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Epidemiologic data have linked chronic low fluid intake (i.e., underhydration) with greater incidence of obesity, but the underlying mechanisms behind this association are unclear. No study has assessed the direct effect of underhydration on *energy balance (EB),* which is inclusive of energy consumed from food or fluid (EI) and energy expended from resting metabolism (RMR), the thermic effect of food (TEF), and physical activity (PAEE). Underhydration increases release of the fluid regulatory hormone arginine vasopressin (AVP) to conserve total body water. However, chronic elevations in AVP may cause metabolic changes including alterations in cortisol release that could influence one's propensity toward developing obesity and metabolic disease. Thus, the purpose of the present study was to characterize the associations between habitual fluid intake and behavioral, perceptual, and physiologic factors influencing energy balance to inform the development of effective intervention strategies promoting adequate hydration for metabolic health. Healthy male participants with low, moderate, and high habitual fluid intake completed measures of EI and fluid intake (TFI), PAEE for seven days, as well as measures of hydration status for four of these days. Participants also came to the lab for assessments of RMR, TEF, fasting and postprandial changes in appetite and thirst, food reward, and salivary and hematological measures of hormonal responses to hydration status. Higher habitual fluid intake was associated with higher RMR and increased PAEE, but there was no effect on overall EB. There was no association between habitual fluid intake and appetite ratings. Lower habitual fluid intake and a flatter diurnal cortisol slope were independently associated with liking of high fat sweet foods and wanting of high fat savory foods, respectively, but twenty-four-hour urinary osmolality was not associated with salivary

cortisol dynamics (peak cortisol, cortisol awakening response, diurnal cortisol slope). These data suggest increased fluid intake is a promising target for future interventions to aid with weight maintenance from both a physiologic and behavioral standpoint.

THE INFLUENCE OF HABITUAL FLUID INTAKE ON ENERGY BALANCE

by

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DEDICATION

To my parents, who always believed in me, even when times were tough.

APPROVAL PAGE

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CHAPTER I: INTRODUCTION

While early sports science literature has focused on the relationship between acute body water deficits and declines in physical performance, more recent evidence suggests associations between chronic inadequate water consumption and adverse health outcomes. Specifically, epidemiologic data have linked chronic low fluid intake with increased incidence of obesity (T. Chang et al., 2016; Enhörning et al., 2013), as well as altered HPA-axis activity (Armstrong et al., 2020), insulin resistance(Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019; Corney, Sunderland, et al., 2015b; Kelly et al., 2012; Pérez-Luco et al., 2019) and increased central adiposity (H. K. Min et al., 2020). Thus, increasing fluid intake has been suggested as a potential strategy to mitigate the substantial economic and health burdens of obesity. Yet, few investigations have assessed the impact of acute changes in fluid intake or hydration status on primary contributors to obesity, such as those influencing energy intake (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019; Corney, Horina, et al., 2015; Corney, Sunderland, et al., 2015; Kelly et al., 2012; Pérez-Luco et al., 2019). No studies have assessed the direct effect of *chronic fluid intake behaviors* on additional factors contributing to *energy balance*.

Energy balance is defined as the difference between energy intake (i.e., calories ingested from food) and energy expenditure (i.e., calories expended at rest or during activity) (J. O. Hill et al., 2013). Over time, a positive energy balance contributes to an increase in body mass and eventual obesity if compensatory physiologic or behavioral adjustments do not occur return to a state of neutral energy balance (Hopkins & Blundell, 2016). Most studies (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019; Corney,

Sunderland, et al., 2015, 2015; Kelly et al., 2012) examining the relationship between hydration status and energy balance have only considered the consciously modifiable individual factors influencing energy balance, such as energy intake or physical activity energy expenditure. Yet energy balance is also dependent upon one's resting metabolic rate (Hopkins & Blundell, 2016) and the energetic cost of digestion (i.e., thermic effect of food) (Calcagno et al., 2019). Studies have also primarily used acute (<24h) protocols meant to induce body water loss as a proxy for habitually low fluid intake, with some confounded by additional factors such as exercise or heat exposure to induce hypohydration (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019; Corney, Horina, et al., 2015; Corney, Sunderland, et al., 2015; Kelly et al., 2012; Pérez-Luco et al., 2019). No studies have considered habitual fluid intake among participants in their design, instead standardizing fluid intake immediately prior to experimental trial to achieve a common state of "euhydration" before either maintaining this state (control condition) or undergoing a dehydration protocol meant to induce a certain percentage of total body water loss via heat exposure, exercise, or a combination. While beneficial from a control perspective, findings from such designs may not be applicable to individuals who chronically under consume fluids.

Chronic *underhydration* induces a preservation of serum osmolality, reduced thirst, and maintenance of total body water (Kavouras, 2019). These effects are driven by an increase in the fluid regulatory hormone arginine vasopressin (AVP), which acts primarily on the kidneys to promote water reabsorption and thus conservation of total body water. By contrast, acute total body water losses caused by common dehydration protocols may result in an increased osmolality that would instead favor *decreased* energy intake (Boyle et al., 2012). However, chronic elevations in AVP have been associated with an increased risk for metabolic disease,

including obesity and diabetes (Carroll et al., 2015; Enhörning et al., 2013, 2021). Different AVP receptor subtypes exist on many organs and in brain regions critical for metabolic function (Koshimizu et al., 2012, p. 1; Oshikawa et al., 2004). AVP binding to these receptors may induce changes in hormonal profiles and responsiveness which could increase obesity risk by influencing one's responsiveness to appetite signals and physiologic factors influencing food wanting and liking. These effects include the action of AVP on the hypothalamic-pituitary-adrenal (HPA) axis through V1a and V1b receptors to ultimately induce an increase in cortisol release. Cortisol, in turn, influences fuel utilization, and chronic elevations in cortisol can influence food selection, food reward, and total energy intake (Duong et al., 2012; Herhaus et al., 2020). Over time, dysfunction in these pathways may help explain the observed associations between low fluid intake and metabolic health outcomes. The influence of habitual fluid intake, and the corresponding regulatory responses, on factors influencing energy balance, is unknown. .

While some literature has found baseline differences in cortisol among "high" and "low" drinkers (E. Perrier, Vergne, et al., 2013), the relationship between habitual fluid intake and the downstream effects of this difference on energy balance and its contributors has not been directly measured. Studies exploring the connection between hydration status and appetite measures have produced mixed effects (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019; Corney, Sunderland, et al., 2015; Kelly et al., 2012), with some finding reduced fullness when in a state of hypohydration (Corney, Sunderland, et al., 2015). The relationship between chronic fluid intake behaviors and appetite, both at baseline and response to a meal, have not been explored. Greater water intake may naturally coincide with other health-promoting behaviors such as increased physical activity and greater fruit and vegetable intake (Popkin et al., 2005). Consuming more plain water instead of sugar-sweetened beverages can

lead to a considerable reduction in total energy intake and body weight among individuals accustomed to consuming more energy dense beverages (J. J. D. Stookey, 2016). Further, water consumption prior to meals may induce gastric distension that could naturally reduce appetite and subsequent energy intake (Corney et al., 2016). Taken together, greater water intake has the potential to reduce one's likelihood of being in a positive energy balance, thereby promoting body weight maintenance.

Fluid consumption is reinforced through stimulation of thirst, which is governed by central and peripheral mechanisms including complex neural networks which sense and relay changes in total body water and its distribution (Armstrong & Kavouras, 2019; Millard-Stafford et al., 2012). Drinking behavior is also guided by social and psychological cues, and thirst does not always translate to adequate fluid intake. Yet thirst seems to follow a more stable pattern throughout the day compared to hunger, which follows natural elevations corresponding with mealtimes (McKiernan et al., 2008). It has been estimated that ~75% of fluid ingestion occurs around mealtimes, with most fluid consumption around meal times coming from energy-yielding beverages (McKiernan et al., 2009). Eating serves as a thirst stimulator to replace fluids used for digestion (e.g. saliva and gastrointestinal water) and to restore blood osmolality to normal levels after consumption of osmolytes from food sources (Leib et al., 2016). Given the intricate links between eating and drinking behavior and the influence of both on physiological indicators of hydration status, the present study seeks to further clarify how these relationships interact to influence energy balance.

The amount of food consumed is driven by both homeostatic and hedonic mechanisms (Bojanowska & Ciosek, 2016; Hopkins & Blundell, 2016). The homeostatic drive to eat is regulated by the hypothalamus based on nutritional status to induce hunger or promote satiety.

The hedonic drive to consume food is related to the brain reward system involving corticolimbic and mesolimbic structures within the brain. This reward system influences one's "liking" of food items, referring to the pleasure obtained from palatable food consumption, as well as one's "wanting", or appetite and motivation to eat a specific food. Only one study has experimentally assessed manipulation of hydration status on food reward ratings, finding an increased preference for food items higher in moisture content and lower in salt following an acute dehydration protocol (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019). However, whether these preferences influence real-time food selection has not been explored in those with different habitual fluid intake. Cluster analyses of NHANES data have shown that water consumers tend to drink fewer soft drinks, eat more fruits and vegetables, low- and medium-fat dairy products, and consume an average of 194 fewer kcals per day (Popkin et al., 2005), though it is not clear what underlying factors are influencing these differences. Perhaps these differences may be attributed to the effects of hydration status directly through an attempt to consume foods aimed at promoting water conservation (i.e., saltier food items, typically higher in energy density) (L. A. J. De Luca et al., 2007; Ma et al., 2015; Rakova et al., 2017), or indirectly through increased cortisol secretion under states of underhydration (R. S. Chang et al., 2022; E. Perrier, Vergne, et al., 2013). It is unclear how chronic low fluid intake and the associated hormonal responses reflecting underhydration influence the homeostatic and hedonic mechanisms regulating eating behaviors.

Thus, the overall purpose of the present study was to characterize the physiological (i.e., hormonal, resting metabolic rate, thermic effect of food), perceptual (i.e., appetite, thirst, food reward), and behavioral (i.e., ad-libitum food selection, energy intake and physical activity energy expenditure) differences impacting energy balance based on fluid intake to inform the

development of effective intervention strategies involving water intake for the promotion of weight management and metabolic health. The following aims were completed to help clarify the links between chronic low fluid intake and risk for positive energy balance and metabolic disease states. The current study adds to this literature by addressing the following specific aims:

- **Specific Aim 1:** Determine the influence of habitual fluid intake on acute (24h) and chronic (over the course of six self-report dietary records) energy balance.
    - **Research Hypothesis 1.1**: Lower habitual fluid intake will predict a greater within-day energy intake relative to estimated energy needs.
    - **Research Hypothesis 1.2:** Lower habitual fluid intake will predict a more positive energy balance across daily measures.
    - **Research Hypothesis 1.3:** Lower habitual fluid intake will predict lower RMR and lower TEF.
    - **Research Hypothesis 1.**4: Lower habitual fluid intake will be associated with reduced physical activity.
- **Specific Aim 2:** Identify the influence of habitual fluid intake on appetite and food reward.
    - **Research Hypothesis 2.1:** Lower habitual fluid intake will predict greater fasting appetite and greater AUC for appetite perceptions.
    - **Research Hypothesis 2.2:** Lower habitual fluid intake will be associated with greater food reward (explicit wanting and explicit liking) for food items associated with a positive energy balance

(i.e., high fat, sweet foods and high fat, savory foods). Lower habitual fluid intake will be associated with greater implicit wanting for high fat, savory food items.

- **Specific Aim 3:** Explore the relationship between habitual fluid intake, HPA-axis activity and energy balance.
    - **Research Hypothesis 3.1:** Higher 24h Urinary osmolality will be associated with higher peak cortisol, blunted salivary cortisol awakening response, and flatter diurnal cortisol slope.
    - **Research Hypothesis 3.2:** Higher peak cortisol, blunted salivary cortisol awakening response, and a flatter diurnal cortisol slope will be associated with greater explicit wanting" and explicit liking of high fat, savory foods.

CHAPTER II: REVIEW OF LITERATURE

Hydration Recommendations

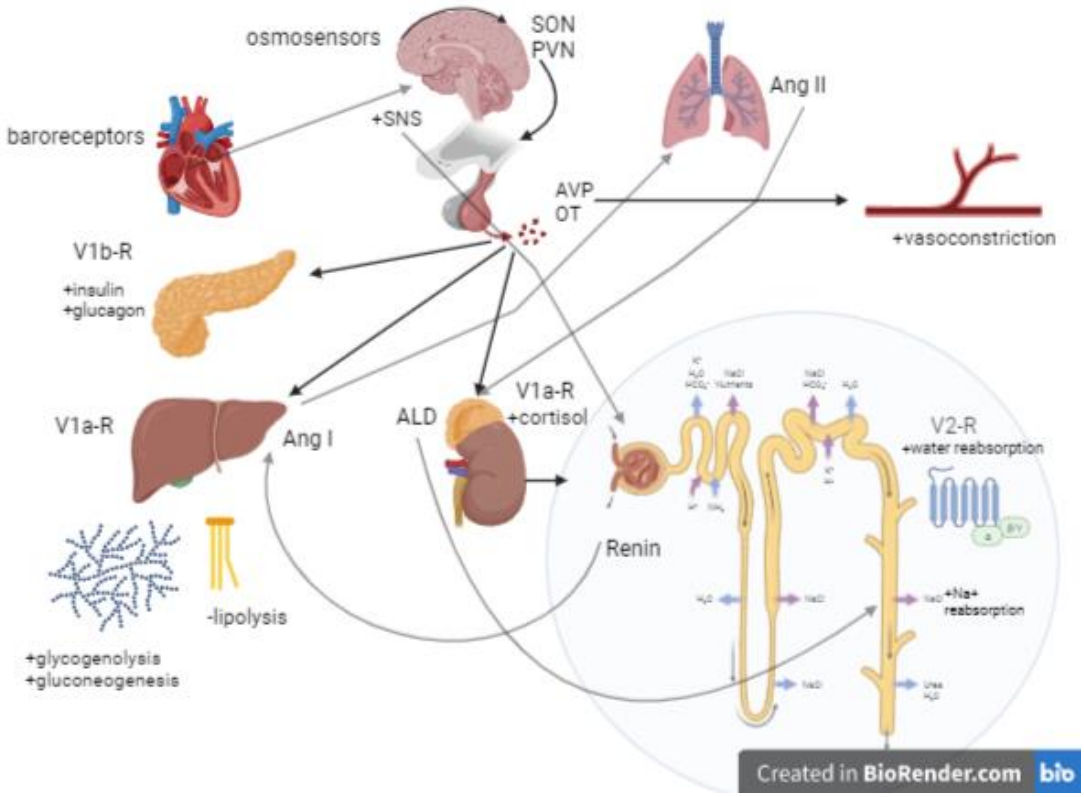
The European Food Safety Authority recommends the consumption of 2.0 L of fluid per day for females and 2.5 L per day for males (EFSA, 2010). These recommendations have been established as a target fluid consumption aimed at attaining a urine concentration (osmolality) of 500mOsm*kg^{-1}, associated with the reduction of adverse health outcomes (EFSA, 2010). Similarly, the Institute of Medicine recommends consumption of 2.7L of fluid for females and 3.7L of fluid for males, which includes fluids both from liquids and food sources (Gandy, 2015). As many as 60% of males and 40% of females do not meet these fluid intake recommendations (Ferreira-Pêgo et al., 2015). Low fluid consumption has been associated with adverse health effects, including impairments in glycemic regulation, chronic kidney disease, and metabolic syndrome (Roncal-Jimenez et al., 2015; J. D. Stookey et al., 2020; Vanhaecke et al., 2020). These adverse health effects extend beyond the typical performance and cognitive declines associated with acute body water losses (e.g., acute body water losses from sweating or heat exposure) and are related to compensatory hormonal responses to conserve body water. In 2019, Dr. Kavouras coined the term “underhydration” to represent low fluid intake leading to an increase in fluid regulatory hormones (e.g., arginine vasopressin), maintenance of plasma osmolality, and decreased thirst (Kavouras, 2019). Over time, chronic elevations in fluid conserving hormones may pose a detrimental impact on long term health. Particularly, the relationship between water intake and metabolic function (i.e., glucose regulation, dyslipidemia, and obesity) has been the focus of recent research. The following subsections discuss the overall regulation of fluid balance before discussing the proposed physiologic and behavioral rationale

for low fluid intake contributing to a positive energy balance and, over the long term, an increased risk of obesity.

Physiology of Underhydration

Human body water regulation and the distribution of water between the intracellular and extracellular fluid space involves a complex series of central (neural) and peripheral (humoral and vascular) mechanisms that work in concert to influence fluid consumption or to conserve or excrete body water **(**Figure 1**)**. The response will vary based on the source (i.e., intracellular vs extracellular water), magnitude, and method of body water change (Armstrong & Kavouras, 2019).

Figure 1. Summary of Primary Fluid Regulatory Pathways Activated in Response to Total Body Water Loss. Darker Pathway Follows the Course Of Hyperosmolality Driven Responses. Gray Pathways Illustrate the Primary Response to Hypovolemia.



Central Mechanisms

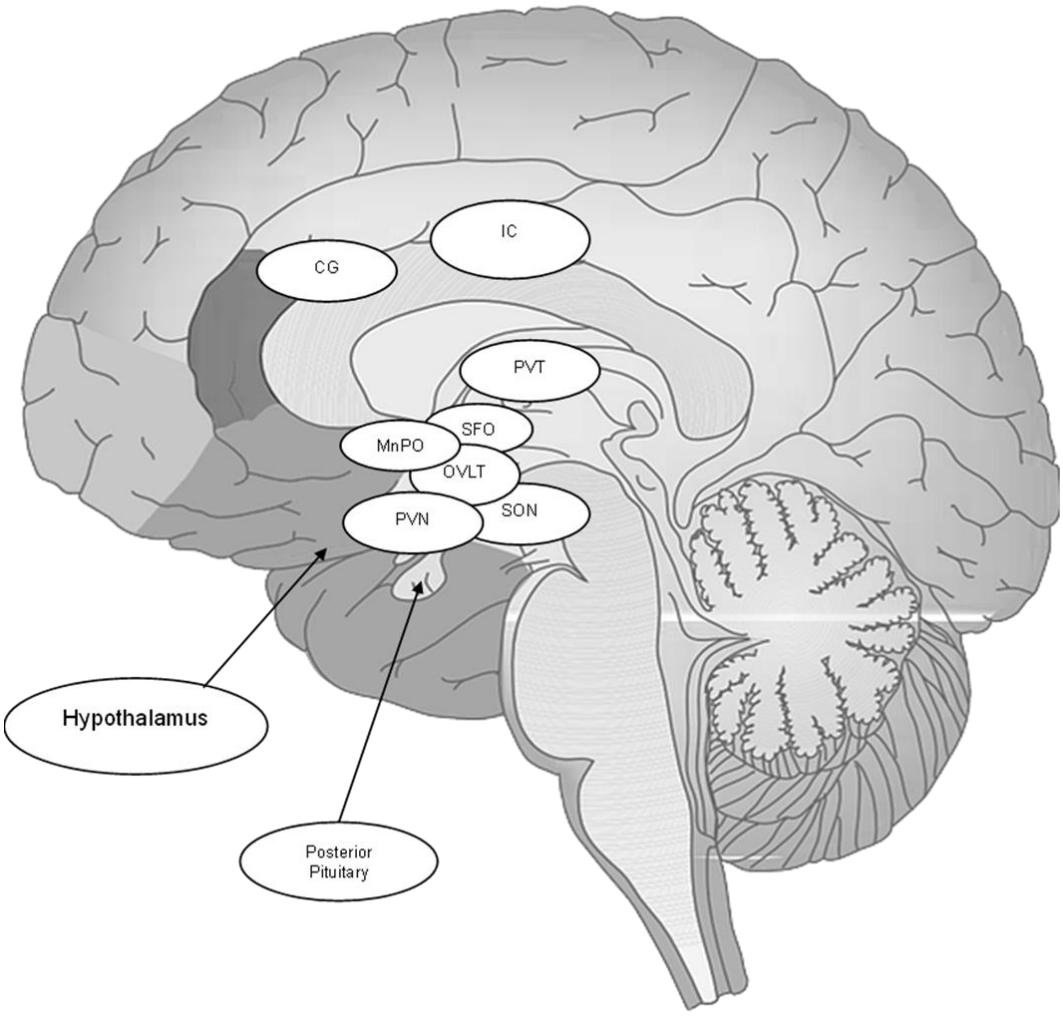
Neural responses to fluid disturbances occur quickly to activate changes in vascular resistance and thus peripheral fluid distribution. Among these, baroreceptors in the heart and vascular system respond to decreases in blood volume (hypovolemic hypohydration). Reduced plasma volume results in reduced venous return to the heart and a decline in central blood pressure and left ventricular stroke volume that ultimately unloads the cardiac baroreceptors in the carotid artery and aortic arch (Baker & Jeukendrup, 2014). This unloading of cardiac

baroreceptors initiates sympathetic nervous system activity that causes peripheral and renal vasoconstriction to restore central venous pressure and mean arterial pressure.

More extended perturbations to fluid balance (> ~20 min) stimulate the release of circulating hormones to modify renal salt and water excretion. With sweat or exercise-induced water loss (hypertonic hypovolemia), increased plasma or cerebrospinal fluid osmolality causes a net movement of water from body cells into the plasma to restore normal osmolality (~280 – 295 $mOsm*kg^{-1}$) (Nose et al., 1988). Reduced intracellular volume within the osmoreceptor cells of the hypothalamus (within the organum vasculosum of the lamina terminalis (OVLT) and the subfornical region (SFO) – circumventricular organs located outside the blood-brain barrier) and heart stimulate the synthesis of arginine vasopressin (AVP, or antidiuretic hormone) by activating the median preoptic nucleus (MnPO) (Antunes-Rodrigues et al., 2004) (Figure 2). The MnPO then stimulates the supraoptic (SON) and paraventricular nuclei (PVN) in the medial aspect of the hypothalamus (McKinley et al., 2015). Magnocellular neurons in the SON and PVN produce the fluid regulatory peptide hormone AVP for storage in the posterior pituitary gland. AVP is released from the posterior pituitary gland to circulation and acts primarily on the kidneys to increase water reabsorption. Neural osmoreceptors also stimulate thirst and salt intake to promote drinking and restoration of vascular volume. Osmoreceptor stimulation in the OVLT and SFO sends efferent signals to the MnPO to activate the paraventricular nucleus of the thalamus (PVT) (Allen et al., 2017). However, there is interindividual variability in the threshold of plasma osmolality change required to initiate this response (Cheuvront et al., 2013). The PVT activates the cingulate gyrus and insular cortex, which stimulates the sensation of thirst, thus promoting water intake to restore normal osmolality (Becker et al., 2017). Thirst is typically alleviated before osmolality restoration, related to peripheral preabsorptive factors that include

oral, oropharyngeal, and gastrointestinal signals relayed to the MnPO, which may act as a "flow-meter" sensing gulping actions and relaying signals for drinking cessation (Augustine et al., 2018). Regardless, an increase in plasma osmolality seems to be the primary driver of the thirst response in mammals, with a 1-2% increase in osmolality inducing water intake; by contrast, a 10% reduction in blood volume is required to induce thirst (Antunes-Rodrigues et al., 2004). The SFO and OVLT can also bind angiotensin II (discussed below), leading to downstream activation of central nervous system structures involved in hypovolemic thirst, sodium appetite and blood pressure control (Fitzsimons, 1998).

Figure 2. Visual Representation Of Major Brain Regions Involved In Thirst And Fluid Regulation. CG = Cingulate Gyrus, IC = Insular Cortex, OVLT = Organum Vasculosum Of The Lamina Terminalis, Mnpo = Median Preoptic Nucleus, PVN = Paraventricular Nuclei; SFO = Subfornical Region, SON = Supraoptic Nuclei.



Peripheral Mechanisms

Peripherally, fluid regulation is controlled by AVP binding to its G-protein coupled receptor subtypes: V1a, V1b, and V2. The kidneys serve as the primary and final organ

governing the maintenance or removal of water and solutes through either glomerular filtration or tubular reabsorption. Specifically, increased AVP secretion in response to an increase in plasma osmolality promotes renal water reabsorption by binding to the V2 receptors (V2-R) on the basolateral membrane of cells in the distal tubule and collecting duct in the kidneys (Schrier, 2008). This binding activates adenylate cyclase to increase cAMP formation, which then activates protein kinase A. Protein kinase A phosphorylates and activates the translocation of aquaporin-2 channels from the cytoplasm to the luminal membrane, which promotes the transport of water from within the kidney lumen to the blood (Antunes-Rodrigues et al., 2004). AVP binding to V2-R also upregulates the transcription of aquaporin-2 genes and the permeability of these channels to water. Combined, this results in fluid retention and excretion of more concentrated urine to maintain or restore normal plasma osmolality during periods requiring water conservation. AVP also binds to vascular V1a-R to promote vasoconstriction and increase blood pressure (Henderson & Byron, 2007). Low-pressure volume receptors in the atria and pulmonary venous system send afferent signals to the brain stem to increase AVP secretion, though this effect on AVP release is less potent than osmotic stimulation. AVP increases systemic blood pressure but also enhances baroreflex control of heart rate by slowing the rate when blood pressure is elevated (Koshimizu et al., 2012); thus, the effects of AVP on cardiovascular control through vascular V1a-R seem minor compared to the renin-angiotensin-aldosterone system (RAAS).

Under hypovolemic conditions (i.e., sweat losses or diuretic administration), RAAS activation helps restore blood volume. The enzyme renin is released by the juxtaglomerular apparatus cells of the kidney in response to increased sympathetic nervous system activity (β-1 adrenergic nerve stimulation), decreased blood pressure, decreased blood sodium, or decreased

renal blood flow (Baker & Jeukendrup, 2014; Cheuvront & Kenefick, 2014). Renin converts the hepatic prohormone angiotensinogen to angiotensin I. Angiotensin-Converting Enzyme (ACE) converts angiotensin I to angiotensin II as it passes through pulmonary circulation; angiotensin II directly increases arterial smooth muscle constriction to maintain blood volume through the creation of hydrostatic and oncotic pressure gradients that promote more water reabsorption (Griendling et al., 1997). Angiotensin II also stimulates aldosterone production in the zona glomerulosa of the adrenal cortex by increasing the transcription of cytochrome P450 Family 11 Subfamily B Member 2 (CYP11B2), the gene encoding aldosterone synthase, which converts 11-deoxycorticosterone to aldosterone (Clyne et al., 1997). Aldosterone promotes increased renal sodium chloride reabsorption by binding to the mineralocorticoid receptor to induce expression of the serum/glucocorticoid regulated kinase 1 (SGK1) gene, which increases the insertion and reduces degradation of the epithelial sodium channel (ENaC) in the principal cells of the collecting duct (Valinsky et al., 2018). Aldosterone also increases the activity of the sodium hydrogen exchanger 3 (NHE3) transporters on the apical membrane of the proximal convoluted tubule in conjunction with increased basolateral membrane Na+/K+-ATPase activity that promotes sodium reabsorption (Musch et al., 2008). As described above, angiotensin II also acts within the CNS to stimulate thirst and increase both water and sodium ingestion. Because it is also released locally in the brain (including the SFO, SON, PVN, central nucleus of the amygdala, and in the brain stem at the nucleus tractus solitarius, parabrachial nuclei, and locus ceruleus (Fitzsimons, 1998), angiotensin II seems involved in learning and memory of sodium appetite and the release of AVP (Fitzsimons, 1998). AVP can also stimulate aldosterone secretion through the adrenal glands via the V1a-R in the macula densa cells by activating neuronal nitric oxide synthase (nNOS) and cyclooxygenase-2 (Aoyagi et al., 2008; Guillon et al.,

1995; Koshimizu et al., 2012). Thus, angiotensin II exerts action as a neurotransmitter and hormone.

While the human body is robust in its maintenance of total body water through the hormonal mechanisms described, prolonged perturbations in these systems may have a negative influence on other physiological processes affecting health.

Hydration and HPA Axis

Conflicting findings regarding AVP and glucose regulation may be mediated by the expression of AVP receptors in the hypothalamic-pituitary-adrenal axis (HPA axis). V1a-R has been found in the adrenal cortex, while V1b-R is expressed in the anterior pituitary and adrenal medulla (Koshimizu et al., 2012). AVP binding to V1a-R on the adrenocortical cells increases cortisol production (Perraudin et al., 1993) and through V1b-R activation in the corticotropes, increases adrenocorticotropic hormone (ACTH) production and release in the anterior pituitary (Tanoue et al., 2004). AVP works in concert with corticotrophin-releasing hormone (CRH) (Gillies et al., 1982) to stimulate glucocorticoid production and secretion from the adrenal cortex. This is more pronounced under stressful conditions, leading some researchers to classify AVP and its surrogate marker copeptin, as stress hormones (Carroll & Melander, 2021). Correlations between an increased number of V1b-R binding sites in the pituitary and increased ACTH responsiveness under conditions of *chronic stress* suggest AVP rather than CRH is the major regulator of ACTH during this more prolonged stress (Koshimizu et al., 2012; Yoshimura et al., 2021), increasing cortisol and subsequently hepatic glucose output. AVP is also locally synthesized and secreted in response to acetylcholine or CRH activation at the adrenal medulla to stimulate catecholamine release, primarily through activation of V1b-R in response to stress (Koshimizu et al., 2012).

Given this relationship, some studies have examined the effect of changes in hydration status on cortisol. Acute osmotic stress from water deprivation increases ACTH and corticosterone in wild type mice compared to V1b-R knockout models (Roberts et al., 2011). In humans, acute hypohydration achieved through exercise, heat exposure, or a combination has been consistently shown to increase cortisol levels (Zaplatosch & Adams, 2020), though the role of habitual fluid intake is less clear. Based on limited data in humans, chronic underconsumption of fluid may increase basal cortisol levels (E. Perrier, Vergne, et al., 2013), while increasing water intake has been associated with decreased ACTH (Enhörning et al., 2021). However, some studies have shown hypohydration achieved via fluid restriction and heat exposure (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Jones, et al., 2019) and hyperosmolality from hypertonic saline administration (Jansen et al., 2019) do not influence cortisol, suggesting AVP may need to work synergistically with other mechanisms to induce a stress response. Perhaps the short-term nature of the latter studies may explain this response, but the time course of habitual fluid intake modification required to induce a change in cortisol has not been explored.

Chronically elevated cortisol affects metabolism (Hewagalamulage et al., 2016; L. Min, 2016), food choices (Duong et al., 2012; Hewagalamulage et al., 2016), energy intake (George et al., 2010; Herhaus et al., 2020) and thermogenesis (Hewagalamulage et al., 2016). Glucocorticoids influence the production of appetite-regulating peptides in the hypothalamus, including neuropeptide Y and agouti-related protein (Hewagalamulage et al., 2016). Yet stressful stimuli, such as acute mental work, seem to increase energy intake despite no change in appetite sensation (Chaput & Tremblay, 2007). These effects could be particularly problematic for emotional eaters (R. S. Chang et al., 2022) and individuals with higher cortisol responsiveness

(Herhaus et al., 2020; Hewagalamulage et al., 2016), but it is unclear how hydration status and AVP influence this response. If adequate fluid intake helps keep basal cortisol levels low, this could significantly impact health by influencing food selections and quantities which favor a neutral energy balance. One study found pharmacologic stimulation of the HPA axis by CRH administration induced both higher cortisol and greater subsequent food intake, independent of psychological stress (George et al., 2010). Yet, it is unclear if chronically elevated basal cortisol produces this effect. The only study examining this relationship with regard to fluid intake or hydration status was acute and may have been confounded by the heat stress induced by both the euhydrated and hypohydrated conditions (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019). By contrast, reduced fasting glucose from increased water intake is associated with decreased ACTH and cortisol (Enhörning et al., 2021).

Angiotensin II may also impact the HPA axis by increasing ACTH (Rivier & Vale, 1983). However, Johnson et al. did not observe any effect of *reduced* water intake in individuals with type 2 diabetes on RAAS (Johnson et al., 2017). Further study is required to determine if RAAS plays a role in HPA axis activity in healthy individuals with varying fluid intake, but based on the volume loss typically required to induce RAAS (Cheuvront & Kenefick, 2014), these hormones are unlikely to be affected under typical daily conditions but may be affected indirectly by AVP.

Stress and cortisol also influence energy expenditure, with variable effects depending on the exposure length as well as whether an organism experiences chronic repeated stress from the same stressor or chronic variable stress occurring in a random, unexpected order (Kuti et al., 2022). In mouse models, chronic repeated stress seems to decrease lean mass (Kuti et al., 2022). While chronic repeated stress increases energy expenditure, this is compensated for by increased

food intake and decreased physical activity (Kuti et al., 2022) In humans, acute cortisol administration increases metabolic rate and protein breakdown (Brillon et al., 1995), with lower lean body mass predicting reduced resting energy expenditure (Muller et al., 2001). Thus, a chronic stressor such as underhydration, through changes in AVP and cortisol, may also influence energy balance through decreased lean body mass and a subsequent reduction in resting energy expenditure.

Given the associations between underhydration and obesity (T. Chang et al., 2016) and elevated cortisol among low drinkers (E. Perrier, Vergne, et al., 2013), further study is warranted to link these pathways with components of energy balance. Excess cortisol may exacerbate the AVP-induced insulin resistance (Rafacho et al., 2014), thereby providing another means by which underhydration may impact metabolism and potentially, subsequent behavior. Although not traditionally an "appetite" hormone, alterations in HPA-axis activity and the glucocorticoid hormone cortisol have been associated with eating behaviors that may favor a positive energy balance (Warne, 2009). While acute increase in cortisol may decrease food intake (Ans et al., 2018), chronic stress-induced cortisol favors selection of more palatable, high calorie foods when available (Pecoraro et al., 2004, 2006). It has been suggested individuals may make these choices in an attempt to blunt their stress response (Dallman et al., 2003). In fact, psychological stress has been associated with eating in the absence of hunger (Rutters et al., 2009) and increased an increased "wanting" for dessert in overweight subjects (Lemmens et al., 2011). Psychological stress corresponding to an increase in cortisol levels has been associated with reduced reward signaling and increased energy intake (Born et al., 2010). Further, chronically elevated cortisol favors visceral fat accumulation in conjunction with insulin through inhibition of lipolysis and through inhibition of lipolytic growth hormone and sex steroids. Exogenous cortisol

administration has been shown to negate the anorectic actions of leptin and led to overeating in animal and human models (Papaspyrou-Rao et al., 1997; Tataranni et al., 1996; Zakrzewska et al., 1997). Given the previously observed correlations between copeptin and cortisol (Katan et al., 2008; Katan & Christ-Crain, 2010), and the potentiating effect of AVP on CRF (Gillies et al., 1982), the present study will assess the influence of underhydration as a chronic physiological stressor to induce higher cortisol and differences in food reward. However, one study in athletes found similar fasting cortisol concentrations among different quartiles of fluid intake (Zhang et al., 2022). Given these discrepancies, and the sensitivity of cortisol to circadian variation (discussed below), the present study assessed the relationship between urinary osmolality and cortisol using a variety of indicators of HPA-axis activity.

Appetite and Satiety

Acute pre-meal water ingestion may reduce energy intake by promoting satiety through increased gastric distension. Gastric distension can decrease hunger and promote fullness, as identified in several studies, both acutely and over the course of an intervention (Corney et al., 2016; McKay et al., 2018; Van Walleghen et al., 2007). It seems the effects of *immediate* pre-meal water consumption are more effective at reducing energy intake for younger and older adults, but timing delays negate this effect in younger adults, perhaps due to slower gastric emptying with age (Van Walleghen et al., 2007)**.** This effect may also be influenced by the temperature of the ingested fluid, with colder water producing later gastric contractions than warmer beverages, which has been associated with reduced energy intake at a meal consumed 60 minutes later (Fujihira et al., 2020). Consuming 500mL of water prior to a meal led to greater weight loss than a hypocaloric diet alone in middle-aged overweight/obese adults throughout a weight-loss intervention (Dennis et al., 2010). However, few have mentioned the habitual fluid

intake habits of participants prior to water preload studies but have instead attempted to standardize fluid intake to reach "euhydration" prior to each trial. This may limit the generalizability of findings to only those who are typically well-hydrated. Overall, greater water intake around mealtimes appears to be beneficial for reducing energy intake in some individuals depending on fluid availability (Corney, Horina, et al., 2015), eating rate (Andrade et al., 2008, 2012), temperature (Fujihira et al., 2020) and timing of fluid ingestion (Corney et al., 2016) although this requires further study with consideration for habitual fluid intake. When fluid is allowed during meals following a period of acute hypohydration (inducing a state of hyperosmolality), there seems to be no difference between energy intake of a homogenous porridge meal compared to when fluid was not available with the meal (Corney, Horina, et al., 2015). It is unclear if differences in food preferences, perhaps driven by fluid regulatory hormonal responses and a preservation of serum osmolality, would influence consumption when participants are allowed free access to a variety of food options.

Downstream physiologic signals may also interact with fluid regulatory processes to influence perceptions of satiety. Hormones associated with eating and satiety have also been proposed to influence neurons associated with thirst and AVP release, including amylin, cholecystokinin, ghrelin, histamines, and leptin (Armstrong & Kavouras, 2019). Specifically, the circumventricular organs (SFO, OVLT, AP) express AVP, Angiotensin II, and oxytocin receptors, along with receptors for hormones associated with energy balance and metabolism (i.e., relaxin, ERα, angiotensin II receptor type 1 (AT1aR), insulin, amylin, CCK, GLP-1, peptide YY, adiponectin, and leptin) (J. K. Jeong et al., 2021). But given the overlap of functions among various brain regions and difficulty in pinpointing precise changes in response to fluid intake,

future work is required with more sophisticated techniques to capture the interactions between food and fluid intake in the brain (Armstrong & Kavouras, 2019).

Increased serum leptin has been observed in V1bR-KO models, suggesting a potential indirect influence of AVP on appetite (Hiroyama et al., 2009). However, limited research has looked at appetite responses in relation to hydration status or habitual fluid intake in humans, and those that did examined the response to acute fluid restriction, heat exposure, and/or exercise-induced sweat losses rather than chronic low fluid intake. With acute hypohydration, there appears to be no significant impact on post-prandial ghrelin (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019; Corney, Sunderland, et al., 2015; Kelly et al., 2012), leptin (Kelly et al., 2012), or PYY (Kelly et al., 2012). However, given the heterogeneity of methods used to induce dehydration as well as the acute nature of body water loss reductions, further study is warranted to explore how *chronic* underconsumption of fluid influences appetite following a standard meal. However, changes in appetite hormone concentrations do not always influence perceptual hunger and fullness signals (Tacad et al., 2021); therefore, the present study focused on subjective ratings of hunger and satiety.

Metabolic Rate

Greater water intake may promote increased thermogenesis (Berneis et al., 1999; Boschmann et al., 2003, 2007; Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019; D. C. Chang et al., 2020; De Jonge et al., 1991; Keller et al., 2003; Rumpler et al., 2001; Sharief & Macdonald, 1982; J. D. Stookey et al., 2012) . Two studies observed increased metabolic rate following 500mL water consumption in both overweight/obese and normal-weight individuals (Boschmann et al., 2003, 2007), independent of the metabolic cost of heating the consumed fluid to body temperature for absorption; drinking an

additional 2L per day could increase energy expenditure by approximately 96kcals. This may function through sympathetic beta-adrenergic activation in response to hypo-osmolality induced cellular swelling (Jordan et al., 2000). Yet this effect is not consistent (Brown et al., 2006) and has not been observed with acute hypohydration from fluid restriction (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019), acute exercise-induced hypohydration (Castro-Sepulveda et al., 2014), or hypoosmotic saline administration (Berneis et al., 1999). Few studies collected any data on habitual fluid intake (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019), with none including this as a covariate in analyses, which may explain discrepancies between studies due to the expected hormonal responses associated with low fluid intake. Differences in resting metabolic rate and the thermic effect of food between individuals with habitually high and low fluid intake are unknown but may, in part, account for some of the variability in metabolism and subsequent energy balance between individuals of similar body composition. Underhydration has been associated with insulin resistance (Enhörning et al., 2013; H. K. Min et al., 2020; Wannamethee et al., 2015), which may influence TEF through decreased glucose uptake into the cells (Calcagno et al., 2019). Thus, the present study examined differences in TEF among individuals of varying fluid intakes.

Behavioral Effects

Variation in water intake may moderate the relationship between energy balance and obesity through indirect effects on energy balance via changes in food reward, food selection, and synergy with other health-promoting behaviors such as physical activity.

Fluid Intake, Hydration Status and Energy Intake

Consuming more water-rich foods (i.e., fruits and vegetables) may reduce the energy density of one's diet and promote improved hydration status and reduced caloric intake throughout the day (Karl & Roberts, 2014). One study compared caloric and macronutrient-matched preloads of water served with the meal (casserole with a glass of water) and water incorporated into the meal (soup), finding greater satiety and decreased energy intake throughout the rest of the day after the soup preload but not when water was provided with the casserole (Rolls et al., 1999). While shorter trials suggest that reducing the energy density of one's diet decreases ad libitum energy intake, longer trials looking at bodyweight changes by manipulating energy density have produced mixed effects (Karl & Roberts, 2014). Others have found the physical form of nutrients alone (solid vs liquids) does not seem to influence energy intake or appetite (Akhavan et al., 2011), but appetite and energy intake may be influenced by perceptions of how the liquid will be digested (Cassady et al., 2012).

Cluster analyses of NHANES data have shown that water consumers tend to drink fewer soft drinks, eat more fruits and vegetables, low- and medium-fat dairy products, and consume an average of 194 fewer kcals per day (Popkin et al., 2005). This is important, considering it has been suggested that relatively small changes in energy balance of just 100kcals per day could alleviate weight gain in most people (J. O. Hill et al., 2013). Increasing the proportion of one's daily fluid intake that comes from plain water has been associated with reduced total daily energy intake and reduced saturated fat, sugar, sodium, cholesterol, and kcals from SSBs (An & McCaffrey, 2016). A systematic review examining the consumption of different beverage categories on dietary patterns found an association between water, unsweetened tea/coffee, low-fat milk, artificially sweetened beverages, and fruit/vegetable juice with a "Prudent" dietary

pattern, characterized by higher consumption of fruits, vegetables, whole grains, and fish (Hedrick et al., 2015). Thus, individuals who consume less calorically dense fluids may also be predisposed toward adopting other healthy dietary behaviors favoring the maintenance of energy balance.

In contrast to the above mechanisms, *acute* manipulation of hydration status (via exercise or heat exposure) seems to have no effect or may even *decrease* energy intake (Corney, Sunderland, et al., 2015; Engell, 1988; Kelly et al., 2012; Shirreffs et al., 2004). Theoretically, this "dehydration anorexia" may be a response to try and restore plasma osmolality to normal levels since eating more would contribute additional osmolytes (Boyle et al., 2012). These effects have also been attributed to slower gastric emptying with acute hypohydration (Neufer et al., 1989). In mouse models, this "dehydration anorexia" has been shown to reduce meal size and duration, though meal frequency is maintained, under conditions of hyperosmolality (Boyle et al., 2012). However, this may present different physiological effects than chronic underhydration, whereby increased AVP would maintain normal plasma osmolality (E. Perrier, Vergne, et al., 2013). In turn, underhydration may induce hormonal changes that can indirectly impact food reward or energy intake (i.e., increased cortisol and insulin resistance). Thus, more work is needed to determine the effects of *chronic* low fluid intake on energy balance (E. Perrier, Vergne, et al., 2013).

Food Reward

Food consumption is a complex process, like thirst, which is driven by both hedonic (i.e., food "liking" or "wanting") and homeostatic (nutritional need) factors that ultimately result in the consumption or cessation of eating. The hedonic factors have been grouped by the term "food reward", which includes both the "liking" and "wanting" for a particular food source. Liking

refers to the affect-driven responses of food reward such as the perceived or expected pleasure-giving value of a food related to its sensory properties, whereas wanting refers to changes in likelihood of consuming the food independent of liking driven by the perception of a food or a food-related cue in the environment (Dalton & Finlayson, 2014; Finlayson et al., 2007). Limited data has analyzed the effect of water or hydration status on food reward. One study found reduced food liking at a lunch buffet (assessed via visual analog scale) when consuming three water bottles throughout the morning (1.5L) (McKay et al., 2018), which led to reduced energy intake in normal-weight individuals but not those who were overweight or obese, but wanting was not assessed in this investigation. Thus, water intake relative to body size should be considered. Carroll et al. (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019) found acute hypohydration influenced thirst and salt preference, but this did not impact energy intake. Few studies (Almiron-Roig & Drewnowski, 2003; Appleton & Blundell, 2007; Black et al., 1991, 1993; Dennis et al., 2010; McKay et al., 2018; Rodin, 1990; Spitzer & Rodin, 1987; Triana et al., 2003) allowed participants to self-select food items, while most (Akhavan et al., 2010, 2011; Akhavan & Anderson, 2007; Canty & Chan, 1991; Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019; Cassady et al., 2012; Chungchunlam et al., 2012; Corney et al., 2016; Corney, Horina, et al., 2015; Corney, Sunderland, et al., 2015; Davy et al., 2008; Flood et al., 2006; Fujihira et al., 2020; J. N. Jeong, 2018; Lavin et al., 1997, 2002; Maersk et al., 2012; Panahi et al., 2013; Pérez-Luco et al., 2019; Rogers et al., 1988, 1990; Shah et al., 2014; Westerterp-Plantenga & Verwegen, 1999; Woodend & Anderson, 2001) restricted intake to a specific food or meal. But while the homeostatic drive to consume foods may not be altered with fluid manipulation, alterations in hedonic preferences could increase energy intake beyond

physiologic need. More work is needed to determine food preference and the actual selection of food items among those with habitual high versus low fluid intake and how this influences total energy intake, particularly when certain nutrients may influence water balance (Adams, Wininger, et al., 2020; Disher et al., 2021). However, the act of actually selecting and consuming a food item may also be influenced by factors beyond both the hedonic desire or physiologic need, such as environment or social factors, habits and experiences, cognitive factors, and sociocultural factors (Chen & Antonelli, 2020).

Hydration Status and Physical Activity Energy Expenditure

A recent scoping review found ~80% of studies assessing energy intake have not considered physical activity energy expenditure (PAEE) (González-Gross et al., 2021). Yet PAEE is the most variable component of TDEE, and in very active individuals PAEE may account for more than 50% of TDEE (Westerterp, 2013). Increased physical activity is associated with improved hydration status (Mora-Rodriguez et al., 2016), suggesting more active individuals compensate for activity-induced sweat losses by consuming more fluids. Beyond a certain point, increased physical activity may result in more energy compensation, whereby decreases in resting metabolic rate result in minimal changes in total energy expenditure (Hall, 2022). For these reasons, physical activity was included in the present study both to capture the effect of PAEE on energy balance and the contribution of PA to hydration status in relation to fluid requirements.

Methodological Considerations

Hydration Assessment

Proper assessment of hydration status is critical to determine associations between water intake and obesity, but hydration assessment is context-specific, with no single universal marker

yet identified that can acutely quantify one's hydration status (Armstrong, 2007). While plasma/serum osmolality has been proposed as an appropriate hydration indicator (Cheuvront et al., 2013), the validity of this marker is impaired when chronic elevations in AVP facilitate water conservation and restore plasma osmolality to normal levels; thus blood osmolality must be used in conjunction with the expensive and impractical measurement of total body water. Urinary measures of hydration status include urinary specific gravity, urine color (Armstrong et al., 1994), urine volume, and urinary osmolality, whereby lower volume and higher values for the other urinary markers suggest hypohydration or inadequate fluid intake (E. Perrier, Rondeau, et al., 2013). Interpretation of urinary hydration markers should also be used with caution, particularly spot urine samples, which can be influenced by circadian effects and acute fluid ingestion (Cheuvront et al., 2015; Tucker et al., 2018). Other measures include body mass change, a practical method for assessing dehydration following acute exercise that may also hold utility as an index of hydration status over several consecutive days when in energy balance. Morning body mass has been used in conjunction with daily perceived thirst and first morning void urine color to characterize the likelihood of achieving adequate hydration (Cheuvront & Kenefick, 2016; Sekiguchi et al., 2021). Other markers such as saliva and tear osmolality require validation beyond acute body water loss (Oliver et al., 2008). Blood markers such as hemoglobin and hematocrit are useful for assessing acute plasma volume change from exercise or heat-stress (Dill & Costill, 1974) but have little utility for day-to-day body water fluctuations. AVP and surrogate biomarker copeptin (Morgenthaler et al., 2006) provide insight into the downstream response of inadequate fluid intake but could be influenced by other factors such as acute exercise, stress, and circadian variation (Rittig et al., 1989). Unfortunately, of the currently published studies directly assessing the relationship between fluid intake and energy intake, only

~20% of the studies examining the role of fluid intake and energy intake actually assessed hydration status (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019; Corney, Horina, et al., 2015; Corney, Sunderland, et al., 2015; Dennis et al., 2010; Kelly et al., 2012; McKay et al., 2018; Pérez-Luco et al., 2019; Shirreffs et al., 2004; Triana et al., 2003). Capturing *both* chronic fluid intake and hydration status in relation to energy balance will provide insight into the utility of fluid modification as a strategy to mitigate weight gain.

Fluid Intake

Accurately measuring fluid intake is essential to develop appropriate conclusions regarding fluid intake and obesity risk. While the most practical means of measuring fluid intake involve direct laboratory measurements, other methods may represent participant fluid intake under free-living circumstances. The Liq.In.7 (Morin et al., 2018) has been used widely to capture participant fluid intake over several days, providing a series of visuals representing beverage container sizes to aid the user in accurate reporting. Another recently developed and validated survey, the BEVQ-15, can estimate one's habitual fluid consumption over longer timeframes (Hedrick et al., 2010, 2012) and can be administered in ~2 minutes. These measures should be used in conjunction with direct measures of hydration status. However, the dietary contribution of water from food sources (moisture content) is not inconsequential (EFSA, 2010). Thus, accurate estimation of participant dietary water intake from food is imperative.

Assessment of Energy Intake

Accurate estimation of energy intake is essential to determine the influence of fluid intake on energy balance. Individuals commonly under-report energy intake (R. J. Hill & Davies, 2001). Thus, in-lab assessments of energy intake are preferable. However, assessing energy

intake in the lab may limit the applicability of findings to free-living scenarios. Thus, incorporating a combination of approaches (i.e., providing participants food items to consume while free-living, in conjunction with 24h urine collection) may be a better approach for energy intake assessment. One such method has previously been utilized via a food menu that was validated to assess energy intake both within and outside of the laboratory setting (McNeil et al., 2012). To the author's knowledge, this has yet to be performed within the context of hydration assessment.

Assessment of Energy Expenditure

Appropriate energy balance assessment also involves measurement or estimation of resting metabolic rate (RMR), or the energy expended at rest in a fasted state in a thermo-neutral environment (Hills et al., 2014). RMR varies depending on age, sex, body size, body composition, and fitness level. Most studies examining the relationship between hydration status and energy intake have not considered energy expenditure. One study (D. C. Chang et al., 2020) observed a significant but weak association between 24-h urine volume and food intake but a positive association between 24-h urine volume and energy expenditure which may have offset this effect. Individuals in this study seemed well-hydrated based on urine volume, but fluid intake was not measured among participants. The present study assessed changes in energy balance rather than caloric intake or expenditure in isolation to further clarify this relationship, with consideration for fluid intake consumed throughout the day and across participants with a range of fluid intakes while using objective measures of total energy expenditure (RMR, TEF, PAEE).

The thermic effect of food (aka diet-induced thermogenesis) is another significant contributor to energy expenditure, defined as the increase in energy expenditure above basal

metabolic rate in response to food ingestion, which varies based on meal energy and macronutrient composition (Westerterp-Plantenga & Verwegen, 1999). No study has assessed changes in the thermic effect of food in individuals of differing hydration status. Given the expected hormonal interactions between AVP and drivers of fuel utilization and storage (i.e., insulin and cortisol), investigation into potential impact of this response to a meal is warranted. Yet even if hydration status does not contribute to a change in TEF, this response to a meal should still be factored into estimates of energy expenditure from meal consumption for the most precise estimation, particularly when a metabolic chamber or the doubly labeled water technique is not feasible or available to estimate energy expenditure.

Physical activity influences both fluid requirements and energy expenditure but has considerable variability within and between individuals. General fluid intake recommendations are designed based on an individual with a "moderate" activity level. But in practice, individuals may exhibit behaviors considerably above and below these criteria which will influence their fluid intake requirements to meet "optimal" hydration criteria. Similarly, energy expenditure from physical activity is often estimated using an activity factor multiplied by resting metabolic rate as a rough estimate of total daily energy expenditure. Direct measures of total energy expenditure (inclusive of physical activity) via metabolic ward studies or using doubly labeled water are expensive and, in the case of the former, may not provide an accurate representation of one's typical real-world behaviors. Thus, alternative methods to assess energy expenditure from physical activity have been used, such as accelerometry, which has shown a strong relationship with physical activity measured using doubly-labeled water (Chomistek et al., 2017). Accurately capturing energy expenditure is essential for accurate estimation of energy balance and was a component of the present study.

Sex

The present study was limited to male participants and thus cannot examine sex differences or the influence of changes in sex hormone concentrations on the relationship between hydration status and energy balance. Sex differences in fluid regulatory responses and energy intake have been identified in previous literature (Giersch et al., 2020, 2021; Lissner et al., 1988; McNeil & Doucet, 2012; Perucca et al., 2007; Tamma et al., 2015). Several of the outcome variables in the present study may be influenced by the expected changes in females sex hormone concentrations throughout the menstrual cycle, which influence fluid regulatory processes (Giersch et al., 2021). Future work should seek to examine the relationship between fluid intake behaviors and energy balance in females across different phases of the menstrual cycle, both in those who are naturally cycling and on oral contraceptives.

Ethnicity

Racial and ethnic differences in fluid intake and the associated hormonal responses should be considered when determining the relationship between water intake and obesity. Some studies have shown that African American participants tend to exhibit worse hydration than Caucasian peers (Adams, Hevel, et al., 2020; Bankir et al., 2007). Differences in the AVP response to hyperosmolality may contribute to the greater hypertension risk (Bankir et al., 2007) and greater obesity risk (Krueger & Reither, 2015) among African Americans. Given these differences, an attempt was made to recruit a diverse sample for the present study.

Circadian Variation

Most research examining the acute impacts of fluid intake or changes in hydration status on energy balance and obesity risk have been isolated to a single meal, without regard for circadian patterns in eating behavior or the expected variation in AVP or copeptin (Beglinger et

al., 2017; Challet, 2019; Darzy et al., 2010). Collecting blood samples at the same time over consecutive days can account for this variation (D. C. Chang et al., 2020), but additional information may be gleaned by measuring copeptin throughout a full day of food intake. When meals are standardized, copeptin follows a trend similar to AVP in individuals with higher baseline copeptin, peaking between 4 am and 6 am and troughing between 5 pm and 7pm (Beglinger et al., 2017). Thus, momentary fluid intake behaviors at certain times of day, when hormonal responses are naturally more or less inclined to conserve body water, may influence other physiological processes such as metabolism and should be considered or controlled for in future research. The present study began all measurements in the morning (0600-0900), but exact arrival time varied based on participants' habitual wake time in order to avoid disturbance to one's usual circadian rhythm.

Other hormones follow a circadian profile, including cortisol, which follows a 24-hour profile of higher concentrations in the early morning, peaking soon after awakening, and decreasing until the evening prior to sleep (Weitzman et al., 1971). Consideration should be made for the timepoints of cortisol sampling to determine whether changes are reflective of an actual change beyond the expected diurnal variation. This may be accounted for by collecting additional samples to capture the expected diurnal cortisol slope for change in this hormone throughout the day (E. K. Adam et al., 2017). There is a significant association between a flatter diurnal cortisol slope and adverse health outcomes, including obesity (E. K. Adam et al., 2017). Yet no literature has examined changes in cortisol profiles throughout the day in relation to hydration status. Thus, the present study sought to capture the relationship between hydration status and changes in cortisol rhythm, as well as any associations with downstream behaviors

such as food reward that may partially explain the observed associations between flatter diurnal cortisol slope and obesity risk.

Summary of the Review of Literature

To summarize, this literature review has discussed 1) regulation of body water with hypohydration and underhydration 2) mechanisms by which underhydration may influence metabolic function 3) the potential direct and indirect effects of hydration status and fluid intake on determinants of energy balance. Chronic underhydration may promote weight gain and increased risk for obesity through hormonal, satiety, and behavioral mechanisms. Additional well-designed studies are needed to assess the longer-term impact of adequate fluid intake on health and determine how to enhance the adoption of such behavior. Findings from the present study will help inform interventions to reduce underhydration, with the goal of reducing obesity risk.

CHAPTER III: THE INFLUENCE OF HABITUAL FLUID INTAKE ON ENERGY BALANCE IN HEALTHY YOUNG ADULT MALES

Abstract

Background: Underhydration resulting from inadequate fluid intake has been associated with obesity. A chronic positive energy balance, whereby energy intake exceeds energy expenditure, is the primary contributor to obesity. However, the mechanisms linking hydration to energy balance and obesity require additional study. **Purpose:** To explore associations between habitual fluid intake and the primary components of energy balance, including total daily energy expenditure (TDEE) (resting metabolic rate [RMR], thermic effect of food (TEF), physical activity energy expenditure (PAEE)) and energy intake (EI). **Methods:** Twenty-seven male participants (age, 23±4y; height, 176 ± 6.5cm; body mass, 78.8 ± 13.0kg; body fat,17.2 ± 9.0%) collected 24h urine samples for 3 days prior to completing an assessment of RMR and TEF following a standard breakfast, and self-selected foods to consume over a 24h period. Participants recorded their food and fluid intake for the three days preceding metabolic measures and for three additional days following the laboratory visit. Linear regressions assessed the relationship between mean habitual fluid intake and RMR, TEF, and the respiratory exchange ratio (RER) for each of these measures. Separate linear mixed models assessed the association of within- and between-person differences in habitual fluid intake, plain water intake, and hydration status on EI, PAEE, and overall energy balance (EB: EI - TDEE). **Results:** Higher habitual fluid intake was associated with higher RMR (β = 69 kcals, p =0.008) and lower RER following breakfast consumption (β = -0.02, p = 0.008). Higher habitual fluid consumption between

participants was associated with both increased EI (β = 194 kcals, p = 0.012) and increased PAEE (β = 212 kcals, p = 0.003). There was no association between habitual fluid intake and total EB (p > 0.05). **Conclusions:** Higher habitual fluid intake is associated with increased RMR and PAEE. However, individuals may compensate for these differences with increased EI.

Introduction

Underhydration and Obesity

Inadequate fluid intake has been reported in as many as 60% of males and 40% of females, contributing to a state of "underhydration" (Ferreira-Pêgo et al., 2015). With underhydration, low fluid consumption contributes to an increase in fluid regulatory hormones (i.e., arginine vasopressin – AVP), maintenance of plasma osmolality and total body water, and an absence of thirst (Kavouras, 2019). Though beneficial for maintaining total body water, the effects of elevations in fluid conserving hormones, particularly AVP and its surrogate marker copeptin (Morgenthaler et al., 2006), on long term health has become the focus of recent research. Epidemiologic literature has identified associations between low water intake and/or high copeptin concentrations and metabolic diseases such as obesity (D. C. Chang et al., 2020; Enhörning et al., 2013), yet the mechanisms underlying these associations are not well defined. Potential rationale for the connection between fluid intake and obesity may be through the influence of fluid intake on energy balance, including metabolism and energy intake.

Water and Metabolism

Water intake may reduce obesity risk through increased thermogenesis following water intake (Berneis et al., 1999; Boschmann et al., 2003, 2007; Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019; D. C. Chang et al., 2020; De Jonge et al., 1991; Keller et al., 2003; Rumpler et al., 2001; Sharief & Macdonald,

1982; J. D. Stookey et al., 2012). Two studies observed increased resting metabolic rate following 500mL water consumption in both overweight/obese and normal-weight individuals (Boschmann et al., 2003, 2007), independent of the metabolic cost of heating consumed fluid to body temperature for absorption. Thus, drinking an additional 2L per day could increase energy expenditure by approximately 96kcals (Boschmann et al., 2003). This may function through sympathetic β-adrenergic activation in response to hypo-osmolality induced cellular swelling (Jordan et al., 2000). Yet this effect is not consistent (Brown et al., 2006), and the opposite effect has not been observed with acute dehydration from fluid restriction (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019), acute exercise-induced dehydration (Castro-Sepulveda et al., 2014), or hyperosmotic saline administration (Berneis et al., 1999). Few studies have measured participants' habitual fluid intake (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019), with none including this as a covariate in analyses, which may explain discrepancies between studies due to the expected hormonal responses to low fluid intake. Underhydration has also been associated with insulin resistance (Enhörning et al., 2013; H. K. Min et al., 2020; Wannamethee et al., 2015), which may negatively influence the post prandial thermic effect of food through decreased glucose uptake into the cells (Calcagno et al., 2019). Differences in resting metabolic rate and the thermic effect of food between individuals with habitually high and low fluid intake are unknown but may help clarify the link between underhydration and obesity.

Water and Energy Intake

Cluster analyses of NHANES data have shown that high water consumers tend to drink fewer soft drinks, eat more fruits, vegetables, and low- and medium-fat dairy products, and

consume an average of 194 fewer kcals per day (Popkin et al., 2005). This is important, considering relatively small changes in energy balance of just 100kcals per day could alleviate weight gain in most people (J. O. Hill et al., 2013). Increasing the proportion of one's daily fluid intake that comes from plain water has been associated with reduced total daily energy intake and reduced saturated fat, sugar, sodium, cholesterol, and kcals from sugar sweetened beverages (SSBs) (An & McCaffrey, 2016), as well as higher consumption of fruits, vegetables, whole grains, and fish (Hedrick et al., 2015). Thus, individuals who consume less calorically dense fluids may also be predisposed toward adopting other health behaviors favoring weight maintenance.

In contrast to the above mechanisms, *acute* manipulation of hydration status (via exercise or heat exposure) seems to have no effect or may even *decrease* energy intake (Corney, Sunderland, et al., 2015; Engell, 1988; Kelly et al., 2012; Shirreffs et al., 2004). This "dehydration anorexia" may be an attempt to restore plasma osmolality to normal levels since eating more would acutely contribute additional osmolytes (Boyle et al., 2012). Dehydration anorexia has also been attributed to increased satiety due to slower gastric emptying with acute hypohydration (Neufer et al., 1989). In mouse models, acute hyperosmolality has been shown to reduce meal size and duration, though meal frequency is maintained (Boyle et al., 2012). However, this may result in different physiological effects than chronic underhydration, where chronic elevations in AVP maintain plasma osmolality within the standard normal range (E. Perrier, Vergne, et al., 2013). In turn, chronic underhydration may induce hormonal changes that indirectly impact food reward or energy intake (Oshikawa et al., 2004; E. Perrier, Vergne, et al., 2013; Wannamethee et al., 2015). Thus, more work is needed to determine the effects of *chronic* low fluid intake on energy balance (E. Perrier, Vergne, et al., 2013).

Assessing Energy Balance

Energy balance may be defined as the difference between total daily energy intake (EI) and energy expenditure (TDEE). Most studies examining the relationship between hydration status and energy intake have not considered energy expenditure (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019; Castro-Sepulveda et al., 2014; Corney, Horina, et al., 2015; Corney, Sunderland, et al., 2015; Kelly et al., 2012; Pérez-Luco et al., 2019). Accurate determination of energy expenditure involves measurement or estimation of resting metabolic rate (RMR), or the energy expended at rest in a fasted state in a thermo-neutral environment (Hills et al., 2014). One study (D. C. Chang et al., 2020) observed a significant but weak association between increased 24h urine volume and increased EI but also a significant association between increased 24h urine volume and increased energy expenditure which may have offset this effect. Individuals in this study seemed well-hydrated based on urine volume, but fluid intake was not measured among participants.

The thermic effect of food (i.e., diet-induced thermogenesis) is another significant contributor to energy expenditure, defined as the increase in energy expenditure above RMR in response to food ingestion, which varies based on meal energy and macronutrient composition (Westerterp-Plantenga & Verwegen, 1999). No study has assessed changes in the thermic effect of food in individuals of differing hydration statuses. Given the expected hormonal interactions between AVP and drivers of fuel utilization and storage (i.e., insulin and cortisol) (Oshikawa et al., 2004; Perraudin et al., 1993), it is plausible that inadequate fluid intake would promote reduced TEF and higher RER, resulting in lower post-prandial energy expenditure and a greater percentage of energy expended from carbohydrates rather than fat.

A recent scoping review found ~80% of studies assessing EI have not considered physical activity energy expenditure (PAEE) (González-Gross et al., 2021). Yet PAEE is the most variable component of TDEE, and in very active individuals PAEE may account for more than 50% of TDEE (Westerterp, 2013). General fluid intake recommendations are designed based on an individual with a "moderate" activity level (EFSA, 2010). But in practice, individuals may exhibit behaviors considerably above or below these criteria which will influence their fluid intake requirements to meet "optimal" hydration criteria. Similarly, energy expenditure from physical activity is often estimated using an activity factor multiplied by resting metabolic rate as a rough estimate of total daily energy expenditure. Direct measures of total energy expenditure (inclusive of physical activity) via metabolic ward studies or using doubly labeled water are expensive and, in the case of the former, may reduce ecological validity. Thus, alternative methods to assess energy expenditure from physical activity have been used, such as accelerometry, which has shown a strong relationship with physical activity measured using doubly-labeled water (Chomistek et al., 2017). Increased physical activity is associated with improved hydration status, (Mora-Rodriguez et al., 2016) suggesting more active individuals compensate for activity-induced sweat losses by consuming more fluids. Beyond a certain point, increased physical activity may result in more energy compensation, whereby decreases in resting metabolic rate and non-exercise physical activity (Fernández-Verdejo et al., 2021; Mansfeldt & Magkos, 2023) result in minimal changes in total energy expenditure (Hall, 2022). For these reasons, physical activity was included in the present study both to capture the effect of PAEE on energy balance and the contribution of PA to hydration status in relation to fluid requirements.

Based on the emerging evidence linking underhydration and obesity (T. Chang et al., 2016; Enhörning et al., 2013; J. D. Stookey et al., 2020), the present investigation sought to determine the influence of habitual fluid intake on acute (24h) and chronic (6 day) energy balance in individuals across a range of fluid intakes. We hypothesized that 1) lower habitual fluid intake compared to one's own mean would be associated with greater within-day energy intake relative to estimated energy needs; 2) lower habitual fluid intake compared to the group mean would be associated with more positive energy balance compared to peers; and 3) lower habitual fluid intake would be associated with lower RMR, TEF, and PAEE.

Methods

Experimental Design

Participants reported to the Exercise Physiology Lab for one baseline Screening Visit to provide their written informed consent, complete baseline anthropometric assessments, and receive materials for the next part of the study. Participants then completed three days of 24h urine collection. Participants also completed two three-day records of food and fluid intake throughout the study, with one record completed during the urine collection period. Participants also completed one Experimental Trial following the urine collection period, with in-lab assessments of metabolism and dietary intake.

Participants: Twenty-nine male participants were recruited for this study. Prior to enrollment, participants completed an electronic questionnaire (Qualtrics, Provo, UT) to ensure they did not meet any of the following exclusionary criteria: 1) no chronic health conditions or disease which would alter body water regulation, 2) no previous surgery of the gastrointestinal tract that could impact body water regulation, 3) no pharmacologic drug treatment in the previous 15 days, 4) not actively attempting to gain or lose body weight, 5) no known or suspected sleep pathologies

(Pittsburg Sleep Quality Index score <5) and 6) no food allergies or severe dietary restrictions. Participants were screened using an electronic version of the Brief 15-Item Beverage Intake Questionnaire (BEVQ-15) survey to estimate habitual fluid intake over the previous month (Fausnacht et al., 2020; Hedrick et al., 2012). A targeted recruitment strategy was used with a goal of recruiting an equal representation of fluid intake fitting into each of the following ranges based on BEVQ-15 results: <1500mL/day, 1500-3000mL/day, >3000mL/day. After verification with fluid intake recorded during the study, 9 participants had "low" fluid intake, 11 had "moderate" intake, and 9 had "high" intake.

Screening Visit

After providing their signed informed consent to participate in the study, participants provided baseline measures of their age, height, nude body mass (NBM), and body composition. Prior to leaving the laboratory, participants also received an activity monitor (Actigraph GT9X-BT, Pensacola, FL) to measure physical activity energy expenditure throughout the study, as well as two opaque 3L containers to collect their urine as part of the pre-experimental trial measurements collected.

Pre-Experimental Trial Measurements

Participants arrived at the exercise physiology lab in the morning (0600-0900) for three consecutive days to provide real-time estimations of habitual fluid intake and urinary measures of hydration status. During each morning visit, participants provided their 24h urine collection containers from the previous day. Participants were then provided clean containers for the rest of that day's 24h urine collection. Participants completed the first of these visits after 24h of collecting their urine, recording their food and fluid intake, and wearing the activity monitor; thus, 3 days of habitual characteristics were collected prior to the Experimental Trial.

Experimental Trial

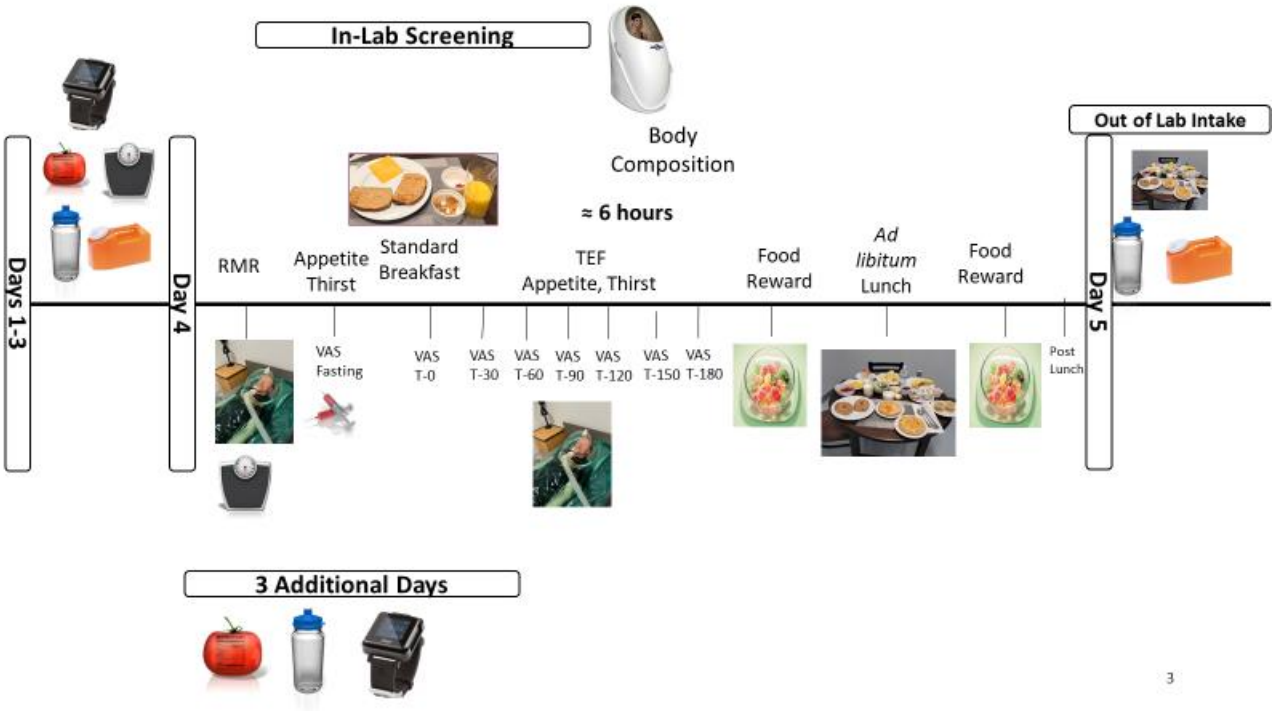
Participants arrived fasted (at least eight hours, with no upper range restriction) to the Exercise Physiology Laboratory in the morning (0600-0900) after the third day of monitoring habitual hydration status, physical activity, and dietary intake (Figure 3). Upon arrival, participants completed a measure of NBM followed by a ~30-minute rest period where they rested on a bed to ensure a true resting state. Participants were then measured for resting metabolic rate (RMR) via the ventilated hood technique (Roffey et al., 2006) using a metabolic cart (Parvo Medics, Salt Lake City, UT). Following RMR measures, participants provided a blood sample from an antecubital vein (14mL). Participants then consumed a standardized meal (78g 100% whole wheat bread, 21g mild cheddar cheese, 17g strawberry jam, 18g peanut butter, 225g orange juice: ~546kcals, 19g protein, 77g carbohydrate, 18g fat) to determine their individual Thermic Effect of Food (TEF) for 30 minutes of every hour throughout the 3-hours following standard meal consumption.

Prior to their final TEF measurement, participants received a food menu from which they chose food items to consume for the 24h following the Experimental Trial as a direct measure of free-living EI (McNeil et al., 2012). Participants were also asked to collect their urine during this 24h period. Participants were provided with a lunchbox containing the food items they selected and were asked to keep any unconsumed portions of the food items in the originally provided containers. Following this final 24h period, participants were asked to stop collecting their urine and return both their 24h urine and any uneaten food items as soon as possible to the lab, typically between 1300-1400.

Post-Trial Monitoring

Participants continued wearing the activity monitor and recorded their food and fluid intake for three additional consecutive days (two weekdays and one weekend day) within the week following the Experimental Trial.

Figure 3. Timeline of Experimental Protocol.



Measures

Body Composition. NBM was measured to the nearest 0.1kg using a digital scale (WB-800S Plus, Tanita Corporation, Tokyo, Japan). Height was measured to the nearest 0.1cm via wall-mounted stadiometer (Model 216, Seca, Chino, CA). Body composition was assessed via air displacement plethysmography (Bodpod, Cosmed, USA Inc., Chicago, IL). Participants were asked to wear only skin-tight clothing within the chamber. Participant lung volumes were estimated based on sex, age, and height (McCrory et al., 1998). Race was used in the estimation

of body density using the Schutte equation (Schutte et al., 1984) for African-Americans and the Brozek equation for other races (Brozek et al., 1963).

RMR. Resting metabolic rate (RMR) and respiratory exchange ratio (RER) were assessed following an overnight fast before the Experimental Trial via indirect calorimetry (Parvo Medics, Salt Lake City, UT). The participant was asked to lie supine for measurements of metabolic gases (oxygen consumption and carbon dioxide production) for 30 minutes to estimate RMR. Twenty minutes of this rest period was used to calculate mean VO2 and VCO2 and used in the Weir equation to estimate RMR (Weir, 1990)**.** The first five minutes of recording were excluded from the analysis to establish a true steady state. The remaining twenty minutes were used for determination of RMR if values for VCO2 were between 0.8-1.2%, per the assessment manual guidelines. If values fell out of this range, additional data from the remaining five minutes were used to replace these aberrant values.

TEF. Participants were instructed to consume the standardized breakfast in its entirety within 15 minutes. Thermic effect of food was assessed by measuring gas consumption for 30 minutes every hour over the course of three hours in the same manner as the RMR measurement. TEF was calculated as the average increase in energy expenditure above RMR for each individual TEF measurement over the course of the 3-hour measurement period:

$$TEFavg = \frac{(TEF1 - RMR) + (TEF2 - RMR) + (TEF3 - RMR)}{3}$$

Physical Activity. Physical activity energy expenditure over the course of the seven monitoring days (6 days free-living + 1 day in lab) was captured from the Actigraph GT9X Link (Actigraph, Pensacola, Fl) and estimated using ActiLife software using the Freedson Combination equation (Sasaki et al., 2011).

Energy Intake. Individuals commonly under-report energy intake (R. J. Hill & Davies, 2001). Thus, in-lab assessments of energy intake are preferable but may limit the applicability of findings to free-living scenarios. In this study we used a previously validated food menu to assess energy intake for 24h following resting metabolic measurements (McNeil et al., 2012). Food items and provided containers were weighed before and after consumption to measure total intake. Quantity of the food items consumed was entered into a nutrition software (Nutritionist Pro, Axxya Systems, Redmond,WA) to obtain total calorie (kcal), macronutrient (g), electrolyte (mg), and moisture (mg) content. Dietary intake for the 3-days preceding the Experimental Trial and for 3 select days (2 weekdays and 1 weekend day) following the experimental trial were obtained using the NIH-ASA24 nutrition assessment tool (Frankenfeld et al., 2012). This tool provides users with visuals to aid in portion size estimation and has shown comparable results to directly weighing food items and compared to the interviewer-administered Automated Multiple-Pass Method (Kirkpatrick et al., 2014). Dietary intake from the provided foods was compared to mean dietary intake over the course of the six self-report days. In the event participants forgot to log into the ASA-24 website to log their foods, they completed a paper dietary recall with foods and portion sizes entered into the nutrition software to estimate energy and nutrient intake.

Energy Balance. Energy balance over the course of six days was calculated as the difference between self-reported kcal intake and kcal expenditure. Total Daily Energy Expenditure (TDEE) was calculated as the sum of energy expenditure measured by the collected actigraphy measures, the individual's resting metabolic rate, and the average thermic effect of food from the three-hour measures. Energy balance was calculated separately for the 24h during which foods were provided.

$$EB = EI - (RMR + TEFavg + PAEE)$$

Hydration Assessment. Each 24h collection container was measured for total urine volume (U_{VOL}), urinary specific gravity (U_{SG}), urinary osmolality (U_{OSMO}) and urine color (U_{COL}). U_{VOL} was measured to the nearest 0.001g (Ranger 3000, Ohaus, Parsippany, NJ). U_{SG} was assessed via digital refractometry (Reichert TS400). U_{OSMO} was measured in duplicate via freezing point depression (Model 3320, Advanced Instruments, Norwood, MA). U_{COL} was rated using a validated and widely used urine color scale (Armstrong et al., 1994).

Biochemical Assessment. A 15mL blood sample was taken after the RMR measure. Tubes were placed upright at room temperature and allowed to clot for approximately 30 minutes prior to being placed in a centrifuge and spun at 3,000 rpm and 2°C for 15 minutes. Serum was then separated into individual aliquots. Serum osmolality was measured in duplicate immediately after separation using the freezing point depression method (Model 3320, Advanced Instruments, Norwood, MA).

Statistical Analyses. Separate random-intercept linear mixed effects models were fit via restricted maximum likelihood to assess the effect of daily within-person and between person differences in fluid intake (person-centered fixed effect) on energy balance and its subcomponents (EI, PAEE) over the course of the seven days of home measurements using the 'lme' function from the 'nlme' package in statistical software R (Pinheiro, J. et al., 2021). The same analysis was repeated with plain water intake as a predictor. Additional analyses assessed the relationship between each beverage category from the Liq.In.7 and self-reported EI. Separate models included person-centered urinary hydration biomarkers from the four days of urine collection as predictors of energy balance and its subcomponents. The mean caloric intake from

the six self-reporting days was compared with the kcals consumed from the provided foods (Day 4) via a paired t-test or Wilcoxon signed-rank test where appropriate. Mean fluid intake from the BEVQ-15 questionnaire was compared to fluid intake reported during the study via the Liq.In.7 using a Wilcoxon signed-rank test. Multiple linear regressions assessed the impact of plain water intake on RMR, TEF, and RER during the in-lab visit. The previous day's urinary hydration biomarkers were used in separate models as predictors of RMR, TEF, and RER. Statistical significance for analyses were set at $\alpha = 0.05$. Additional covariates of age, race/ethnicity, and the previous day's physical activity were explored given the influence of age on RMR (Zampino et al., 2020), observed racial differences in hydration status (Adams, 2019), and the influence of exercise on resting energy expenditure (Speakman & Selman, 2003). Covariates were omitted from the final models when not statistically significant. For significant relationships, fluid intake was further divided into tertiles based on the average self-reported fluid intake throughout the study to examine the influence of between group differences in metabolic and behavioral determinants of energy balance. In response to observed findings, an additional exploratory linear mixed model was used to assess the influence of within- and between-person changes in PAEE on total intake of calorie containing beverages (sum of milk, soft drinks, alcohol, and hot drinks). Results for magnitude differences are presented as mean differences (MD) and 95% confidence intervals (95% CI) throughout.

Results

Participant Characteristics

Demographic characteristics of participants are presented in Table 1. Across all observation days, 82% of the expected days captured both fluid and food intake, leaving 161 participant-days of analysis; the missing values were omitted from analysis via listwise deletion. One participant lost their fluid log and was unable to recall or repeat recording of their fluids; this participant was excluded from all analyses and tables presented below. One participant retrospectively reported attempting to gain weight during the study and logged caloric intakes considerably above the rest of the participants (>2 SDs); given the primary aims of this study, this participant was excluded from all analyses.

Table 1. Demographic Characteristics of All Participants.

	Overall (N=27)
Height (cm)	
Mean (SD)	176 (6)
Median [Min, Max]	176 [160, 188]
Body Mass (kg)	
Mean (SD)	78.8 (13.0)
Median [Min, Max]	75.2 [61.9, 109.0]
BMI ($kg*m^2$)	
Mean (SD)	25.6 (4.2)
Median [Min, Max]	24.6 [19.2, 35.1]
BF (%)	
Mean (SD)	17.2 (9.0)

	Overall (N=27)
Median [Min, Max]	16.1 [2.3, 36.5]
Age (y)	
Mean (SD)	23 (4)
Median [Min, Max]	23 [18, 34]
Race/Ethnicity	
African American	6 (22.2%)
Asian	2 (7.4%)
Caucasian	15 (55.6%)
Hispanic	4 (14.8%)
Resting Metabolic Rate (RMR) - kcals/day	
Mean (SD)	1850 (220)
Median [Min, Max]	1790 [1510, 2400]
Thermic Effect of Food - kcals	
Mean (SD)	220 (128)
Median [Min, Max]	217 [5, 603]
PAEE - kcals	**N = 26**
Mean (SD)	1400 (722)
Median [Min, Max]	1212 [304, 3852]
Total Daily Energy Expenditure - kcals	**N = 26**

	Overall (N=27)
Mean (SD)	3555 (818)
Median [Min, Max]	3363 [2406, 5409]

Note: Demographic data excludes participant who did not log fluids and the one participant who reported an active attempt to gain weight.

Food and Fluid Intake

Table 2 illustrates differences between mean calorie (EI), macronutrient, total fluid (TFI), and plain water intake during home observations compared to the food consumed when food was provided for 24h. EI was significantly higher during the day of provided food intake; thus, separate analyses were run for this day compared to the self-report measurement days. On average, participants ate more calories when foods were provided (MD, [95% CI]: 751kcals [297, 1206], p = 0.002), primarily from increased carbohydrates (183g [126, 240], p = 9.7e-7). The difference in carbohydrate intake was primarily derived from increased sugar intake when foods were provided (73g [47, 99], p = 5.7e-6). Total fat and protein intake was not significantly different between the mean self-reported dietary intake and provided foods (p = 0.891, p = 0.763, respectively). Total fluid intake and water intake was similar between the lab-provided and self-reported intake (p = 0.905, p = 0.822, respectively). The intraclass correlation coefficient (ICC) for the effects of within and between person changes in variables of interest was moderate-high (0.36-0.76), warranting the use of a linear mixed model to account for the heterogeneity of responses to within- and between-person variations in fluid intake.

Fluid intake among participants was positively skewed, with a median fluid intake of 2550mL [range, 380 - 7570], while plain water intake was 1820mL [range, 50 -6120] . This fluid intake was slightly above the median intake captured by the BEVQ-15 pre-screening questionnaire (2194 mL [range, 798 - 3697], MD, [95% CI]: 810 [113, 1547], p = 0.014]) . The mean fluid intake among participants was 3040 ±1810mL, exceeding the European Food Safety Authority recommendations for fluid intake for males of 2500mL per day (EFSA, 2010), which also exceeded the mean captured by the BEVQ-15 screening survey (mean ± SD: 2046 ± 854mL).

Table 2. Total Kcal, Fluid, and Macronutrient Breakdown of the Average Self-Reported Intake and the One Day of Laboratory Provided Food.

	Home (N=27)	Lab (N=24)[f]	Overall (N=51)
EI (Kcals)			
Mean (SD)	2330 (729)	2950 (1040)**	2620 (931)
Median [Min, Max]	2230 [1430, 4620]	3060 [793, 4700]	2570 [793, 4700]
Protein (g)			
Mean (SD)	116 (48.4)	113 (49.8)	114 (48.6)
Median [Min, Max]	109 [49.3, 278]	126 [31.7, 200]	110 [31.7, 278]
Fat (g)			
Mean (SD)	96.4 (32.0)	91.9 (45.2)	94.3 (38.4)
Median [Min, Max]	91.6 [39.3, 188]	93.8 [5.16, 209]	92.9 [5.16, 209]
Carbohydrates (g)			
Mean (SD)	251 (92.0)	417 (138)***	329 (142)

	Home (N=27)	Lab (N=24)[f]	Overall (N=51)
Median [Min, Max]	237 [117, 466]	426 [142, 689]	305 [117, 689]
Fluid (mL)*			
Mean (SD)	3120 (1590)	2940 (2060)	3040 (1810)
Median [Min, Max]	2550 [800, 7570]	2530 [380, 6710]	2550 [380, 7570]
Water (mL)[§]			
Mean (SD)	2510 (1500)	2300 (1910)	2410 (1700)
Median [Min, Max]	1820 [500, 5680]	2400 [50.0, 6120]	2250 [50.0, 6120]

*Denotes total of all fluids consumed as liquid.
**p<0.01
***p<0.001
§Denotes just plain water intake.
f Three participants either forgot to record their fluid on the laboratory provided foods day (n=1) or lost their fluid log (n =2).

Hydration Status

Table 3 demonstrates participant urinary hydration indices. Mean 24h urinary osmolality across all participants was slightly worse than the recommendations for health of <500mOsm*kg^{-1}, despite, on average, meeting fluid intake recommendations.

Table 3. Average Urinary and Hematologic Hydration Biomarkers of Participants Collected Across 4 Days (3 Days Free-Living Conditions, 1 Day of Laboratory Provided Foods).

	Overall (N= 27)
Fasting Serum Osmolality (mOsm*kg^{-1})	
Mean (SD)	292 (3.69)

	Overall (N= 27)
Median [Min, Max]	293 [285, 297]
Missing	1 (3.7%)
24h U_{VOL} (L)	
Mean (SD)	1.76 (0.768)
Median [Min, Max]	1.64 [0.608, 3.81]
24h U_{OSMO} (mOsm*kg-1)	
Mean (SD)	560 (199)
Median [Min, Max]	542 [199, 1030]
24h U_{SG}	
Mean (SD)	1.02 (0.01)
Median [Min, Max]	1.02 [1.01, 1.03]
24h Urine Color	
Mean (SD)	4.36 (1.08)
Median [Min, Max]	4.25 [2.75, 7.50]

Note: Average was obtained from all 4 collection days (3 days free living, 1 day laboratory provided foods).

Physical Activity Energy Expenditure

PAEE from the six days of free-living Actigraph measurements (excluding the day of provided foods) was 1400 ± 722 kcals per day. One participant swam for exercise for three days during the study and as instructed, did not wear the Actigraph during this time period. The energy cost of the swimming was estimated by using the Compendium of Physical Activities

(Ainsworth et al., 2000) and multiplying the metabolic equivalent for each swimming bout by the participant's body weight to obtain an estimated kcal expenditure during that time frame.

Associations Between Fluid or Hydration Status and Markers of Metabolism

RMR and RER

Regression models assessing the relationships between habitual fluid intake (TFI) and metabolic measurements were influenced by two outliers with high RMR values (>2 SDs above the mean), despite normal FFM and TFI. To account for this, a sensitivity analysis was conducted that included or excluded (adjusted model) these observations, but overall results were stable between the models. Individual resting metabolic rate was higher among individuals with higher average fluid intake where every 1L increase in TFI was associated with ~69 more kcals expended at rest ($p = 0.008$) (Table 4, Figure 4). TFI was not significantly associated with RER in the un-adjusted ($p = 0.291$) or adjusted ($p = 0.050$) models. 24h Urinary hydration markers were not associated with RMR (U_{OSMO}: $p = 0470$; U_{VOL}: $p = 0.360$) or resting RER (U_{OSMO}: $p = 0.214$,U_{VOL}: $p = 0.067$) (Table 6, Table 7). When dividing fluid intake into tertiles, the highest tertile had, on average, higher RMR compared to the lowest tertile (Table A45, $p = 0.0004$), but RMR in the middle tertile was not significantly different from the lowest tertile ($p = 0.151$). Fasting RER did not differ by tertile in the middle tertile ($p = 0.234$) or the highest tertile ($p = 0.234$) compared to the lowest tertile. Fasting RER did not differ in the second tertile ($p = 0.234$) or the third tertile ($p = 0.234$), compared to the first tertile.

TEF

On average, participant metabolism increased 220 kcals (11.9%) following ingestion of the standard breakfast. Three participants did not complete the standard breakfast in its entirely due to: later disclosing they were lactose intolerant (n = 1), unable to complete the meal within

the allotted time due to very low appetite (n = 1) or discarding the remainder of their breakfast (n = 1). Thus, TEF analyses were completed with and without these participants included to assess stability of the results. Regardless of the scenario, these participants still consumed at least 85% of the meal in terms of total caloric content. Overall effects were stable with the omission of these participants (Tables 5-6). Habitual fluid intake was not significantly associated with TEF (p = 0.279); results were similar in the adjusted model (p = 0.123). Higher habitual fluid intake was associated with a small but significantly lower post-prandial RER regardless of the model used (p = 0.008 un-adjusted, p = 0.019 adjusted), where every additional liter of fluid intake between participants was associated with a 0.02 reduction in RER following the standard breakfast. Urinary hydration markers were not associated with TEF (U_{OSMO}, p = 0.057; U_{VOL}, p = 0.603) or post-prandial RER (U_{OSMO}, p = 0.090; U_{VOL}:, p = 0.383) (Table 6, Table 7), p-values for models with participants completing entire breakfast).When dividing fluid intake into tertiles (Table A45), TEF was not significantly different in the middle (p = 0.810) or highest (p = 0.364) tertiles compared to the lowest tertile. However, post-prandial RER was lower in both the middle tertile (p = 0.008) and highest tertile (p = 0.002), compared to the first tertile.

For all measures of metabolism (RMR, TEF, and the RER for each measure), the inclusion of age, race/ethnicity, and raw activity counts (based on Actigraph measurements taken from the previous 24h) were tested for inclusion as covariates using step forward selection. Model fit did not significantly improve with the inclusion of age, race/ethnicity, or activity counts. Thus, only the raw, unadjusted models are presented.

Table 4. Models Assessing The Relationship Between Habitual Fluid Intake and Resting Metabolism.

	Dependent variable:			
	RMR (kcals)		RER	
	(1)	(2)	(3)	(4)
Fluid (L)	69.4***	71.6***	-0.01	-0.01**
	(22.52, 116.24)	(40.0, 103.2)	(-0.02, 0.01)	(-0.03, -0.00)
Constant	1,632.1***	1,584.7***	0.86***	0.87***
	(1,468.6, 1,795.5)	(1,473.3, 1,696.2)	(0.81, 0.90)	(0.82, 0.91)
Observations	27	25	27	26
R^2	0.252	0.462	0.045	0.152
Adjusted R^2	0.222	0.438	0.006	0.116
Residual Std. Error	194.170 (df = 25)	130.611 (df = 23)	0.057 (df = 25)	0.049 (df = 24)
F Statistic	8.421*** (df = 1; 25)	19.711*** (df = 1; 23)	1.168 (df = 1; 25)	4.288** (df = 1; 24)

Note: *p<0.1**p<0.05***p<0.01

RMR = Respiratory resting metabolic rate.; RMR RER = Average respiratory exchange ratio during RMR assessment;

Table 5. Models Assessing the Relationship Between Habitual Fluid Intake and Thermic Effect of Food Measurements.

	Dependent variable:			
	TEF (kcals)	TEF RER	TEF (kcals)	TEF RER
	(1)	(2)	(3)	(4)
Fluid (L)	-17.4	-0.02***	-23.6	-0.02**
	(-48.1, 13.4)	(-0.03, -0.01)	(-52.3, 5.2)	(-0.03, -0.00)
Constant	274.3***	0.92***	316.0***	0.92***
	(167.2, 381.5)	(0.89, 0.96)	(214.5, 417.4)	(0.88, 0.96)
Observations	27	27	24	24
R^2	0.047	0.253	0.105	0.229
Adjusted R^2	0.009	0.224	0.064	0.194
Residual Std. Error	127.266 (df = 25)	0.044 (df = 25)	114.293 (df = 22)	0.047 (df = 22)
F Statistic	1.227 (df = 1; 25)	8.489*** (df = 1; 25)	2.580 (df = 1; 22)	6.525** (df = 1; 22)
Note:				*p<0.1**p<0.05***p<0.01

TEFavg = average thermic effect of food across the three post-prandial measurements; TEF RER = average respiratory exchange ratio across the three TEF measurements.

Figure 4. The Relationship Between Average Daily Total Fluid Intake (TFI) and Resting Metabolic Rate (RMR).

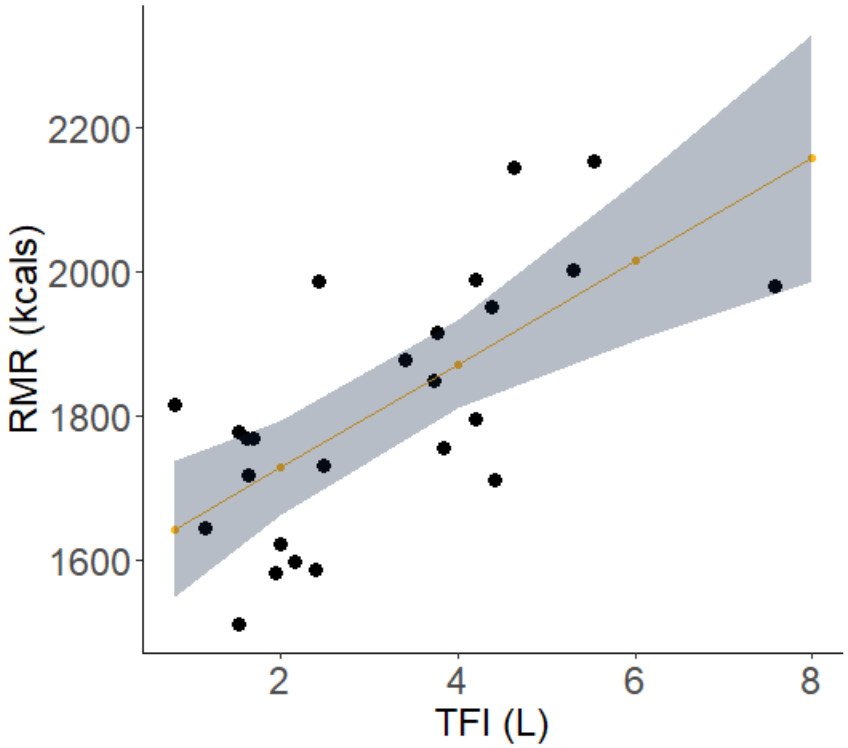


Table 6. Models Assessing the Relationship Between Average Urinary Osmolality and Metabolic Measurements.

	Dependent variable:					
	RMR	RMR RER	TEFavg		TEF RERavg	
	(1)	(2)	(3)	(4)	(5)	(6)
Urine Osmolality ($mOsm*kg^{-1}$)	-0.2	0.0001	0.2	0.2*	0.00*	0.00*
	(-0.6, 0.3)	(-0.00, 0.00)	(-0.0, 0.4)	(0.0, 0.4)	(0.00, 0.00)	(-0.00, 0.00)
Constant	1,938.6***	0.792***	113.0	118.545*	0.82***	0.82***
	(1,683.9, 2,193.2)	(0.73, 0.86)	(-29.7, 255.7)	(-9.302, 246.392)	(0.768, 0.877)	(0.767, 0.882)
Observations	27	27	27	24	27	24
R^2	0.021	0.061	0.089	0.156	0.134	0.125
Adjusted R^2	-0.018	0.024	0.052	0.117	0.100	0.086
Residual Std. Error	222.115 (df = 25)	0.056 (df = 25)	124.436 (df = 25)	111.011 (df = 22)	0.048 (df = 25)	0.050 (df = 22)
F Statistic	0.540 (df = 1; 25)	1.630 (df = 1; 25)	2.433 (df = 1; 25)	4.055* (df = 1; 22)	3.885* (df = 1; 25)	3.154* (df = 1; 22)
Note:						*p<0.1**p<0.05***p<0.01

RMR = Respiratory resting metabolic rate.; RMR RER = Average respiratory exchange ratio during RMR assessment; TEFavg = average thermic effect of food across the three post-prandial measurements; TEF RER = average respiratory exchange ratio across the three TEF measurements.

Table 7. Models Assessing the Relationship Between Average Urine Volume and Metabolic Measurements.

	Dependent variable:					
	RMR	RMR RER	TEFavg		TEF RERavg	
	(1)	(2)	(3)	(4)	(5)	(6)
Urine Volume (L)	52.6	-0.03*	-1.4	-16.5	-0.01	-0.01
	(-57.8, 163.0)	(-0.05, 0.00)	(-66.6, 63.8)	(-77.9, 44.8)	(-0.05, 0.01)	(-0.04, 0.02)
Constant	1,755.9***	0.88***	222.6***	271.5***	0.90***	0.90***
	(1,544.8, 1,967.0)	(0.83, 0.93)	(97.9, 347.3)	(151.1, 391.9)	(0.85, 0.95)	(0.84, 0.95)
Observations	27	27	27	24	27	24
R^2	0.034	0.129	0.0001	0.013	0.046	0.035
Adjusted R^2	-0.005	0.094	-0.040	-0.032	0.008	-0.009
Residual Std. Error	220.682 (df = 25)	0.054 (df = 25)	130.346 (df = 25)	120.049 (df = 22)	0.050 (df = 25)	0.053 (df = 22)
F Statistic	0.873 (df = 1; 25)	3.687* (df = 1; 25)	0.002 (df = 1; 25)	0.280 (df = 1; 22)	1.219 (df = 1; 25)	0.793 (df = 1; 22)

Note: *p<0.1**p<0.05***p<0.01

RMR = Respiratory resting metabolic rate.; RMR RER = Average respiratory exchange ratio during RMR assessment; TEFavg = average thermic effect of food across the three post-prandial measurements; TEF RER = average respiratory exchange ratio across the three TEF measurements.

Associations Between Daily Fluid Intake or Hydration Status and Energy Balance

Energy Intake

Simple linear regressions assessing the relationship between total fluid intake and plain water intake and 24h urinary hydration biomarkers on energy intake from the provided foods are presented in Table 8**.** There was no significant association between TFI ($p = 0.228$), plain water intake ($p = 0.198$), U_{OSMO} ($p = 0.648$) or U_{VOL} ($p = 0.100$) and ad libitum EI from the laboratory provided foods.

Table 8. Simple Linear Regressions Assessing the Impact of Fluid Intake or Urinary Hydration Biomarkers on EI From the 24h of Laboratory Provided Foods.

	Dependent variable:			
	EI			
	(1)	(2)	(3)	(4)
Fluid (L)	128.235			
	(-74.642, 331.111)			
Water (L)		147.498		
		(-70.050, 365.046)		
Urine Osmolality ($mOsm*kg^{-1}$)			0.428	
			(-1.387, 2.243)	
Urine Volume (L)				524.629*
				(-75.313, 1,124.571)
Constant	2,569.317***	2,606.288***	2,637.919***	1,941.893***
	(1,845.970, 3,292.664)	(1,960.195, 3,252.381)	(1,508.959, 3,766.880)	(792.100, 3,091.686)
Observations	24	24	25	25
R^2	0.065	0.074	0.009	0.113
Adjusted R^2	0.023	0.032	-0.034	0.075
Residual Std. Error	1,023.791 (df = 22)	1,018.802 (df = 22)	1,077.149 (df = 23)	1,019.017 (df = 23)
F Statistic	1.535 (df = 1; 22)	1.766 (df = 1; 22)	0.213 (df = 1; 23)	2.938* (df = 1; 23)

Note: *p<0.1**p<0.05***p<0.01

Models 3 and 4 include 1 participant with available urinary measures but no fluid log.

Linear mixed models assessing the relationship of between-person (bs) and within-person (ws) differences in total fluid intake and water intake on EI are presented in Table 9. Various random effects structures with the inclusion of a random slope effect of the person or group mean centered fluid intake predictors did not improve model fit; thus, a random intercept model

was maintained for analyses. Individuals with higher mean fluid intake overall tended to consume more calories (β = 193.87, [43.74, 344.01], p = 0.012); day-to-day changes in total fluid intake (wsFluid) were not related to changes in EI (p = 0.812). On days when individuals consumed more plain water than was typical, there was no significant difference in EI (β = -235.27 [-500.94, 30.40], p = 0.082) (Figure 5).

Table 9. Results of Linear Mixed Effects Model Examining the Association Between Within-Person (ws) and Between-Person (bs) Differences in Daily Total Fluid Intake (Fluid) and Plain Water Intake (Water) on Daily Self-Reported energy Intake (EI). Models Did Not Include the 24h of Laboratory Provided Foods.

	EI			EI		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	2312.66	2064.31 – 2561.01	**<0.001**	2320.87	2047.05 – 2594.69	**<0.001**
wsFluid	30.81	-225.83 – 287.45	0.812			
bsFluid	193.87	43.74 – 344.01	**0.012**			
wsWater				-235.27	-500.94 – 30.40	0.082
bsWater				120.22	-64.16 – 304.59	0.199
Random Effects						
σ^2	537749.89			544159.50		
τ_{00}	299933.16 $_{Subject}$			388724.81 $_{Subject}$		
ICC	0.36			0.42		
N	27 $_{Subject}$			27 $_{Subject}$		
Observations	130			130		
Marginal R^2 / Conditional R^2	0.126 / 0.439			0.041 / 0.441		
AIC	2125.656			2132.093		

Figure 5. Association Between Within Person Changes in Plain Water Intake and Energy Intake.

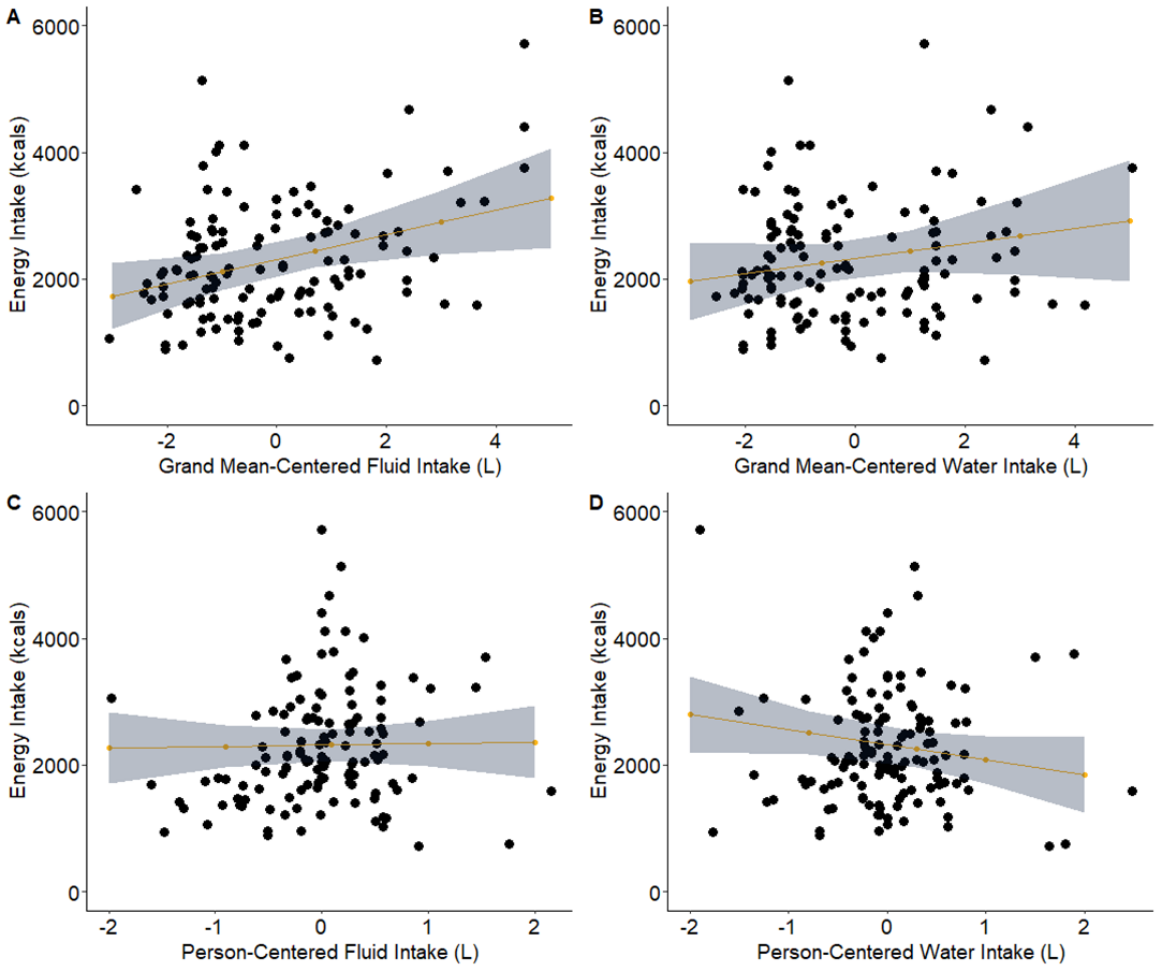


Table 10 shows model results for the linear mixed models examining the associations for the within and between person changes in urinary osmolality and urine volume on EI. Within and between person differences in 24h hydration biomarkers were not associated with changes in EI, EE, TDEE, or EB (ps > 0.05).

Table 10. Results of Linear Mixed Effects Model Examining the Association Between Within-Person (ws) and Between-Person (bs) Differences in Daily Urinary Osmolality (U_{OSMO}) (Column 1) and Urine Volume (U_{VOL}) (Column 2) on energy Intake (EI). Models Did Not Include the 24h of Laboratory Provided Foods.

	EI			EI		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	2405.36	2101.42 – 2709.30	**<0.001**	2409.51	2106.31 – 2712.72	**<0.001**
wsU_{OSMO}	-1.14	-3.29 – 1.02	0.294			
bsU_{OSMO}	0.67	-0.79 – 2.13	0.360			
wsU_{VOL}				-36.24	-539.48 – 467.01	0.885
bsU_{VOL}				165.92	-178.34 – 510.19	0.337
Random Effects						
σ^2	569130.08			567811.64		
τ_{00}	379541.99 $_{Subject}$			376766.16 $_{Subject}$		
ICC	0.40			0.40		
N	26 $_{Subject}$			26 $_{Subject}$		
Observations	72			72		
Marginal R^2 / Conditional R^2	0.023 / 0.414			0.026 / 0.415		
AIC	1192.373			1192.123		

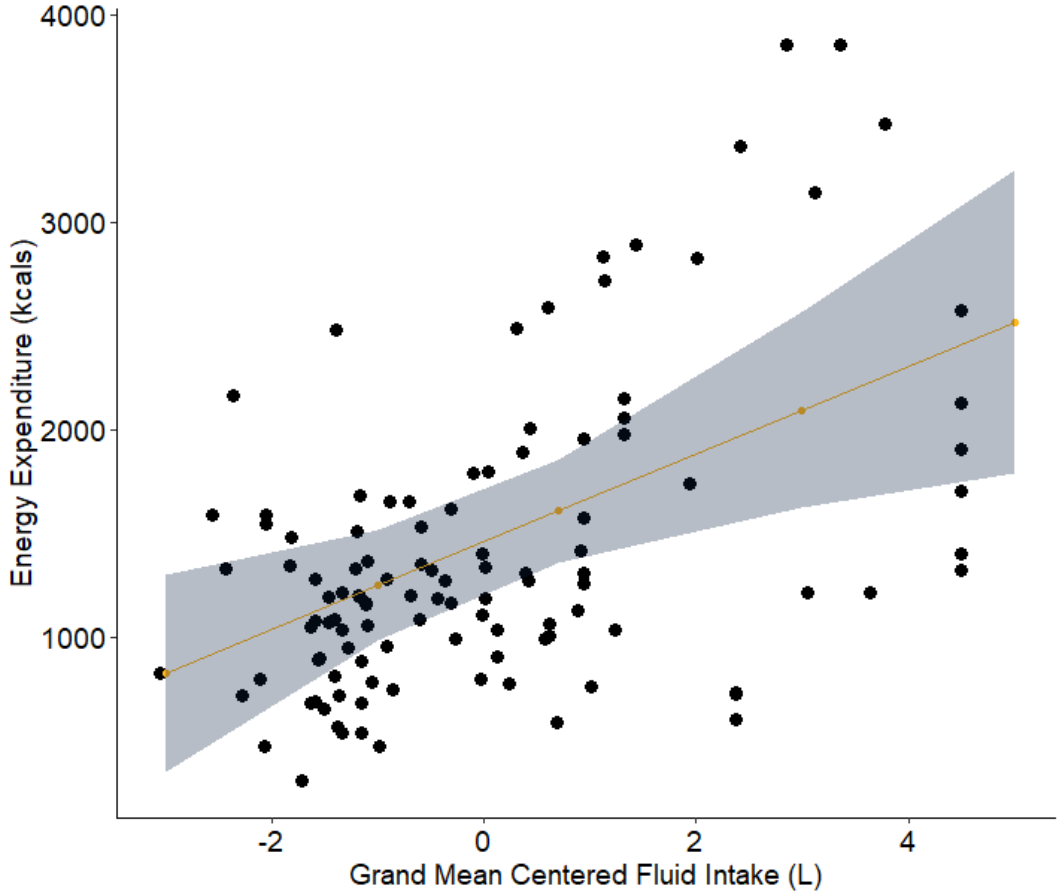
Actigraph data was not included for one participant (out of the initial 27) who had insufficient wear time for the Actigraph monitor for all fluid and food recording days (kcals expended <200/day and visible periods of non-wear time observed in Acti-Life software > 85%), leaving 26 participants for the PAEE analyses. Higher mean fluid intake overall (between-subject effects) was associated with increased PAEE (p = 0.003) (Table 11, Figure 6); every 1L higher fluid intake above the overall mean was associated with an additional 212 kcals expended

from physical activity. There was no association between higher average plain water intake across participants and PAEE ($p = 0.067$). PAEE was not significantly associated with within-person changes in fluid intake ($p = 0.176$) and water intake ($p = 0.379$)**;** on days when participants modified their fluid intake, physical activity behaviors were similar. However, more PAEE between individuals was significantly associated with consuming more calorie containing beverages ($\beta = 0.38$, [0.11 – 0.66], $p = 0.007$), though there was no relationship between daily within-person changes in PAEE on consumption of calorie containing beverage ($\beta = -0.30$, [-0.79, 0.19], $p = 0.223$).

Table 11. Linear Mixed Models Examining the Relationship Between Within-Person (ws) and Between-Person (bs) Differences in Daily Fluid Intake (Fluid - Column 1) and Water Intake (Water – Column 2) and Actigraph-Measured Physical Activity Energy Expenditure (PAEE).

		PAEE			**PAEE**	
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	1461.30	1231.11 – 1691.49	**<0.001**	1472.55	1219.46 – 1725.64	**<0.001**
wsFluid	-117.38	-288.48 – 53.72	0.176			
bsFluid	211.50	72.22 – 350.79	**0.003**			
wsWater				-85.08	-276.28 – 106.12	0.379
bsWater				156.86	-11.54 – 325.26	0.067
Random Effects						
σ^2	105625.34			105949.03		
τ_{00}	318181.65 $_{Subject}$			390266.37 $_{Subject}$		
ICC	0.75			0.79		
N	26 $_{Subject}$			26 $_{Subject}$		
Observations	112			112		
Marginal R^2 / Conditional R^2	0.246 / 0.812			0.112 / 0.810		
AIC	1686.951			1692.108		

Figure 6. Relationship Between Grand Mean Centered Fluid Intake and PAEE.



Overall energy balance was not influenced by within-person changes in TFI ($p = 0.262$), between person changes in TFI ($p = 0.499$) or plain water intake stats (within – $p = 0.632$, between – $p = 0.442$) (Table 12). Using the addition of the Actigraph-assessed energy expenditure, RMR, and TEF measurements to calculate TDEE, most participants were in negative energy balance (intercept: $\beta = -1135$ [-1469, -801], $p < 0.001$, with 23 out of 26 participants in negative EB, and 85% of observed days from all participants in negative EB), with large variability between participants (-1173 ± 897kcals) and between individual observation days (-1036 ± 1089kcals). There was no significant influence of TFI ($p = 0.991$) or water intake ($p = 0.818$) on overall EB when foods were provided to participants.

Table 12. Linear Mixed Models Examining the Relationship Between Within-Person (ws) and Between-Person (bs) Differences in Daily Fluid Intake (Fluid) and Water Intake (Water) and Overall Energy Balance (EB) Over the Course of the Six Self-Reported Dietary and Physical Activity Assessment Days.

		EB			**EB**	
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	-1137.54	-1485.48 – -789.60	**<0.001**	-1139.45	-1485.82 – -793.07	**<0.001**
wsFluid	178.00	-135.57 – 491.58	0.262			
bsFluid	-71.29	-280.09 – 137.50	0.499			
wsWater				-75.47	-387.92 – 236.98	0.632
bsWater				-89.13	-318.61 – 140.35	0.442
Random Effects						
σ^2	590783.16			581086.68		
τ_{00}	634619.65 $_{\text{Subject}}$			629363.19 $_{\text{Subject}}$		
ICC	0.52			0.52		
N	26 $_{\text{Subject}}$			26 $_{\text{Subject}}$		
Observations	112			112		
Marginal R^2 / Conditional R^2	0.017 / 0.526			0.026 / 0.532		
AIC	1856.655			1854.973		

Discussion

This study assessed associations between habitual fluid intake and indicators of energy balance using objective measures of dietary intake and total energy expenditure (RMR, TEF, PAEE). Results from the present observational study suggest greater fluid intake is associated with a higher resting metabolic rate. The observed lower post-prandial RER in individuals with higher habitual fluid intake suggest greater fat oxidation in individuals with higher fluid intake.

Previous studies have suggested an elevation in RMR in response to acute water ingestion (Boschmann et al., 2003, 2007), but it is unclear if modifications in water intake affect whole-day RMR predictions. By contrast, acute *dehydration* in the context of exercise has also been shown to increase metabolic rate above that experienced following exercise alone (Castro-Sepulveda et al., 2014). Our study is the first to suggest an association between *chronic* fluid intake behaviors and resting metabolic rate. The magnitude of the observed effect was similar to prior research suggesting the potential of an increase in metabolic rate by approximately 48kcals for every 1L increase in fluid intake (Boschmann et al., 2003, 2007), as compared to 69kcals in the present study. Thus, it may be that the beneficial effects of fluid intake on metabolic rate extend beyond the acute post-ingestion period. However, given the positive association observed between TFI and PAEE, differences in RMR may also have been driven by higher levels of activity. It is possible that between person differences in PAEE on the day prior to their lab visit influenced both fluid intake the day prior and their metabolic rate the morning of the RMR measurement. However, we assessed this by including the previous day's activity counts as a potential covariate in our step forward selection procedure, and this did not improve model fit, suggesting the effects of fluid intake on this relationship are independent of activity levels. The long-term effects of physical activity, or exercise specifically, on RMR, have primarily been

attributed to changes in body composition (Karstoft et al., 2017), including strength training specifically for the preservation of FFM during weight loss interventions (Stiegler & Cunliffe, 2006). However, the slow phase of the excess post-exercise O2 consumption (EPOC) following activity may persist for up to 48h (Speakman & Selman, 2003), which was not accounted for in the present study, although participants were restricted from exercising the morning of their laboratory visit. It may also be that some individuals are water "responders", as has been observed in studies examining the relationship between fluid intake and glucose regulation (Enhörning, Tasevska, et al., 2019). Future investigation should explore the mediating role of fluid intake on RMR with more rigorous control of physical activity and/or exercise. However, the overarching purpose of this study was to explore the relationships between fluid intake and behavioral components of energy balance. If individuals are accustomed to regular physical activity participation, then their morning RMR when exercise is not restricted the day prior is likely more reflective of their day-to-day metabolism; we would expect a lower RMR than is typical if individuals who are habitually active are asked to restrict activity.

In the present study, habitual TFI was associated with post-prandial RER and a higher resting metabolic rate. The effects observed in our study equate to ~5-6% postprandial fat oxidation (Carpenter, 1921) per liter increase in fluid intake, which, if consistent day to day over the course of a month, would equate to an additional 2100kcals expended, and ~116 extra kcals expended post prandially from fat per month for an average RMR and TEF, respectively. Interestingly, this is in line with prior observations of the acute effects of increased water intake promoting primarily an increase in lipid utilization (a 100% increase 40 minute post-ingestion) following 500mL of water consumption among male participants (Boschmann et al., 2003). Low water intake and the associated increase in AVP can influence lipid metabolism both centrally

and peripherally. Centrally, AVP stimulates sympathetic nervous system activity, potentially *increasing* hepatic and adipose lipid metabolism (Yoshimura et al., 2021). However, AVP seems to exert antilipolytic effects depending on the feeding state of the individual (Nakamura et al., 2009). Animal studies suggest AVP inhibits β-oxidation of fatty acids (Hiroyama et al., 2007; Koshimizu et al., 2012), and may promote triglyceride synthesis (Pollard & Brindley, 1984). AVP may also decrease insulin signaling in adipocytes through reduced Akt phosphorylation (Hiroyama et al., 2009). These mechanistic studies provide some potential rationale for the relationship between fluid intake and a reduction in RER observed following breakfast consumption. More study in humans is required to verify these mechanisms, but one study by Chang et al. observed contrasting relationships between 24h urine volume and 24-h RQ, and copeptin and 24-h RQ (D. C. Chang et al., 2020). In their study, lower urine volume (suggestive of worse hydration) was associated with lower RQ, yet higher copeptin, a surrogate biomarker for AVP elevated with underhydration, was associated with *higher* RQ, independent of urine volume. However, their study did not measure participant fluid consumption, and participants seemed largely well-hydrated in the study based on mean urine volume (>3L). Further experimental study in humans is required to clarify the relationship between fluid intake and metabolism.

Our results suggest an association between increased fluid intake and increased EI. However, the strength of this relationship was relatively small, and overall energy balance was *not* related to within- or between-subject differences in fluid intake or water intake, supported by the weight stability of the participants recruited. For the models assessing EI from provided foods, while the overall effect was non-significant, the magnitude of the coefficient and variability of intake when foods were provided was substantial (β, [95% CI]: 128.2 kcals, [-74.6,

331.1]. This suggests the relationship between fluid intake and energy intake is not consistent across individuals. By contrast, increased water intake has previously been used as a successful addition to a weight loss intervention in older adults, producing ~2kg greater weight loss over the course of 12 weeks when water was ingested before meals compared to a hypocaloric diet alone (Dennis et al., 2010). It is possible that increased fluid intake is primarily beneficial towards energy balance when combined with other dietary efforts to maintain or reduce body weight, as has been suggested by a systematic review (Muckelbauer et al., 2013). Additional proposed mechanisms for increased water facilitating weight loss include improved satiety (J. N. Jeong, 2018). The observed effects may also be partially due to replacement of caloric containing beverages with water, since within-person increases in total fluid intake were not significantly associated with energy intake. Interestingly, higher total fluid intake but not plain water intake between individuals was associated with increased physical activity energy expenditure. Perhaps individuals who are more active in general tend to compensate for increased activity by consuming additional calorie containing beverages (i.e., sports drinks, milks, etc.), which is supported by the positive association of between-person fluid intake and EI and the lack of association for within-person changes in total fluid intake and EI. This was the case in the present study, as individuals who were more physically active, on average, had a greater consumption of calorie containing beverages. Physiological differences in hydration status are unlikely to explain this effect, as changes in urine volume and urinary osmolality did not match these results; however, other factors may influence these urinary hydration biomarkers beyond fluid intake, such as high consumption of dietary osmolytes (i.e., amino acids, sugars, polyols) (Armstrong, 2007; Manz & Wentz, 2003) or consuming nutrients that influence fluid retention (i.e., electrolytes, caffeine, alcohol, protein) (Maughan et al., 2016). Additional analyses will

explore the contribution of these other dietary factors from both the self-reported measures and direct observations of dietary intake from the provided foods.

The difference between mean self-reported food intake and the laboratory-provided foods is worth noting. Although the provided menu offered a variety of nutrient-dense food options (i.e., fruits and vegetables – Appendix B), the menu also provided access to other sugary food items that perhaps participants enjoyed but did not typically purchase (i.e., juices, candy, cookies). Thus, this novelty effect could have influenced acute dietary intake. It is unclear if such behavioral differences would persist if foods were provided for a longer term, though other factors such as finances and convenience likely played a large role in food selection and consumption, particularly in this predominately younger population, which included many student participants.

Limitations

The observational nature of the study precludes us from making any causal inferences regarding the influence of total fluid or plain water intake on energy balance or metabolism. However, this study provides insight into the wide range of natural variability in fluid intake that occurs across participants, regardless of whether they habitually consumed a high or low amount of fluid. Though there are inherent limitations with self-reported dietary data, we attempted to rectify these limitations both by providing participants with foods to consume for one of the seven days and the use of a digital food record which used images to aid participants with portion size estimation. Additional days of food provision may have enhanced the internal validity of the study but perhaps at the expense of external validity, as other factors influencing access to foods may influence behaviors (i.e., finances, environment, time constraints). Other physiological variables may have influenced day-to-day changes in energy intake which

obscured the effects of fluid intake such as sleep, environmental temperature, or individual stress levels. Future analyses may examine the role of sleep and stress on these outcome variables with consideration for hydration status.

This study is strengthened by objective measures of total daily energy expenditure, including RMR, TEF, and PAEE, and while we were able to make multiple assessments of many of our variables, we were limited to a single time point for assessment of RMR. Thus, the magnitude of effects of day-to-day changes in fluid intake on energy balance may have been obscured by missing alterations in daily RMR measures depending on the variability in daily fluid intake. Further, while participants were instructed not to exercise prior to coming to the lab on the RMR measurement day, they were not explicitly instructed to refrain from activity the day prior to prevent any interference with their habitual behaviors that could impact energy balance measures on that day. To establish a causal relationship, future work may explore day-to-day changes in RMR in response to experimental manipulation of hydration status, when controlling for additional variables known to influence metabolic rate. Another consideration is that the accuracy of wrist-worn accelerometry for measurement of PAEE may vary depending on the nature of activities performed among participants. Specifically, accelerometer-assessed physical activity may have reduced utility for measuring resistance training (Stec & Rawson, 2012) and cycling based energy expenditure (Herman Hansen et al., 2014)**,** as well as energy expended while swimming, as occurred in one participant. Additionally, discrepancies have been observed between wrist- and hip-worn activity monitors, with wrist-worn monitors tending to overestimate PAEE (Guediri et al., 2021); thus, the absolute calculation of energy balance from these measures should be interpreted with caution (Mandigout et al., 2019).

Another potential limitation is the power to detect an effect in the present study. An attempt was made to power this study based on the expected within-person variability anticipated over the course of the six days of self-reported measurements based on prior data in our lab examining the influence of within- and between- person changes in fluid intake on total kcal intake. It was assumed the effect size for changes in energy balance would be slightly higher than observed in our pilot data due to the suggested increase in RMR anticipated for every 2L increase in water intake, as well as an expected within subject increase in fluid intake concurrent with increases in PAEE. The study was originally designed with the intent to have participants record measurements for the entirety of four weeks, thus, initial power calculations were generated using this observation number via power simulations using the "simR" package in R for power simulations of linear mixed models (Green & MacLeod, 2016). This initial simulation suggested ~21 subjects would be needed to achieve ~93% power for detection of a within-person decrease in energy balance of 250kcals for every 1L increase in water. To alleviate participate burden, observation days were reduced to two sets of three-day recording periods (6 total collection days + 1 in-lab recording day). However, an error was made in adjusting the power simulation which was not identified until we were near the end of the planned data collection period. The revised, corrected power, using the same assumptions as the first model but with seven observation days, suggested ~80% power to detect this effect could be detected by recruiting 49 participants. However, the assumption that increased physical activity would be additive in the effect size for the relationship between fluid intake and energy balance was also incorrect, as evidenced by the results for the within-person changes in PAEE. Thus, the overall effect size may have been smaller than predicted. Regardless, given the observed coefficients from the present sample size and the null effects observed from this collection period, it may be

speculated that even with a longer collection interval the results would remain the same; perhaps the intraindividual variability of energy balance is too large for this time frame to adequately capture small differences that would contribute to weight gain over the long-term. Regardless, this study is an important first step to examine the relationships between habitual fluid intake and energy balance both as a whole and its individual subcomponents, which have previously only been studied in isolation. Future studies may seek to perform more longitudinal work relevant to this question, perhaps taking similar sets of measures over the course of different months or years to determine the time course of these changes as well as other contextual factors specific to life stage that may influence energy balance itself.

Strengths

Despite these weaknesses, this study also has several strengths. We successfully assessed multiple components of energy balance through objective measures of EI and TDEE. Further, with our pre-screening procedures we were able to recruit individuals from a range of habitual fluid intakes in a relatively even distribution. We were able to expand upon prior investigations investigating the relationship between hydration status and metabolism by using a validated tool (Liq.In.7) to measure participant fluid intake. Our approach also allowed us to examine the change in metabolic rate following a specific meal rather than a full day's expenditure, as has been done previously. Our sample also contained individuals from a variety of racial/ethnic backgrounds, enhancing the generalizability of our findings. The repeated measure nature of our study also allowed us to look at the contributions of both inter- and intra-individual differences in fluid intake on energy balance and the associated subcomponents. Inclusion of the provided foods also allowed us to obtain a direct, accurate measurement of participant energy intake during that 24h period.

Conclusion

Greater habitual fluid intake is associated with higher resting metabolic rate, lower post-prandial RER, and higher physical activity energy expenditure. Fluid intake does not seem to substantially impact overall energy balance, suggesting the potential for within-day energy compensation to offset these differences. Further experimental investigation is required to determine the acute and chronic influence of modifications in fluid intake on metabolism.

CHAPTER IV: THE ASSOCIATION BETWEEN HABITUAL FLUID INTAKE AND THE HEDONIC AND HOMEOSTATIC CONTRIBUTORS TO FOOD CONSUMPTION IN HEALTHY YOUNG ADULT MALES

Abstract

Evidence linking hydration behaviors to other dietary behaviors is lacking but important considering emerging associations between chronic low fluid intake and metabolic diseases, including obesity. Prior research examining the relationship between hydration and energy intake has primarily focused on acute dehydration followed by consumption of a homogenous meal, which may not translate to scenarios of chronic underhydration. The purpose of this study was to examine associations between habitual fluid intake and the homeostatic (appetite) and hedonic (food reward) aspects of food intake. **Methods:** 27 male participants (age, 23±4y; height, 176 ± 6.5cm; body mass, 78.8 ± 13.0kg; body fat,17.2 ± 9.0%) consumed a standard breakfast and completed electronic visual analog scales assessing appetite and thirst ratings prior to and every 30 minutes over the course of the three hours following the meal; participants did not have access to food or water during this time. Participants then completed an electronic food reward task (Leeds Food Preference Questionnaire) to assess explicit liking, explicit wanting, and implicit wanting of food items that were high fat sweet (HFSW), high fat savory (HFSA), low fat sweet (LFSW), and low-fat savory (LFSA) before and after consuming an ad libitum lunch. Multiple regression models assessed the relationship between habitual fluid intake and fasting appetite and thirst and AUCs for the change in these ratings over the course of three hours. Multiple regression or linear mixed effects models assessed the relationship between habitual fluid intake and food reward before and after the ad libitum lunch. **Results:** There was no

association between habitual fluid intake and indicators of appetite and baseline or total AUC for any ratings throughout the 3-hour measurement period. Higher habitual fluid intake was associated with lower explicit liking and explicit wanting of HFSW food items, both before (Liking: β = -6.939 [-11.700, -2.129], p = 0.009; Wanting: -6.879 [-11.794, -1.963], p = 0.011**)** and after the ad libitum meal (Liking: β = -7.191 [-12.012, -2.370], p = 0.005, Wanting: β = -6.051 [-10.884, 1.219], p = 0.018. **Conclusions:** Results suggest thirst and appetite perceptions after a standardized meal are similar for individuals regardless of varying habitual fluid intakes. However, the greater explicit liking and explicit wanting for HFSW foods among those with lower habitual fluid intake warrants further investigation.

Introduction

Recent interest in the health benefits of adequate hydration has identified a relationship between chronic fluid intake or hydration status and obesity (T. Chang et al., 2016; Enhörning et al., 2013; J. D. Stookey et al., 2020). Yet current clinical practice guidelines for obesity treatment do not include water intake recommendations (Wharton et al., 2020). Within-day factors that may influence energy intake such as appetite sensations and thirst have previously been studied after acute periods of experimentally induced dehydration. Acutely, pre-meal water ingestion may reduce energy intake by promoting gastric distension, thereby increasing fullness both acutely and over the course of an intervention (Corney et al., 2016; McKay et al., 2018; Van Walleghen et al., 2007). The effects of *immediate* pre-meal water consumption are more effective at reducing energy intake for younger than older adults, but a longer delay between drinking and eating has proven more effective for reducing energy intake in older adults, perhaps due to slower gastric emptying with age (Van Walleghen et al., 2007)**.** This effect may also be influenced by the temperature of the ingested fluid, with colder water resulting in reduced energy

intake at a meal consumed 60 minutes later compared to warmer or room temperature fluids—likely because colder water produces later gastric contractions than warmer beverages (Fujihira et al., 2020). However, few have considered the habitual fluid intake habits of participants prior to water preload studies, and it is unclear if this could confound results. Instead, most studies attempt to standardize fluid intake to reach "euhydration" prior to each trial. While this adjusts for baseline difference in hydration status, it fails to account for the inter-individual differences in thirst and fluid regulatory hormones that would be experienced across individuals prior to meal initiation. Chronic high fluid intake (and low concentrations of the fluid regulatory hormone arginine vasopressin - AVP) may reduce expression of aquaporin-2 channels in the kidneys responsible for water reabsorption (Knepper & Star, 2008), potentially predisposing individuals with high fluid intake toward more adverse effects from acute dehydration. This may limit the generalizability of prior experimental studies to only those who are chronically well-hydrated. However, Perrier observed a stabilization of urinary hydration biomarkers within 24-h following an acute increase or decrease in fluid intake among participants with low or high fluid intake, respectively (E. Perrier, Demazières, et al., 2013). Overall, greater water intake around mealtimes appears to be beneficial for reducing energy intake in some individuals depending on fluid availability (Corney, Horina, et al., 2015), eating rate (Andrade et al., 2008, 2012), temperature (Fujihira et al., 2020) and timing of fluid ingestion (Corney et al., 2016).

Increased serum leptin has been observed in vasopressin 1B receptor knockout models (V1bR-KO), suggesting a potential indirect influence of the fluid regulatory hormone arginine vasopressin (AVP) on appetite (Hiroyama et al., 2009). However, limited research has looked at appetite responses in relation to hydration status or habitual fluid intake in humans, and those that did, examined the response to acute fluid restriction, heat exposure, and/or exercise-induced

sweat losses rather than chronic low fluid intake (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019; Corney, Sunderland, et al., 2015; Kelly et al., 2012; Pérez-Luco et al., 2019). With acute dehydration, there appears to be no significant impact on post-prandial ghrelin (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019; Corney, Sunderland, et al., 2015; Kelly et al., 2012), leptin (Kelly et al., 2012), or PYY (Kelly et al., 2012). Providing additional water (three 500mL bottles) prior to meals decreased perceived hunger in females (McKay et al., 2018), and dehydration achieved through exercise and heat exposure was associated with reduced fullness (Corney, Sunderland, et al., 2015), though another study found no effect of exercise dehydration on appetite ratings (Kelly et al., 2012). Given the heterogeneity of methods used to induce dehydration as well as the acute nature of body water loss reductions, further study is warranted to explore how *chronic* underconsumption of fluid influences appetite following a standard meal. However, changes in appetite hormone concentrations do not always influence perceptual hunger and fullness signals (Tacad et al., 2021); therefore, the present study focused first on subjective ratings of hunger and satiety.

Food Reward

The concept of 'food reward' considers hedonic aspects of eating behavior that extend beyond biological need. Food reward includes "liking" and "wanting" of food items which govern the motivational drive to consume a specific food. The Leeds Food Preference Questionnaire (LFPQ) is a tool commonly used to assess these traits (Finlayson et al., 2007). Greater hedonic responses to higher calorie food items have been shown to encourage overconsumption and may contribute to obesity over time (Beaulieu et al., 2018). Limited data has analyzed the effect of water or hydration status on food reward. One study found reduced

food liking at a lunch buffet when consuming a large volume of water throughout the morning (1.5L) (McKay et al., 2018), which led to reduced energy intake in normal-weight individuals but not those who were overweight or obese. Thus, water intake relative to body size (instead of total water intake) should be considered as a potential influence on caloric consumption at meals. Carroll et al. (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019) found acute hypohydration influenced thirst and salt preference, but this did not impact energy intake. Some studies (Almiron-Roig & Drewnowski, 2003; Appleton & Blundell, 2007; Black et al., 1991, 1993; Dennis et al., 2010; McKay et al., 2018; Rodin, 1990; Spitzer & Rodin, 1987; Triana et al., 2003) have allowed participants to self-select food items, while the majority (Akhavan et al., 2010, 2011; Akhavan & Anderson, 2007; Canty & Chan, 1991; Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019; Cassady et al., 2012; Chungchunlam et al., 2012; Corney et al., 2016; Corney, Horina, et al., 2015; Corney, Sunderland, et al., 2015; Davy et al., 2008; Flood et al., 2006; Fujihira et al., 2020; J. N. Jeong, 2018; Lavin et al., 1997, 2002; Maersk et al., 2012; Panahi et al., 2013; Pérez-Luco et al., 2019; Rogers et al., 1988, 1990; Shah et al., 2014; Westerterp-Plantenga & Verwegen, 1999; Woodend & Anderson, 2001) restricted intake to a specific food or meal. More work is needed to determine food preference among those with habitual high versus low fluid intake and how this influences total energy intake, particularly when certain nutrients may influence water balance (Adams, Wininger, et al., 2020; Disher et al., 2021).

The nutrient composition of one's diet influences the conservation and distribution of fluids throughout the body (Adams, Wininger, et al., 2020; Maughan et al., 2016; Millard-Stafford et al., 2021; Ray et al., 1998). Specifically, caloric content, electrolyte composition, tonicity, and presence of diuretics influence the retention of an ingested beverage (Bechke et al.,

2022; Maughan et al., 2016; Millard-Stafford et al., 2021). But while certain nutrients may favor improved hydration, it is unclear if habitually low fluid intake contributes to a preference for these nutrients. Sodium, specifically, favors greater extracellular fluid retention, but has been named a nutrient to limit in the 2020-2025 Dietary Guidelines for Americans (Snetselaar et al., 2021). While the role of sodium intake in health and disease is controversial (Mente et al., 2021), sodium is often consumed in the form of items with high energy density (i.e., processed foods), which may favor the development of obesity (Pérez-Gimeno et al., 2020). Thus, minimizing states that favor increased sodium consumption, such as low water intake (L. A. De Luca et al., 2010), may indirectly help reduce energy intake. When fluid consumption is allowed during meals after a period of acute dehydration (inducing a state of hyperosmolality), there seems to be no difference between energy intake of a homogenous porridge meal compared to when fluid was not available with the meal (Corney, Horina, et al., 2015). Yet it is unclear if differences in food preferences, perhaps driven by fluid regulatory hormonal responses and a drive to preserve serum osmolality, would influence consumption when participants are allowed free access to a variety of food options.

The purpose of this study was to assess the influence of habitual fluid intake on appetite and food reward. It was predicted that lower habitual fluid intake would be associated with greater fasting appetite and a greater AUC for appetite perceptions. By contrast, lower habitual fluid intake would be associated with lower fasting thirst and a lower AUC for thirst perceptions. We also predicted lower habitual fluid intake would be associated with greater food reward (explicit liking and explicit wanting) for food items associated with a positive energy balance (i.e., high fat and/or high sugar foods). Last, we predicted lower habitual fluid intake would be associated with greater implicit wanting for high fat, savory food items.

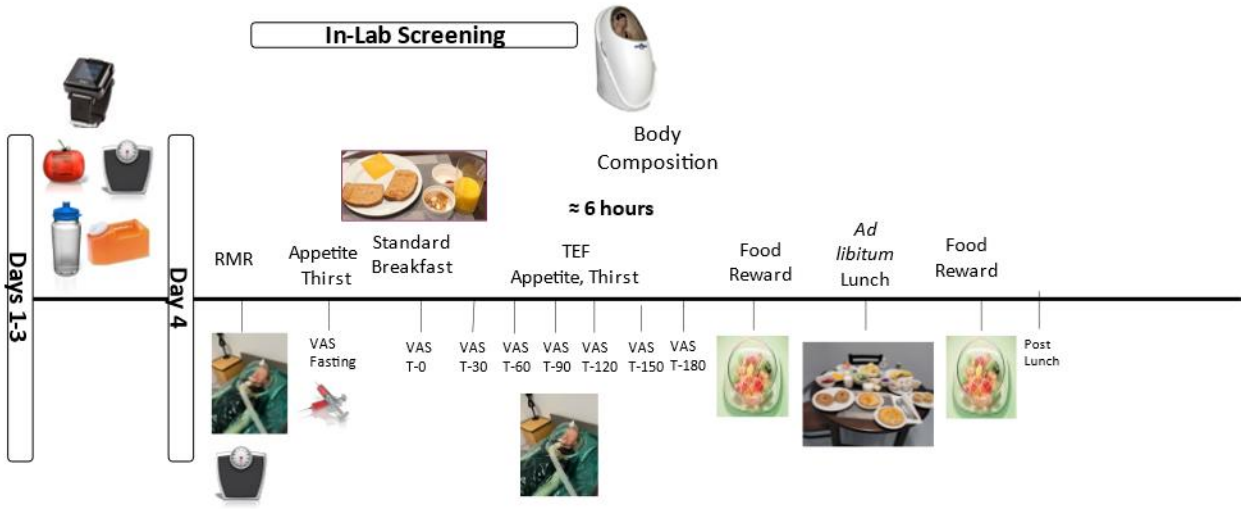
Methods:

This project is part of a larger study which assessed the relationship between habitual fluid intake and energy balance. In the larger study, participants completed measurements of resting metabolic rate (RMR) and thermic effect of food (TEF) over three hours to estimate energy expenditure over the course of several days. The present study describes additional measures taken during the three-hour timeframe that may contribute to within-day energy intake, specifically appetite and food reward. Participants were specifically recruited based on habitual fluid intake with pre-screening through an electronic version of the BEVQ-15 questionnaire (Fausnacht et al., 2020), which captures participants' habitual fluid intake behaviors over the course of a month. To recruit a wide range of fluid intakes, a recruitment goal of 10 participants from each of the following total fluid consumption categories was used: <1500mL/day; 1500-3000mL/day; >3000mL/day, with no upper limit placed on fluid intake. After verification with reported fluid intake, 9 participants had "low" fluid intake, 11 had "moderate" intake, and 9 had "high" intake. Participants were measured for age, height, nude body mass (NBM) and body composition on a separate screening day to ensure they had 1) no chronic health conditions or diseases which would alter body water regulation, 2) no previous surgery of the gastrointestinal tract that could impact body water regulation, 3) no pharmacologic drug treatment in the previous 15 days, 4) not exercising more than 10 hours per week, 4) no known or suspected sleep pathologies (Pittsburgh Sleep Quality Index (PSQI) <5), and 5) no food allergies or severe dietary restrictions.. All participants completed written informed consent prior to participation in the study.

Following written informed consent, participants arrived fasted (at least 8 hours) to the exercise physiology lab in the morning (0600-0900). After the RMR assessment, participants

completed fasted ratings of appetite and thirst before consuming a standardized breakfast (78g 100% whole wheat bread, 21g mild cheddar cheese, 17g strawberry jam, 18g peanut butter, 225g orange juice: ~546kcals, 19g protein, 77g carbohydrate, 18g fat) and had their gas consumption measured again to determine their Thermic Effect of Food (TEF) for 30 minutes once per hour for 3 hours. During this time, participants provided ratings of thirst and appetite every 30 minutes for 3 hours. Participants were allowed to perform any type of sedentary activities in between TEF measurements (i.e., reading, computer work, etc.). Any urine produced during the study was collected in a specimen container and weighed for determination of urine volume. Participants were not permitted to consume fluids during this time. Following the TEF assessment, participants completed an electronic food reward task, the Leeds Food Preference Questionnaire (Finlayson et al., 2007) to assess their liking and wanting for various food items. Prior to their final TEF measurement, participants received a food menu (Appendix B) from which they selected items to consume ad-libitum over the course of thirty minutes. Participants consumed this meal after their final TEF measurement and then repeated measures of thirst, appetite, and food reward after lunch (Figure 7).

Figure 7. Timeline Of Experimental Protocol.



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Measures

Appetite and Thirst.

Participants indicated their responses to the following questions by clicking on a 10 cm line using computer generated visual-analog scales (VAS) with anchor terms on either end of the lines (Marsh-Richard et al., 2009): Questions assessed Desire ("How strong is your desire to eat?": very weak - very strong), Hungry ("How hungry do you feel?"; Not hungry at all - As hungry as I have ever felt), How Full ("How full do you feel?"; Not full at all - Very full), and prospective food consumption (How Much) ("How much food do you think you could eat?"; Nothing at all - A large amount).

Thirst was assessed every 30 minutes using a series of prompts on a 10 cm VAS evaluating various domains of thirst (Adams et al., 2019). The thirst scale followed a similar format, with participants asked to indicate: "How thirsty do you feel right now?"; Not at all

thirsty - Very thirsty, “How pleasant would it be to drink some water right now?”; Very unpleasant - Very pleasant, “How dry does your mouth feel right now?”; Not at all dry - Very dry, “How would you describe the taste in your mouth?”; Normal - Very unpleasant, “How full does your stomach feel right now?”; Not at all full - Very full, and “How sick to your stomach do you feel right now?”; Not at all sick - Very sick.

Food Reward.

Food Reward was assessed with the Leeds Food Preference Questionnaire (LFPQ) (Finlayson et al., 2007; Oustric et al., 2020). The LFPQ is a computerized task that measures multiple domains of food reward, including explicit liking and wanting, and implicit wanting, using food items from four food categories (high-fat savory (HFSA), low-fat savory (LFSA), high-fat sweet (HFSW) and low-fat sweet (LFSW)). The task was customized to each participant by having them select their top four food items from each category from the large catalog of food items validated for this task. Explicit liking was assessed by presenting single food images from each category and asking participants to rate “How pleasant would it be to taste some of this food now?” using a 100-unit VAS scale, while explicit wanting was assessed with the prompt “How much do you want some of this food now?”. Scores were averaged by category, with higher scores indicating greater explicit liking and wanting for each food category, respectively. Participants then completed a forced choice task where they were presented with food image pairs from different categories and asked, “Which food do you most want to eat now?” The frequency of choosing items from each category and the reaction time for each pairing was recorded and used to calculate implicit wanting using a frequency-weighted algorithm (Oustric et al., 2020):

$$Frequency - weighted\ algorithm: I_A = \sum_{i=1}^{N_{choice}} \frac{\bar{t}}{t_i} - \sum_{j=1}^{N_{non-choice}} \frac{\bar{t}}{t_j}$$

In the above equation, IA = Implicit wanting for category A; N_{choice} = number of times category was selected; $N_{non\text{-}choice}$ = number of times category A was not selected; t = mean of all reaction times. A more positive score reflects more rapid preference for one category over the other, negative indicates the opposite, and a zero score indicates equal preference for food categories.

Ad Libitum Lunch

Participants were allowed to select food items from a validated food menu to consume during the ad libitum lunch (McNeil et al., 2012). Items were initially served as a double-portion size, with participants allowed to select additional items if desired. Participants were instructed to 'eat as little or as much as you want'. Food items were weighed to the nearest gram before serving using an electronic scale (AvaWeigh PCOS20**),** Nutrient composition of foods consumed during the meal was determined and analyzed with the nutrition analysis software Nutritionist Pro (Axxya Systems, Stafford, Texas). Participants were allowed to request additional portions if desired.

Statistical Analyses

The influence of habitual fluid intake on appetite was assessed using a multiple regression with habitual fluid intake averaged from six days of self-reported fluid intake (three preceding the trial and three following the trial) as a predictor of the area under the curve (AUC) for appetite ratings. AUC was calculated using the trapezoidal rule for each appetite and thirst subscale with respect to a '0' starting rating, with all AUC calculations starting at time '0' and proceeding until time '180' (Doucet et al., 2003).

A random intercept linear mixed model was used to assess changes in appetite response before and after the ad-libitum meal, with habitual fluid intake and water consumed during the test meal as predictors of appetite response.

Separate ordinary least squares regressions were used to assess the relationship between habitual fluid intake or plain water intake on average explicit wanting and explicit liking scores for each food category in the LFPQ (high fat sweet, high fat savory, low fat sweet, low-fat savory). These analyses were repeated for "implicit wanting" scores using the frequency-weighted algorithm. A random intercept linear mixed model was used to assess changes in explicit liking and explicit wanting ratings before and after the ad-libitum lunch meal, with habitual fluid intake, water intake during lunch, and calories consumed during the meal as independent variables.

Exploratory Pearson (for normally distributed variables) or Spearman (for non-normally distributed variables) correlations were run to assess the relationships between food reward metrics, habitual fluid intake, and nutrient intake during the ad libitum lunch.

Mean fluid intake from the BEVQ-15 questionnaire was compared to fluid intake reported during the study via the Liq.In.7 using a Wilcoxon signed-rank test. The above analyses were repeated with total fluid intake from the BEVQ-15 as the predictor variable for all outcomes to assess the stability of the results.

Results

Twenty-seven participants completed the study. Due to a late participant arrival and a schedule conflict at the end of the visit, one participant did not complete the in-lab lunch and was omitted from analyses for post-lunch appetite and food reward ratings. Participant characteristics

are described in Table 13. On average, participants met current fluid intake recommendations (mean: 3.1 ± 1.7L) based on the EFSA guidelines of 2.5 L per day (EFSA, 2010).

Table 13. Participant Demographic Characteristics.

	Overall (N=27)
Height (cm)	
Mean (SD)	176 (6.48)
Median [Min, Max]	176 [160, 188]
Body Mass (kg)	
Mean (SD)	78.8 (13.0)
Median [Min, Max]	75.2 [61.9, 109]
BMI (kg/m^2)	
Mean (SD)	25.6 (4.21)
Median [Min, Max]	24.6 [19.2, 35.1]
BF (%)	
Mean (SD)	17.2 (9.04)
Median [Min, Max]	16.1 [2.30, 36.5]
Age (y)	
Mean (SD)	23 (4)
Median [Min, Max]	23 [18, 34]
Race/Ethnicity	
African American	6 (22.2%)
Asian	2 (7.4%)
Caucasian	15 (55.6%)
Hispanic	4 (14.8%)

	Overall (N=27)
Total Fluid Intake (L)	
Mean (SD)	3.12 (1.65)
Median [Min, Max]	2.49 [0.740,7.57]

Standard Meal Results

Appetite and Thirst

Table 14 displays model results for associations between habitual fluid intake and hunger ratings at baseline (fasted). There was no association between average habitual fluid intake and ratings for each aspect of hunger (all ps > 0.05).

Table 14. Model Results For The Relationship Between Habitual Fluid Intake and Fasted Hunger Ratings.

	Dependent variable:			
	Desire	Hungry	Full	How much
	(1)	(2)	(3)	(4)
Average Fluid (L)	2.634	-0.001	-4.291*	2.204
	(-1.891, 7.159)	(-3.149, 3.148)	(-8.444, -0.138)	(-1.303, 5.710)
Constant	62.295***	57.965***	35.707***	59.145***
	(46.397, 78.192)	(46.904, 69.026)	(21.116, 50.297)	(46.825, 71.465)
Observations	27	27	27	27
R^2	0.049	0.000	0.141	0.057
Adjusted R^2	0.011	-0.040	0.107	0.020
Residual Std. Error (df = 25)	19.400	13.498	17.805	15.034
F Statistic (df = 1; 25)	1.301	0.00000	4.101*	1.517
Note:				*p<0.1**p<0.05***p<0.01

Table 15 displays results for regressions examining the association between habitual fluid intake and baseline dimensions of thirst. There were no significant differences in thirst across all measures (ps > 0.05).

Table 15. Model Results For The Relationship Between Habitual Fluid Intake and Fasted Baseline Thirst Ratings.

	Dependent variable:					
	Thirsty	Pleasant	Dryness	Taste	Fullness Thirst	Sick
	(1)	(2)	(3)	(4)	(5)	(6)
Average Fluid (L)	0.57	1.70	-0.33	0.98	-4.29*	-1.23
	(-2.39, 3.53)	(-1.16, 4.56)	(-6.11, 5.44)	(-5.30, 7.26)	(-8.44, -0.14)	(-5.85, 3.38)
Constant	68.01***	73.63***	54.14***	28.04**	35.71***	18.75**
	(57.62, 78.40)	(63.59, 83.68)	(33.84, 74.44)	(5.98, 50.11)	(21.12, 50.30)	(2.54, 34.96)
Observations	27	27	27	27	27	27
R^2	0.01	0.05	0.001	0.004	0.14	0.01
Adjusted R^2	-0.03	0.01	-0.04	-0.04	0.11	-0.03
Residual Std. Error (df = 25)	12.68	12.26	24.77	26.93	17.81	19.78
F Statistic (df = 1; 25)	0.14	1.35	0.01	0.09	4.10*	0.27

Note: *p<0.1**p<0.05***p<0.01

Figure 8 illustrates the mean AUC and inter-individual variability of appetite response for each domain following the standard breakfast, including the 3 hours following breakfast and pre-post ad libitum lunch. Higher habitual fluid intake was not associated with AUC for any of the hunger indices (β, [95% CI], β_{Desire} = 321.7, [-409.0, 1052.5], p = 0.372), β_{Hungry} = 82.5, [-577.5, 742.4], p = 0.799; β_{Full} = -322.5, [-932.8, 287.8], p = 0.287); $\beta_{Howmuch}$ = 286.1, [-312.4, 884.6], p = 0.334). Appendix A, Table A48, displays the average AUC values for each hunger and thirst subscale.

Figure 8. Changes in Appetite Ratings Throughout the Laboratory Visit. Plotted as Overall Mean (Blue Line and Gray Error Bars) and Individual Profiles.

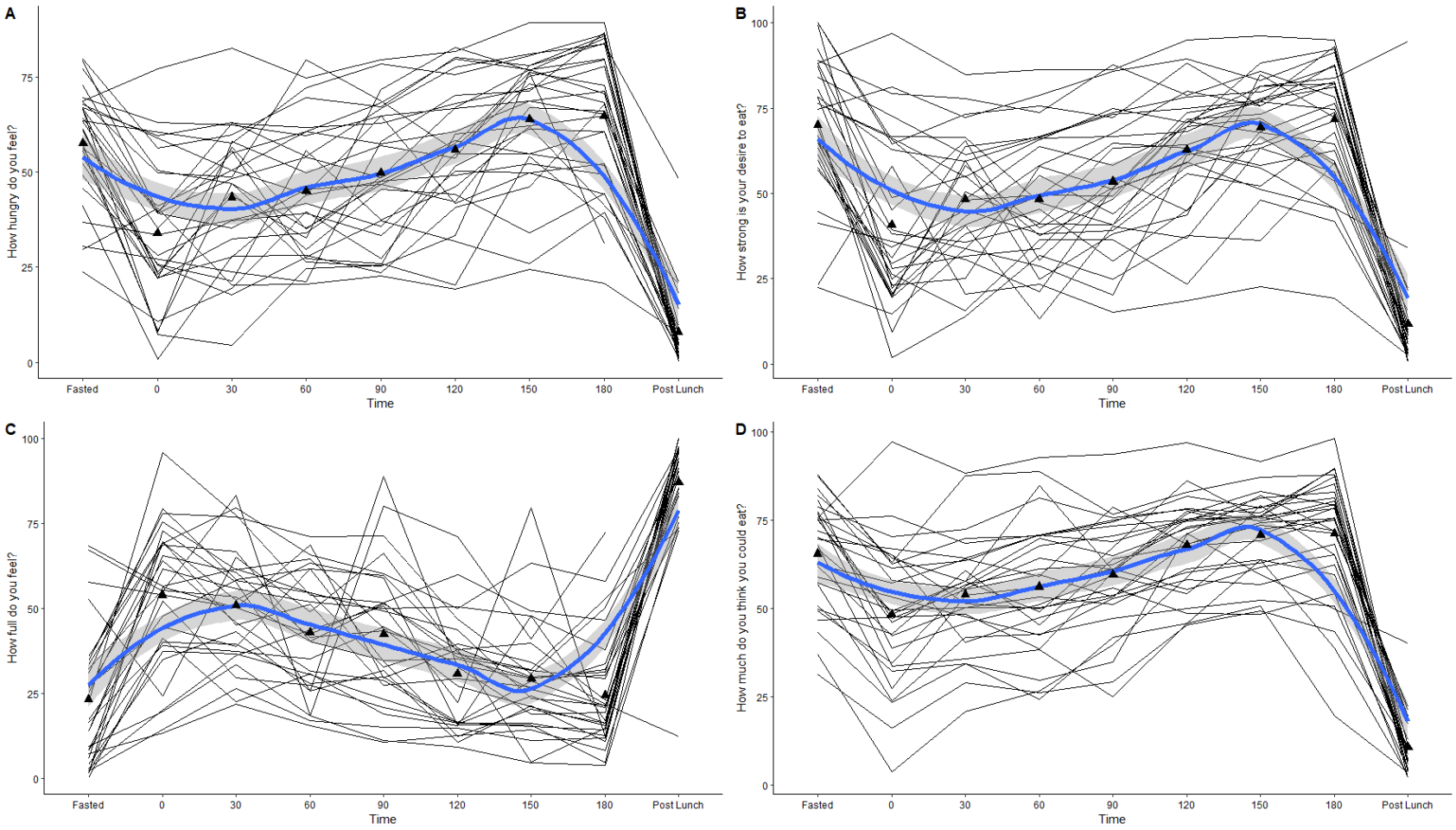
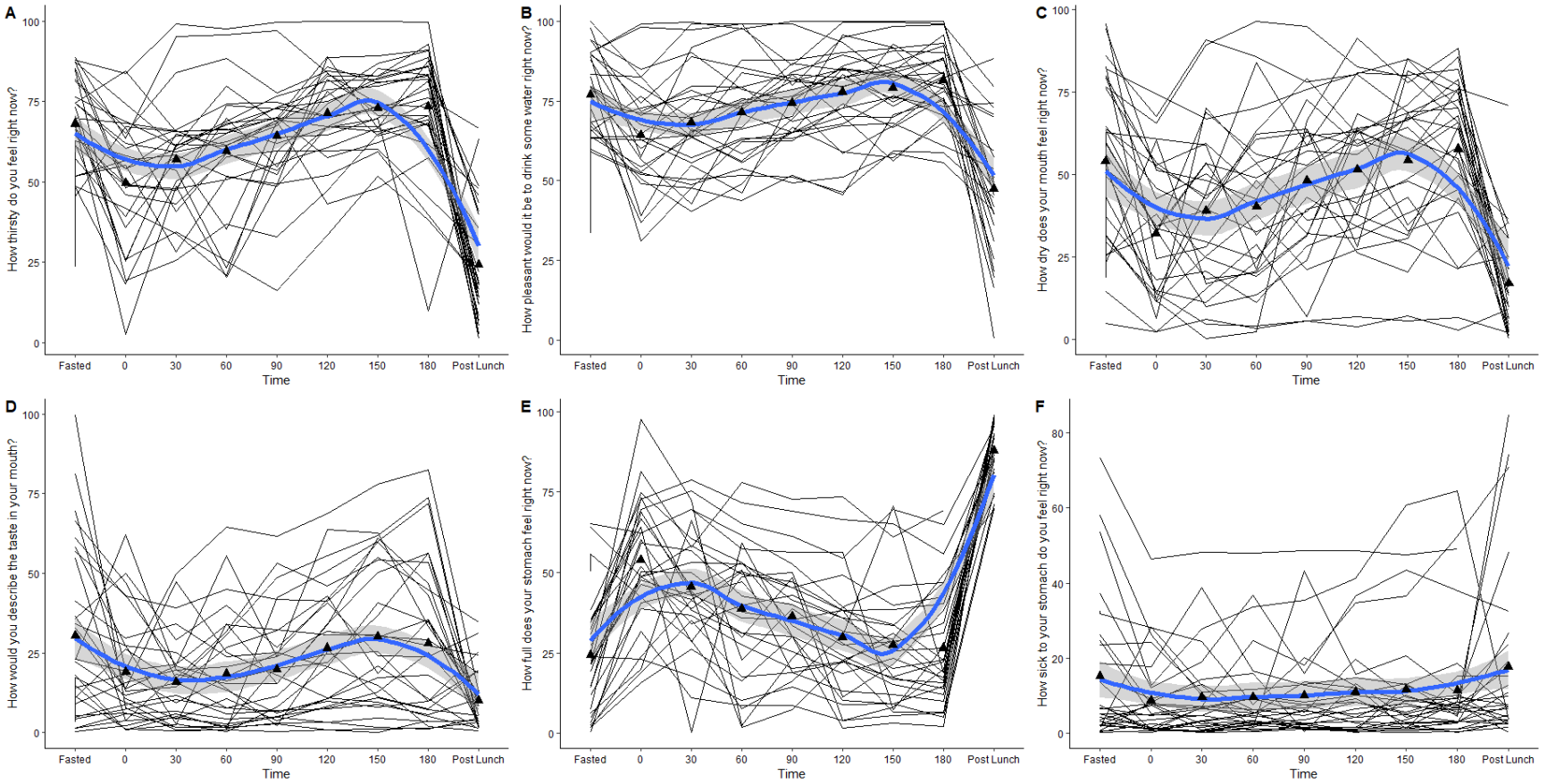


Figure 9 illustrates the AUC and interindividual changes in thirst ratings throughout the visit across different domains. Higher average fluid intake was not associated with overall thirst rating AUC throughout the visit for any of the subdomains of thirst: (β, [95% CI], $\beta_{Thirsty}$ = 492.9, [-99.7, 1085.5], p = 0.099; $\beta_{Pleasant}$ = 425.8, [-147.5, 999.1], p = 0.139; $\beta_{Dryness}$ = 325.4, [-522.2, 1173.1], p = 0.437; β_{Taste} = 455.3, [-220.4, 1131.1], p = 0.177; β_{Full}; -474.1, [-1140.7, 192.6], p = 0.156; β_{Sick} = 10.0 [-512.2, 532.2], p = 0.969).

Figure 9. Changes in Thirst Ratings Throughout the Laboratory Visit. Plotted as Overall Mean (Blue Line and Gray Error Bars) and Individual Profiles.



Ad Libitum Meal Results

Appetite

The intraclass correlation coefficients (ICCs) for the linear mixed models assessing the responses pre- and post-ad libitum test meal were generally low, suggesting most of the variance in appetite response was accounted for by within-person variability. All appetite ratings were significantly reduced following the meal except fullness, which was significantly increased (all ps < 0.001) (Table 16). Habitual fluid intake and plain water intake at lunch were not associated with post-lunch appetite ratings (all ps < 0.05, Table 16).

Table 16. Regression Results for the Interaction of Kcals and Water Consumed at Lunch and Change in Appetite Following Lunch.

	Desire			Hungry			Full			How Much		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	73.81	58.56 – 89.05	**<0.001**	69.10	57.56 – 80.64	**<0.001**	30.14	17.51 – 42.77	**<0.001**	77.13	67.69 – 86.57	**<0.001**
Time [Post Lunch]	-59.70	-68.93 – 50.47	**<0.001**	-57.64	-65.17 – 50.12	**<0.001**	62.90	54.30 – 71.51	**<0.001**	-62.97	-69.47 – 56.46	**<0.001**
Average Fluid (L)	0.16	-3.38 – 3.69	0.929	-0.58	-3.24 – 2.07	0.659	-1.62	-4.51 – 1.27	0.265	0.09	-2.07 – 2.24	0.935
Water at Lunch (L)	-8.18	-34.49 – 18.13	0.534	-4.74	-24.50 – 15.03	0.632	-4.72	-26.26 – 16.82	0.661	-13.75	-29.83 – 2.32	0.092
Random Effects												
σ^2	266.39			177.25			231.98			132.70		
τ_{00}	74.18 $_{Subject}$			28.06 $_{Subject}$			22.25 $_{Subject}$			10.57 $_{Subject}$		
ICC	0.22			0.14			0.09			0.07		
N	26 $_{Subject}$			26 $_{Subject}$			26 $_{Subject}$			26 $_{Subject}$		
Observations	51			51			51			51		

Marginal R^2 / Conditional R^2	0.727 / 0.787	0.805 / 0.832	0.800 / 0.818	0.876 / 0.885

Food Reward

Overall, average habitual total fluid intake and habitual plain water intake, respectively, were not significantly associated with explicit liking or explicit wanting of HFSA, LFSA, or LFSW food items (Table 17). However, higher habitual fluid intake was associated with lower overall mean explicit liking (β, [95% CI]: β = -6.94, [-11.99, -1.89], p = 0.009) and explicit wanting (β = -6.88, [-10.87, -2.88], p = 0.002) of HFSW food items (Figure 10). Greater plain water intake was not associated with explicit liking (p = 0.058) or explicit wanting (p = 0.067) of HFSW food items.

Table 17. Model Results for the Relationship Between Average Total Fluid Intake or Plain Water Intake on Explicit Liking of Foods From Each Category.

	Dependent variable:							
	ML HFSA (1)	ML HFSW (2)	ML LFSA (3)	ML LFSW (4)	ML HFSA (5)	ML HFSW (6)	ML LFSA (7)	ML LFSW (8)
Average Total Fluid (L)	-0.966	-6.939***	-0.136	0.383				
	(-3.604, 1.671)	(-11.749, -2.129)	(-2.946, 2.673)	(-2.875, 3.641)				
Average Water (L)					-1.561	-5.800*	0.745	0.039
					(-4.461, 1.339)	(-11.511, -0.089)	(-2.367, 3.858)	(-3.589, 3.667)
Constant	80.950***	81.152***	71.666***	75.481***	81.850***	74.057***	69.371***	76.579***
	(71.685, 90.215)	(64.254, 98.050)	(61.794, 81.539)	(64.036, 86.927)	(73.443, 90.257)	(57.501, 90.613)	(60.347, 78.395)	(66.062, 87.096)
Observations	27	27	27	27	27	27	27	27
R^2	0.020	0.242	0.0004	0.002	0.043	0.137	0.009	0.00002
Adjusted R^2	-0.019	0.212	-0.040	-0.038	0.004	0.102	-0.031	-0.040

Residual Std. Error (df = 25)	11.306	20.621	12.047	13.967	11.176	22.010	11.997	13.982
F Statistic (df = 1; 25)	0.516	7.996***	0.009	0.053	1.113	3.962*	0.220	0.0004

Note: *p<0.1;**p<0.05;***p<0.01

ML = Mean Liking, MW = Mean Wanting, HFSA = High Fat Savory, HFSW = High Fat Sweet, LFSA = Low Fat Savory, LFSW = Low Fat Sweet

Table 18. Model Results for the Relationship Between Average Total Fluid Intake or Plain Water Intake on Explicit Wanting of Foods From Each Category.

	Dependent variable:							
	MW HFSA (1)	MW HFSW (2)	MW LFSA (3)	MW LFSW (4)	MW HFSA (5)	MW HFSW (6)	MW LFSA (7)	MW LFSW (8)
Average Fluid (L)	-0.679	-6.879**	0.996	0.416				
	(-3.409, 2.052)	(-11.794, -1.963)	(-1.977, 3.969)	(-2.999, 3.830)				
Average Water (L)					-1.058	-5.701*	1.773	0.405
					(-4.081, 1.965)	(-11.524, 0.122)	(-1.489, 5.035)	(-3.395, 4.204)
Constant	78.709***	80.381***	65.773***	75.231***	79.246***	73.225***	64.432***	75.513***
	(69.117, 88.301)	(63.111, 97.650)	(55.328, 76.217)	(63.235, 87.227)	(70.482, 88.010)	(56.345, 90.105)	(54.975, 73.890)	(64.498, 86.527)
Observations	27	27	27	27	27	27	27	27
R^2	0.009	0.231	0.017	0.002	0.018	0.128	0.043	0.002
Adjusted R^2	-0.030	0.201	-0.022	-0.038	-0.021	0.094	0.005	-0.038
Residual Std. Error (df = 25)	11.705	21.074	12.746	14.639	11.651	22.441	12.573	14.643
F Statistic (df = 1; 25)	0.237	7.523**	0.431	0.057	0.470	3.682*	1.135	0.044

Note: *p<0.1; **p<0.05; ***p<0.01

ML = Mean Liking, MW = Mean Wanting, HFSA = High Fat Savory, HFSW = High Fat Sweet, LFSA = Low Fat Savory, LFSW = Low Fat Sweet

Figure 10. Relationship Between Mean Fluid Intake or Mean Water Intake on Explicit "Wanting" and Explicit "Liking" Scores for High Fat, Sweet Food Items From The LFPQ.

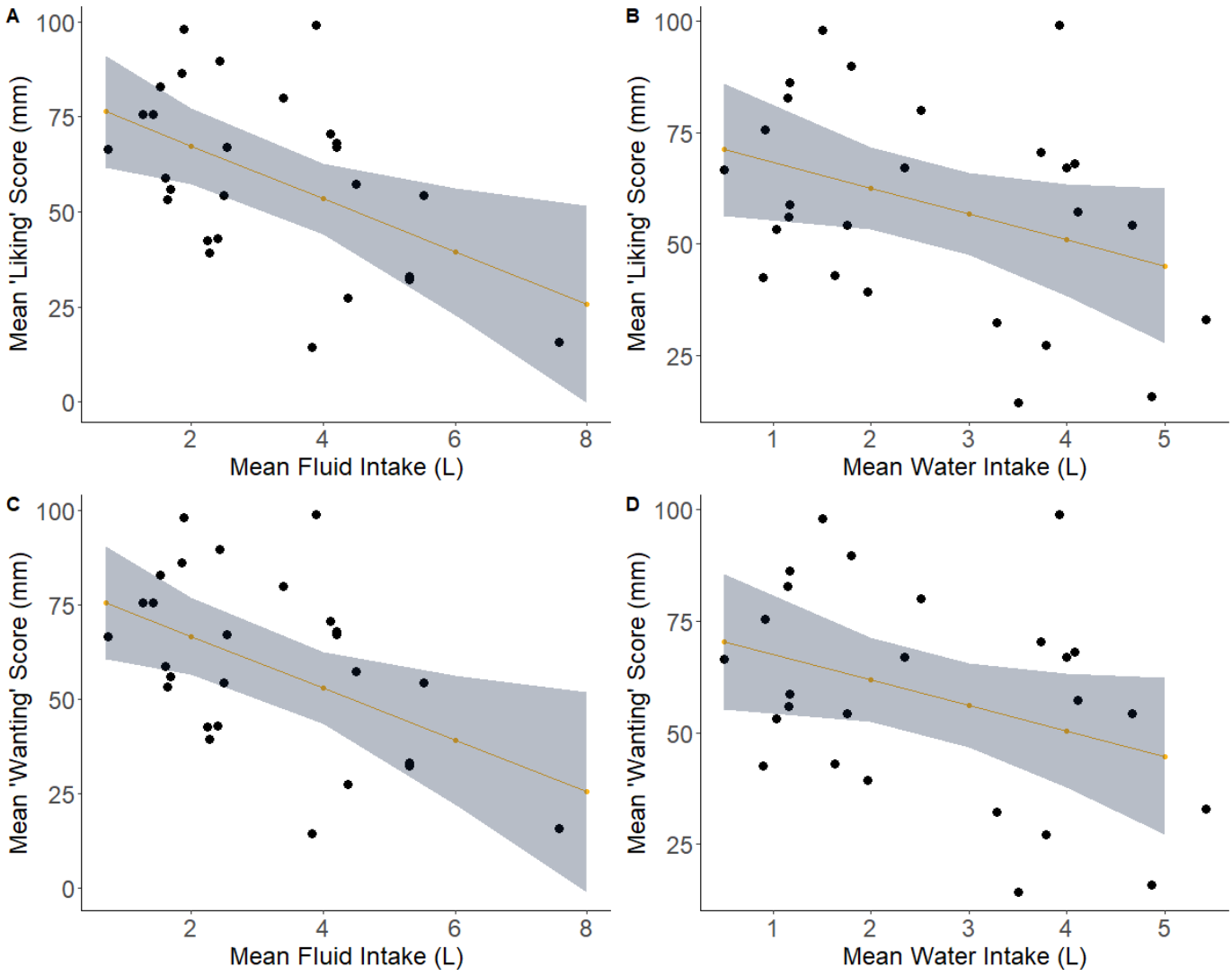


Table 19. Model Results for the Relationship Between Average Total Fluid Intake or Plain Water Intake and Implicit Wanting (Frequency Weighted Algorithm – FWA) of Food Items From Each Category.

	Dependent variable:							
	FWA HFSA	FWA HFSW	FWA LFSA	FWA LFSW	FWA HFSA	FWA HFSW	FWA LFSA	FWA LFSW
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Average Fluid (L)	-0.891	-5.049	0.007	5.933*				
	(-6.307, 4.524)	(-11.640, 1.542)	(-5.369, 5.384)	(-0.847, 12.713)				
Average Water (L)					-1.876	-4.726	1.261	5.341
					(-7.868, 4.116)	(-12.154, 2.702)	(-4.699, 7.221)	(-2.352, 13.035)
Constant	24.827**	-8.805	0.774	-16.795	26.752***	-12.700	-2.366	-11.686
	(5.801, 43.852)	(-31.961, 14.350)	(-18.114, 19.662)	(-40.613, 7.024)	(9.382, 44.122)	(-34.233, 8.834)	(-19.645, 14.913)	(-33.990, 10.618)
Observations	27	27	27	27	27	27	27	27
R^2	0.004	0.083	0.00000	0.105	0.015	0.059	0.007	0.069
Adjusted R^2	-0.036	0.046	-0.040	0.070	-0.025	0.021	-0.033	0.032
Residual Std. Error (df = 25)	23.217	28.257	23.050	29.067	23.092	28.627	22.971	29.651
F Statistic (df = 1; 25)	0.104	2.255	0.00001	2.942*	0.377	1.555	0.172	1.851
Note:								*p<0.1 **p<0.5 ***p<0.01

FWA = Frequency Weighted Algorithm for Implicit Wanting, HFSA = High Fat Savory, HFSW = High Fat Sweet, LFSA = Low Fat Savory, LFSW = Low Fat Sweet

Change in Food Reward Post Ad Libitum Meal

Singular ICCs for many of the pre-post models assessing change in explicit liking and explicit wanting suggested linear-mixed effects models were not required; ordinary least squares regression models were used as an alternative, with trial (post lunch as referent category), water

intake at lunch, and average habitual fluid intake as predictors of post-meal food reward ratings. Only models assessing mean liking of HFSW and LFSA and mean explicit wanting for HFSW food items were retained as linear mixed effects models (ICC of 0.35, 0.09, and 0.04, respectively, Table 20).

Table 20. Change in Mean Explicit Liking "ML" and Mean Explicit Wanting "MW".

	Dependent variable:							
	ML HFSA *OLS* (1)	ML HFSW *linear mixed-effects* (2)	ML LFSA *linear mixed-effects* (3)	ML LFSW *OLS* (4)	MW HFSA *OLS* (5)	MW HFSW *linear mixed-effects* (6)	MW LFSA *linear mixed-effects* (7)	MW LFSW *OLS* (8)
Trial 1	55.240***	29.606***	43.231***	38.644***	56.375***	31.413***	43.000***	40.519***
	(46.897, 63.584)	(19.920, 39.292)	(34.794, 51.667)	(27.779, 49.510)	(47.982, 64.768)	(20.996, 41.831)	(34.271, 51.729)	(29.217, 51.822)
Water at Lunch (L)	-8.615	-5.924	-4.529	-4.714	-5.183	-0.683	1.184	6.009
	(-27.597, 10.368)	(-37.847, 26.000)	(-24.466, 15.407)	(-29.433, 20.006)	(-24.277, 13.911)	(-32.551, 31.186)	(-20.611, 22.978)	(-19.704, 31.723)
Average Fluid (L)	-0.524	-6.636***	-1.102	-1.993	-0.060	-5.694**	-0.368	-1.537
	(-3.105, 2.056)	(-10.976, -2.296)	(-3.813, 1.608)	(-5.354, 1.367)	(-2.656, 2.536)	(-10.027, -1.361)	(-3.331, 2.595)	(-5.033, 1.959)
Constant	27.310***	52.822***	32.489***	45.702***	21.868***	45.415***	26.971***	39.052***
	(15.936, 38.685)	(34.379, 71.265)	(20.601, 44.376)	(30.890, 60.515)	(10.426, 33.309)	(26.901, 63.928)	(14.062, 39.881)	(23.644, 54.459)
Observations	52	52	52	52	52	52	52	52
R^2	0.779			0.511	0.783			0.512
Adjusted R^2	0.765			0.480	0.770			0.481
Log Likelihood		-221.061	-206.781			-222.842	-209.681	
Akaike Inf. Crit.		454.122	425.562			457.684	431.363	
Bayesian Inf. Crit.		465.829	437.269			469.392	443.070	
Residual Std. Error (df = 48)	15.349			19.988	15.439			20.792
F Statistic (df = 3; 48)	56.461***			16.716***	57.873***			16.759***

Note: *p<0.1**p<0.05***p<0.01

Both explicit liking and explicit wanting for food items decreased after lunch across all food categories ($p < 0.001$). Similar to pre-meal results, higher habitual fluid intake was associated with lower explicit liking ($\beta = -6.64$ [-11.09, -2.18], $p = 0.004$) and explicit wanting ($\beta = -5.69$ [-10.14, -1.24], $p = 0.013$) of HFSW food items. Other post-meal food preferences were not related to habitual fluid intake ($p > 0.05$).

Implicit wanting was higher after the lunch for HFSW and LFSW foods but was significantly lower for HFSA foods (**Table 21**). Implicit wanting of LFSA foods was not affected by the lunch. Average habitual fluid intake was not associated with change in implicit wanting for HFSA, LFSW, or LFSA foods, but higher average habitual fluid intake was associated with lower implicit wanting of HFSW foods following lunch ($\beta = -6.74$ [-12.98, -0.50], $p = 0.035$) (Model 1), when controlling for water intake at lunch.

Table 21. Change in Mean Implicit Wanting Following Lunch.

	FWA HFSW			FWA HFSA			FWA LFSW			FWA LFSA		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	21.11	-4.59 – 46.81	0.105	-33.13	-50.34 – -15.92	**<0.001**	15.73	-5.22 – 36.69	0.138	-3.71	-21.42 – 14.01	0.676
Trial [1]	-22.15	-36.65 – -7.64	**0.004**	45.78	33.49 – 58.07	**<0.001**	-33.48	-42.86 – -24.10	**<0.001**	9.85	-2.30 – 22.00	0.110
Water at Lunch (L)	-4.67	-49.32 – 39.98	0.834	5.52	-23.59 – 34.63	0.705	6.89	-30.09 – 43.88	0.710	-7.74	-37.88 – 22.39	0.608
AVERAGE L	-6.74	-12.98 – -0.50	**0.035**	2.40	-1.67 – 6.47	0.241	5.02	-0.15 – 10.19	0.057	-0.68	-4.89 – 3.53	0.747
Random Effects												
σ^2	702.62			504.40			293.97			492.72		
τ_{00}	309.13 $_{\text{Subject}}$			28.57 $_{\text{Subject}}$			306.11 $_{\text{Subject}}$			54.46 $_{\text{Subject}}$		
ICC	0.31			0.05			0.51			0.10		
N	27 $_{\text{Subject}}$			27 $_{\text{Subject}}$			27 $_{\text{Subject}}$			27 $_{\text{Subject}}$		
Observations	54			54			54			54		
Marginal R^2 / Conditional R^2	0.196 / 0.442			0.508 / 0.535			0.372 / 0.693			0.051 / 0.145		

FWA = Frequency Weighted Algorithm for Implicit Wanting. ML = Mean Liking, MW = Mean Wanting, HFSA = High Fat Savory, HFSW = High Fat Sweet, LFSA = Low Fat Savory, LFSW = Low Fat Sweet

Fluid intake from the Liq.In.7 measures was slightly above the median intake captured by the BEVQ-15 pre-screening questionnaire (2194 mL [range: 798 - 3697], MD: 810 [113, 1547], p = 0.014]). Thus, additional analyses were run with BEVQ-15 as a predictor (Appendix Table A46, 47, 49). Overall thirst results were stable both for most fasting and AUC ratings, but there was a significant positive association between BEVQ-15 reported habitual fluid intake for fasted and AUC for ratings of "How Much" (p = 0.013, p = 0.003). The effects on food reward were no longer significant for liking of HFSW foods, but wanting of HFSA foods was still related to fluid intake from the BEVQ-15.

Exploring the Effect of Preferences on Behavior

Ad Libitum Nutrient Intake

Table 22 presents the dietary composition of the ad libitum test meal. The meal was generally high in kcals, sodium, and carbohydrates. Among the participants, 19 (70%) chose to drink plain water with their meal. Average habitual fluid consumption was not correlated with plain water intake at lunch (rho = -0.87, p = 0.674). Plain water consumption at lunch was not correlated with kcal consumption at lunch (rho = -0.19, p = 0.361). Average habitual fluid intake was associated with higher kcal consumption at lunch (r = 0.39, p = 0.047).

Table 22. Nutrient Intake at the Ad Libitum Test Meal. Values Presented as Means (SD). One Participant Did Not Complete the Lunch.

	Overall (N=26)
Kcals	1300 (468)
Protein (g)	57.9 (27.2)
Carbohydrates (g)	171 (69.6)
Fat (g)	39.7 (22.1)
Sugar (g)	58.7 (39.3)
Sodium (mg)	2090 (741)
Water (mL)	305 (225)

Table 23 illustrates exploratory correlations assessing the relationships between LFPQ ratings and nutrient intake during the ad libitum lunch. Of note, water intake at lunch was negatively correlated with sugar intake at lunch (rho = -0.406, p = 0.0394). Water intake at lunch was not correlated with liking or wanting scores for food items from any of the categories (ps > 0.05).

Table 23. Exploratory Correlations Between LFPQ Ratings and Consumption at Lunch. Correlations Involving 'Water' Consumed at Lunch Used Spearman Correlations Due to the Non-Normality of This Variable. Other Analyses Used Pearson Correlations.

	Kcals	CHO	Fat	Sodium	Sugar	Water	HFSA Liking	LFSA Liking	HFSW Liking	LFSW Liking	HFSA Wanting	LFSA Wanting	HFSW Wanting	LFSW Wanting
Kcals	1													
CHO	0.866***	1												
Fat	0.624***	0.231	1											
Sodium	0.828***	0.625***	0.546**	1										
Sugar	0.690***	0.863***	0.062	0.371	1									
Water	-0.187	-0.316	0.113	-0.1464	-0.406*	1								
HFSA Liking	0.335	0.467*	-0.011	0.007	0.598**	-0.370	1							
LFSA Liking	-0.176	0.095	-0.490*	-0.398*	0.308	-0.149	0.536**	1						
HFSW Liking	-0.035	0.199	-0.166	-0.264	0.16	-0.08	0.392*	0.411*	1					
LFSW Liking	0.14	0.342	-0.232	-0.236	0.398*	-0.275	0.373	0.375	0.224	1				
HFSA Wanting	0.37	0.457*	0.08	0.05	0.572**	-0.29	0.942***	0.496**	0.408*	0.315	1			
LFSA Wanting	0.017	0.268	-0.373	-0.233	0.433*	-0.029	0.560**	0.898***	0.419*	0.388	0.580**	1		
HFSW Wanting	-0.064	0.154	-0.135	-0.292	0.113	-0.148	0.397*	0.412*	0.978***	0.223	0.430*	0.425*	1	
LFSW Wanting	0.058	0.255	-0.235	-0.321	0.343	-0.159	0.388	0.388	0.242	0.922***	0.412*	0.477*	0.273	1

*p<0.05, **p<0.01, ***p<0.001

Discussion

In this study we examined appetite, thirst, and food reward among individuals across a range of habitual fluid intake volumes. Overall, higher habitual fluid intake was not associated with appetite ratings at baseline or changes throughout the visit following a standard breakfast. In addition, habitual fluid intake outside the laboratory and plain water intake in the laboratory at lunch were not associated with changes in appetite ratings following the ad libitum lunch. However, individuals with higher habitual fluid intake expressed lower explicit liking and explicit wanting for food items high in fat and sugar content, both before and after an ad libitum lunch. Higher habitual fluid intake was also moderately correlated with calorie intake at lunch.

Appetite and Thirst

The relationships observed between habitual fluid intake and hunger ratings are contrary to our hypotheses of greater appetite ratings with lower fluid intake. This was the case both throughout the visit and following lunch, where water intake at lunch did not influence post-meal appetite ratings. This is similar to the effects observed with acute fluid restriction (-1.8% hypohydration), where there were no differences in hunger, fullness, or ad libitum energy intake of a porridge meal at breakfast when the same individuals were hypohydrated compared to euhydrated and when fluid intake was available or not available under both conditions (Corney, Horina, et al., 2015). Thus, our study also suggests no difference in appetite ratings even when given access to a more diverse meal with self-selected choices as compared to the porridge meal used by Corney et al (Corney, Horina, et al., 2015), which may have satisfied thirst due to the naturally high water content of the porridge meal.

A similar post-prandial thirst response was observed among individuals with varying habitual fluid intake. This suggests other factors beyond thirst may contribute to habitual fluid

consumption, such as habits, beliefs about health benefits, or immediate availability of water (Sims et al., 2022). Although individuals were permitted to consume water the morning prior to coming to the laboratory visit, no fluid intake was permitted throughout the visit itself until the lunch meal. Despite this, only six individuals reported consuming fluid the morning of the visit, with a maximum intake of 600mL and mean intake of 463mL. Additional analyses on the interactions between thirst and hunger ratings and experimental investigation are warranted, as pre-meal water ingestion has been found to promote satiety in this population (i.e., young, healthy males) when consumed close to a meal (Corney et al., 2016; J. N. Jeong, 2018). It is plausible that if these same individuals were allowed to consume fluids ad-libitum, likely in response to thirst throughout the lab visit, we might see differential effects on changes in appetite ratings. This has been investigated in one study, which found individuals reduced their food intake when fluid intake was restricted to 40% of usual consumption, with a negative correlation between thirst intensity and food intake (Engell, 1988). Yet it is unclear if *increasing* fluid intake would contribute to appetite sensations and subsequent energy intake, particularly in underhydrated individuals, or how a water preload before meals would differentially affect people who are or are not accustomed to consuming more fluids throughout the day. Interestingly, higher habitual fluid intake was correlated with greater energy intake at lunch. This may be related to the aforementioned factors, where allowing fluids ad-libitum may serve as an appetite suppressant between meals in those who habitually drink greater amounts of fluid; thus, in the absence of fluid intake, this inhibition may have been removed. Furthermore, the present study standardized the timing of the lunch meal. The present study cannot delineate if total nutrient intake would be altered if individuals had been given free access to fluids throughout the day and then allowed to naturally select the timing of their lunch.

Downstream physiologic signals may interact with fluid regulatory processes to influence perceptions of satiety. Hormones associated with eating and satiety have also been proposed to influence neurons associated with thirst and release of the fluid regulatory hormone arginine vasopressin (AVP), including amylin, cholecystokinin, ghrelin, histamines, and leptin (Armstrong & Kavouras, 2019)**.** But given the overlap of functions among various brain regions and difficulty in pinpointing precise changes in response to fluid intake, future work with more sophisticated techniques is required to capture the interactions between food and fluid intake on the neurological pathways associated with hunger and thirst in the brain so that synergies and discrepancies between these sensations and the subsequent effects on behavior can be teased apart (Armstrong & Kavouras, 2019). This may involve interventions directly modifying hydration status intravenously, as well as in response to caloric or non-caloric beverages, and dry food items, and observing the neurological responses to the separate interventions.

Food Reward

Contrary to our hypothesis, habitual fluid intake was not associated with preferences for HFSA food items. Given the role that sodium plays in fluid conservation (Maughan et al., 2019), we hypothesized that individuals with habitually low fluid intake would have greater preference for high fat savory food items to assist with fluid retention. It may be that larger acute water loss is needed to induce a change in salt preference (i.e., from exercise or heat exposure), perhaps via stimulation of aldosterone (L. A. De Luca et al., 2010), yet the present study did not measure aldosterone concentrations. A previous study found a decrease in water consumption among individuals with increased salt intake, perhaps related to greater fluid conservation (Rakova et al., 2017), but these results should be interpreted with caution since the number of participants included in this study was small (n = 10).

Both explicit liking and explicit wanting of HFSW foods were lower among individuals with higher habitual fluid intake at baseline and following lunch. It may be that individuals with higher habitual fluid intake, when eating sweet foods, tend to choose those with higher water content and lower fat (i.e., fruits) to attenuate adverse thirst sensations. Only one previous study has investigated relationships between fluid intake and food reward (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019); this study used an acute dehydrating protocol among participants, which led to an increased preference for foods high in water content when dehydrated. Carroll et al. then permitted participants to consume an ad libitum pasta meal, which resulted in no difference in energy intake between conditions when participants were acutely dehydrated compared to euhydrated. The present study allowed participants to self-select their food intake, but correlations between the wanting ratings for the food items did not match the expected food consumption across categories. HFSW liking and wanting ratings were not related to kcals, fat, carbohydrate, or sugar consumption at lunch; but HFSA liking and wanting scores were both positively associated with carbohydrate and sugar intake. LFSW and LFSA ratings were also positively associated with sugar intake, perhaps related to more sugar intake from fruits offered at the ad libitum lunch.

The differences in food preferences observed may be related to alterations in taste sensitivity to salty and sweet food items. One study observed a decreased preference for salty and fatty and sweet and fatty food items following at least five days of a water-only fast, as well as an increase in vegetable consumption and decrease in sugar sweetened beverage intake after the fast (Myers et al., 2022). The authors attribute the difference in salt sensitivity potentially to alterations in oral microbiota (Cattaneo et al., 2019). While recent research has shown some connection between hydration biomarkers and intestinal microbiota (Willis et al., 2021), to the

author's knowledge the influence of hydration on oral microbiota has not been explored. Sweet taste perception follows a complex series of central and peripheral pathways, but among these responses is an increase in insulin (Lee & Owyang, 2017). Underhydration has been associated with insulin resistance (H. K. Min et al., 2020); thus, insulin may be a mediator in the link between habitual fluid intake and preference for sweet food items, but this requires further study. It is unclear if these taste perceptions would be altered by the addition of plain water intake to one's habitual intake, particularly in individuals who are chronically underhydrated, and if this would translate to changes in dietary intake.

Another possible rationale for the link between low fluid intake and differences in the hedonic value of food is alterations in the activity of the hypothalamic-pituitary-adrenal axis. Some studies have found an association between underhydration and cortisol (E. Perrier, Vergne, et al., 2013), which may be alleviated with increased fluid intake (Seal et al., 2021). Chronic activation of the HPA-axis can contribute to altered glucose metabolism, insulin resistance, and affect neurological appetite signals (Yau & Potenza, 2013). Chronic stress has also been shown to increase preference and consumption of hyperpalatable foods high in fat and sugar (Ans et al., 2018), making this a promising pathway to explain these relationships.

Exploratory correlations suggest these preferences did not translate to differences in total kcal consumption during the ad libitum lunch, but greater water intake was associated with reduced sugar consumption at lunch. One systematic review found consistently higher energy intake at meal times when sugar-sweetened beverages were consumed with meals (Daniels & Popkin, 2010). However, in our study individuals seemed to adjust for lower sugar intake with increased kcal intake from other sources. Some prior evidence suggests pre-meal water intake may support a reduction in calories. Our findings suggest, at least acutely, greater water intake at

a specific meal is not correlated with overall total kcal intake or post-meal appetite sensations. Consuming 500mL of water prior to a meal led to ~2kg greater weight loss than a hypocaloric diet alone in middle-aged overweight/obese adults throughout a 12-week weight-loss intervention (Dennis et al., 2010). Perhaps water intake alone is not sufficient to induce a notable change in energy intake without a conscious effort to reduce caloric intake. The ad libitum nature of the test meal provided in our study allows for greater external validity compared to previous studies similar in nature which permitted individuals to consume only a homogenous meal such as pasta (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019) or porridge (Corney, Sunderland, et al., 2015).

Limitations

This study provided a standardized breakfast meal which was consistent across participants. While beneficial from a between-subject perspective, variability in individual energy requirements may have influenced the satiating nature of the meal. This was evident from anecdotal statements from some participants stating this was less than their typical breakfast intake. However, we were able to account for this in our linear mixed models; in most cases this was not necessary, and the homeostatic and hedonic responses to meals were similar across participants.

It is also possible that individuals may have modified their fluid intake behaviors simply because they were participating in this study. Although participants were not informed of the purpose or hypotheses of the study, an additional Wilcoxon signed-rank test revealed significantly higher fluid consumption from the Liq.In.7 survey compared to the BEVQ-15. Thus, the “Hawthorne effect” (McCambridge et al., 2014) cannot entirely be ruled out for this study, particularly for the “How Much” ratings, where greater fluid intake from the BEVQ-15

predicted higher perceptions of prospective food intake, both fasting and over the course of the visit. These findings are opposite to our hypotheses, but if true, may be a product of the lack of fluid provided between the standard meal and lunch. Given the reported satiating nature of fluid intake found in some studies, it may be that restricting fluid intake prevented what might otherwise be a signal for satiety in individuals with habitually higher fluid intake. Conversely, this may also occur because individuals with higher habitual fluid intake due to higher body mass or activity levels also have higher kcal requirements; thus, the standardized meal may not have been as satiating for these individuals. Results for the food reward task also differed slightly when using the BEVQ-15 results as a predictor of food preferences. Notably, there was no longer a significant relationship between fluid intake from the BEVQ15 and explicit liking of HFSW food items. Thus, there may be some discrepancy in the relationship between short (7 days) and longer-term fluid intake (over the course of a month) and food reward. Additional longitudinal studies are warranted to help clarify these effects.

Strengths

Strengths of this study enhance our understanding of the relationship between fluid consumption and the hedonic and homeostatic factors contributing to food consumption. This study successfully recruited individuals from a range of habitual fluid intakes. Previous work which has standardized hydration status among participants have unintentionally produced lower fasted hunger ratings among participants because of greater gastric distention from increasing fluid intake among those who were typically underhydrated. Our study is strengthened by the objective markers of appetite and food reward taken under real-world conditions, with fluid intake and hydration status prior to the lab visit allowed to vary based on habitual consumption. This allows for more direct translation of findings to practice, where behavior may be influenced

by recent exposures to more, or less, fluid consumption. In this study we were able to capture several domains of appetite and thirst over the course of several repeated measures. The inclusion of both the standardized meal and the ad libitum meal allowed us to independently assess differences in appetite when a specific meal is provided as compared to free access to a variety of different foods of varying nutrient and moisture content.

Conclusion

Habitual fluid intake is not associated with fasting or postprandial appetite ratings in healthy, young adult males. However, habitual fluid intake in this population is associated with some hedonic aspects of food consumption, particularly preference for high fat, sweet foods. Future intervention studies may assess the efficacy of increasing fluid intake on the hedonic aspects of food consumption.

CHAPTER V: THE RELATIONSHIP BETWEEN HYDRATION STATUS, HPA AXIS ACTIVITY, AND FOOD REWARD IN HEALTHY YOUNG ADULT MALES

Abstract

Mechanistic studies have established a link between release of the fluid regulatory hormone arginine vasopressin (AVP), elevated in response to low fluid intake, and alterations in HPA-axis activity, including release of the stress hormone cortisol. Elevations in both hormones have been associated with increased risk for obesity. However, relationships between hydration status in individuals across a range of habitual fluid intake and cortisol dynamics have been limited to single measurements. The purpose of the present study was to explore associations between hydration status and indicators of the circadian pattern of cortisol. A secondary purpose was to explore the links between cortisol and food reward as a factor which may predispose one toward higher risk of developing obesity. **Methods:** 30 male participants with low (<1500mL/day), moderate (1500-3000mL/day), or high (>3000mL/day) habitual fluid intake (Age: 23±4y, Height 175±6.4 cm, body mass 79.8±14.2 kg, body fat 17.4±9.4%) collected their urine for 24 hours for assessment of urinary osmolality (U_{osmo}) the day before and day after a 6 hour in-lab visit. Several home and in-lab saliva samples were used to assess salivary peak cortisol (C_{peak}), cortisol awakening response (CAR), and diurnal cortisol slope (DCS). Participants also completed an in-lab assessment of food reward, and the Leeds Food Preference Questionnaire (LFPQ) before and after an ad libitum lunch. **Results:** 24h U_{OSMO} from the previous day was not significantly associated with any marker of HPA axis activity assessed in the current study ($p > 0.05$) but the 24h U_{OSMO} collected after the in-lab visit was negatively associated with C_{peak} ($p = 0.008$). When controlling for activity and total sleep time, there was no

relationship between 24h U_{OSMO} and $C_{peak.}$ A flatter DCS was associated with greater explicit liking of High Fat Sweet foods (β = 31.87, [2.07,61.68] p = 0.046) and significantly greater explicit wanting for High Fat Savory foods (β = 16.22, [1.75, 30.69] p = 0.037). Higher C_{peak} was associated with lower explicit liking of HFSW foods (β = -0.95, [-1.65, -0.25], p = 0.014, HFSA foods (β = -0.433, [-0.776, -0.090], p = 0.021) and lower explicit wanting of HFSW (β = -0.883, [-1.600, 0.166], p = 0.024) and HFSA foods (β = -0.430, [-0.779, -0.080], p = 0.024).

Conclusion: The natural circadian pattern of cortisol release may be robust against normal changes in fluid regulatory responses. However, the associations between some HPA axis indices and food reward warrant further experimental investigation to elucidate the role of acute changes in stress hormones on food reward.

Introduction

Habitual low fluid intake, or underhydration (Kavouras, 2019), activates hormonal pathways designed to conserve available fluids. Specifically, increased secretion of arginine vasopressin (AVP) in response to an increase in plasma osmolality promotes renal water reabsorption by binding to the V2 receptors (V2-R) on the basolateral membrane of cells in the distal tubule and collecting duct in the kidneys (Schrier, 2008). Yet AVP also activates other receptors (V1a and V1b) throughout the rest of the body related to cardiovascular homeostasis, hormone secretion, and social behavior (Koshimizu et al., 2012). Prolonged activation of some of these pathways has been associated with increased risk of obesity, diabetes, and cardiovascular disease (Riphagen et al., 2013; Roussel et al., 2016; Wannamethee et al., 2015). Understanding the pathways by which underhydration contributes to these risks may better inform the development of intervention strategies designed to increase fluid intake for health promotion and disease prevention.

Hydration Status

There is no ubiquitous "gold standard" indicator of hydration status (Armstrong, 2007). However, 24h urinary osmolality has been used as an adequate representation of sufficient fluid intake to reduce one's risk of chronic disease, with a value of <500mOsm*kg^{-1} as a daily urinary osmolality target (E. T. Perrier et al., 2015). In response to inadequate fluid consumption, the action of AVP stimulates pathways which lead to renal fluid conservation, resulting in a decrease in free water clearance and an increase in urinary osmolality.

HPA Axis Activity

AVP receptors are expressed in the hypothalamic-pituitary-adrenal axis (HPA axis). V1a-R has been found in the adrenal cortex, while V1b-R is expressed in the anterior pituitary and adrenal medulla (Koshimizu et al., 2012). AVP binding to V1a-R on the adrenocortical cells increases cortisol production (Perraudin et al., 1993) and through V1b-R activation in the corticotropes, increases adrenocorticotropic hormone (ACTH) production and release in the anterior pituitary (Tanoue et al., 2004). AVP works in concert with corticotrophin-releasing hormone (CRH) (Gillies et al., 1982) to stimulate glucocorticoid production and secretion from the adrenal cortex. This is more pronounced under stressful conditions, leading some researchers to classify AVP as a stress hormones (Carroll & Melander, 2021). Correlations between an increased number of V1b-R binding sites in the pituitary and increased ACTH responsiveness under conditions of *chronic stress* suggest AVP rather than CRH is the major regulator of ACTH during this more prolonged stress (Koshimizu et al., 2012; Yoshimura et al., 2021), increasing cortisol and subsequently hepatic glucose output. AVP is also locally synthesized and secreted in response to acetylcholine or CRH activation at the adrenal medulla to stimulate catecholamine release, primarily through activation of V1b-R in response to stress (Koshimizu et al., 2012).

Given this relationship, some studies have examined the effect of changes in hydration status on cortisol. Acute osmotic stress from water deprivation increases ACTH and corticosterone in wild type mice compared to V1b-R knockout models (Roberts et al., 2011). In humans, acute hypohydration achieved through exercise, heat exposure, or a combination has been consistently shown to increase cortisol concentrations (Zaplatosch & Adams, 2020), though the role of habitual fluid intake without a dehydrating stimulus (i.e., removing both exercise and heat stress) is less clear. Based on limited data in humans, chronic underconsumption of fluid may increase basal cortisol concentrations (E. Perrier, Vergne, et al., 2013), while increasing water intake has been associated with decreased ACTH (Enhörning et al., 2021) and decreased cortisol (Seal et al., 2021). However, other studies have shown hypohydration achieved via fluid restriction and heat exposure (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Jones, et al., 2019) and hyperosmolality from hypertonic saline administration (Jansen et al., 2019) do not influence cortisol, suggesting AVP may need to work synergistically with other mechanisms to induce a stress response. Perhaps the short-term nature of the latter studies may explain this response, but the time course of habitual fluid intake modification required to induce a change in cortisol has not been explored.

Circadian Variation

The circadian profile of cortisol is well defined, following a 24-hour profile with higher concentrations in the early morning, peaking soon after awakening, and decreasing until the evening prior to sleep (Weitzman et al., 1971). Consideration should be made for the timepoints of cortisol sampling to determine whether any intervention effects are reflective of an actual change beyond the expected diurnal variation. This may be accounted for by collecting additional samples to capture the expected diurnal cortisol slope for change in this hormone

throughout the day (E. K. Adam et al., 2017). There is a significant association between a flatter diurnal cortisol slope and adverse health outcomes, including obesity (E. K. Adam et al., 2017), making this an important area of study.

When meals are standardized, AVP and its surrogate marker copeptin, tend to follow a circadian pattern in some individuals. The pattern is most prominent in individuals with higher baseline copeptin, peaking between 4 am and 6 am and troughing between 5 pm and 7 pm (Beglinger et al., 2017). Yet no literature has examined associations between hydration status and cortisol profiles among individuals of varying fluid intakes.

Downstream Effects on Behavior

Chronically elevated cortisol has been shown to affect metabolism (Hewagalamulage et al., 2016; L. Min, 2016), food choices (Duong et al., 2012; Hewagalamulage et al., 2016), energy intake (George et al., 2010; Herhaus et al., 2020) and thermogenesis (Hewagalamulage et al., 2016). Glucocorticoids influence the production of appetite-regulating peptides in the hypothalamus, including neuropeptide Y and agouti-related protein (Hewagalamulage et al., 2016). These effects could be particularly problematic for emotional eaters (R. S. Chang et al., 2022) and individuals with higher cortisol responsiveness (Hewagalamulage et al., 2016). If adequate fluid intake helps keep basal cortisol levels low, this could significantly impact health, especially in individuals who are high responders to stress. However, the only study examining this relationship with regard to fluid intake/hydration was acute and may have been confounded by the heat stress induced by both the euhydrated and hypohydrated conditions (Carroll, Templeman, Chen, Edinburgh, Burch, Jewitt, Povey, Robinson, Dooley, Buckley, et al., 2019). By contrast, reduced fasting glucose from increased water intake is associated with decreased ACTH and cortisol (Enhörning et al., 2021). Given the associations between underhydration and

obesity (T. Chang et al., 2016) and elevated cortisol among low drinkers (E. Perrier, Vergne, et al., 2013), this study also explored associations between these pathways and food reward.

Although not traditionally an "appetite" hormone, alterations in HPA-axis activity and the glucocorticoid hormone cortisol have been associated with eating behaviors that may favor a positive energy balance (Warne, 2009). While acute increase in cortisol may decrease food intake (Ans et al., 2018), chronic stress-induced cortisol favors selection of more palatable, high calorie foods when available (Pecoraro et al., 2004, 2006). It has been suggested that individuals may make these choices in an attempt to blunt their stress response (Dallman et al., 2003). In fact, psychological stress has been associated with eating in the absence of hunger (Rutters et al., 2009) and an increased "wanting" for dessert in overweight subjects (Lemmens et al., 2011). Psychological stress corresponding to an increase in cortisol levels has been associated with reduced reward signaling and increased energy intake (Born et al., 2010). Further, chronically elevated cortisol favors visceral fat accumulation in conjunction with insulin through inhibition of lipolysis and through inhibition of lipolytic growth hormone and sex steroids. Exogenous cortisol administration has been shown to negate the anorectic actions of leptin and led to overeating in animal and human models (Papaspyrou-Rao et al., 1997; Tataranni et al., 1996; Zakrzewska et al., 1997). Given the previously observed correlations between copeptin and cortisol (Katan et al., 2008; Katan & Christ-Crain, 2010), and the potentiating effect of AVP on CRF (Gillies et al., 1982), the present study assessed the relationship between chronic low fluid intake resulting in underhydration as a chronic physiological stressor and cortisol, and the impact this has on food reward. Given the sensitivity of cortisol to circadian variation, the present study evaluated the relationship between a biomarker for hydration status (24h urinary osmolality) and the diurnal pattern of cortisol.

Prior literature has not examined the associations between chronic low fluid and fluctuations in cortisol rhythm throughout the day, or the subsequent effects these relationships may have on the hedonic aspects of food consumption. The purpose of this study was to explore the relationship between hydration status, HPA-axis activity, and food reward. We predicted hydration biomarkers indicative of chronic low fluid intake would be associated with elevated peak salivary cortisol, blunted salivary cortisol awakening response, and a flatter salivary diurnal cortisol slope. We also predicted these salivary cortisol differences would be associated with greater explicit wanting and explicit liking of high fat, savory foods.

Methods

This was part of a larger study assessing the relationship between fluid intake and energy balance. In brief, participants came to the Exercise Physiology lab for one screening visit, recorded food and fluid intake for three days, then came to the lab for one Experimental Trial. In the larger study, participants were screened to ensure they had 1) no chronic health conditions or diseases which would alter body water regulation, 2) no previous surgery of the gastrointestinal tract that could impact body water regulation, 3) no pharmacologic drug treatment in the previous 15 days, 4) not exercising more than 10 hours per week, 4) no known or suspected sleep pathologies (Pittsburgh Sleep Quality Index (PSQI) <5), and 5) no food allergies or severe dietary restrictions. To ensure a range of hydration statuses were obtained, participants were also screened using an electronic version of the Brief 15-Item Beverage Intake Questionnaire (BEVQ-15) to estimate habitual fluid intake over the previous month in order to obtain participants from a range of fluid intakes (goal of 10 per fluid intake category < 1500mL/day, 1500-3000mL/day, >3000mL/day) (Fausnacht et al., 2020; Hedrick et al., 2010). After verification of fluid intake, this ended up being 10 participants <1500mL/day, 11 participants

1500-3000mL/day, and 9 participants >3000mL/day, although these designations were not used for the analyses presented in this paper. All participants completed written informed consent prior to participation in the study. Participants also completed a brief survey to assess individual chronotype, the reduced Morningness-Eveningness Questionnaire (rMEQ) (Danielsson et al., 2019).

Pre-Trial Period

Participants arrived at the exercise physiology lab in the morning (0600-0900) for two days prior to their Experimental Trial. During each of these morning visits, participants completed a brief survey of thirst, nude body mass (NBM), and provided a 24h urine sample collected the previous day for assessment of urinary hydration markers.

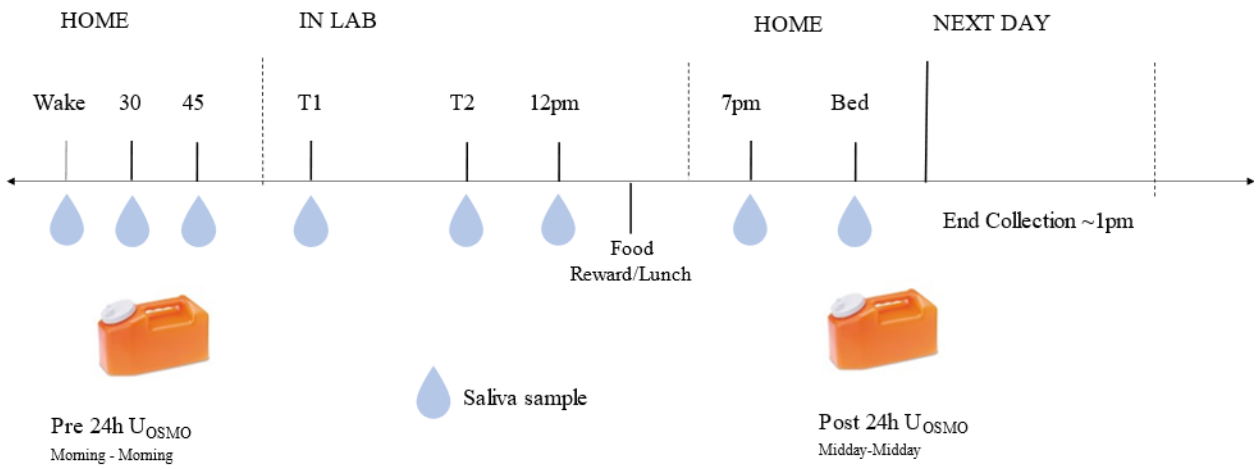
Experimental Trial

Participants arrived fasted (at least 8 hours) to the Exercise Physiology Lab in the morning (0600-0900) after their third day of 24h urine collection. Prior to arriving at the laboratory, participants collected saliva samples via passive drool (Duplessis et al., 2010). Participants collected samples in provided containers upon awakening the morning of the experimental trial, 30 minutes after awakening, and 45 minutes after awakening and then brought all samples with them to the lab. Participants then consumed a standardized meal that included: 78g 100% whole wheat bread, 21g mild cheddar cheese, 17g strawberry jam, 18g peanut butter, 225g orange juice (~546kcals, 19g protein, 77g carbohydrate, 18g fat). Participants then provided repeated measures of saliva and blood throughout the Experimental Trial (see – "Salivary" below).

Approximately 3.5 h following breakfast, participants completed an electronic food reward task, the Leeds Food Preference Questionnaire (LFPQ) (Finlayson et al., 2007), to assess

explicit liking and explicit wanting of various food items. Participants completed this task before and after an ad libitum lunch. Participants then collected their urine for another 24h following the lab visit and collected saliva at 7pm and immediately prior to bed the evening of their lab visit.

Figure 11. Timeline of Experimental Protocol. T1 = 30 minutes Post-Breakfast, T2 = 2 Hours Post Breakfast.



Measures

Urinary Hydration Measures

Each 24h collection container, and individual urine samples throughout the experimental visits, was measured for urinary osmolality (U_{OSMO}) in duplicate via freezing point depression (Model 3320, Advanced Instruments, Norwood, MA), with higher values indicative of worse hydration.

Biochemical Analyses

Salivary

Saliva samples were collected using the "passive drool" technique (Granger et al., 2012) upon awakening, 30- and 45-minutes post-awakening the morning of the Experimental Trial, and approximately 30 minutes (T1), and 120 minutes (T2) post breakfast, with an additional sample collected at 12pm for all participants regardless of start time. After leaving the laboratory, participants were asked to collect two additional samples at 7pm and immediately prior to bed on that day (Figure 11). Participants were asked to refrain from brushing their teeth, eating, or drinking during this morning collection period. Participants were also asked to refrain from exercise prior to coming to the laboratory. Samples were stored at -80°C upon receiving.

Salivary measures were assessed via commercial ELISA for salivary cortisol (IBL America, Minneapolis, MN). These samples were used to estimate the circadian profile of cortisol among participants (Weitzman et al., 1971). Saliva samples were thawed and centrifuged at 14,000 rpm for 15 minutes before completing the assays. All samples were assayed in duplicate, with samples reanalyzed if the coefficient of variation exceeded 25%. All participant samples were analyzed on the same day and same plate where possible. The intraassay CV was 7.69%; the interassay CV was 18.7%.

HPA-axis Activity Metrics

Individual circadian cortisol profiles were calculated for the cortisol awakening response (CAR) and diurnal cortisol slope. To determine the CAR, the Δ score was calculated as the difference between the first awakening sample and the greatest concentration during the awakening period (time 30 or 45). Calculation of the diurnal cortisol slope (DCS) was determined using a linear mixed model with individual salivary observations at level-1 and

participant at level-2, similar to methods of Doane et al. (Doane et al., 2013), using all available samples for each participant to estimate the coefficient for time via maximum likelihood estimation.

Food Reward

Food reward was measured via the previously utilized Leeds Food Preference Questionnaire (LFPQ) (Finlayson et al., 2007), which measures the wanting and liking of food items of various composition (i.e., high-fat savory [HFSA], low-fat savory [LFSA], high-fat sweet [HFSW], and low-fat sweet [LFSW]). Items are rated for which item participants "most want to eat now" from a series of paired food items and the extent to which they "like" or "want" items (on a 100mm VAS). This task was adjusted per participant based on individualized ratings of food items prior to the task administration. This task has shown good test-retest reliability to detect implicit wanting and explicit liking (Dalton & Finlayson, 2014). Liking refers to the affect-driven responses of food reward such as the perceived or expected pleasure-giving the value of a food related to its sensory properties, whereas wanting refers to changes in likelihood of consuming the food independent of liking driven by the perception of a food or a food-related cue in the environment (Dalton & Finlayson, 2014; Finlayson et al., 2007).

Statistical Analyses

An ordinary least squares regression was used to predict salivary CAR, from 24h U_{OSMO} of the previous day. Separate ordinary least squares regressions were used to predict salivary C_{peak} from the 24h urine collected before and after the laboratory visit. To determine the relationship between 24h urinary osmolality and DCS, the 24h urine sample from both the previous day and the 24h following the laboratory visit were used in separate linear mixed effects models as a predictor of salivary cortisol at each available time point (Wake, 30, 45, T1,

T2, noon, 7pm, Bed) (E. K. Adam et al., 2017); in these models a significant interaction effect between U_{OSMO} and time would indicate an effect of hydration status on the DCS. Time was re-coded in this analysis to represent total minutes of the day. This model was run with and without covarying for time spent awake and individual chronotype, which has shown some influence on cortisol profiles (Bailey & Heitkemper, 2001), assessed via the rMEQ questionnaire where available. The participant-level 'Time' coefficient from a model with only Time as a predictor and allowing for a random slope effect was retained and used as a predictor of food reward (see below). Additional analyses controlled for raw physical activity counts assessed via Actigraph the day prior to the laboratory visit and total sleep time on HPA axis indices, given the influence of vigorous exercise participation (Anderson & Wideman, 2017) and/or altered sleep patterns (Anderson et al., 2021) on CAR.

Exploratory Welch's two sample t-tests were used to compare HPA-axis indices between individuals who were "underhydrated", based on a mean 24h U_{OSMO} from both urine collection periods > 500mOsm*kg^{-1}.

Separate multiple regressions were used to assess the influence of these HPA-axis activity indices on baseline ratings and changes in explicit liking, and explicit wanting of food items from each food category from the LFPQ (high fat, sweet (HFSW); high fat, savory (HFSA); low fat, sweet (LFSW); low fat, savory (LFSA) before and after an ad-libitum lunch meal. To assess the independent effects of these HPA axis indicators and hydration status, separate models also controlled for 24h U_{OSMO} from both the previous 24h and the 24h following the in-lab visit for DCS and C_{peak}, and the previous day's 24h U_{OSMO} for models with CAR.

One participant displayed values considerably higher than the values of the remaining participants (C_{peak} = 435 ng/mL compared to the mean of 21.2 ng/mL). Further investigation

revealed this participant was taking isotretinoin, commonly used for the treatment of acne, which may also be associated with alterations in HPA-axis activity (Bremner et al., 2012). Given this large difference in values, this participant was a high leverage outlier, where inclusion contributed to biased regression model results; thus, this participant was excluded from all analyses.

Results

Demographic characteristics are presented in Table 24**.** One participant left the study early (before lunch); thus, his data were not used for the models assessing change in food reward before and after the lunch meal.

Table 24. Demographic Characteristics of Participants.

	Overall (N=29)
Age (y)	
Mean (SD)	23.4 (4.35)
Median [Min, Max]	23.0 [18.0, 34.0]
Body Mass (kg)	
Mean (SD)	79.8 (14.5)
Median [Min, Max]	75.2 [61.9, 117]
BMI (kg/m^2)	
Mean (SD)	26.0 (4.75)
Median [Min, Max]	24.6 [19.2, 38.5]
Body Fat (%)	
Mean (SD)	17.4 (9.56)
Median [Min, Max]	16.1 [2.30, 36.5]
Race/Ethnicity	
African American	6 (20.7%)
Asian	2 (6.9%)
Caucasian	17 (58.6%)
Hispanic	4 (13.8%)
24h Urinary Osmolality Pre-Lab ($mOsm*kg^{-1}$)	
Mean (SD)	580 (253)
Median [Min, Max]	600 [186, 1000]

	Overall (N=29)
24h Urinary Osmolality Post-Lab (mOsm*kg^{-1})	
Mean (SD)	599 (252)
Median [Min, Max]	617 [167, 1010]
rMEQ Total	
Mean (SD)	24.0 (3.09)
Median [Min, Max]	24.0 [19.0, 29.0]
Missing	5 (17.2%)
Total Sleep Time (min)	
Mean (SD)	264 (88.2)
Median [Min, Max]	252 [134, 449]
Missing	11 (37.9%)

Urinary Hydration Status

Table 24 includes the average hydration status of participants from the 24h prior to and the 24h following the laboratory visit. On average, participants' hydration status was slightly above the 500 mOsm*kg^{-1} threshold recommended for health, with 20 participants with more concentrated urine ("Underhydrated") and 9 participants with less concentrated urine ("Hydrated").

HPA Axis Activity

Table 25 presents mean values for salivary cortisol measurements. There was no significant difference in the binary classification of "Underhydrated" or "Hydrated" between participants for CAR ($p = 0.658$), C_{peak} = ($p = 0.087$), or DCS ($p = 0.286$).

Table 25. Mean Values for HPA Axis Indices. CAR = Cortisol Awakening Response, Calculated as: Morning Peak (30 Or 45min Sample) – Awakening Sample.

	Hydrated (N=9)	Underhydrated (N=20)	Overall (N=29)
CAR (ng/mL)			
Mean (SD)	10.7 (19.8)	7.62 (7.38)	8.59 (12.3)
Median [Min, Max]	12.8 [-25.4, 41.9]	6.46 [-10.1, 24.0]	6.48 [-25.4, 41.9]
C_{peak} (ng/mL)			
Mean (SD)	28.3 (15.5)	18.0 (6.60)	21.2 (11.0)
Median [Min, Max]	25.9 [6.42, 53.5]	17.1 [7.91, 33.9]	20.9 [6.42, 53.5]
Diurnal Slope (ng/mL)			
Mean (SD)	-0.104 (0.378)	0.0469 (0.203)	0.000 (0.272)
Median [Min, Max]	-0.0589 [-0.807, 0.324]	0.0979 [-0.487, 0.346]	0.0817 [-0.807, 0.346]

Cortisol Peak

Higher 24h U_{OSMO} from urine collected in the 24h after leaving the lab was associated with a lower C_{peak} (β = -0.021 [-0.036, -0.007], p = 0.008, Table 26). However, C_{peak} tended to occur during the CAR collection period for most participants (69%) and did not overlap with the second urine collection period.

Table 26. Association Between 24h U_{osmo} Following The Lab Visit and C_{peak}.

	Dependent variable:
	C_{peak}
U_{OSMO}	-0.021^{***}
	(-0.036, -0.007)
Constant	21.166^{***}
	(17.590, 24.742)
Observations	29
R^2	0.234
Adjusted R^2	0.206
Residual Std. Error	9.826 (df = 27)
F Statistic	8.264^{***} (df = 1; 27)
Note:	$^{*}p<0.1$ $^{**}p<0.05$ $^{***}p<0.01$

CAR

Simple linear regressions showed no association between prior day U_{OSMO} and CAR (p = 0.932), Table 27). When expressing CAR as the percentage increase following awakening to morning peak (CAR%), effects were similar (p = 0.346, Table 27, Model 2).

Table 27. Association between 24-hour urinary osmolality the day prior and absolute and relative CAR the next morning.

	Dependent variable:	
	CAR	CAR %
	(1)	(2)
U_{OSMO}	-0.001	-0.149
	(-0.019, 0.018)	(-0.453, 0.155)
Constant	9.060	252.998
	(-2.522, 20.642)	(61.019, 444.976)
Observations	29	29
R^2	0.0003	0.033
Adjusted R^2	-0.037	-0.003
Residual Std. Error (df = 27)	12.532	207.722
F Statistic (df = 1; 27)	0.008	0.922
Note:		*p<0.1**p<0.05***p<0.01

Diurnal Cortisol Slope (DCS)

Salivary cortisol declined over time regardless of the 24h U_{OSMO} used for analysis ($p < 0.001$) (Figure 12). A quadratic effect of time was tested in each model to account for the CAR response of these measures (i.e., an expected rise in the morning followed by a decline), but this effect did not improve overall model fit based on AIC or the likelihood ratio test for models with or without including total time awake. There was no interaction effect between 24h U_{OSMO} the day prior to the lab visit and change in salivary cortisol throughout the day ($p = 0.779$) (Table 28). There was no interaction effect between 24h U_{OSMO} the day following the laboratory visit and salivary cortisol change over time, indicating no effect of hydration status on DCS ($p = 0.491$) (Table 29). Controlling for individual chronotype using the total score from the rMEQ did not significantly influence the relationship between hydration status and DCS, regardless of the

hydration timepoint used, though five fewer observations were available for this control measure (all ps > 0.05, Table A51).

Table 28. Relationship Between 24h U_{OSMO} the Day Prior to the Laboratory Visit and DCS.

	Cortisol Concentration (ng/mL)											
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	8.04	6.52 – 9.57	**<0.001**	7.32	5.56 – 9.08	**<0.001**	8.05	4.44 – 11.66	**<0.001**	5.12	0.84 – 9.39	**0.020**
Time (hours)	-0.58	-0.74 – -0.42	**<0.001**	-0.70	-0.92 – -0.48	**<0.001**	-0.70	-1.29 – -0.11	**0.020**	-3.66	-6.04 – -1.28	**0.003**
U_{OSMO}	-0.00	-0.01 – 0.01	0.779	-0.00	-0.01 – 0.01	0.710	0.01	-0.01 – 0.02	0.467	-0.00	-0.02 – 0.02	0.844
Time * U_{OSMO}	-0.00	-0.00 – 0.00	0.781	-0.00	-0.00 – 0.00	0.777	0.00	-0.00 – 0.00	0.354	-0.01	-0.02 – 0.00	0.126
$Time^2$				0.02	-0.00 – 0.05	0.110				-0.42	-0.75 – -0.10	**0.011**
$Time^2$ * U_{OSMO}				0.00	-0.00 – 0.00	0.832				-0.00	-0.00 – 0.00	0.054
Time Awake (hours)							-0.65	-1.94 – 0.64	0.316	-0.44	-1.72 – 0.84	0.500
Random Effects												
σ^2	40.95			40.81			45.27			42.67		
τ_{00}	10.17 $_{Subject}$			10.16 $_{Subject}$			23.03 $_{Subject}$			22.00 $_{Subject}$		
ICC	0.20			0.20			0.34			0.34		
N	29 $_{Subject}$			29 $_{Subject}$			16 $_{Subject}$			16 $_{Subject}$		

Observations	180	180	91	91
Marginal R^2 / Conditional R^2	0.191 / 0.352	0.202 / 0.361	0.076 / 0.387	0.128 / 0.425
AIC	1239.159	1264.788	652.519	664.032

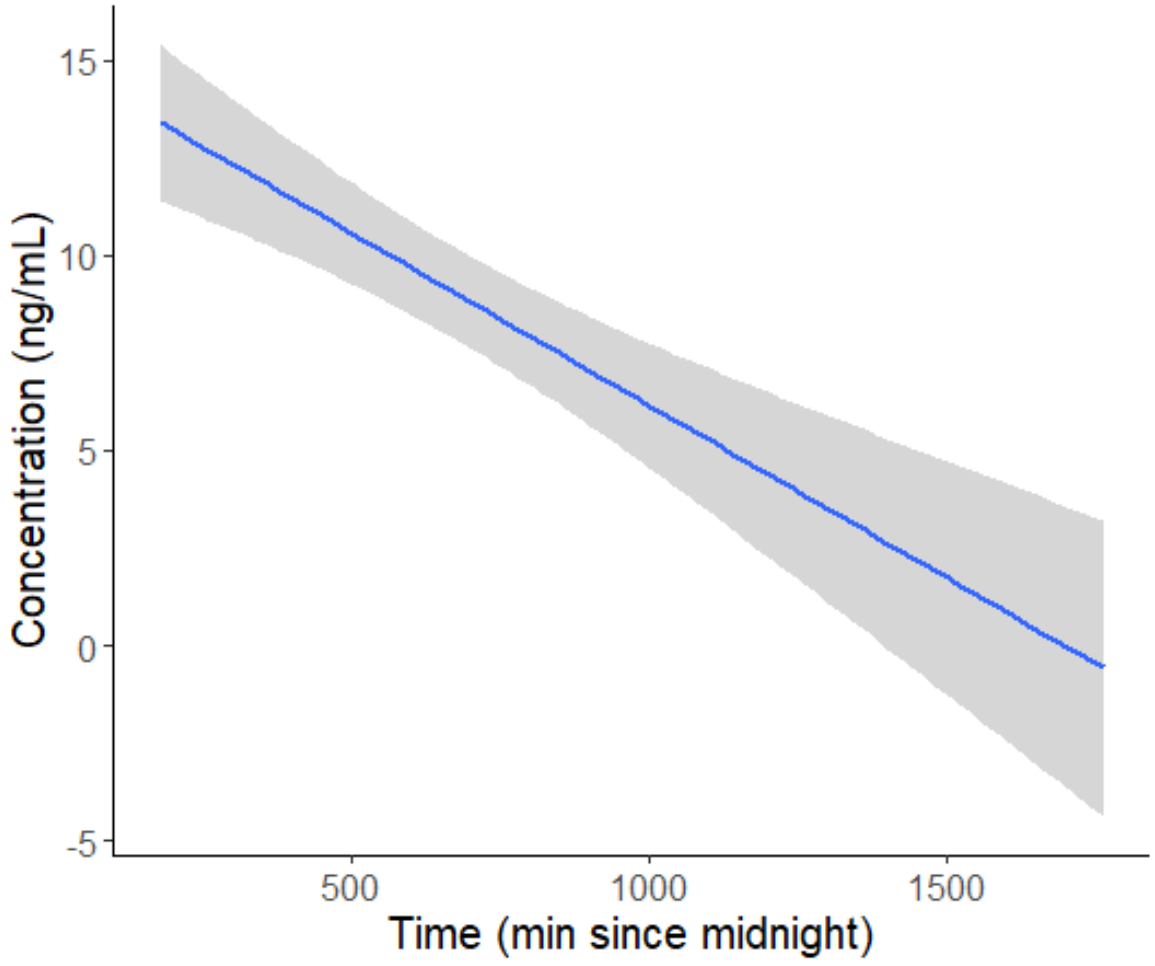
Note: Variables were mean centered for ease of interaction coefficient interpretation.

Table 29. Relationship Between 24h Urine Osmolality the Day Following the Lab Visit and Salivary Cortisol Concentrations Throughout the Day (DCS).

	Cortisol Concentration (ng/mL)											
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	8.118	6.692 – 9.543	**<0.001**	7.346	5.675 – 9.017	**<0.001**	8.159	4.754 – 11.564	**<0.001**	6.716	2.636 – 10.797	**0.002**
Time (hours)	-0.590	-0.750 – -0.429	**<0.001**	-0.717	-0.937 – -0.497	**<0.001**	-0.739	-1.326 – -0.151	**0.014**	-2.002	-4.070 – 0.065	0.057
U_{OSMO}	-0.006	-0.012 – 0.000	0.054	-0.006	-0.013 – 0.000	0.068	-0.004	-0.021 – 0.012	0.598	-0.011	-0.032 – 0.011	0.314
Time * U_{OSMO}	0.000	-0.000 – 0.001	0.491	0.000	-0.001 – 0.001	0.669	0.002	-0.002 – 0.005	0.340	-0.006	-0.020 – 0.009	0.438
$Time^2$				0.023	-0.003 – 0.049	0.089				-0.165	-0.421 – 0.091	0.202
$Time^2$ * U_{OSMO}				0.000	-0.000 – 0.000	0.804				-0.001	-0.003 – 0.001	0.303
Time Awake (hours)							-0.719	-1.852 – 0.414	0.211	-0.479	-1.626 – 0.668	0.409
Random Effects												
σ^2	40.86			40.56			45.27			44.72		
τ_{00}	8.12 $_{Subject}$			8.15 $_{Subject}$			17.24 $_{Subject}$			16.51 $_{Subject}$		
ICC	0.17			0.17			0.28			0.27		
N	29 $_{Subject}$			29 $_{Subject}$			16 $_{Subject}$			16 $_{Subject}$		
Observations	180			180			91			91		
Marginal R^2 / Conditional R^2	0.219 / 0.348			0.231 / 0.360			0.145 / 0.381			0.167 / 0.392		
AIC	1235.262			1260.249			649.619			664.498		

Note: Variables were mean centered for ease of interaction coefficient interpretation.

Figure 12. Average DCS Throughout the Lab Visit Day and Home Saliva Collection, Plotted as Change in Cortisol Concentration From "Wake" to "Bed"



Physical Activity and Sleep

We observed no significant influence of total activity counts from the previous day or total sleep time, either alone or in combination, on the relationships between U_{OSMO} and HPA axis indices (Appendix A, Table A52-A50), though total sleep time was negatively associated with CAR ($p = 0.011$). An outlier was also found in these models, and analyses were run with and without this individual, suggesting other factors such as individual stress are likely to influence these relationships. The observed relationship between the following day's 24 U_{OSMO}

and C_{peak} was no longer significant when controlling for total sleep time and raw Activity Counts from the day prior in the analysis (p = 0.362).

Food Reward

One participant was aware of the study aims/hypotheses; thus, this participant was excluded from analyses involving the measures of food reward.

C_{peak} and Hydration on Food Reward

Greater C_{peak} was associated with lower explicit liking of HFSW foods (β = -0.946, [-1.647, -0.245], p = 0.014), HFSA foods (β= -0.433, [-0.776, -0.090], p = 0.021) and lower explicit wanting of HFSW (β = -0.883, [-1.600, 0.166], p = 0.024) and HFSA foods (β = -0.430, [-0.779, -0.080], p = 0.024)(Table 30). There was no association between C_{peak} and explicit liking of LFSW (p = 0.432), LFSA (p = 0.732) or explicit wanting of LFSW (p = 0.992) or LFSA (p = 0.684).

There was no association between C_{peak} and preference for HFSA food items before lunch [β = -0.34 [-0.7,0.012], p = 0.069), when controlling for the 24h U_{OSMO} the day prior to the lab visit. Higher C_{peak} was associated with lower explicit liking of HFSW food items before lunch (β = -0.857 [-1.609, -0.106], p = 0.0344). There was no relationship between C_{peak} or U_{OSMO} on 'liking' of LFSW (p = 0.747, p = 0.201, respectively) or LFSA foods (p = 0.794, p = 0.865, respectively) at baseline (Table 31).

Similarly, higher C_{peak} was associated with lower mean explicit wanting at baseline for HFSW food items (β = -0.824, [-1.597, -0.052] p = 0.047) and HFSA food items (β = -0.396 [-0.772,0.021], p = 0.049), controlling for 24h U_{OSMO} from the day prior. C_{peak} and the previous day's 24h U_{OSMO} were not significantly associated with explicit wanting of LFSW (p = 0.659, p = 0.201, respectively) or LFSA foods (p = 0.715, p = 0.971, respectively) before lunch (Table

32). Overall effects were similar for explicit liking and explicit wanting when controlling for the following day's 24h $U_{OSMO,}$ though the relationship between C_{peak} and wanting of HFSA items was no longer significant ($p = 0.102$).

Table 30. Association Between C_{peak} on Mean Liking (ML) and Mean Wanting (MW) of Foods From Each Category of The LFPQ.

	Dependent variable:							
	ML HFSW	ML HFSA	ML LFSW	ML LFSA	MW HFSW	MW HFSA	MW LFSW	MW LFSA
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Cpeak	-0.946**	-0.433**	-0.189	-0.067	-0.883**	-0.430**	0.003	-0.081
	(-1.647, -0.245)	(-0.776, -0.090)	(-0.653, 0.275)	(-0.447, 0.313)	(-1.600, -0.166)	(-0.779, -0.080)	(-0.482, 0.487)	(-0.467, 0.304)
Constant	80.115	86.635	80.245	72.682	77.990	85.539	76.099	70.155
	(63.471, 96.760)	(78.491, 94.779)	(69.235, 91.255)	(63.664, 81.700)	(60.970, 95.011)	(77.250, 93.828)	(64.592, 87.605)	(61.002, 79.308)
Observations	28	28	28	28	28	28	28	28
R^2	0.212	0.191	0.024	0.005	0.183	0.183	0.00000	0.007
Adjusted R^2	0.182	0.160	-0.014	-0.034	0.152	0.151	-0.038	-0.032
Residual Std. Error (df = 26)	20.810	10.182	13.766	11.275	21.280	10.363	14.386	11.444
F Statistic (df = 1; 26)	6.991**	6.129**	0.638	0.120	5.827**	5.816**	0.0001	0.170

Note: *p<0.1**p<0.05***p<0.01

Table 31. Association Between U_{OSMO} (24h Prior) and C_{peak} On Mean Liking (ML) and Mean Wanting (MW) of Foods From Each Category of The LFPQ. All Independent Variables Were Centered With Respect to the Grand Mean.

	Dependent variable:							
	ML HFSW	ML HFSA	ML LFSW	ML LFSA	MW HFSW	MW HFSA	MW LFSW	MW LFSA
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
U_{OSMO}	0.012	0.012	0.014	0.002	0.008	0.004	0.015	0.0003
	(-0.022, 0.045)	(-0.004, 0.028)	(-0.007, 0.036)	(-0.017, 0.020)	(-0.027, 0.042)	(-0.012, 0.021)	(-0.007, 0.038)	(-0.018, 0.019)
C_{peak}	-0.858**	-0.344*	-0.081	-0.055	-0.824**	-0.396**	0.116	-0.079
	(-1.609, -0.106)	(-0.700, 0.012)	(-0.566, 0.404)	(-0.466, 0.355)	(-1.597, -0.052)	(-0.772, -0.021)	(-0.391, 0.623)	(-0.496, 0.338)
Constant	47.969***	72.601***	75.109***	70.479***	47.644***	70.843***	77.795***	67.324***
	(34.723, 61.215)	(66.325, 78.877)	(66.554, 83.665)	(63.239, 77.720)	(34.024, 61.264)	(64.219, 77.466)	(68.854, 86.737)	(59.970, 74.677)
Observations	28	28	28	28	28	28	28	28
R^2	0.227	0.255	0.087	0.006	0.190	0.192	0.064	0.007
Adjusted R^2	0.165	0.196	0.014	-0.074	0.125	0.127	-0.010	-0.073
Residual Std. Error (df = 25)	21.022	9.960	13.578	11.491	21.615	10.511	14.190	11.670
F Statistic (df = 2; 25)	3.664**	4.289**	1.190	0.073	2.924*	2.963*	0.861	0.083

Note: *p<0.1**p<0.05***p<0.01

Table 32. Association Between U_{OSMO} (24h Post) and C_{peak} on Mean Liking (ML) and Mean Wanting (MW) of Foods From Each Category of The LFPQ. All Independent Variables Were Centered With Respect to the Grand Mean.

	Dependent variable:							
	ML HFSW	ML HFSA	ML LFSW	ML LFSA	MW HFSW	MW HFSA	MW LFSW	MW LFSA
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
U_{OSMO}	-0.005	0.009	0.020	-0.002	-0.006	0.006	0.018	-0.007
	(-0.043, 0.034)	(-0.009, 0.028)	(-0.004, 0.044)	(-0.023, 0.019)	(-0.046, 0.033)	(-0.013, 0.025)	(-0.008, 0.044)	(-0.028, 0.014)
C_{peak}	-1.001**	-0.326	0.046	-0.089	-0.956**	-0.362	0.214	-0.167
	(-1.846, -0.156)	(-0.732, 0.081)	(-0.487, 0.578)	(-0.547, 0.369)	(-1.819, -0.092)	(-0.781, 0.056)	(-0.350, 0.778)	(-0.628, 0.294)
Constant	60.140***	77.663***	76.585***	71.238***	59.313***	76.591***	76.437***	68.331***
	(52.265, 68.015)	(73.876, 81.450)	(71.622, 81.547)	(66.969, 75.507)	(51.266, 67.360)	(72.691, 80.490)	(71.182, 81.692)	(64.035, 72.627)
Observations	28	28	28	28	28	28	28	28
R^2	0.214	0.220	0.116	0.006	0.186	0.194	0.070	0.025
Adjusted R^2	0.151	0.158	0.046	-0.074	0.121	0.129	-0.004	-0.053
Residual Std. Error (df = 25)	21.197	10.193	13.357	11.491	21.661	10.496	14.145	11.564
F Statistic (df = 2; 25)	3.397**	3.530**	1.646	0.074	2.860*	3.008*	0.947	0.315
Note:								*p<0.1**p<0.05***p<0.01

CAR and Hydration on Food Reward

There was no association between CAR and food reward prior to lunch for any category (ps > 0.05, Table 33**)**. There was no relationship between CAR or 24h U_{OSMO} from the previous day on explicit liking or explicit wanting of any food categories prior to lunch (Table 34). Results were similar when CAR was expressed as a relative increase (CAR%, Table 35).

Table 33. Associations Between CAR and Explicit Liking (ML) and Explicit Wanting (MW) of Foods From Each Category of the LFPQ.

	Dependent variable:							
	ML HFSW	ML HFSA	ML LFSW	ML LFSA	MW HFSW	MW HFSA	MW LFSW	MW LFSA
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
CAR	-0.074	-0.300*	-0.033	-0.037	-0.087	-0.178	0.126	0.026
	(-0.783, 0.635)	(-0.622, 0.023)	(-0.454, 0.389)	(-0.379, 0.305)	(-0.799, 0.625)	(-0.518, 0.163)	(-0.307, 0.559)	(-0.322, 0.373)
Constant	60.831***	80.014***	76.539***	71.580***	60.135***	77.980***	75.101***	68.234***
	(50.334, 71.327)	(75.236, 84.791)	(70.298, 82.781)	(66.520, 76.640)	(49.595, 70.675)	(72.943, 83.017)	(68.694, 81.508)	(63.090, 73.377)
Observations	28	28	28	28	28	28	28	28
R^2	0.002	0.113	0.001	0.002	0.002	0.039	0.012	0.001
Adjusted R^2	-0.037	0.079	-0.038	-0.037	-0.036	0.002	-0.026	-0.038
Residual Std. Error (df = 26)	23.422	10.661	13.927	11.291	23.519	11.240	14.296	11.477
F Statistic (df = 1; 26)	0.042	3.308*	0.023	0.046	0.057	1.047	0.326	0.021

Note: *p<0.1**p<0.05***p<0.01

Table 34. Association Between U_{OSMO} From The Previous 24h and CAR on Mean Liking (ML) and Mean Wanting (MW) of Foods From Each Category of The LFPQ. All Independent Variables Were Centered With Respect to the Mean.

	Dependent variable:							
	ML HFSW	ML HFSA	ML LFSW	ML LFSA	MW HFSW	MW HFSA	MW LFSW	MW LFSA
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
U_{OSMO}	0.024	0.016*	0.015	0.002	0.020	0.010	0.013	0.001
	(-0.012, 0.060)	(0.001, 0.032)	(-0.006, 0.036)	(-0.016, 0.021)	(-0.016, 0.055)	(-0.008, 0.027)	(-0.009, 0.035)	(-0.018, 0.020)
CAR	-0.139	-0.327*	-0.070	-0.093	-0.148	-0.215	0.082	-0.041
	(-0.851, 0.572)	(-0.638, -0.015)	(-0.490, 0.350)	(-0.462, 0.275)	(-0.864, 0.568)	(-0.564, 0.133)	(-0.357, 0.521)	(-0.425, 0.344)
Constant	61.552***	78.241***	77.052***	72.172***	60.629***	77.267***	76.957***	69.474***
	(52.912, 70.192)	(74.455, 82.028)	(71.947, 82.156)	(67.693, 76.652)	(51.930, 69.328)	(73.033, 81.502)	(71.626, 82.288)	(64.807, 74.141)
Observations	29	29	29	29	29	29	29	29
R^2	0.069	0.251	0.078	0.012	0.049	0.096	0.056	0.002
Adjusted R^2	-0.003	0.193	0.007	-0.065	-0.024	0.027	-0.017	-0.074
Residual Std. Error (df = 26)	23.739	10.403	14.026	12.308	23.901	11.634	14.648	12.823
F Statistic (df = 2; 26)	0.964	4.354**	1.098	0.152	0.670	1.387	0.765	0.030

Note: *p<0.1**p<0.05***p<0.01

Table 35. Association Between U_{OSMO} From the Previous Day and CAR% and Mean Liking (ML) and Mean Wanting (MW) of Foods From Each Category of the LFPQ. All Independent Variables Were Centered With Respect to the Mean.

	Dependent variable:							
	ML HFSW (1)	ML HFSA (2)	ML LFSW (3)	ML LFSA (4)	MW HFSW (5)	MW HFSA (6)	MW LFSW (7)	MW LFSA (8)
U_{OSMO}	0.025	0.014	0.014	0.001	0.022	0.009	0.014	0.001
	(-0.011, 0.062)	(-0.002, 0.031)	(-0.007, 0.036)	(-0.018, 0.020)	(-0.015, 0.059)	(-0.009, 0.028)	(-0.008, 0.037)	(-0.018, 0.021)
CAR%	0.006	-0.015	-0.007	-0.005	0.013	-0.004	0.006	-0.0002
	(-0.038, 0.049)	(-0.034, 0.005)	(-0.033, 0.018)	(-0.028, 0.017)	(-0.031, 0.056)	(-0.026, 0.017)	(-0.021, 0.033)	(-0.024, 0.023)
Constant	61.552***	78.241***	77.052***	72.172***	60.629***	77.267***	76.957***	69.474***
	(52.897, 70.206)	(74.316, 82.166)	(71.968, 82.135)	(67.689, 76.656)	(51.956, 69.303)	(72.928, 81.606)	(71.630, 82.283)	(64.803, 74.145)
Observations	29	29	29	29	29	29	29	29
R^2	0.066	0.195	0.085	0.010	0.055	0.051	0.057	0.001
Adjusted R^2	-0.006	0.133	0.015	-0.066	-0.018	-0.022	-0.015	-0.076
Residual Std. Error (df = 26)	23.778	10.785	13.968	12.318	23.830	11.922	14.635	12.833
F Statistic (df = 2; 26)	0.918	3.147*	1.214	0.131	0.752	0.701	0.790	0.009
Note:								*p<0.1**p<0.05***p<0.01

DCS and Hydration on Food Reward

Raw results examining the relationship between DCS and pre-lunch food reward ratings are presented in Table 36. A flatter (more positive) DCS was associated with greater explicit liking of HFSW foods ($p = 0.046$) and greater explicit wanting of HFSA foods ($p = 0.037$). DCS was not associated with food reward from the other categories (all $ps > 0.05$).

Controlling for the previous 24h U_{OSMO}, a flatter (more positive) DCS was not associated with Liking of HFSW foods ($\beta = 30.18$, [0.695,59.673] $p = 0.056$) but was significantly associated with explicit wanting of HFSA foods ($\beta = 15.529$, [1.065, 29.993] $p = 0.046$) (Table 37). Higher 24h U_{OSMO} from the previous day was not associated with explicit liking of HFSA food items ($p = 0.050$).

Results were influenced slightly when controlling for the 24h U_{OSMO} from the following 24h (Table 38). There was no effect of 24h U_{OSMO} the day following the visit on measures of food reward before lunch (Table 38). The relationship between DCS and Liking of HFSW foods when controlling for the following day's 24 U_{OSMO} remained non-significant ($p = 0.09$), as was the relationship between the following day's 24 U_{OSMO} and Wanting of HFSA foods ($p = 0.105$). DCS was not associated with pre-meal ratings of food items from the other categories.

Table 36. Associations Between DCS and Explicit Liking (ML) and Explicit Wanting (MW) of Foods From Each Category of the LFPQ.

	Dependent variable:							
	ML HFSW (1)	ML HFSA (2)	ML LFSW (3)	ML LFSA (4)	MW HFSW (5)	MW HFSA (6)	MW LFSW (7)	MW LFSA (8)
DCS	31.871**	14.018*	4.166	-1.643	27.477*	16.220**	-2.511	2.487
	(2.068, 61.675)	(-0.579, 28.615)	(-14.921, 23.252)	(-17.165, 13.879)	(-3.117, 58.071)	(1.747, 30.693)	(-22.263, 17.240)	(-13.267, 18.241)
Constant	60.266***	77.541***	76.275***	71.265***	59.456***	76.527***	76.148***	68.450***
	(52.236, 68.297)	(73.608, 81.474)	(71.132, 81.418)	(67.083, 75.448)	(51.212, 67.699)	(72.627, 80.426)	(70.825, 81.470)	(64.206, 72.695)
Observations	28	28	28	28	28	28	28	28
R^2	0.145	0.120	0.007	0.002	0.106	0.157	0.002	0.004
Adjusted R^2	0.112	0.086	-0.031	-0.037	0.072	0.124	-0.036	-0.035
Residual Std. Error (df = 26)	21.681	10.618	13.885	11.291	22.256	10.528	14.369	11.460
F Statistic (df = 1; 26)	4.393**	3.543*	0.183	0.043	3.099*	4.825**	0.062	0.096

Note: *p<0.1**p<0.05***p<0.01

Table 37. Relationship Between DCS and 24h U_{OSMO} From the Day Prior on Food Reward.

	Dependent variable:									
	ML HFSW	ML HFSA		ML LFSW		MW HFSW	MW HFSA	MW LFSW		MW LFSA
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
DCS	30.184*	12.788*	4.531	2.970	18.436*	26.104	15.529**	-3.578	9.773	2.387
	(0.695, 59.673)	(-1.026, 26.601)	(-11.779, 20.841)	(-15.766, 21.705)	(-2.418, 39.289)	(-4.558, 56.767)	(1.065, 29.993)	(-23.183, 16.026)	(-12.947, 32.492)	(-13.730, 18.504)
U_{OSMO}	0.022	0.016*	0.015**	0.015	0.016*	0.018	0.009	0.014	0.015	0.001
	(-0.010, 0.054)	(0.001, 0.031)	(0.001, 0.030)	(-0.005, 0.036)	(-0.002, 0.035)	(-0.016, 0.051)	(-0.007, 0.025)	(-0.008, 0.035)	(-0.006, 0.035)	(-0.016, 0.019)
Constant	60.460***	77.682***	78.404***	76.412***	75.059***	59.613***	76.606***	76.270***	75.102***	68.462***
	(52.537, 68.382)	(73.970, 81.393)	(74.735, 82.073)	(71.378, 81.445)	(70.368, 79.750)	(51.375, 67.851)	(72.720, 80.492)	(71.003, 81.537)	(69.992, 80.213)	(64.132, 72.792)
Observations	28	28	27	28	27	28	28	28	27	28
R^2	0.200	0.248	0.163	0.087	0.207	0.143	0.196	0.062	0.105	0.004
Adjusted R^2	0.137	0.187	0.094	0.014	0.141	0.075	0.131	-0.013	0.030	-0.075
Residual Std. Error	21.375 (df = 25)	10.013 (df = 25)	9.645 (df = 24)	13.580 (df = 25)	12.332 (df = 24)	22.225 (df = 25)	10.484 (df = 25)	14.210 (df = 25)	13.435 (df = 24)	11.682 (df = 25)
F Statistic	3.135* (df = 2; 25)	4.113** (df = 2; 25)	2.345 (df = 2; 24)	1.185 (df = 2; 25)	3.131* (df = 2; 24)	2.089 (df = 2; 25)	3.043* (df = 2; 25)	0.823 (df = 2; 25)	1.405 (df = 2; 24)	0.056 (df = 2; 25)

Note: *p<0.1 **p<0.05 ***p<0.01

Models run with and without inclusion of a high leverage observation if results were influenced with the inclusion of this participant (n=28 vs n=27). All independent variables centered with respect to the grand mean across all observations.

Table 38. Association Between 24U$_{OSMO}$ From the Following 24h and DCS and Mean Liking (ML) and Mean Wanting (MW) of Foods From Each Category of the LFPQ. All Independent Variables were Centered With Respect to the Mean.

	Dependent variable:									
	ML HFSW	ML HFSA		ML LFSW		MW HFSW	MW HFSA	MW LFSW		MW LFSA
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
DCS	29.324*	9.791	-0.576	-2.167	12.636	25.238	13.256	-7.591	5.266	4.038
	(-3.104, 61.752)	(-5.462, 25.044)	(-18.323, 17.170)	(-21.822, 17.489)	(-9.807, 35.080)	(-8.082, 58.559)	(-2.212, 28.724)	(-28.431, 13.249)	(-19.324, 29.856)	(-13.082, 21.158)
U$_{OSMO}$	0.008	0.013	0.015*	0.020*	0.017	0.007	0.009	0.016	0.014	-0.005
	(-0.028, 0.044)	(-0.004, 0.030)	(-0.001, 0.031)	(-0.002, 0.042)	(-0.004, 0.038)	(-0.030, 0.044)	(-0.008, 0.027)	(-0.007, 0.039)	(-0.009, 0.036)	(-0.024, 0.014)
Constant	60.388***	77.743***	78.626***	76.577***	75.316***	59.563***	76.668***	76.390***	75.295***	68.376***
	(52.210, 68.566)	(73.896, 81.590)	(74.879, 82.373)	(71.620, 81.534)	(70.577, 80.054)	(51.159, 67.966)	(72.767, 80.569)	(71.134, 81.646)	(70.103, 80.487)	(64.059, 72.694)
Observations	28	28	27	28	27	28	28	28	27	28
R^2	0.151	0.194	0.134	0.117	0.197	0.111	0.192	0.069	0.083	0.013

Adjusted R^2	0.083	0.130	0.062	0.046	0.130	0.040	0.128	-0.006	0.007	-0.066
Residual Std. Error	22.029 (df = 25)	10.362 (df = 25)	9.811 (df = 24)	13.352 (df = 25)	12.408 (df = 24)	22.635 (df = 25)	10.508 (df = 25)	14.157 (df = 25)	13.595 (df = 24)	11.630 (df = 25)
F Statistic	2.220 (df = 2; 25)	3.013* (df = 2; 25)	1.863 (df = 2; 24)	1.657 (df = 2; 25)	2.945* (df = 2; 24)	1.566 (df = 2; 25)	2.973* (df = 2; 25)	0.924 (df = 2; 25)	1.092 (df = 2; 24)	0.170 (df = 2; 25)

Note: *p<0.1**p<0.05***p<0.01

Post-Prandial Changes in Food Reward

All measures of food reward significantly decreased immediately following the ad libitum lunch meal, as evidenced by the significant, negative intercepts in these models ('Constant' in Table 39, all ps < 0.01). None of the HPA axis indices were predictive of the *change* in Food Reward from Pre-Post meal (Tables 39, 41, 43, all ps > 0.05). Hydration status assessed by 24h U_{OSMO} from the day prior or the day following the laboratory visit was not associated with the absolute change in any measures of explicit wanting or explicit liking across food categories (Tables 40, 42, 44). Results are presented with the following day's 24h U_{OSMO} with C_{peak} and the previous 24h U_{OSMO} for CAR and DCS.

Table 39. Association Between C_{peak} Change in Liking (DL) and Change in Wanting (DW) for Each Food Category From the LFPQ Pre and Post Lunch.

	Dependent variable:							
	DL HFSW (1)	DL HFSA (2)	DL LFSW (3)	DL LFSA (4)	DW HFSW (5)	DW HFSA (6)	DW LFSW (7)	DW LFSA (8)
C_{peak}	0.389	0.118	-0.378	-0.124	0.473	0.090	-0.343	0.008
	(-0.443, 1.220)	(-0.629, 0.865)	(-1.329, 0.574)	(-0.851, 0.602)	(-0.420, 1.365)	(-0.643, 0.824)	(-1.300, 0.614)	(-0.712, 0.728)
Constant	-38.795***	-57.079***	-29.806**	-40.981***	-42.314***	-58.126***	-32.240**	-43.066***
	(-58.715, -18.875)	(-74.969, -39.189)	(-52.585, -7.027)	(-58.379, -23.583)	(-63.694, -20.934)	(-75.684, -40.568)	(-55.152, -9.328)	(-60.315, -25.816)
Observations	27	27	27	27	27	27	27	27
R^2	0.032	0.004	0.024	0.004	0.041	0.002	0.019	0.00002
Adjusted R^2	-0.006	-0.036	-0.015	-0.035	0.003	-0.038	-0.020	-0.040
Residual Std. Error (df = 25)	24.636	22.126	28.173	21.518	26.442	21.716	28.337	21.334
F Statistic (df = 1; 25)	0.838	0.096	0.606	0.112	1.076	0.058	0.494	0.0005

Note: *p<0.1**p<0.05***p<0.01

Table 40. Association Between C_{peak} and the Following Day's 24h U_{OSMO} and Change in Liking (DL) and Change in Wanting (DW) for Each Food Category From the LFPQ Pre and Post Lunch.

	Dependent variable:							
	DL HFSW (1)	DL HFSA (2)	DL LFSW (3)	DL LFSA (4)	DW HFSW (5)	DW HFSA (6)	DW LFSW (7)	DW LFSA (8)
Cpeak	0.479	-0.155	-0.596	-0.243	0.474	-0.199	-0.615	-0.085
	(-0.521, 1.480)	(-1.032, 0.722)	(-1.731, 0.539)	(-1.114, 0.629)	(-0.602, 1.550)	(-1.056, 0.657)	(-1.750, 0.521)	(-0.951, 0.780)
U_{OSMO}	0.008	-0.023	-0.019	-0.010	0.0001	-0.025	-0.023	-0.008
	(-0.038, 0.054)	(-0.064, 0.017)	(-0.071, 0.033)	(-0.050, 0.030)	(-0.049, 0.049)	(-0.064, 0.014)	(-0.075, 0.029)	(-0.048, 0.032)
Constant	-30.565***	-54.574***	-37.806***	-43.611***	-32.306***	-56.213***	-39.509***	-42.898***
	(-40.027, -21.102)	(-62.867, -46.281)	(-48.539, -27.072)	(-51.852, -35.370)	(-42.485, -22.126)	(-64.315, -48.111)	(-50.246, -28.772)	(-51.084, -34.712)
Observations	27	27	27	27	27	27	27	27
R^2	0.037	0.056	0.044	0.015	0.041	0.063	0.050	0.007
Adjusted R^2	-0.043	-0.023	-0.036	-0.067	-0.039	-0.015	-0.029	-0.076
Residual Std. Error (df = 24)	25.086	21.987	28.457	21.847	26.987	21.481	28.465	21.703
F Statistic (df = 2; 24)	0.460	0.708	0.548	0.180	0.517	0.805	0.633	0.079
Note:								*p<0.1**p<0.05***p<0.01

Table 41. Association Between CAR and Change in Liking (DL) and Change in Wanting (DW) for Each Food Category From the LFPQ.

	Dependent variable:							
	DL HFSW	DL HFSA	DL LFSW	DL LFSA	DW HFSW	DW HFSA	DW LFSW	DW LFSA
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
CAR	-0.023	0.363	0.053	0.314	0.111	0.157	0.028	0.268
	(-0.783, 0.737)	(-0.294, 1.020)	(-0.812, 0.918)	(-0.329, 0.956)	(-0.707, 0.929)	(-0.499, 0.814)	(-0.840, 0.896)	(-0.371, 0.907)
Constant	-30.380***	-57.545***	-38.238***	-46.179***	-33.215***	-57.501***	-39.739***	-45.092***
	(-41.690, -19.070)	(-67.330, -47.760)	(-51.110, -25.365)	(-55.744, -36.613)	(-45.393, -21.037)	(-67.276, -47.725)	(-52.661, -26.817)	(-54.599, -35.585)
Observations	27	27	27	27	27	27	27	27
R^2	0.0001	0.045	0.001	0.035	0.003	0.009	0.0002	0.026
Adjusted R^2	-0.040	0.007	-0.039	-0.003	-0.037	-0.031	-0.040	-0.013
Residual Std. Error (df = 25)	25.044	21.667	28.504	21.181	26.967	21.646	28.613	21.051
F Statistic (df = 1; 25)	0.003	1.171	0.014	0.915	0.071	0.220	0.004	0.677

Note: *p<0.1**p<0.05***p<0.01

Table 42. Association Between CAR and Previous Day's 24h U_{OSMO} and Change in Liking (DL) and Change in Wanting (DW) for Each Food Category From the LFPQ.

	Dependent variable:							
	DL HFSW	DL HFSA	DL LFSW	DL LFSA	DW HFSW	DW HFSA	DW LFSW	DW LFSA
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
CAR	0.116	0.443	0.187	0.415	0.231	0.239	0.160	0.381
	(-0.725, 0.957)	(-0.243, 1.130)	(-0.736, 1.110)	(-0.270, 1.100)	(-0.641, 1.102)	(-0.448, 0.926)	(-0.764, 1.084)	(-0.315, 1.076)
U_{OSMO}	-0.013	-0.008	0.005	0.006	-0.016	-0.007	0.004	0.003
	(-0.056, 0.029)	(-0.042, 0.027)	(-0.041, 0.051)	(-0.028, 0.040)	(-0.060, 0.027)	(-0.041, 0.028)	(-0.042, 0.051)	(-0.031, 0.038)
Constant	-32.907***	-56.037***	-39.943***	-45.275***	-34.409***	-57.635***	-41.604***	-44.755***
	(-43.278, -22.536)	(-64.503, -47.571)	(-51.325, -28.561)	(-53.719, -36.832)	(-45.159, -23.660)	(-66.110, -49.161)	(-53.000, -30.208)	(-53.336, -36.174)
Observations	28	28	28	28	28	28	28	28
R^2	0.019	0.069	0.008	0.057	0.032	0.025	0.006	0.045
Adjusted R^2	-0.060	-0.006	-0.072	-0.019	-0.045	-0.053	-0.074	-0.032
Residual Std. Error (df = 25)	27.999	22.855	30.726	22.795	29.019	22.878	30.765	23.166
F Statistic (df = 2; 25)	0.237	0.923	0.099	0.750	0.417	0.320	0.072	0.588

Note: *p<0.1**p<0.05***p<0.01

Table 43. Association Between DCS and Previous Day's 24h U_{OSMO} and Change in Liking (DL) and Change in Wanting (DW) for Each Food Category From the LFPQ.

	Dependent variable:							
	DL HFSW	DL HFSA	DL LFSW	DL LFSA	DW HFSW	DW HFSA	DW LFSW	DW LFSA
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
DCS	-16.213	-3.640	16.397	7.809	-16.574	-3.327	14.607	1.649
	(-50.055, 17.630)	(-34.085, 26.805)	(-22.271, 55.066)	(-21.682, 37.300)	(-53.128, 19.981)	(-33.189, 26.534)	(-24.316, 53.530)	(-27.675, 30.973)
Constant	-30.565***	-54.574***	-37.806***	-43.611***	-32.306***	-56.213***	-39.509***	-42.898***
	(-39.850, -21.280)	(-62.927, -46.221)	(-48.415, -27.197)	(-51.702, -35.520)	(-42.335, -22.277)	(-64.406, -48.020)	(-50.188, -28.830)	(-50.943, -34.853)
Observations	27	27	27	27	27	27	27	27
R^2	0.034	0.002	0.027	0.011	0.031	0.002	0.021	0.0005
Adjusted R^2	-0.005	-0.038	-0.012	-0.029	-0.008	-0.038	-0.018	-0.039
Residual Std. Error (df = 25)	24.616	22.145	28.126	21.451	26.588	21.720	28.311	21.329
F Statistic (df = 1; 25)	0.882	0.055	0.691	0.269	0.790	0.048	0.541	0.012

Note: $^{*}p<0.1$ $^{**}p<0.05$ $^{***}p<0.01$

Table 44. Association Between DCS and Previous Day's 24h U_{OSMO} and Change in Liking (DL) and Change in Wanting (DW) for Each Food Category From the LFPQ.

	Dependent variable:							
	DL HFSW	DL HFSA	DL LFSW	DL LFSA	DW HFSW	DW HFSA	DW LFSW	DW LFSA
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
DCS	-15.197	-2.945	16.194	7.489	-15.316	-2.734	14.441	1.475
	(-49.549, 19.156)	(-33.971, 28.080)	(-23.410, 55.798)	(-22.688, 37.666)	(-52.314, 21.681)	(-33.204, 27.736)	(-25.427, 54.309)	(-28.556, 31.506)
U_{OSMO}	-0.013	-0.009	0.003	0.004	-0.016	-0.008	0.002	0.002
	(-0.051, 0.025)	(-0.043, 0.025)	(-0.041, 0.046)	(-0.029, 0.037)	(-0.057, 0.025)	(-0.041, 0.026)	(-0.042, 0.046)	(-0.031, 0.035)
Constant	-30.565***	-54.574***	-37.806***	-43.611***	-32.306***	-56.213***	-39.509***	-42.898***
	(-39.954, -21.175)	(-63.054, -46.094)	(-48.630, -26.981)	(-51.859, -35.363)	(-42.418, -22.193)	(-64.541, -47.885)	(-50.406, -28.612)	(-51.106, -34.690)
Observations	27	27	27	27	27	27	27	27
R^2	0.052	0.013	0.027	0.013	0.054	0.010	0.022	0.001
Adjusted R^2	-0.027	-0.070	-0.054	-0.069	-0.025	-0.073	-0.060	-0.082
Residual Std. Error (df = 24)	24.893	22.482	28.698	21.867	26.809	22.079	28.890	21.761
F Statistic (df = 2; 24)	0.654	0.155	0.338	0.158	0.683	0.120	0.264	0.014

Note: *p<0.1**p<0.05***p<0.01

Discussion

In this study we examined associations between 24h urinary hydration status and different indicators of HPA axis activity associated with health, and the relationship between these markers and food reward. Our findings suggest no cross-sectional association between hydration status and the circadian pattern of salivary cortisol secretion (CAR, DCS). However, higher 24h U_{OSMO} collected in the 24h period following the visit to the lab, suggestive of underhydration, was associated with lower C_{peak} from the day prior. Also, a flatter (more positive) DCS was associated with greater food reward from HFSA food items, when controlling for the following day's 24h U_{OSMO}, which was more pronounced when controlling for the previous day's 24h U_{OSMO}. Overall, differential effects were observed in the relationship between HPA-axis indices and aspects of food reward depending on the metric utilized and, in some instances, the timing of 24h U_{OSMO} measure.

In contrast to our hypotheses, 24h U_{OSMO} was not associated with CAR or DCS. Interestingly, 24h U_{OSMO} from the 24h urine collected after the lab visit was associated with *decreased* C_{peak}. Discrepancies between our findings on hydration and cortisol and prior studies may be related to the medium used to quantify cortisol concentrations. While salivary cortisol strongly correlates with the unbound, free portion of the hormone (without corticosteroid binding globulin – CBG (Vieira-Correa et al., 2019)), this molecule undergoes rapid conversion to cortisone by 11β-hydroxysteroid dehydrogenase II (11β-HSD2) (Gröschl, 2008). Most prior studies associating hydration status and cortisol have used serum cortisol (E. Perrier, Vergne, et al., 2013). It may be that hydration status, as measured by 24h urinary hydration status, is more strongly associated with total cortisol secretion rather than the free hormone, but we did not measure serum cortisol in the present study.

Perrier et al. compared two groups ("Low Drinkers" and "High Drinkers") and found significantly higher fasting plasma cortisol concentrations among Low Drinkers (545 vs 459 nmol/L) but did not directly assess the influence of differences in U_{OSMO} between these groups (767 mOsm*kg^{-1} vs 371 mOsm*kg^{-1}) on cortisol values. However, one small study (n = 5) did find reductions in first morning salivary cortisol with an increase in water intake (J. D. Stookey et al., 2013) among 4 out of the 5 individuals with low water intake. By contrast, we did not find any significant differences in CAR, C_{peak}, or DCS between individuals who were underhydrated or hydrated during the two days of urinary measurements used for the present study. It may be that we were underpowered for this between group comparison, since most individuals in the present study (69% of participants), were underhydrated. Perhaps the timing of our urinary measures in relation to salivary cortisol may also explain the lack of association both in the group comparisons and when treating U_{OSMO} as a continuous variable, though we attempted to correct for this by testing both timeframes for analyses involving C_{peak} and DCS. There may be a delay in the association between hydration status and these metrics as suggested by the significant relationship between the 24h urine collection period following the laboratory visit and $C_{peak,}$ which was not observed when using the previous day's U_{OSMO} as a predictor. Perhaps the reverse relationship is true (i.e., the previous day's C_{peak} influencing the subsequent day's U_{OSMO}). Stress and the corresponding increase in sympathetic nervous system activation are known to influence fluid retention through additional stimulation of the renin-angiotensin-aldosterone system under hypovolemic conditions, which is unlikely in free-living scenarios without a dehydrating stimulus (Espiner, 1987). But if the directionality of effect were reversed, we should have observed a positive relationship between these two variables in our study. This discrepancy may be related to the competition between cortisol and aldosterone for the

mineralocorticoid receptor, for which both molecules have a high affinity. Perhaps short term increases in cortisol acutely result in additional cortisol binding to the mineralocorticoid receptor in the kidney beyond the ability of the enzyme 11β-HSD2 to convert cortisol to the inactive cortisone (Kubzansky & Adler, 2010). Thus, when cortisol is lower, 11β-HSD2 may be able to adequately prevent cortisol from binding to the mineralocorticoid receptor, perhaps allowing aldosterone to bind and promote more fluid retention (i.e., an increase in urinary osmolality).

Our results suggest studies examining the circadian profile of cortisol alone may not need to control for hydration status among participants in cross-sectional studies. When controlling for several additional factors thought to influence cortisol secretion patterns (i.e., sleep, physical activity, chronotype), we found that urinary hydration biomarkers were unrelated to CAR or DCS. This may be true of a younger, healthy population, as these results match one study in athletes which found similar fasting cortisol concentrations among different quartiles of fluid intake (Zhang et al., 2022). Additionally, one study in individuals with obesity also found no association between copeptin and either hair cortisol or cortisone, reflective of long-term cortisol exposure, although copeptin was related to both BMI and waist circumference (van der Valk et al., 2020). However, during repeated measures designs, consideration of hydration status may still be warranted, especially in individuals who are underhydrated. Seal et al. found a reduction in serum cortisol following increased water intake among "Low Drinkers" (Seal et al., 2021) but did not assess changes in circadian patterns. Perhaps a certain threshold of underhydration is required before differences in cortisol at a single time point are observed, as seems to be the case for associations between increased water intake and glucose regulation (Enhörning, Brunkwall, et al., 2019). Further, our findings should be verified with the inclusion of some measure of individual psychological stress levels.

Alternatively, the effects of hydration on cortisol may require pairing with additional stress such as exercise, heat exposure, or a combination, as has been used in most investigations assessing acute effects of dehydration on cortisol (Zaplatosch & Adams, 2020), since this relationship has not always been observed (Carroll & Melander, 2021). Specifically, in a study by Jansen et al., osmotic stress alone was sufficient to induce an increase in AVP but not cortisol (Jansen et al., 2019). It may be the *combination* of acute psychological stress associated with dehydration or underhydration (i.e., unpleasant thirst sensation), in addition to the physiological response of increased fluid regulatory hormones, which synergistically increase cortisol levels. However, individuals who are underhydrated also tend to report low thirst ratings (Kavouras, 2019). More complicated is the divergent effects of different types of uncontrolled stressors on circadian profiles of cortisol, particularly the CAR, with some forms of stress either increasing (job stress and general life stress) or decreasing (fatigue, burnout, or exhaustion) this metric (Chida & Steptoe, 2009).

The present study did not measure the major fluid regulatory hormone arginine vasopressin (AVP), or its surrogate biomarker, copeptin. As both cortisol and copeptin are shown to be influenced by acute (Siegenthaler et al., 2014) and chronic stress (Carroll & Melander, 2021), it may be that previously observed associations between fluid intake and cortisol hormones are driven by a combination of the stress response and hydration changes rather than osmotic stimulation from underhydration alone. Thus, while acutely increasing fluid intake may naturally suppress AVP, and by extension, cortisol due to AVP inhibition, the reverse is less likely in the presence of the additional confounding stressors. This may be confirmed through a comparison of these same models with copeptin as a predictor of these HPA axis indices, while

collecting measures of additional external psychological stressors that may impact cortisol results.

This study also did not measure other hormones involved in fluid regulation, such as renin, angiotensin II, or aldosterone. Angiotensin II may also impact the HPA axis by increasing ACTH (Rivier & Vale, 1983). However, Johnson et al. did not observe any effect of *reduced* water intake in individuals with type 2 diabetes on RAAS (Johnson et al., 2017). Further study is required to determine if RAAS plays a role in HPA axis activity in healthy individuals with varying fluid intake, but based on the volume loss typically required to induce RAAS (Cheuvront & Kenefick, 2014), these hormones are unlikely to be affected under typical daily conditions but may be affected indirectly by AVP activation. Despite this, there is also preliminary evidence of an "aldosterone awakening response", which follows a similar secretion pattern to cortisol, which may operate regardless of plasma volume changes (Gideon et al., 2022). Although we did not observe an association between 24h urinary osmolality and CAR, perhaps the relationship between hydration status and cortisol during the morning period may be more aldosterone-driven as compared to AVP mediated. Thus, it may be that more pronounced disturbances to fluid balance that lead to a significant reduction in plasma volume, such as those induced by exercise, heat exposure, or a combination, may be more likely to influence the circadian pattern of aldosterone and, perhaps, cortisol, whereas psychological stress paired with osmotic stimulation may initiate an AVP stimulated rise in cortisol.

We observed a greater explicit liking of HFSW foods and explicit wanting of HFSA foods in individuals with a flatter diurnal cortisol slope. This contrasts with the observed lower preference for HFSW in individuals with a higher C_{peak}. It appears C_{peak} and DCS may provide different information about the link between HPA axis activity and health behaviors. A flatter

diurnal slope has been associated with adverse health outcomes (Charles et al., 2020); our findings support this association, as chronic consumption of calorically dense, high-fat, high-sugar foods elevate one's risk of chronic disease (Huang et al., 2023). Our findings support a possible rationale for the notion that disruptions in HPA axis activity, as indicated by a flatter DCS, may be an antecedent to metabolic disease (E. K. Adam et al., 2017). The association between DCS and food reward from HFSW and HFSA compliment prior findings suggesting an interplay between cortisol and neural reward systems (T. C. Adam & Epel, 2007). Other investigations have primarily used a single morning serum cortisol sample, with one study finding an association between higher baseline morning cortisol and weight gain over the course of 6-months (Chao et al., 2017). Generally, our sample had a healthy weight and body composition, but a prior investigation suggests obese individuals with high cortisol reactivity (i.e., a greater rise in cortisol in response to a stressor), tended to consume more kcals (Herhaus et al., 2020). These same individuals also had higher cortisol levels prior to introduction of a stressor, which seems to facilitate increased food intake (la Fleur et al., 2004). From our results it seems the hedonic desire to eat is an important contributor to this observation, particularly through the preference for more salient, calorie dense food items. One study tested this hypothesis in adult male rats, finding both the frequency and duration of consuming a sweeter food were more effective in reducing HPA-axis activity compared to the amount of calories consumed, suggesting the hedonic properties of food contribute to stress-induced food intake (Ulrich-Lai et al., 2011). Taken together, it may be that both cortisol reactivity and differences in cortisol circadian rhythms influence the hedonic aspects of food intake and subsequent risk for obesity.

To our knowledge, the present study is the first to examine associations between the DCS and food reward. Interestingly, one study in children found an opposing response; larger CAR and steeper DCS were associated with higher sweet, fatty, and snack food consumption over two separate days (Michels et al., 2013). By contrast, flatter DCS has been associated with poorer overall health, particularly in males (Dmitrieva et al., 2013). Another investigation assessed the impact of acute stress in students either during an examination period over 4-5 days or several weeks after examination, finding no association between this form of stress on cortisol awakening response or money spent on high reward foods (Berg Schmidt et al., 2018); it may be that the stress in that study was not sufficient in either magnitude or duration to induce a change in either CAR or behavior. The observed relationship in our study between flatter DCS and food reward for HFSW foods warrants further investigation, but if true, developing targeted strategies to reduce HPA axis dysfunction through the antecedent stressors may be a means of reducing one's risk for obesity and diabetes (Hackett et al., 2016).

We also predicted that a flatter DCS would also be associated with greater food reward from HFSA foods, mainly due to expected associations between hydration status and preference for these food items, as well as associations between flatter diurnal cortisol slope and poorer health. Interestingly, this association was observed between DCS and wanting of HFSA foods but was not significant for explicit liking of HFSA foods. Thus, it would seem DCS is more related to the immediate desire to consume a food item. However, these relationships were not observed for CAR. CAR is believed to aid in preparation for the tasks in the post awakening period, though its precise function is unknown (E. K. Adam et al., 2006; Anderson & Wideman, 2017), and CAR responds both positively and negatively to different types of psychological stressors (Chida & Steptoe, 2009). It may be that differences in habitual fluid intake and

hydration status are insufficient as a stressor to influence CAR. Direct, graded manipulation of hydration status can help further clarify potential relationships between this outcome and the hedonic desire to eat. Together, these observations suggest hydration status and the HPA axis may have an influence on food reward but perhaps through independent mechanisms and depending on the HPA axis metric utilized.

Limitations

The cross-sectional nature of our study precludes us from making causal inferences regarding the relationship between underhydration and the circadian pattern of cortisol release. However, the free-living nature of our study provides greater ecological validity, particularly for the observed relationships between flatter diurnal cortisol slope and food reward from HFSW and HFSA foods.

Our selection of biomarker for hydration status may have also influenced these results. While 24h urinary osmolality has been proposed as an adequate indicator of hydration status and has been used in the determination of fluid intake guidelines (EFSA, 2010; E. T. Perrier et al., 2015), it does have limitations. Specifically, sodium, osmolyte, and protein consumption affect the water requirements to maintain normal osmolality (Armstrong, 2007). Dietary oxalate consumption may also influence the relationship between fluid intake and urinary osmolality, as does obesity (Rosinger et al., 2019), which may further affect the accuracy of this marker (Perinpam et al., 2016). Further, the time frame of urine collection relative to the cortisol measurements may have factored into the null associations between these biomarkers. It may be that a shorter urine collection interval during the time of the salivary cortisol measurements (~12 hours, rather than the day prior or the 24h after the lab visit) would provide a better representation of the relationship between these two variables. There is limited data on time

delays between AVP and cortisol, and the clearance rate of copeptin, more commonly used as an indicator of AVP release in humans, is unclear (Mu et al., 2022). For most analyses the selection of which 24h period to use in the prediction of HPA axis indices did not matter. However, the U_{OSMO} from the 24h following the lab visit was a better predictor of C_{peak} than the prior day's osmolality, where higher urinary osmolality was associated with *decreased* C_{peak} in the previous 24h ($p = 0.008$). This contrasts the finding of no effect observed when the day prior was used in the analysis. Regardless, this effect was diminished when controlling for activity count and total sleep time, highlighting the important role of these factors for total stress response. The effects of DCS on food reward were increased when using the previous day's 24h U_{OSMO} compared to the next 24h. This may have been the result of the time delay between each urine collection periods, where urinary osmolality was not measured during the 6 hour in-lab protocol.

Because participants collected the saliva samples in their home environment prior to coming to the lab, we were reliant upon their adherence to the prescribed timeline. Small delays in collection of the cortisol measurements may drastically impact the CAR results; if participants delayed collecting the morning sample, this may have influenced the true magnitude of the CAR (Elder et al., 2014). Additional, uncontrolled factors may also have influenced study results, such as any vigorous exercise participation the day before (Anderson & Wideman, 2017) and/or altered sleep patterns (Anderson et al., 2021), as well as psychological stress (Chida & Steptoe, 2009; Joseph & Golden, 2017). Depending on the intensity, duration, environmental conditions, and hydration strategies utilized during activity, we would anticipate a more pronounced increase in cortisol if dehydrated following activity (Zaplatosch & Adams, 2020). However, we found no significant influence of total activity counts from the previous day or total sleep time, either

alone or in combination, on the relationships between U_{OSMO} and HPA axis indices though total sleep time was negatively associated with CAR.

Although an attempt was made to have participants report to the lab shortly after their habitual wake time, scheduling conflicts with participants at times necessitated they wake up slightly earlier than normal (Clow et al., 2004). Weekday measurement of CAR tends to be higher in anticipation of stressful days (Clow et al., 2004). While we did not control for this in our analyses, the nature of the laboratory visit (approximately 6 hours in the lab), typically necessitated participants either came on a weekend day or had fewer obligations scheduled for the day of the visit and so, was less likely to be a factor in the present study. However, some suggest these variations in sleep time may not impact the overall patterns of cortisol release (Bowles et al., 2022), and the individuals in our study generally had good sleep habits based on pre-screening with the PSQI. The conditions under which participants awoke may contribute to this response, such as waking up in response to an alarm compared to a natural awakening response. Also, while it was not a pre-determined aim of the study, from these analyses, a potential relationship between hydration status and total cortisol exposure could not be ruled out. Thus, as a final exploratory analysis, we also assessed whether total morning cortisol exposure (the addition of the awakening, 30-, and 45-minute salivary cortisol samples) was related to 24h hydration status but found no effect with or without controlling for activity counts and total sleep time (Appendix A, Table A55).

Further, our population consisted of young, healthy males. The circadian profiles of diseased individuals may be more influenced by changes in water intake or hydration status, as appears to be the case for hydration and glucose regulation (Johnson et al., 2017). Additionally, although women were not included in the current study, menstrual cycle hormones are likely to

influence both fluid regulatory hormones in females. Verifying these findings in females is warranted, particularly given some observed differences in food cravings (Souza et al., 2018), energy intake (McNeil & Doucet, 2012) and diet composition throughout the menstrual cycle (Gorczyca et al., 2016).

Strengths

Despite these limitations, the present study was strengthened by the participant recruitment, sample collection, and objective measures of hydration status. First, our sample, while limited to males, included individuals from diverse racial and ethnic backgrounds, enhancing the generalizability of these findings to multiple racial and ethnic groups. Our participant pre-screening strategy enabled us to capture participants across a wide range of habitual fluid consumption behaviors and, by extension, a range of hydration statuses. Next, we successfully obtained saliva samples for several timepoint throughout the day, enabling us to capture a range of HPA axis indices from both the morning (CAR), and the whole day (C_{peak} and DCS), which has not been previously investigated in relation to hydration status. Our methodology also allowed participants the ability to collect these samples on their own after instruction on proper collection protocols from the investigators. The use of salivary collection provided us with a better representation of the free, active form of cortisol rather than total cortisol, where the latter may have some bound to the binding protein CBG when measured from serum.

Conclusion

Hydration status, as assessed by the prior day's 24h urinary osmolality, is not associated with multiple indices of HPA axis activity. However, a flatter diurnal cortisol slope, an indicator of HPA axis dysfunction, is associated with a greater food reward from high fat, sweet foods and

high fat, savory foods in young adult males. Future analyses will examine the association between copeptin and the pattern of cortisol release.

CHAPTER VI: CONCLUSIONS AND FUTURE DIRECTIONS

To clarify the associations between underhydration and obesity, this dissertation examined the relationship between chronic fluid intake and physiological and behavioral components associated with energy balance in individuals across a range of habitual fluid intakes.

In Aim 1 (Chapter 3) we assessed the association between habitual fluid intake and both energy intake and energy expenditure. We observed a negative association between habitual fluid intake and postprandial respiratory exchange ratio, whereby increased fluid intake was associated with expending more calories from fat following a standard meal. We also observed a positive relationship between habitual fluid intake and resting metabolic rate, where an additional liter of fluid intake was associated with an extra 69 kcals burned at rest. The effect on postprandial RER observed in our study equate to ~5-6% higher postprandial fat oxidation (Carpenter, 1921) per liter increase in fluid intake. For some individuals, these effects could be the difference between weight gain compared to weight maintenance or weight loss. Similarly, we observed a positive association between habitual fluid intake and physical activity energy expenditure. Despite these differences, overall energy balance was not influenced by daily fluctuations in fluid intake or differences in mean fluid intake between individuals. Under free-living conditions, energy compensation may be occurring to offset these effects, likely through increased EI.

In Aim 2 (Chapter 4) we examined the association between habitual fluid intake and hedonic and homeostatic mechanisms contributing to energy intake. Fasting and postprandial appetite ratings were similar across a range of fluid intakes. Future cross-sectional studies in healthy, young adult males may not need to control for habitual fluid intake when assessing

changes in appetite. However, greater fluid intake was associated with a lower food reward from high fat, sweet foods. While it may simply be that individuals who are more health conscious tend to both drink more water and have reduced preferences for foods higher in fat and sugar, additional studies should seek to examine the influence of increased fluid intake on food reward.

In Aim 3 (Chapter 5) we explored the associations between hydration status and indicators of HPA axis activity. While urinary osmolality was not associated with the cortisol awakening response or diurnal cortisol slope, higher urinary osmolality from the next day was associated with lower peak cortisol from the previous morning. The mechanisms for this are unclear but point to discrepancies in the relationship between hydration status and cortisol which may depend on the hydration biomarker used, medium used to assess cortisol (i.e., serum versus saliva), and the time course of measurement collection in relation to different cortisol samples. However, there was an association between flatter DCS and greater explicit liking of HFSW foods and greater explicit wanting of HFSA foods.

Rationale and Potential Impact

While the present findings cannot directly establish a cause-effect relationship, they may have important implications for practice as well as future research. However, it should be noted that these findings are presently limited in their application to young, healthy males, with the potential for results to differ across the lifespan, between differences in biological sex, and in clinical populations. Findings from Aim 1 suggest increased fluid intake may be a promising adjunct to existing weight management and weight loss strategies, both through increased resting metabolic rate and increased postprandial fat oxidation. This provides additional rationale for promoting adequate water intake in a largely underhydrated population (Ferreira-Pêgo et al., 2015). The joint effects of increased fluid intake and exercise and/or other dietary interventions

may assist with improvements in metabolic health. Specifically, increasing plain water intake should support these effects without the added caloric load from energy-yielding beverages and could help alleviate the expected decline in resting metabolic rate expected with weight loss. Additionally, studies should assess the effects of a water intake intervention in addition to a traditional weight loss program (reduction in kcals alone) on changes in metabolism and body mass over time in underhydrated individuals.

Our findings from Aim 2 suggest a connection between fluid intake and food reward. It was somewhat surprising that the association between greater fluid intake and reduced preference for high fat, sweet foods was present for total fluid intake rather than plain water alone, especially when many other beverages captured by the study are likely to be sweeter (i.e., sugar-sweetened beverages, milks, juices). While our data do not support a direct effect of increasing fluid intake on food reward, this may be a promising area of future study. It may be that altered fluid intake may interact with pathways associated with taste perceptions, though this will require further experimental investigation. It may also be that individuals who drink more fluid tend to naturally prefer foods lower in energy density, unrelated to the physiological effects of fluid intake itself. Regardless, results for this aim provide preliminary evidence for a synergy between this health behavior and hedonic responses.

Our findings from Aim 3 suggest no relationship between hydration status as assessed by 24h urinary osmolality and indices of HPA axis activity (C_{peak}, CAR, DCS). Our results suggest that future cross-sectional studies assessing the CAR may not need to control for 24h urinary hydration status; thus, this additional participant burden may be omitted–at least in young healthy males. This does not rule out the potential for other more acute indicators of hydration status influencing these indices (i.e., copeptin, plasma osmolality). However, the timing between

these measures and cortisol assessments warrants further study, inclusive of the number of measurements taken for each index (hydration status and cortisol) and controlling for additional confounders (such as total sleep time for CAR). Findings from this aim also warrant investigation into the utility of strategies that impact DCS, with hopes that this may translate to less food reward from foods high in fat and sugar content or high in fat and salt content.

Lessons Learned

The insights obtained from this project provide valuable considerations for future research. Each component of this dissertation included numerous measurements and prolonged participation time that was burdensome on both the participants and researchers. Overall, the complete study included a 6-hour laboratory visit preceded by three days of 24h urine collection, food and fluid recording, and physical activity monitoring. The burden of this protocol likely affected our ability to obtain complete data for all participants for each aim. Particularly, observations during the second three days of food and fluid recording were missed most frequently, perhaps because of the longer delay in direct contact between researcher and participant (though reminders were provided via email) and perhaps related to fatigue from the rigorous lead-in protocol to the in-lab visit (three days of urine collection, food and fluid recording, activity monitoring) in addition to the 6-hour in lab visit itself. We may have obtained more complete data across participants by reducing the timeframe for the laboratory visit and instead placing more emphasis on reinforcing accurate reporting of food and fluid intake.

In hindsight, it may also have been more advantageous to fully control some parameters such as vigorous activity to capture a more accurate representation of differences in participant metabolism at different levels of habitual fluid intake; this would have also permitted better control of an additional factor impacting the pattern of cortisol secretion. However, this likely

would have been at the expense of our ability to capture habitual energy balance parameters across participants. A remedy for this may have been to include an additional day in between habitual fluid intake measures, whereby participants would collect urine and record food and fluid intake for an additional day prior to the laboratory visit with vigorous physical activity limited for only that day. Then the day prior to metabolic and cortisol measurements could be omitted from energy balance assessments but would still provide insight into the relationship between the previous day's fluid intake, hydration status, metabolism and indices of HPA axis activity.

The limited staff on this project (primarily the principal investigator and one assistant for most visits), made this study particularly intense for all involved with data collection. Future investigations of this scope will seek to obtain additional resources to recruit and train additional staff to facilitate the data collection process. Alternatively, where this is not possible, efforts will be made to simplify protocol design to ease burden both on the participants and the staff involved in data collection. For example, a future design could remove the TEF and appetite assessment protocol from the laboratory testing day, or reserving assessment of TEF to a separate, individual study without the inclusion of pre- or post-trial monitoring of food, fluids, and urinary hydration status. While the relationship between habitual fluid intake and postprandial RER in the present study was interesting, the rise in post-prandial metabolic rate was consistent with other estimations suggesting an 8-15% postprandial increase in metabolism following meal consumption (~11% in our study) (von Loeffelholz & Birkenfeld, 2000). Thus, TEF could be estimated as an assumed 11% increase in metabolic rate rather than directly measured to alleviate the extra three hours of participant laboratory time required for this measurement. The TEF obtained in our study is also an *average estimate* of response to meals

under free-living conditions since the TEF is influenced by factors such as meal size and macronutrient composition (Calcagno et al., 2019), which will vary person-to-person and meal to meal. This would reduce participant burden so that approximately 1 hour would be required for the in-lab assessment day, which would allow for collection of multiple participants on the same day. The modified protocol would also remove the in-lab lunch component of the study but could retain the 24h of provided food component as a direct measurement of food intake. However, given the large discrepancy in kcal consumption between the laboratory provided foods and self-report metrics, the role that additional psychological factors played in influencing food consumption behaviors such as convenience and cost should be assessed following both types of dietary reporting measures (self-report vs provided foods).

Future Directions

This dissertation provides support for future investigations in this area. First, future studies may seek to directly examine the effect of increased fluid intake on resting and postprandial metabolism in underhydrated individuals, including assessment of potential mechanisms such as increased sympathetic nervous system activity (i.e., increased epinephrine or norepinephrine) which has only been explored following acute water consumption (Çıtar Dazıroğlu & Acar Tek, 2023). Secondly, additional studies could test the efficacy of a chronic fluid intake intervention (i.e., increased fluid intake for 6-12 weeks) on food reward, dietary intake, and body weight change. A longitudinal design assessing participant fluid and dietary intake at 3-month intervals over the course of a year may identify more subtle differences in the relationship between these behaviors over time that may not have been captured by the week-long dietary assessment in the present study.

Third, more precise measurements of the timing between cortisol measurements and both hydration status and fluid intake may be necessary to explain discrepancies observed between fluid intake, hydration, and HPA axis activity, when controlling for physical activity throughout the entirety of the measurement period (Moyers & Hagger, 2023) and with consideration for individual psychological stressors. In a water intake intervention among seven low-drinkers, Seal observed an overall significant reduction in cortisol over 11 hours with an acute increase in water intake (Seal et al., 2021). Although post hoc pairwise differences between individual timepoints for each fluid intake period (usual intake vs recommended fluid intake) were not statistically significant, the most pronounced difference in cortisol between high and lower water intake appeared to begin around 1pm and persisted through 6pm, times when both cortisol and AVP should naturally be lower. This suggests that perhaps the morning rise (CAR) and subsequent fall in cortisol concentration into the afternoon may be robust against minor disturbances in fluid balance, but the early evening period may be more responsive to fluid modification.

Our study design could not capture the relationship between within-day changes in hydration status and the pattern of cortisol release, since 24h urine collection from the day prior included the CAR (wake, 30 min, 45 min samples) but not the entirety of the DCS samples, whereas the 24h urine collection following the laboratory visit only overlapped with a portion of the DCS samples (7pm and bedtime cortisol samples). These findings may be rectified with adjusted collection and reporting procedures. For one, dividing the urine collection time periods into 12-hour periods (wake – noon and noon-bed), at the very least, could tease out the potential differences in the relationship between hydration status and cortisol captured during the morning compared to the evening period. Alternatively, for a more comprehensive assessment, each void throughout the day could be collected and measured separately before combining into a final 24h

sample to capture both the temporal fluctuations of urinary osmolality in relation to cortisol dynamics as well as the complete 24h profile of each measure. Additionally, participants could record the time they collected their samples on the instruction sheet for sample collection; this was not captured in the present study but was estimated based on actigraphy-derived wake times, but we cannot be certain these align with the prescribed collection times. Novel technologies could be used to capture this information while reducing participant burden, such as the utilization of "smart water bottles" (Cohen et al., 2022) to directly estimate fluid consumption, and the use of mobile devices to capture dietary intake (Bekelman et al., 2022). Thus, a simplified design aiming to maximize the amount of data collected while minimizing participant burden could include three days of 24h urine collection, each with at-home measures of CAR (0, 30, and 45 minutes) and additional saliva samples collected at noon and immediately prior to bed (to assess DCS). This protocol would allow us to better assess both between- and within-person differences in salivary cortisol responses using an analysis similar to Aim 1 for the daily energy balance changes. Participants would not need to record fluid in this protocol, as this would be captured by the smart water bottle and time-stamped for each fluid consumption period. This would then allow one to examine the within-day influence of acute fluid intake preceding each cortisol collection period (i.e., fluid consumed up to the point of collection) on cortisol changes, as well as the overall daily effect. The separate days of collection would also determine whether there is a lag in the daily relationship between the hormonal changes and urinary hydration biomarkers. It may also be that the relationship between habitual fluid intake and cortisol responses is either more subtle than first anticipated or requires a certain threshold of underhydration to observe a difference. The threshold effect has been described with regard to exercise, whereby lower intensity exercise reduces circulating cortisol concentrations, but

moderate-high intensity exercise (60-80% of VO2max) increases cortisol (E. E. Hill et al., 2008) Thus, a future study could seek to establish a dose-response between increases in habitual fluid intake (and changes in the corresponding hydration biomarkers 24h urinary osmolality and copeptin) and HPA axis indices through the provision of 6 weeks of increased fluid intake (similar to a previous intervention targeting glucose reduction (Enhörning et al., 2021)) in underhydrated individuals (24h Urinary osmolality < 500mOsm*kg^{-1}) at varying dosages (i.e., an increase of 0.5L, 1L, or 1.5L on top of typical intake) compared to repeated measurements of these variables in a control condition. This intervention would also measure individual perceived stress levels as a potential confounding variable (van Eck et al., 1996).

Last, the results from the present study should be verified and expanded upon through investigation into other segments of the population. Specifically, perhaps females would display differential effects on metabolism and behavior in response to differences in fluid intake, as the female sex hormones estrogen and progesterone are also known to influence body water regulation (Giersch et al., 2021). Future work should also investigate these relationships in older individuals, who maintain both a reduced thirst response and a lower metabolism associated with aging (Kenney & Chiu, 2001). Clinical populations may see benefit from inclusion in studies of this nature as well, particularly conditions have shown some associations with hydration status (i.e., diabetes and chronic kidney disease) (Enhörning, Brunkwall, et al., 2019; Enhörning et al., 2021; Jansen et al., 2019; Johnson et al., 2017; Riphagen et al., 2013).

While many more questions have been raised, overall, this dissertation provides support for further exploration into the connections between habitual fluid intake and health (Armstrong et al., 2020; E. T. Perrier et al., 2020), particularly with regard to metabolism and food reward.

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Table A45. Total Fluid Intake Tertiles Category as a Predictor of Metabolic Measurements.

	Dependent variable:			
	RMR	RMR RER	TEFavg	TEF RERavg
	(1)	(2)	(3)	(4)
Moderate Fluid§	224.4**	-0.04	-2.13	-0.06***
	(44.0, 404.8)	(-0.09, 0.01)	(-121.7, 117.5)	(-0.10, -0.02)
High Fluid§	252.8**	-0.04	-62.5	-0.07***
	(72.4, 433.2)	(-0.09, 0.01)	(-182.1, 57.1)	(-0.11, -0.04)
Constant	1,689.3***	0.86***	241.7***	0.92***
	(1,561.8, 1,816.8)	(0.82, 0.89)	(157.2, 326.3)	(0.89, 0.95)
Observations	27	27	27	27
R^2	0.274	0.111	0.053	0.408
Adjusted R^2	0.213	0.037	-0.025	0.359
Residual Std. Error (df = 24)	195.239	0.056	129.437	0.040
F Statistic (df = 2; 24)	4.528**	1.495	0.677	8.275***

Note: *p<0.1**p<0.05***p<0.01

§Low Fluid used as reference category.

Table A46. BEVQ-15 Average Fluid Intake as a Predictor Of Pre-Lunch Hunger and Thirst Ratings.

	Dependent variable:									
	Desire	Hungry	Full	How much	Thirsty	Pleasant	Dryness	Taste	Fullness Thirst	Sick
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
BEVQ15 (L)	5.93	5.43	-7.24	9.66**	3.85	1.97	-2.06	2.13	-7.24*	-5.39
	(-2.45, 14.32)	(-0.29, 11.14)	(-15.25, 0.76)	(4.05, 15.27)	(-1.62, 9.32)	(-3.39, 7.34)	(-13.29, 9.17)	(-9.90, 14.16)	(-15.25, 0.76)	(-13.78, 3.00)
Constant	58.78	47.43	36.61	46.29	62.39	74.83	58.91	28.05	36.61	25.62
	(39.94, 77.62)	(34.60, 60.26)	(18.63, 54.59)	(33.70, 58.89)	(50.09, 74.68)	(62.78, 86.88)	(33.69, 84.14)	(1.03, 55.07)	(18.63, 54.59)	(6.78, 44.47)
Observations	28	28	28	28	28	28	28	28	28	28
R^2	0.07	0.12	0.11	0.30	0.07	0.02	0.005	0.005	0.11	0.06
Adjusted R^2	0.03	0.08	0.07	0.28	0.03	-0.02	-0.03	-0.03	0.07	0.02
Residual Std. Error (df = 26)	19.11	13.02	18.24	12.78	12.47	12.22	25.58	27.40	18.24	19.12
F Statistic (df = 1; 26)	1.92	3.46*	3.14*	11.40***	1.90	0.52	0.13	0.12	3.14*	1.59

Note: *p<0.1 **p<0.05 ***p<0.01

Table A47. BEVQ-15 Average Fluid Intake as a Predictor of the AUC for Hunger and Thirst Ratings.

	Dependent variable:									
	Hungry AUC	How much AUC	Desire AUC	Full AUC	Thirsty AUC	Full AUC thirst	Dry AUC	Taste AUC	Pleasant AUC	Sick AUC
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
BEVQ15 (L)	1,094.760	1,358.057	1,314.367	-678.012	119.267	-788.709	-1,030.760	787.601	-160.761	75.832
	(-40.420, 2,229.939)	(372.370, 2,343.744)	(51.531, 2,577.204)	(-1,793.738, 437.713)	(-1,030.152, 1,268.686)	(-2,026.399, 448.980)	(-2,554.101, 492.581)	(-462.688, 2,037.891)	(-1,259.947, 938.426)	(-882.687, 1,034.352)
Constant	6,933.779	8,303.206	7,364.805	8,440.485	11,284.070	8,279.299	10,273.770	2,322.134	13,637.620	1,630.981
	(4,424.231, 9,443.327)	(6,124.142, 10,482.270)	(4,573.045, 10,156.570)	(5,973.944, 10,907.020)	(8,743.042, 13,825.100)	(5,543.132, 11,015.470)	(6,906.110, 13,641.430)	(-441.888, 5,086.156)	(11,207.640, 16,067.600)	(-488.024, 3,749.986)
Observations	27	27	27	27	27	27	27	27	27	27
R^2	0.125	0.226	0.143	0.054	0.002	0.059	0.066	0.057	0.003	0.001
Adjusted R^2	0.090	0.195	0.108	0.016	-0.038	0.021	0.028	0.020	-0.037	-0.039
Residual Std. Error (df = 25)	2,522.115	2,189.977	2,805.741	2,478.892	2,553.753	2,749.869	3,384.523	2,777.864	2,442.148	2,129.617
F Statistic (df = 1; 25)	3.573*	7.292**	4.161*	1.419	0.041	1.560	1.759	1.524	0.082	0.024

Note: *p<0.1**p<0.05***p<0.01

Table A48. Mean AUC Ratings For Hunger and Thirst Throughout the Laboratory Visit.

	Overall (N=27)
HungryAUC	
Mean (SD)	9170 (2640)
Median [Min, Max]	9030 [4140, 14700]
FullAUC	
Mean (SD)	7050 (2500)
Median [Min, Max]	7100 [2310, 11500]
HowmuchAUC	
Mean (SD)	11100 (2440)
Median [Min, Max]	11100 [6270, 16800]
DesireAUC	
Mean (SD)	10100 (2970)
Median [Min, Max]	9850 [4170, 16300]
ThirstyAUC	
Mean (SD)	11500 (2510)
Median [Min, Max]	12000 [5810, 17600]
PleasantAUC	
Mean (SD)	13300 (2400)
Median [Min, Max]	13600 [9380, 17900]
DryAUC	
Mean (SD)	8170 (3430)
Median [Min, Max]	8520 [975, 15300]
TasteAUC	
Mean (SD)	3930 (2810)
Median [Min, Max]	3840 [139, 11200]
FullAUCthirst	
Mean (SD)	6670 (2780)
Median [Min, Max]	6590 [1710, 12600]

Table A49. BEVQ-15 Average Fluid Intake as a Predictor of Baseline Liking (ML) and Wanting (MW) of Food Categories From the LFPQ.

	Dependent variable:							
	ML HFSW	MW HFSW	ML HFSA	MW HFSA	ML LFSW	MW LFSW	ML LFSA	MW LFSA
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
BEVQ15 (L)	-0.477	-0.066	4.873*	5.246**	-0.741	-1.634	-1.012	0.545
	(-11.138, 10.185)	(-10.884, 10.753)	(0.099, 9.646)	(0.369, 10.124)	(-7.028, 5.545)	(-8.199, 4.931)	(-6.421, 4.397)	(-5.237, 6.327)
Constant	60.484***	59.060***	67.967***	65.861***	78.192***	79.871***	73.311***	67.764***
	(36.916, 84.053)	(35.143, 82.977)	(57.415, 78.519)	(55.078, 76.643)	(64.295, 92.090)	(65.357, 94.385)	(61.354, 85.269)	(54.982, 80.547)
Observations	27	27	27	27	27	27	27	27
R^2	0.0003	0.00001	0.138	0.151	0.002	0.009	0.005	0.001
Adjusted R^2	-0.040	-0.040	0.104	0.117	-0.038	-0.030	-0.034	-0.039
Residual Std. Error (df = 25)	23.686	24.037	10.605	10.837	13.967	14.586	12.017	12.846
F Statistic (df = 1; 25)	0.008	0.0001	4.003*	4.444**	0.053	0.238	0.135	0.034

Note: *p<0.1**p<0.05***p<0.01

Table A50. BEVQ-15 Average Fluid Intake as a Predictor Of Implicit Wanting (FWA) of Food Categories From the LFPQ.

	Dependent variable:			
	FWA HFSW	FWA HFSA	FWA LFSW	FWA LFSA
	(1)	(2)	(3)	(4)
BEVQ15 (L)	4.106	5.240	-7.613	-1.733
	(-9.075, 17.288)	(-5.028, 15.508)	(-21.118, 5.892)	(-12.085, 8.619)
Constant	-32.954**	11.327	17.285	4.342
	(-62.095, -3.814)	(-11.373, 34.027)	(-12.572, 47.141)	(-18.543, 27.228)
Observations	27	27	27	27
R^2	0.015	0.038	0.047	0.004
Adjusted R^2	-0.025	0.00001	0.008	-0.036
Residual Std. Error (df = 25)	29.286	22.814	30.006	23.000
F Statistic (df = 1; 25)	0.373	1.000	1.221	0.108
Note:				*p<0.1**p<0.05***p<0.01

Table A51. Additional Analyses of Interactions Between 24h Urinary Osmolality and Diurnal Cortisol Slope When Controlling for Total Time Awake and Total Score on the rMEQ. All Variables are Grand-Mean Centered.

	Cortisol Concentration (ng/mL)					
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	9.674	6.313 – 13.035	**<0.001**	9.499	6.182 – 12.815	**<0.001**
Time (hours)	-0.555	-1.189 – 0.078	0.085	-1.546	-3.298 – 0.206	0.083
U_{OSMO}	-0.011	-0.028 – 0.006	0.194	-0.010	-0.026 – 0.007	0.237
Time Awake (hours)	-0.748	-2.097 – 0.601	0.273	-0.528	-1.867 – 0.810	0.434
rMEQ (Total Score)	-0.135	-1.033 – 0.763	0.766	-0.225	-1.124 – 0.674	0.619
Time * U_{OSMO}	0.000	-0.003 – 0.003	0.973	-0.004	-0.014 – 0.007	0.491
$Time^2$				-0.200	-0.521 – 0.122	0.220
$Time^2$ * U_{OSMO}				-0.001	-0.003 – 0.001	0.418
Random Effects						
σ^2	44.61			44.45		
τ_{00}	21.46 $_{Subject}$			19.87 $_{Subject}$		
ICC	0.32			0.31		
N	15 $_{Subject}$			15 $_{Subject}$		
Observations	85			85		
Marginal R^2 / Conditional R^2	0.120 / 0.406			0.142 / 0.407		
AIC	609.353			624.147		

Table A52. The Relationship Between The Previous Day's 24h U_{OSMO} and Change in Cortisol Concentration to Determine DCS, When Controlling For Actigraph Activity Counts From the Day Prior and/or Total Sleep Time (TST). All Variables are Grand-Mean Centered.

	Cortisol Concentration (ng/mL)								
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	4.748	1.687 – 7.809	**0.003**	8.877	-0.481 – 18.235	0.063	5.877	-3.015 – 14.768	0.193
Time (hours)	-0.910	-1.372 – -0.448	**<0.001**	-0.827	-1.384 – -0.270	**0.004**	-0.842	-1.399 – -0.285	**0.003**
U_{OSMO}	0.003	-0.007 – 0.012	0.579	0.002	-0.012 – 0.017	0.746	0.003	-0.011 – 0.017	0.686
Activity Counts $*10^3$	0.004	-0.000 – 0.008	0.060				0.005	0.000 – 0.011	**0.036**
Time * U_{OSMO}	0.000	-0.002 – 0.002	0.795	0.000	-0.002 – 0.003	0.762	0.000	-0.002 – 0.003	0.795
TST (minutes)				-0.005	-0.037 – 0.026	0.742	-0.005	-0.034 – 0.023	0.708
Random Effects									
σ^2	37.46			44.64			44.80		
τ_{00}	11.73 $_{Subject}$			21.97 $_{Subject}$			15.86 $_{Subject}$		
ICC	0.24			0.33			0.26		
N	27 $_{Subject}$			18 $_{Subject}$			18 $_{Subject}$		
Observations	128			102			102		
Marginal R^2 / Conditional R^2	0.148 / 0.351			0.065 / 0.373			0.148 / 0.370		
AIC	891.531			734.665			742.679		

Table A53. The Relationship Between 24h U_{OSMO} from the Previous Day and CAR, When Controlling for Actigraph Activity Counts from the Day Prior and/or Total Sleep Time (TST). Models Were Run With (Model 1, Model 3, Model 5) and Without an Extreme Outlier (Model 2, Model 4, Model 6). All Variables are Grand-Mean Centered.

	Dependent variable:					
	CAR					
	(1)	(2)	(3)	(4)	(5)	(6)
U_{OSMO}	-0.008	-0.005	-0.006	0.0005	-0.004	0.0003
	(-0.025, 0.009)	(-0.020, 0.009)	(-0.030, 0.019)	(-0.015, 0.016)	(-0.026, 0.017)	(-0.015, 0.016)
Activity Counts*10^3	0.005	-0.001			0.012**	0.004
	(-0.004, 0.015)	(-0.010, 0.008)			(0.002, 0.022)	(-0.004, 0.012)
TST (minutes)			-0.053	-0.059**	-0.053*	-0.058**
			(-0.117, 0.012)	(-0.099, -0.019)	(-0.110, 0.004)	(-0.098, -0.019)
Constant	7.328**	9.297***	9.633***	7.198***	3.235	5.333*
	(0.893, 13.763)	(3.731, 14.863)	(4.195, 15.071)	(3.740, 10.657)	(-4.091, 10.560)	(0.144, 10.522)
Observations	27	26	18	17	18	17
R^2	0.075	0.026	0.145	0.404	0.375	0.443
Adjusted R^2	-0.002	-0.059	0.031	0.319	0.241	0.314
Residual Std. Error	10.777 (df = 24)	9.107 (df = 23)	11.345 (df = 15)	6.946 (df = 14)	10.039 (df = 14)	6.971 (df = 13)

F Statistic	0.975 (df = 2; 24)	0.309 (df = 2; 23)	1.271 (df = 2; 15)	4.746** (df = 2; 14)	2.801* (df = 3; 14)	3.440** (df = 3; 13)

Note: *p<0.1**p<0.05***p<0.01

Table A54. The Relationship Between The Next Day's 24h U_{OSMO} and C_{peak}, When Controlling For Actigraph Activity Counts From the Day Prior and/or Total Sleep Time (TST). Models Were Run With (Model 2, Model 5) and Without an Extreme Outlier (Model 4, Model 6). All Variables are Grand-Mean Centered.

	Dependent variable:					
	C_{peak}					
	(1)	(2)	(3)	(4)	(5)	(6)
U_{OSMO}	-0.021***	-0.013	-0.010*	-0.009	-0.011	-0.008
	(-0.036, -0.007)	(-0.028, 0.002)	(-0.021, 0.001)	(-0.025, 0.007)	(-0.034, 0.012)	(-0.025, 0.008)
Activity Counts*10^3		0.005	-0.001		0.009	0.002
		(-0.003, 0.013)	(-0.008, 0.005)		(-0.002, 0.020)	(-0.007, 0.011)
TST (minutes)				-0.023	-0.014	-0.022
				(-0.062, 0.016)	(-0.071, 0.042)	(-0.063, 0.018)
Constant	21.166***	18.154***	20.063***	18.309***	15.366***	17.404***
	(17.590, 24.742)	(12.751, 23.557)	(15.920, 24.205)	(14.817, 21.801)	(7.483, 23.249)	(11.711, 23.097)
Observations	29	27	26	17	18	17
R^2	0.234	0.158	0.120	0.138	0.272	0.148
Adjusted R^2	0.206	0.087	0.043	0.015	0.116	-0.048
Residual Std. Error	9.826 (df = 27)	9.027 (df = 24)	6.776 (df = 23)	7.031 (df = 14)	10.213 (df = 14)	7.251 (df = 13)
F Statistic	8.264*** (df = 1; 27)	2.245 (df = 2; 24)	1.562 (df = 2; 23)	1.118 (df = 2; 14)	1.743 (df = 3; 14)	0.755 (df = 3; 13)
Note:						*p<0.1**p<0.05***p<0.01

Table A55. Relationships Between 24 U_{OSMO} From the Day Prior and Total CAR (Sum of Wake, 30, and 45 Minute Salivary Cortisol Samples). Analysis Run With and Without Controlling for Activity Counts and/or Total Sleep Time (TST) From the Previous Night.

	Dependent variable:			
	Total CAR			
	(1)	(2)	(3)	(4)
U_{OSMO}	-0.012	-0.008	-0.006	-0.004
	(-0.043, 0.020)	(-0.040, 0.025)	(-0.057, 0.045)	(-0.052, 0.044)
Activity Counts*10^3		0.011		0.019
		(-0.007, 0.029)		(-0.004, 0.042)
TST (minutes)			-0.029	-0.029
			(-0.163, 0.106)	(-0.157, 0.098)
Constant	41.495***	36.567***	38.677***	28.354***
	(33.860, 49.131)	(24.334, 48.799)	(27.364, 49.990)	(12.034, 44.673)
Observations	28	27	18	18
R^2	0.021	0.060	0.012	0.172
Adjusted R^2	-0.017	-0.018	-0.120	-0.005
Residual Std. Error	20.586 (df = 26)	20.486 (df = 24)	23.602 (df = 15)	22.366 (df = 14)
F Statistic	0.545 (df = 1; 26)	0.772 (df = 2; 24)	0.092 (df = 2; 15)	0.970 (df = 3; 14)
Note:				*p<0.1**p<0.05***p<0.01

APPENDIX B: DATA COLLECTION TOOLS

Food Menu

☐ Plain Bagel
☐ Whole Wheat Bagel
☐ White Bread
☐ Whole Wheat Tortilla

☐ Orange
☐ Apple
☐ Banana
☐ Strawberries
☐ Pineapple Cups

☐ Raisin Bran
☐ Corn Flakes
☐ Honey Nut Cheerios
☐ Oats and Honey Granola

☐ Nature Valley Granola Bar
☐ Strawberry Nutrigrain Bar
☐ KIND Bar – Dark Chocolate

☐ Skittles
☐ Kit Kat
☐ Hershey Dark Chocolate Kisses
☐ Oreos
☐ Lays Plain Chips
☐ Goldfish Crackers
☐ Almonds
☐ Triscuits
☐ Smartfood Popcorn

☐ Chobani Strawberry Non-fat Greek Yogurt
☐ Chobani Vanilla Non-fat Greek Yogurt
☐ Cottage Cheese

☐ Baby Peppers
☐ Cherry Tomatoes
☐ Baby Carrots
☐ Cucumber

☐ Cheddar Cheese
☐ Swiss Cheese
☐ Deli Ham
☐ Deli Turkey

☐ Four Cheese Pizza
☐ Meat Lasagna
☐ Creamy Spinach & Tomato pasta
☐ Chicken Parmesan
☐ Chicken Lo Mein
☐ Beef & Broccoli

☐ Creamy Peanut Butter
☐ Plain Cream Cheese
☐ Strawberry Jam
☐ Butter
☐ Mustard
☐ Mayonnaise
☐ Ranch Dressing
☐ Hummus - Plain

☐ Water
☐ 2% Milk
☐ Chocolate Milk
☐ Apple Juice
☐ Orange Juice
☐ Coca Cola

Appetite Scales

How strong is your desire to eat?

Very weak _____ Very strong

How hungry do you feel?

Not hungry at all _____ As hungry as I have ever felt

How full do you feel?

Not full at all _____ Very full

How much food do you think you could eat?

Nothing at all _____ A large amount

Thirst Scales

How thirsty do you feel right now?

Not at all thirsty _____ Very Thirsty

How pleasant would it be to drink some water right now?

Very unpleasant _____ Very pleasant

How dry does your mouth feel right now?

Not at all dry _____ Very dry

How would you describe the taste in your mouth?

Normal _____ Very unpleasant

How full does your stomach feel right now?

Not at all full _____ Very full

How sick to your stomach do you feel right now?

Not at all sick _____ Very sick