 1996; Watts, 1999), and it may be that the level of hypohydration at which human eating behaviour is affected is greater than 2-3%. Whilst hypohydration of >3% body mass is not a common occurrence, some athletes in training or competition (Cheuvront and Haymes, 2001) or military personnel during field exercise or sustained operations (Lieberman *et al*., 2005) might be exposed to these levels of hypohydration. A decrease in appetite and/ or food intake might therefore impair recovery from exercise, training adaptation or military duties.

If fluid is not available during feeding, then a reduction in energy intake might be observed with hypohydration (Engell, 1988; Shirreffs *et al.,* 2004), although the present

study suggests this might depend on the nature of the food available. Only a few studies have examined the effect of fluid intake during or in close proximity to meals, but alterations in hydration status that influence thirst sensation might have the potential to influence eating behaviour. If sufficient, water intake causes gastric distension and thus might attenuate food intake. This effect has been demonstrated in animal models (Share *et al*., 1952), and is likely to be explained by activation of the vagal nerve due to gastric distension (Paintal *et al*., 1954). In young healthy adults, ingestion of a bolus of water (~500 mL) 30 min (Van Walleghen *et al*., 2007) or 60 min (Rolls *et al*., 1990) before an *ad-libitum* meal does not influence eating behaviour. However, immediate pre-meal water intake that produces gastric distension might reduce food intake (Corney *et al*., 2014). If thirst is greatly increased due to hypohydration it seems likely that at least some water ingestion will occur immediately prior to eating. Although whether this is sufficient to influence eating behaviour is likely to depend on the volume of fluid required to satiate thirst prior to eating, which was not determined in the present study.

There are limitations to the present study that need to be acknowledged. The study design was limited in scope, in that only one level of hypohydration (~2% body mass loss) was examined and the measurement of energy intake was only determined at a single meal. It seems from this and previous studies that future investigations should seek to examine the effects of larger losses of body water (i.e. >3% body mass). Additionally, future studies should examine situations where larger deviations in hydration status are likely to occur such as prolonged endurance exercise with inadequate fluid intake (Cheuront and Haymes, 2001) or military training (Lieberman *et al*., 2005). Although also limited in scope, previous studies in humans that have reported reductions in energy intake with reductions in fluid intake and hydration status have examined energy intake over an extended period (Engel, 1988; Shirreffs et al., 2004). Therefore, future studies should

examine the influence of hypohydration on energy intake at meals other than breakfast and/ or multiple meals.

In conclusion, these results demonstrate that in a laboratory setting there appears to be little effect of hypohydration or fluid availability on *ad-libitum* intake. These findings are likely explained by the use of a semi-solid breakfast meal, which might be more palatable to the hypohydrated/ fluid restricted individual.

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Table 1. Body mass change relative to 0 h (%), 24 h urine (mL), plasma volume change relative to 0 h (%). Values are mean (standard deviation). ^ Significantly different from EU-F and EU-NF.

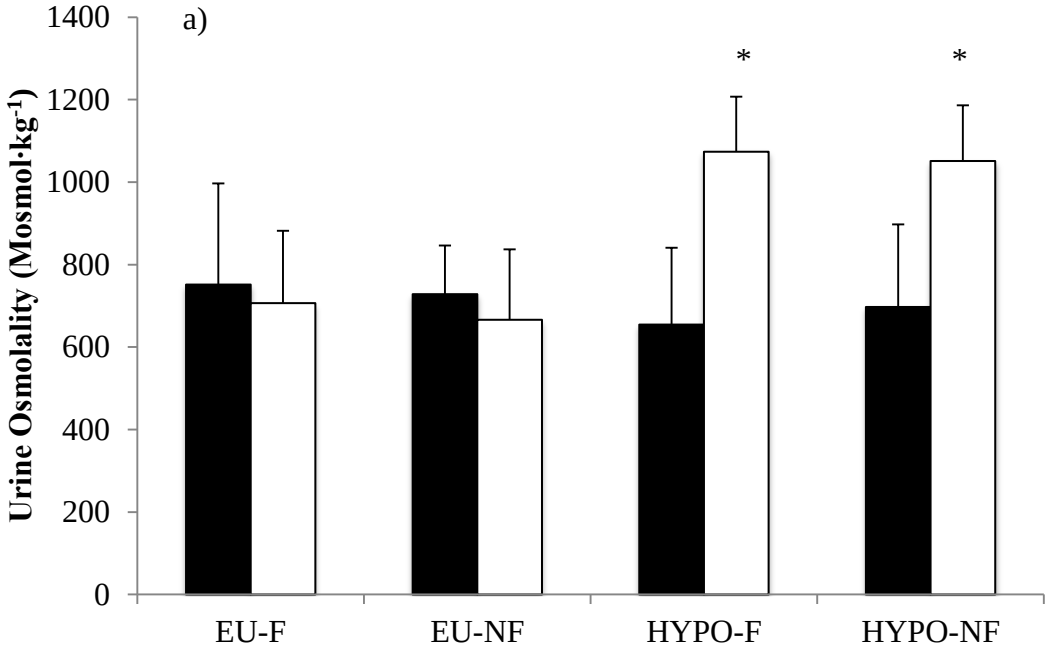
	EU-F	EU-NF	HYPO-F	HYPO-NF
Body mass change %	-0.28 (0.59)	-0.35 (0.51)	-1.78 (0.53)^	-1.89 (0.45)^
24 h urine volume (mL)	2262 (494)	2478 (494)	724 (272)^	806 (201)^
Plasma volume change (%)	+0.3 (3.9)	+2.0 (3.5)	-2.9 (2.8)^	-4.1 (2.3)^

Table 2. Acylated ghrelin ($pg \cdot mL^{-1}$). Values are median (range). * Significantly different from 0 h.

	0 h	24 h
EU-F	122 (29-292)	105 (21-263)
EU-NF	97 (24-295)	88 (5-267)
HYPO-F	147 (15-542)	103 (18-473)*
HYPO-NF	149 (17-311)	112(19-303)

514 **Table 3.** Subjective feelings reported using 100 mm visual analogue scales for thirst (a),
515 fullness (b) and hunger (c). Values are median (range). ^ Significantly different from EU-
516 F and EU-NF. # Significantly different from EU-NF and HYPO-NF. * Significantly
517 different from 0 h.

		0 h	24 h	24.5 h
a) Thirst	EU-F	56 (16-100)	52 (5-78)	9 (0-67)*#
	EU-NF	51 (31-85)	56 (15-100)	73 (14-86)
	HYPO-F	47 (19-96)	91 (69-100)*^	12 (0-75)*#
	HYPO-NF	67 (18-86)	92 (29-100)*^	74 (4-92)
b) Fullness	EU-F	26 (0-51)	22 (12-50)	80 (61-95)*
	EU-NF	25 (3-51)	35 (2-52)*	82 (67-96)*
	HYPO-F	31 (6-49)	15 (4-75)	87 (54-100)*
	HYPO-NF	29 (0-66)	22 (6-85)	79 (50-94) *
c) Hunger	EU-F	52 (3-100)	70 (14-85)	7 (0-37)*
	EU-NF	69 (25-92)	61 (13-87)	13 (0-54)*
	HYPO-F	68 (32-90)	70 (27-94)	8 (0-28)*
	HYPO-NF	66 (40-86)	75 (6-96)	7 (0-45)*



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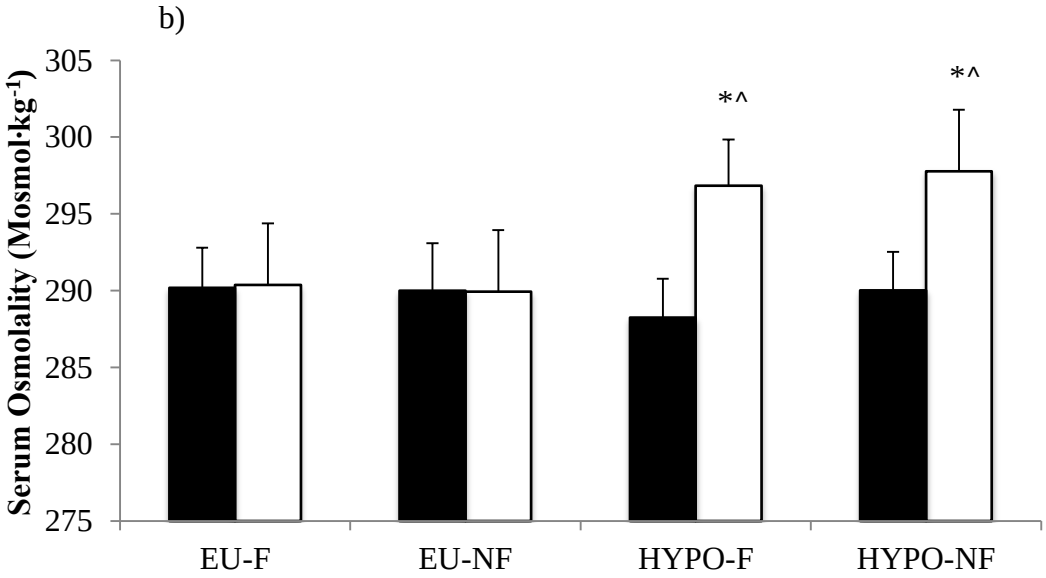

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520 **Fig 1.** Osmolality (mosmol·kg^{-1}) of serum (a) and urine (b) of samples collected at 0 h
521 and 24 h. Bars represent mean values and error bars are SD. * Significantly different
522 from 0 h. ^ Significantly different from EU-F and EU-NF.

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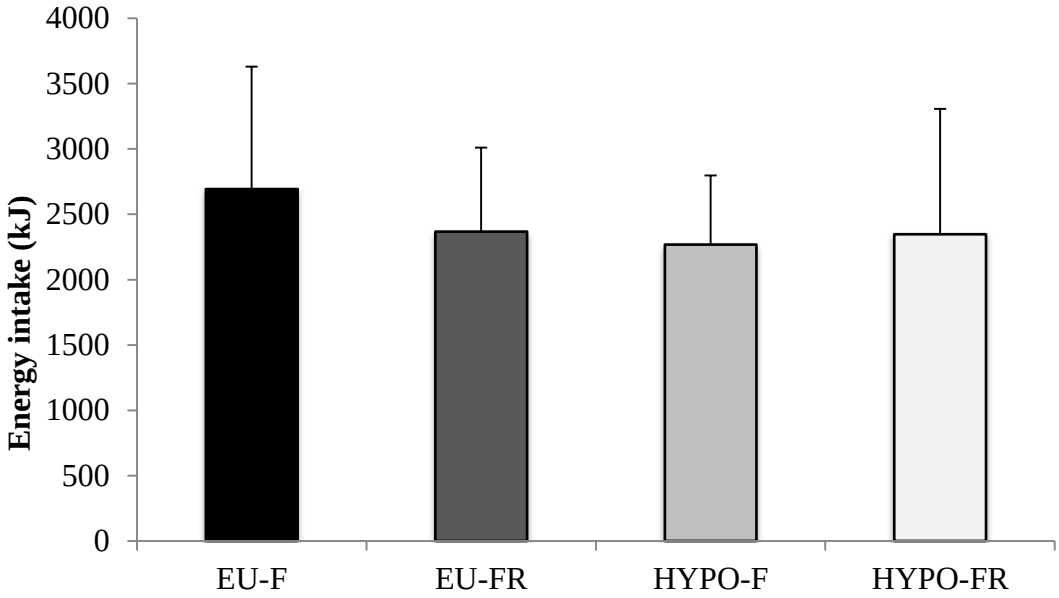

Fig 2. Energy intake (kJ) at the *ad libitum* breakfast. Bars are mean and error bars are SD.