Energy Intake and Appetite Responses Following Manipulation of Fluid Balance and Intake

by

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CERTIFICATE OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this thesis, that the original work is my own except as specified in acknowledgments or in footnotes, and that neither the thesis nor the original work contained therein has been submitted to this or any other institution for a degree.

....(Signed)

....(Date)

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Abstract

Fluid intake and regulation are implicated in the control of energy balance and appetite. The studies in this thesis have examined the effects of fluid manipulation on appetite and energy intake. Fifty-eight young, predominantly Caucasian males were recruited to five studies. The age, height and body mass of the subjects were: 24.9 ± 3.8 y, 1.79 ± 0.1 m, 80.1 ± 14.8 kg (mean $\pm$ SD) respectively.

In Chapter 3, 13 h of hypohydration after exercise in the heat did not influence energy intake at an *ad-libitum* buffet meal ($P=0.436$) compared to a euhydrated trial, although greater thirst ($P<0.001$) and lower fullness ($P<0.01$) was reported in the hypohydration trial.

Chapter 4 demonstrated that there was no difference in energy intake or appetite after 24 h of hypohydration either with or without fluid during a semi-solid *ad-libitum* breakfast. Thirst and fluid intake were greater during the hypohydrated with fluid (HYPO-F; 618 (251) mL) than the euhydrated with fluid (EU-F; 400 (247) mL) trials ($P<0.01$).

Chapter 5 and 6 showed that a bolus of water (500 mL) immediately before an *ad-libitum* porridge breakfast reduced energy intake in both healthy and overweight and obese subjects *(P<0.001)*. The water preload increased fullness and decreased hunger compared to pre-trial in both studies (P<0.001).

In Chapter 7, 75 minutes before an *ad-libitum* lunch a post-exercise milk (MILK) based drink reduced energy intake (6746 (2035) kJ) compared to an isoenergetic flavoured carbohydrate (CHO) and water based drink (7762 (1921) kJ; 7672 (2005) kJ) ($P<0.05$).

This thesis has shown that when subjects are hypohydrated, either after exercise or after 24 h of fluid restriction energy intake is not different at an *ad-libitum* meal. However, there is an increased thirst and subsequent fluid intake before an *ad-libitum* meal (chapter 3 and 4). This effect was more acutely displayed when a bolus of water was provided immediately before an *ad-libitum* breakfast meal and subsequently decreased energy intake in both normal and overweight/ obese subjects (chapter 5 and 6). The possible mechanism for this was gastric fill and distension creating satiety before a meal. Chapter 7 has showed that when subjects consume isoenergetic drinks with different energy densities (milk vs CHO

and water), before an *ad-libitum* lunch, energy intake was decreased when milk was consumed. Milk having an increased energy density due to larger protein fractions (casein) may further explain the decrease in energy intake found in chapters 5 and 6 by a similar mechanism. Therefore, gastric fill before a meal decreases *ad-libitum* energy intake by either the intake of water immediately before a meal or by milk as a more delayed response (75 min). The hydration status however, did not affect energy intake directly in our finding, although it did affect subsequent fluid ingestion, which may have affected findings in chapters 3 and 4.

Key words: Fluid restriction, appetite, energy intake, hypohydration, exercise, dehydration.

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List of Publications and Presentations

Papers from the following studies have been peer reviewed and have been accepted
for publication as follows:

- Corney RA, Sunderland C, James LJ. The Effect of Hydration Status on Appetite and Energy Intake. Journal of Sports Sciences 2015;33(8):761-768 (Chapter 3).
- Corney RA, Horina A, Sunderland C, James LJ. Effect of Hydration Status and Fluid Availability on *Ad-Libitum* Energy Intake of a Semi-Solid Breakfast. Appetite, 2015;91:399-404 (Chapter 4).
- Corney RA, Sunderland C, James LJ. Immediate Pre-Meal Water Ingestion Decreases Voluntary Food Intake in Lean Young Males. European Journal of Nutrition, 2016;55(2):815-819 (Chapter 5).

Abstracts from the following studies have been peer reviewed and have been accepted
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- Effect of hydration status on appetite and energy intake International Sport and Exercise Nutrition Conference – Newcastle 2012 (poster presentation) (Chapter 3).
- Effect of Hydration Status and Fluid Availability on *ad-libitum* energy intake of a semi-solid breakfast. Nutrition Society Annual Summer Meeting - Newcastle 2013 (poster presentation), (Chapter 4).
- The Effect a preload of water either immediately before a meal or *ad-libitum* during a meal on energy intake and subjective perceptions British Feeding and Drinking Group Conference – Portsmouth, UK 2015 (poster presentation), (Chapter 5).
- Pre-meal water ingestion reduces energy intake in overweight/ obese adults, British Feeding and Drinking Group Conference – Amsterdam, Netherlands 2015 (oral-presentation), (Chapter 6).
- Effect of Post-Exercise Skimmed Milk or Sucrose Drink Ingestion on Subsequent Appetite and Energy Intake, American College of Sports Medicine Conference – San Diego USA 2015 (oral presentation), (Chapter 7).

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List of Abbreviations

AgRP   (agouti-related peptide)

αMSU   (alpha-melanocyte-stimulating hormone)

ANOVA   (analysis of variance)

BCAA   (branched chain amino acids)

BMI   (body mass index)

BMR   (basal metabolic rate)

CCK   (Cholecystokinin)

EU   (euhydrated)

K_2EDTA   (potassium ethylenediaminetetraacetic acid)

g   (gram)

GLP-1   (glucagon-like-peptide 1)

h   (hour)

HYPO   (hypohydrated)

kg   (kilogram)

kJ   (kilojoule)

l   (litre)

m   (metre)

mg   (milligram)

min   (minute)

mL   (millilitre)

mmol   (millimole)

mosmol   (milliosmole)

NPY   (neuropeptide Y)

NF   (no fluid)

OXM   (oxyntomodulin)

Pg/mL   (pictograms per milliliter)

POMC   (pro-opiomelanocortin)

PP   (pancreatic polypeptide)

PYY   (peptide-YY)

RE   (rehydrated)

RPE   (rating of perceived exertion)

RPM   (revolutions per minute)

XV

RMR   (resting metabolic rate)

s   (second)

SD   (standard deviation)

SPQ   (subjective perceptions questionnaire)

SDQ   (subjective drink questionnaire)

VO$_2$ max   (maximum rate of oxygen uptake)

VO$_2$ peak   (peak rate of oxygen uptake

Chapter 1
General Introduction and Literature Review

Energy Balance

To function appropriately, the human body needs energy and the nutrients contained within food provide this energy (Yaniv, Rosin and Tobol, 2009). Energy is expended in the process of daily functioning and unspent energy is accumulated in the body, mainly in the form of fat tissues that increase body mass. Obesity theory owes its foundations to the study of energy (thermodynamics), whereby the first law asserts that the change in the internal energy of a closed system is equal to the amount of heat supplied to the system, minus the amount of work done by the system on its surroundings. Taking this law into account and applied to obesity, an individual will gain weight (energy) if the total energy consumed exceeds the total energy expended (Culter et al. 2003; Yaniv et al. 2009).

Basal metabolic rate (BMR) is the largest source of energy expenditure in the general population, which can be described as the rate at which a person expends energy to sustain life at complete rest (blood circulation, respiration and daily maintenance of body temperature) (Yaniv et al. 2009). Assessing energy expended at rest is important for establishing caloric prescriptions and must be determined accurately. The best predictor of 24-h energy expenditure is described by Mifflin et al. (1990) to be determined by indirect calorimetric measurement, which accounts for 65-70% of total 24-h energy expenditure. Resting metabolic rate (RMR) is determined by sex, age, weight and height, but the thermic effect of food and physical activity, which will account for the remaining 10-15% and 20-30% respectively are subject to choice and therefore changeable (Mifflin et al. 1990). Thermic effect of food and physical activity are highly changeable and can make the difference to a positive (excess energy), negative (minus energy) or neutral (equal energy) energy balance.

It has been proposed that as physical demands of an individuals job have decreased and car ownership and labour saving devices have increased, physical activity levels are lower and hence so is energy expenditure (Hardman and Stensel, 2009). Also, as poor food and drink choices have become more widely available and affordable, the strive for convenience and relief from hunger may supersede health objectives (Manchino and Kinsley, 2004). As foods that are convenient are commonly high in energy, fat, simple carbohydrates, cholesterol and sodium, an ancillary increase in food and drink more commonly than not results in excess

energy intake (Manchino and Kinsley, 2004). This therefore means that people seem to be getting heavier due to consuming more energy or expending less energy. The development of obesity therefore lends itself to the economic model of obesity and the aim for a person to lose weight would be to decrease energy.

Subjective Hunger and Appetite Perceptions

Appetite is defined as the sensory and qualitative aspects of eating and includes the responsiveness to both physiological and environmental influences (Blundell et al. 2010). Visual analogue scales are the most commonly used tool in appetite research and are strongly supported as the selected method to confirm the effects of perceptions of fullness and hunger and eating motivations (Blundell et al. 2010). Questionnaires are used to determine the degree of response to a selected question plotted on a horizontal line 100mm in length with descriptive statements at each end (Flint et al. 2000). The amount individuals consume is related to their motivational state and individuals eat more when they are hungry compared to when they are full. Hunger can be defined as the discomfort, weakness or pain caused by lack of food. With this, it is important that subjects start a meal in a similar state of hunger and fullness for repeatability (Blundell et al. 2010).

Satiation and Satiety

Satiation is defined as a short-term signal arising directly from the meal or food just consumed creating a feeling of fullness or being satisfied after eating. Satiety is the feeling or state of being satiated. Rolls and colleagues observed attenuation of the responses of the lateral hypothalamic neurons in response to the sight and smell of food in animals (Burton, Rolls and Mora, 1976; Rolls, Burton and Mora, 1976; Rolls, Burton and Mora, 1980). Drewnowski (1998) defines sensory specific satiety as reduced palatability of the just-consumed food relative to other foods. Therefore the supplying of food to excess or to fullness can cause satiety by decreasing the pleasantness of the food consumed. This response seems specific to the food consumed as when individuals eat one food to satiation, the pleasantness of the taste of that food decreases more than that of other foods tasted, but not eaten (Rolls, Rolls, Rowe and Sweeney, 1980). Therefore, this implies that more food will be eaten of a varied meal than of a single food item based meal and although variety is

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important for a nutritionally balanced diet for food assessment purposes, intake may be amplified with varied food based meals

Incidence of Obesity

Throughout the world, the incidence of obesity has increased by an epidemic proportion, to the point that in 2008 it was estimated that over 1.4 billion adults (20 years and older) were overweight (BMI of 25-30 kg·m^{-2}) and 500 million qualifying as obese (BMI >30 kg·m^{-2}) (World Health Organisation, 2012). This picture is not dissimilar in the United Kingdom (UK) as obesity rates have trebled since 1980 (National Audit Office, 2001). In England, 24.8% of adults are obese and 61.7% are either overweight or obese according to the (Health and Social Care Information Centre, 2013). If this trend continues, it is predicted 50% of the UK population could be deemed as being obese by 2030, which is worrying and must be addressed (Wang et al. 2011).

Susceptibility to an increased body mass is difficult to predict, but can be explained by a number of factors including those of genetic, physiological, metabolic, behavioural and psychological origin (Blundell and King, 2000). The incidence of obesity has dramatically increased in last 50 years and this therefore may suggest that human genetic influences do not have a substantial contribution (Farooqi and O'Rahilly, 2006). It therefore seems far more likely that environmentally driven changes have compelled the rise in the prevalence of adult and childhood obesity. The current environment promotes excessive food intake through increased portion sizes, a diet abundant in sugar, fat and subsequent energy density (Hill et al. 2003; The House of Commons Select Committee, 2004). Furthermore, lifestyle has changed such that there are fewer manual jobs, as well as increased car ownership and a rise in labour saving devices at home and at work (Government Office for Science, 2007).

Economic and Health Consequences of Obesity

Positive energy balance and the consequent overweight and obesity that developed have potentially severe implications for an individual's health, as well as impacting the economy. Specifically, being overweight or obese is associated with significant metabolic changes and subsequent health conditions such as heart disease, diabetes mellitus, hypertension,

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reproductive dysfunction, osteoarthritis, gall bladder disease and certain forms of cancer (Bray, 2004). Given the specific impact of overweight and obesity on human health, the current obesity epidemic is overwhelming health resources. Reporting the estimates of the costs and consequences of obesity in England, it is stated that the net cost is 2.47 billion , which is 0.3% of the UK governments total budget in 2016 (Tovey, 2017).

Surgical Interventions for Obesity

With the need to attend to obesity as a progressive disease, losing weight can substantially improve or cure the related health conditions. That said, clinical reports only present a 10% reduction in body weight after weight loss drugs with assistance of a diet (Kerrigan, Magee and Mitchell, 2011). Furthermore, long-term studies have reported that weight loss will typically plateau at 6 months and be difficult to maintain despite multiple interventions (Franz et al. 2007). Losing weight is therefore a challenge for the vast majority of individuals and difficult to sustain in the long-term. Due to this need for drastic action, the number of bariatric surgeries has increased dramatically in the recent years (Patkar et al. 2017). There are two main types of bariatric surgery available: laparoscopic adjustable gastric band (LAGB) and the Roux-en-Y gastric bypass (RYGB) surgery. LAGB surgery implements a tightening mechanism to slow and limit the amount of food entering the stomach at the top of the stomach, whereas the RYGB surgery re-arranges the small intestine to bypass 90% of the stomach into a Y-configuration. In both cases, the end result is the rapid-onset of stomach fullness perceptions, followed by a growing satiety (or "indifference" to food) shortly after the start of a meal (Kerrigan et al. 2011). RYGB is currently deemed the obesity intervention gold standard. After this surgery, portion size is controlled by the creation of a small pouch at the bottom end of the gullet, separate from the distal stomach. This bypasses the fundus (the site of production of ghrelin), distal stomach and speeds up delivery to the jejunum. The result of this surgery is increased stomach distention, leading to smaller portion sizes through increased perceptions of fullness. The main ghrelin production site bypassed (fundus) leads to decreased ghrelin (hunger hormone) levels and faster food delivery to the jejunum increases appetite suppressing gut hormones such as peptide YY (PYY), glucagon-like peptide-1 (GLP-1) and oxyntomodulin (Kerrigan et al. 2011). Bariatric surgeries, particularly RYGB surgery, have rapidly advanced to be the most effective available long-term treatment for morbidly obese individuals (Berthoud,

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Zheng and Shin, 2012). However, due to the cost and risks of surgery it is only considered for patients with a BMI greater than 40 kg·m^2, or greater 35 kg·m^2 with one or more comorbid conditions. Also, the concomitant risks associated with surgery may result in long-term complications such as nutritional deficiencies, internal hernias, anastomotic stenosis, and emotional disorders (Pories, 2008). Consequently, restrictions are in place to limit surgery to those who need it most and once all other therapeutic options have been exhausted. Hence, bariatric surgery does not currently offer a viable therapeutic option for the majority of overweight or modestly obese individuals within any given population. It therefore remains as a cure to an extreme situation to a small population who need it most.

Pharmacological Interventions for Obesity

Although numerous anti-obesity drugs have been investigated and prescribed over the years, health side-effects and medical effectiveness have limited their use, resulting in the removal of certain types of anti-obesity drugs from the commercial markets (Bray, 2008; Encinosa et al. 2005). Sibutramine, a commonly prescribed anti-obesity drug operates as a neurological inhibiter of serotonin and norepinephrine causing the reuptake of these neurotransmitters in the brain. Following a European review, it was removed in January 2010 from the commercial market due to an increased risk of stroke and myocardial infarction (Williams, 2010). Consequently, Orlistat (Xenical) remains the only drug prescribed by GP practices in England for obese patients with prescriptions lasting for up to two years. It functions by reducing intestinal digestion of fat by working as a potent inhibitor of pancreatic lipase in the intestines (Bray, 2008). During the first year of a 4-yr double-blind, randomised and placebo-controlled trial, when prescribed Orlistat, body mass decreased by ~11% below baseline in the 3304 overweight subjects. In the following three years there was a small weight regain (6.9% below baseline) with the total amount of body mass loss compared to the placebo trial resultantly small (~2%; Bray, 2008). The average number of days Orlistat was supplied by GP's per patient in the UK was 110 days, although this drug is recommended for long-term use (2 y) (Encinosa et al. 2005). Orlistat is commonly associated with unpleasant side effects due to its mechanism of action (oily stools and faecal urgency, fat soluble vitamin deficiencies), which might help to explain the low adherence levels. Taking this into account, presently the pharmacological drugs available remain limited in their effectiveness along with the fact that there are still health and safety

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concerns for the patient.

Exercise, Physical Activity and Links to Body Mass Loss and Appetite

Regular physical activity has been demonstrated as a significant component to assist with overweight and obesity levels along with maintaining cardiovascular health and prevent disease (Myers, 2003; Schubert et al. 2013). However, in the absence of energy restriction, a long-term exercise programme may only confer modest body weight losses (McTiernan et al. 2007) or assist in the maintenance of a person's present weight (Catenacci and Wyatt, 2007; Donnelly et al, 2009; Martins, Morgan and Truby, 2008). Regardless of body weight losses, other benefits of exercise can be gained. Johnson et al. (2009) demonstrated reduced hepatic and visceral lipids in previously sedentary obese individuals following aerobic exercise training. Also, suppressions in hunger and perturbed appetite hormone levels affect energy intake after intense exercise (King et al. 2011). This has been termed 'exercise-induced anorexia' and is defined as a brief suppression of hunger accompanied by the delayed onset of energy intake (King, Burley and Blundell, 1994). Numerous studies have investigated this effect and have shown transient decreases in perceptions of hunger when intense exercise is performed (Broom et al. 2007; Pomerleau et al. 2004).

Cross-sectional research has observed an inverse relationship between body weight and body mass index and levels of physical activity over a lifespan (McTiernan et al. 2007). Data pooled from a minimum-1-year weight-management intervention, based on 80 studies and eight different interventions, found that attention to food intake provides the most encouraging short-term weight loss results, whereas exercise alone was far less encouraging (Franz et al. 2007). Similar results have been reported by Chaput et al. (2011) where exercise alone was considered a disappointing intervention for weight loss compared to diet and exercise and diet trials.

Dietary Manipulation

The over consumption of energy is one of the main contributory factors to ever increasing obesity levels (Beaulieu et al. 2017). Diet has changed significantly over the past 50 years with diets containing higher amounts of sugars and fats (French et al. 2012). Prentice and

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Jebb (1995) reported each kJ of carbohydrate in 1940 was associated with 0.6 kJ of fat, which increased to 0.9 kJ of fat per kJ of carbohydrate in 1990, presenting a ~50% rise over 50 years. Estimates from the National Diet and Nutrition Survey's rolling programme 2012-2014 reports sugar intake of 74.2 g/d in England, which is 15.6% of total daily energy consumed from food, nearly double the recommended intakes. The largest single source of added sugar intake for young boys aged 11-18 years is sugar-sweetened beverages and accounts for ~10% of their total energy intake (Gibson, 2008). Consumption of these beverages, such as colas, lemonade and fruit drinks, was shown to increase by 135% between 1977 and 2001 (Malik, Schulze and Hu, 2006). The way in which food and drinks are consumed, has had a big impact on the eating environment. Changes in behaviour related to energy intake and energy expenditure have occurred over a relatively short period of time and therefore physiological mechanisms that defend against positive energy balance may become weakened and weight gain becomes common (Karra and Batterham, 2010). Therefore, restricting the amount of energy consumed seems to be the most effective method for controlling and decreasing body weight (Spiegelman and Flier, 2001).

Types of Diets and Weight Regain

An energy surplus of 3-4% in adults or 350 kJ/d (equivalent to a slice of bread) would produce a weight gain of about 1 kg per year (Stubbs and Lee, 2004). Estimates on Data in Europe from the MONICA study indicate that energy supply per capita correlates with the prevalence of obesity (Silventoinen et al. 2004). Thus, with easy access to high fat and sugar foods in diets, it is not a surprise that manipulating these dietary components is commonly prescribed as a dietary method for controlling body weight (Malik, Schulze and Hu, 2006).

A low-fat diet (~30% of total energy from fat) and concomitant reduction in total energy intake, has been shown to reduce the risk of cardiovascular disease (Yu-Poth et al. 1999) and body mass (Klem et al. 1997; Klein et al. 2004). Low-fat diets are typically recommended over a period of 6 months and above for combatting obesity (Gardener et al. 2016), heart disease and certain cancers. Interestingly, low fat foods were found to be advantageously accepted when comparing foods similar in appearance and palatability with a higher fat content, leading to subjects consuming 11.3% less energy when consuming the low-fat diet option (Lissner et al. 1987). Furthermore, a meta-analysis containing 19

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controlled, *ad-libitum,* low-fat, 2-12 month intervention studies reported a 3.2 kg decrease in body weight, representing a viable choice for weight loss (Astrup et al. 2000). That said, observational research in this area may be confounded through lack of control of physical activity, smoking and food recording errors (Astrup et al. 2000).

A low-carbohydrate diet (~20-40%) is determined by deriving a large proportion of energy intake from protein or fat with a specific focus on decreasing simple sugars and refined carbohydrates (Nordmann et al. 2006). Recently, there has been increased interest in low-carbohydrate diets as a means of controlling body weight, although the safety and efficiency is still to be concluded (Bravata et al. 2003). In a 2-year trial assessing 272 moderately obese subjects, it was found that a low fat diet reduced body mass by 2.9 kg whereas a low carbohydrate diet, reduced it by 4.7 kg. Thus, a low-carbohydrate diet may be an effective alternative to a low-fat diet (Normann et al. 2006; Shai et al. 2008). However, a systemic review by Bravata et al. (2003) reported any weight loss recorded while on a low carbohydrate diet may be associated with a decreased caloric consumption and not necessarily the reduced carbohydrate content per se.

The optimal dietary macronutrient composition that facilitates a lasting and safe level of weight loss is unknown. Some diets and interventions have been advocated (Astrup et al. 2000; Hill, Melanson and Wyatt, 2000; Bray, 2008) and they have the potential to induce short-term weight loss in determined individuals, butweight regain often occurs (Crujeiras et al. 2010; Franz et al. 2007). This can render such dietary practices relatively ineffective at producing a sustained weight loss over the longer duration (~6 months-1 year). Franz et al. (2007) affirms that weight loss interventions involving attention to food intake (e.g. diet alone, meal replacements and weight loss medications) seem to produce the most encouraging results, although at approximately six months, weight loss begins to plateau in nearly all interventions. It therefore, seems warranted to explore interventions to encourage lifestyle changes and a more prolonged weight loss and maintenance.

A variety of dietary approaches have been proposed, but it is unlikely that one diet is optimal for all individuals. Dietary guidance should be individualised to allow for specific food preference, lifestyles and environments to be considered (Klein et al. 2004). A variety of strategies are available to induce a reduction in energy intake, without specifically

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focusing on energy control. For example, lowering energy density and/ or portion size of foods (consuming water dense foods such as soups, fruits, vegetables and limiting foods high in fat) (Ello-Martin, Ledikwe and Rolls, 2005) or drinking more around meals (water or high protein) (Abreu et al. 2012; Stookey et al. 2008), may provide an effective way to induce weight loss over a prolonged period as a behavioural intervention (Mucklebauer et al. 2013).

Eating Behaviour

Making long-term changes to eating and activity behaviours is difficult for most individuals to contend with. It seems that once a person has gained weight, it becomes difficult to then return to a normal body weight (Franz et al. 2007). Therefore, it appears further emphasis should be encouraged to facilitate the maintaining of a healthy body weight. Ultimately, eating behaviours influence energy intake through the amount and type of food chosen, including the decision to stop eating (Blundell and Cooling, 2000). Thus, a vast amount of research has been invested in ways to better understand food choices (Wansink, 2004), and it is now becoming more important to determine what drives food consumption and more importantly the cessation of food consumption.

Gastrointestinal Regulation of Appetite and Energy Intake

Despite large fluctuations in daily energy intake and expenditure a person's body weight remains remarkably constant. This shows the presence of a powerful and complex physiological system that balances energy intake and energy expenditure and is composed of afferent signals and efferent effectors (Wynne et al. 2005). These signals are integrated to operate as a synergistic system between the central nervous system and peripheral tissues to affect appetite and energy intake over both an acute (meal-to-meal) and chronic basis (Morton et al. 2006). The signaling of satiation after the consumption of foods involves the integration of both gastric and intestinal feedback signaling, although volumetric changes may be the initial and overriding factor towards creating initial cessation of food intake (Phillips and Powley, 1996). Although nutrient or chemical properties may not have a primary effect on satiation, signaling from the intestines results from a nutrient detection, which then consequently triggers the release of various anorectic gut peptides and neurotransmitters (Ritter, 2004). Many of these intestinally derived anorectic signals

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stimulate vaso-vagal reflexes, reduce gastric emptying and subsequently result in retention of gastric contents. Therefore, meal termination has been stipulated to occur in response to neuronal integration of the stomach and intestinal negative feedback signaling (Powley and Phillips, 2004).
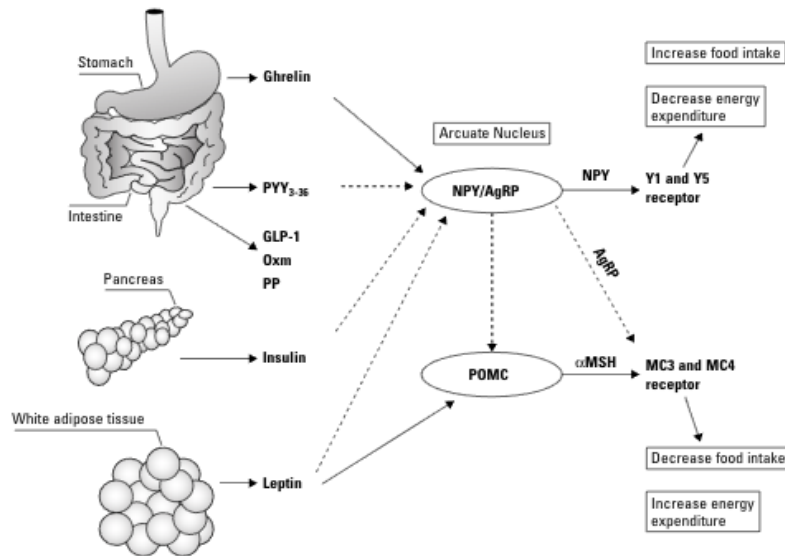
Figure 1.1. Circulating hormones affecting energy balance via the arcuate nucleus
Continuous lines indicate stimulation of hunger effects and dashed lines indicate inhibitory effects hunger (Adapted from Murphy and Bloom, 2004).

Gut-Brain Interactions and Appetite

The central nervous system integrates and interprets numerous signals to establish the energy requirement of the body and initiates relevant behavioural actions (Harrold et al. 2012). Circulating hormones, along with other factors generated by the sensory experience of eating (sight, smell, taste and mouth feel) initiate the consumption of food and utilisation of nutrients (absorption, metabolism and storage of energy; Harrold et al. 2012). The regulation of appetite and energy intake is initiated by peptide hormones to communicate acute and chronic energy status of the bodies reserves. These peptides are secreted into the circulation from peripheral structures, such as the stomach, intestines, pancreas and adipose tissue to signal to regulatory appetite centres within the brain via entry through the partially permeable blood-brain barrier (Badman and Flier, 2005). The area postrema and the nucleus tracts solitaries are the target for circulating hormones (e.g. GLP-1 and Amylin) and create neural interaction with the signals from the vagal nerve in the gastrointestinal tract to the

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brain stem (Berthoud, 2002). However, the arcuate nucleus (ARC) within the hypothalamus is the key location for receiving circulating factors related to total energy stores in fat, energy availability and nutrients present in the gut (Williams et al. 2000; Badman and Flier, 2005). Within the ARC there are two distinct neuronal populations responsible for integrating afferent signals and regulating the expression of appetite stimulating/ inhibiting neuropeptides. Neurons expressing neuropeptide Y (NPY) and agouti-related peptide (AgRP) stimulate higher order neurons which promote appetite and energy intake, while reducing energy expenditure. Conversely, neurons expressing pro-opiomelanocortin (POMC) and cocaine and amphetamine-related transcript (CART) induce the expression of neuropeptides responsible for reducing appetite and food intake while increasing energy expenditure (Morton et al. 2006; Harold, 2012) The gastrointestinal tract responds to a number of signals that originate in the lumen including nutrient (chemical) and non-nutrient chemicals (mechanical). The presence of nutrients (carbohydrates, fats and proteins) lead to the discharge of sensory afferent fibres that may be independent of the energy densities and mechanical changes (French, 2004).

Gastric Distension

Gastric distension can activate both gastric stretch receptors (Paintal, 1954; Schwartz, McHugh, Moran, 1993) and mechanoreceptors that detect intragastric pressure to signal satiety (Iggo, 1955). This occurs through ingestion of a meal and activation of vagal afferent signals from the stomach to the brain, which results in the perception of fullness and satiety (Wang et al. 2008). This suggests that stretch receptors, situated in the smooth muscle of the stomach, respond to the degree of distension of the stomach (Paintal, 1954). Paintal (1954) investigated gastric stretch receptors and their role in satiation of hunger in cats. They found that stomach stretch receptors exerted increased nerve impulses as the stomach was distended by a balloon constituting a peripheral mechanism for satiation of hunger. It is, therefore apparent that at meal times, where food or water is ingested, a large number of these impulses would be excited and increase as the meal or water intake increases. Share et al. (1952) investigated sham feeding in dogs and found it was the distension of the stomach through bulk feeding rather than caloric content that limited food intake. Furthermore, the observed satiety remained as long as the distension was present. It is, therefore, likely that gastric stretch receptors make neural connections to exert a regulatory function on food intake (Brobeck, 1946). The solitary nucleus receives a host of gastrointestinal mechanic

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and chemosensory information via the vagus nerve (Rogers et al. 1996). Vrang et al. (2002) confirms gastric distension created by a gastric balloon in rats produced an increase of c-fos-expressing neurons of the solitary tract, specifically GLP-1 neurons ($21 \pm 9\%$ to $32 \pm 5\%$).

Hormonal Regulation of Appetite

Leptin, which was discovered in 1994, (Zhang et al. 1994) has been identified as one of the most important 'adiposity' signals responsible for informing the central nervous system of the status of adipose tissue. It is primarily involved in energy homeostasis and satiety, whereby leptin levels in the circulation are increased in proportion to fat mass. This was implicated in early studies by the discovery of *Ob/ob* mice, who do not produce functional leptin and exhibit hyperphagia and weight gain (Gale, Castracane and Mantzoros, 2004; Schwartz et al. 1999). Circulating leptin conveys information to the hypothalamus regarding the amount of energy stored in adipose tissue, suppressing appetite and affecting energy expenditure (Gale et al. 2004). Consistent with this, findings report plasma leptin concentrations to be highly correlated with body mass index in rodents and humans (Maffei et al. 1995). Although administration of leptin can reverse this weight gain in rare genetic mutations, obesity is widespread and only a handful of patients have a mutation in leptin or leptin receptor genes (Berthoud, 2002). Therefore, as circulating concentrations of leptin are elevated in most obese individuals, a level of leptin resistance in the obese population is suggested to explain this hyperphagia and weight gain (Considine et al. 1996).

Insulin, produced by beta cells of the pancreas, shares many properties with leptin as it also acts as an adiposity signal informing the brain of long-term energy reserves. Like leptin, insulin also crosses the blood-brain barrier and interacts with specific receptors in the ARC to suppress food intake (Gale et al. 2004). Insulin and leptin both reduce food intake although insulin secretion is stimulated acutely in response to meals, whereas leptin is not. As body weight increases, insulin secretion must also increase to compensate for insulin resistance, whereby failure of pancreatic beta cells to achieve this adaptive increase can lead to hyperglycaemia and type-2 diabetes with obesity (Schwarz et al. 2000).

The long-term signals (leptin and insulin) communicate information regarding the energy status in the body, whereas short-term signals regulate meal initiation and termination

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(Murphy and Bloom, 2006). Peptides including ghrelin, PYY, CCK and GLP-1 are released in response to the presence of food in the GI tract. Ghrelin is the only orexigenic hormone that stimulates eating, whereas the others function to reduce hunger or initiate satiety.

During the search for an endogenous growth hormone secretagogue, ghrelin was discovered by Kojima et al. (1999). Ghrelin is a 28 amino acid peptide produced by cells primarily located in the fundus. Two forms of ghrelin exist in the circulation being acylated and desacylated ghrelin. When fasting and/ or low circulating levels of glucose and insulin are present in the blood stream, ghrelin is secreted into the circulation with desacylated ghrelin (70-90%) representing the predominant form. Ghrelin becomes active when caprylic acid (octanoic) is linked to serine at the 3-position by the enzyme ghrelin O-acyltransferase (GOAT) (Yang et al. 2008). Acylated ghrelin, the less predominant form, has only a short circulating half-life (~25 min) to exert its effects on food intake (Liu et al. 2008). Ghrelin has been reported to have central and peripheral actions including gastric motility and gastric acid secretion (Asakawa et al. 2001; Date et al. 2001), that contribute to ghrelin's orexigenic properties and its role in energy homeostasis (Karra and Batterham, 2010).

The stimulatory effects of ghrelin on appetite and food intake are mediated through the hypothalamic ARC. Circulating levels along with hunger scores, peak before meals during fasting, and fall shortly after food ingestion (Cummings et al. 2004). The postprandial plasma ghrelin concentration has been reported to decrease in proportion to the ingested caloric load of a meal (Callaghan et al. 2004) and the accumulation of energy throughout the day (Leidy and Williams, 2006).

Currently, ghrelin is the only circulating hormone that is known to stimulate appetite and food intake, whilst other circulating hormones acutely control appetite after food intake to prompt satiety. After endogenous administration of ghrelin in rodents, research has reported enhanced appetite and energy intake in a dose dependent fashion (Wren et al. 2001). In humans, circulating concentrations of ghrelin enhance appetite, which has been shown to increase energy intake in individuals with anorexia/ bulimia nervosa (Wren et al. 2001; Tanaka et al. 2002) and decrease energy intake in obese individuals (Shiiya et al. 2002).

Peptide YY (PYY) is an anorectic gut hormone that is part of the PP fold peptide family

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(Murphy and Bloom, 2004). PYY is predominantly found in the endocrine pancreas and is released in response to the presence of food in the GI tract. The majority of PYY is found stored in L cells and present in the circulation in the form of PYY $_{(3-36)}$. PYY$_{(3-36)}$ is a 34 amino acid peptide (due to the cleavage of two amino acids), released from the distal intestinal endocrine cells (L cells) and is the most abundant pancreatic polypeptide in the circulation in both a fed and fasted state (Batterham et al. 2006). PYY$_{(3-36)}$ has been shown to be released in response to the presence of food in the GI tract (Gale et al. 2004) and induces a decrease in gastric emptying and a consequential reduction in food intake (Allen et al. 1984). In rodents (Batterham et al. 2002) and humans (Batterham et al. 2003) exogenous administration of PYY$_{(3-36)}$ reduces appetite and *ad-libitum* energy intake. Also, findings in lean and obese subjects are similar, exerting a 30% reduction in energy intake and hunger ratings at a buffet meal provided 2 h after the end of a 90 min intravenous infusion of PYY$_{(3-36)}$ (Batterham et al. 2003). These findings indicate that there is no evidence of a relationship between obesity and PYY$_{(3-36)}$ resistance and this may provide a target for research into obesity pharmacotherapy (Gale et al. 2004).

Cholecystokinin (CCK) was the first recognised peptide hormone as an inhibitor of food intake and it is secreted by duodenal-jejunal endocrine L-cells of the intestinal mucosa ~15 min after eating. Meal termination and satiety is mediated with CCK levels peaking at ~25 min after a meal and remaining elevated for ~3 h after (Paik et al. 2007). CCK is released post-prandially from the small intestine, mainly in the presence of fats and proteins and seems to reduce food intake through CCK1 receptors on the vagal nerve (Kissileff et al. 2003; Murphy and Bloom, 2006). CCK has been shown to relax the stomach, constrict the pylorus and inhibit gastric emptying (Debas, Farooq and Grossman, 1975; Liddle et al. 1986) when food is in the stomach, thus playing a key role in increasing gastric distension. After research in rats presented a decrease in food intake (Gibbs et al. 1973), human studies in both lean and obese subjects have reported similar effects (Kissileff et al. 1981). This makes CCK an interesting candidate for the research of an appetite-suppressing agent for reduction of energy intake and elevating perceptions of fullness via gastric distension.

Glucagon-like peptide 1 (GLP-1) and oxyntomodulin are both produced by the precursor protein preproglucagon which is synthesised in the intestine. GLP-1 is synthesised by intestinal L-cells located in the distal ilium and colon and exists in several forms with the

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most common being the release of GLP-1. GLP-1 responds to eating, peaking at ~30 min and remaining elevated for several hours. GLP-1 effects on the gastrointestinal system are mediated through its distinct receptors on the vagal nerve and are received through the ARC (Vrang et al. 2002). Appetite inhibiting effects in rats have been reported when GLP-1 is administered intracerebroventricularly (Tang-Christiansen et al. 1996; Turton et al. 1996). Furthermore, an increased satiety and decrease food intake is reported in normal weight diabetic and obese subjects after peripheral administration of GLP-1 (Gutzwiller et al. 1999). GLP-1 is also effective at potentiating the production of insulin, controlling appetite and blood glucose concentration (Murphy and Bloom, 2006).

Human Body Water

Water constitutes approximately 60% of a male's weight and 50% of a female's weight due to the differences in white adipose tissue present. The difference in body composition accounts for the biggest variation whereby some overweight and obese individuals can be as little as 45% water by relative weight (Hall, 2015). Water storage is in two compartments; the intracellular water 65% (25 L) and the extracellular water 35% (15 L) (Sawka, Cheuvront and Carter, 2005).

Physiological Control of Water Balance

The maintenance of water in the body is efficiently regulated despite its abundance. Therefore, even small deviations in body water away from normal resting levels can affect a person's health and performance. Achieving daily water balance is the management of water losses and water gains. In a normal healthy population total body water is regulated to ±0.22% body mass in temperate environmental conditions and ±0.48% body mass in the heat and during exercise (Greenleaf, 1992). For the sedentary person in a temperate living environment, daily water turnover is ~2-3 L, meaning 5-10% of total body water content is turned over each day (Maughan, 2003). The main routes of water loss are the urinary system (~1400 mL·d^{-1}) and the skin (~500ml·d^{-1}) with lower levels from the gastrointestinal tract (~200 mL mL·d^{-1}) and the respiratory surfaces (~400 mL·d^{-1}) (Sawka et al. 2007). Total daily water loss can therefore be ~2500mL, but can vary greatly dependent on the individual, environmental condition or an individual's activity level. Body water losses are

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replenished on a daily bases principally through water intake in fluids and food, as well as metabolic water production through metabolism of dietary nutrients. Whilst the majority of water ingestion is normally consumed through fluid, solid foods can still contribute significant water intake depending on the water content of the food or the amount consumed (Sawka et al. 2005). Being in a state of water balance is termed euhydration, whereas reduced body water stores is termed hypohydration. Rehydration refers to the process of gaining water from a state of hypohydration whereby the aim is to alleviate thirst and return water stores to a euhydrated level (Greenleaf, 1992; Sawka et al. 2005). Therefore daily water balance is mainly determined by the balance of water consumption with renal and perspiratory water losses.

As ingesting fluid is the main way to help maintain body water homeostasis, thirst is a key regulatory mechanism. McKinley and Johnson (2004) define thirst as a subjective perception that provides the urge for humans and animals to drink fluid. Sensations of thirst may arise due to a regulatory response to reductions in fluid content of various bodily compartments (hypohydration), hypertonicity of the extracellular fluid or a rise in the circulating levels of some dipsogenic hormones (McKinley and Johnson, 2004). During a transition away from euhydration, ranges of compensatory responses are initiated when the intra- or extracellular compartments are depleted (e.g. vasopressin secretion, stimulation of the renin-antiotensin-aldosterone system, sympathetic activation and reduced renal solute and water excretion). The drive to drink and the renal system will regulate mechanisms within the endocrine system to maintain body water homeostasis. Thus thirst is an important component of the co-ordinated effort to maintain the volume and composition of body fluids (McKinley and Johnson, 2004). Specifically, the main physiological factors involved to drive thirst are increases in serum osmolality and decreases in plasma volume (Adolph et al. 1954; Kenney, Wilmore and Costil, 2015). Although these physiological mechanisms stimulate thirst, they do not appropriately restore body fluids to their original state in the initial few hours after hypohydrating exercise (Shirreff et al. 2004). This therefore means that a longer time period (8-24 h) is required to adequately replenish water deficits (Institute of Medicine, 2005).

A fundamental part of the renal system is anti-diuretic hormone (ADH), also named arginine vasopressin (AVP) secreted from the posterior lobe of the pituitary gland primarily in

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response to hyperosmolality (Bayliss, 1987; Kumar and Berl, 1998; McKinley and Johnson, 2004). ADH acts on the kidney via the adenylyl cyclase-coupled vasopressin receptor (V_2 receptor) to regulate water excretion (Nielson et al. 1995). Vasopressin reduces urinary water output by in-part increasing water permeability of the renal collecting ducts and distal convoluted tubule by inducing translocation of aquaporin-CD (AQP-CD or AQP-2) water channels in the plasma membrane of collecting ducts (Nielson et al. 1995). This promotes water reabsorption in the kidneys from urine to the blood stream, which can lead to smaller volumes of more concentrated urine being excreted. In healthy individuals, urine osmolality is in the range of 442-1052 mosmol·kg^{-1} (although it can be concentrated up to ~1400 mosmol.kg^{-1} (Armstrong et al. 1994). When individuals are hypohydrated there is a resultant positive correlation in serum osmolality and AVP and an inverse relationship between plasma volume and AVP (Robertson and Athar, 1972; Bayliss, 1987). Baylis (1998) reported that a 1 mosmol·kg^{-1} increase in plasma osmolality results in 0.41 pmol·l^{-1} increase in plasma AVP. Also, 1 pmol·l^{-1} increase in plasma AVP is equal to 235 mosmol·kg^{-1} increase in urine osmolality (until urine concentration is maximal) resulting in a change of serum osmolality will consequence a change in urine osmolality of 100 mosmol·kg^{-1} (Bayliss, 1987; 1998). The other important hormone in water balance regulation is aldosterone, which is involved in the renal control of blood pressure. Aldosterone is the main mineralcorticoid hormone released by the zona glomerulosa of the adrenal cortex in the adrenal gland and its primary action is to promote sodium reabsorption in the distal convoluted tubules and collecting ducts of the kidneys (Martini, 2006). As part of the renin angiotensin system, renin is an enzyme released in response to a decreased blood pressure or blood volume to mediate the extracellular volume and arterial vasoconstriction. Renin participates in the conversion of a plasma protein angiotensinogen into an active form called angiotensin I. In the blood stream angiotensin I is converted to angiotensin II by the action of angiotensin converting enzyme (ACE), which operates as a potent vasoconstrictor, increasing blood pressure and releasing aldosterone to induce its actions upon the kidneys.

Measurement of Hydration Status

Many measurement techniques have been used to determine hydration status in laboratory and field settings, including body mass changes, urinary indices and blood markers (Sawka,

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2007). Hydration markers should be sensitive enough to determine a change in total body water of ~3% (equivalent to a ~2% change in body mass) as this is the most commonly reported level of hypohydration after exercise (Sawka et al. 2007). Acute changes in body mass during exercise can be used to calculate permutations in hydration status, assuming that 1 kg of mass lost equates to 1 L of sweat loss (Maughan, Shireffs and Leiper, 2007; Sawka et al. 2007). Pre-exercise body mass compared with post-exercise body mass along with urine losses and drink/ food consumption can be used to estimate water lost during exercise. Therefore, body mass can be used as a substitute marker for changes in body water stores where other markers prove difficult during exercise (Maughan et al. 2007; Sawka et al. 2007; Shirreffs et al. 2004). Although this method is a simple and effective tool to monitor water balance, when used in combination with other hydration markers such as urine indices, it has greater merit (Sawka et al. 2007). Urine markers include urine osmolality, specific gravity, volume and colour. An 8-colour scale is used to determine hydration status by the colour of the urine (Armstrong, 2000), however, this can be misleading when consuming large volumes of hypotonic fluid and large urine volumes are excreted before hydration is re-established (Sawka et al. 2007). Urine osmolality is a quantifiable measurement commonly used to determine hydration status. Shirreffs and Maughan (1998) have effectively assessed urine osmolality as a measure of hydration status in athletes training in a hot environment. Values greater than 900 mosmol·kg^{-1} were typically observed in subjects who had lost 1.9% of body mass due to water losses. Blood measurements of hydration status include haematocrit in combination with haemoglobin to determine percentage change in plasma volume, as long as postural and dietary changes are standardised (Harrison, 1985). Plasma osmolality may be the best potential static (at one-time point) hydration marker when compared to standard reference values along with other hydration markers (Cheuvront et al. 2010). Popowski et al. (2001) reported plasma osmolality increases linearly by five mosmol·kg^{-1} for every 2% reduction in body mass up to 5% body mass loss.

Daily Water Intake

Water is an essential nutrient for life, however despite its importance it is often ignored as a dietary constitute. Public attention has deemed drinking water key for health and performance, although currently the dietary guidelines remain unclear (Sawka et al. 2005).

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Normal water needs may differ due to some factors including metabolism, diet, climate and clothing (Sawka et al. 2007). Mild hypohydration, defined as a 1% to 2% loss of body mass caused by water loss, impairs exercise performance, affects overall health in the elderly and increases the risk of urinary stone disease (Shirreffs et al. 2004). The goal of drinking water throughout the day is to maintain water balance and therefore anything under daily requirements will lead to a level of hypohydration. Fluid balance studies have reported that daily water needs increase with age from early infancy (~0.6 L) throughout childhood (~1.7 L) (Sichert-Hellert, Kersting and Manz, 2001). When a person reaches adulthood, the daily water needs of men increase to 2.5 L if sedentary (Adolph 1954; Newbugh, Woodwell and Falcon-Lesses, 1930). It will increase to ~3.2 L if performing a modest physical activity level and ~6 L with higher activity in a warm environment (Ramen et al. 2004; Welch, Buskirk and Ianpietro, 1958). Sawka et al. (2005) reported a modest daily exercise sweat loss of 1.4 L will increase daily water requirements by 45%. Thus, it may prove difficult to replace fluid where small volume consumption and larger fluid losses can result in negative water balance (Shirreffs and Maughan, 1998).

Hypohydration and Exercise Performance

Athletes are commonly reported to start exercise hypohydrated, whereby a high starting urine specific gravity, urine osmolality or darker urine colour is reported (Bland et al. 2009; Volpe, Poule). This may be explained as poor pre-exercise drinking practices and/or the close proximity of previous exercise bouts, not enabling the opportunity to properly rehydrate between exercise bouts. Starting exercise hypohydrated can further exacerbate water losses during exercise and lead to increased physiological strain and decreased exercise performance, especially when exercise is performed in temperate, warm or hot conditions (Cheuvront et al. 2005; Maughan and Shirreffs, 2004; Sawka, Montain and Latzka, 2001). When hypohydrated to 2% or more of body mass cognitive abilities are shown to decline compared to a euhydrated control (Cian, Melin and Raphel, 2001; Gopinathan et al. 1988). Furthermore, effects on the ability of the central nervous system driven by hypohydration may also affect musculature and thus exercise performance. Judelson et al. (2007) reported a decrease in central activation decrements as 4-8% hypohydration possibly implying alterations in the central nervous system, the peripheral nervous system and/ or excitation-contraction coupling related to hydration-related

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decrements in endurance or resistance exercise performance.

Rehydration After Exercise

Exercise is associated with high rates of heat production, resulting in sweat losses that may exceed 2 l/h. The consequential water deficit is detrimental to exercise performance, cognition and potentially health (Gopinathan et al. 1988; Judeelson et al. 2007; Sawka et al. 2007). Therefore, optimising adequate fluid replacement is essential after exercise. However, even when fluids are available the volume ingested is seldom sufficient to match the rate of sweat loss, thus starting the next bout of exercise in fluid deficit is common place (Maughan and Shirreffs, 2004). Volume and composition are deemed the main factors influencing post-exercise rehydration (Shirreffs et al. 2004). Recommendations are to consume fluid in a greater volume than the fluid lost through sweating to account for ongoing sweat losses and the increased diuresis during the post-exercise period, particularly if a large volume of fluid is ingested in a short time period. Furthermore, a volume equivalent to 150-200% of the sweat losses is required during the immediate post-exercise period (~1 h) (Shirreffs et al. 1998). Fluid losses can be difficult to replace and retain in a short period of time. Additionally, spacing fluid intake over several hours of recovery after exercise reduces urine losses and is more effective in restoration of fluid balance (Archer and Shirreffs, 2001). Therefore, if time permits between training sessions, post-exercise fluid intake should be provided in a metered manner.

The addition of electrolytes to drinks (Merson, Maughan and Shirreffs, 2008; Nose et al. 1988) or consumption of food in the recovery period (Maughan, Lieper and Shirreffs, 1996) is also an effective way to retain ingested fluid compared to ingestion of only water (Ray et al. 1998; Sawka et al, 2005). Sodium is the primary cation lost in sweat, with ~35 mmol·l^{-1} (range 10–70 mmol·l^{-1}) typically lost during exercise (Sawka et al. 2007). Also, the addition of sodium to rehydration drinks or salty snacks remains an effective method of rehydration.

Macronutrients and Rehydration

Research studies have reported that the addition of macronutrients is an effective way to increase fluid retention of a rehydration drink and decrease urine output (Seifert et al. 2006;

Evans et al. 2009; Osterberg et al. 2010; Watson et al. 2008). An electrolyte-free solution usually results in a large diuresis during the hours following drinking due to the fast gastric emptying and appearance in the peripheral circulation (Shirreffs, Watson and Maughan, 2007; Vist and Maughan, 1994). When consuming large volumes of fluid, haemodilution, (a decrease in serum electrolyte concentrations) results in the initiation of diuresis (Shirreffs et al. 2007). Macronutrients will slow this process by decreasing the rate at which the fluid empties from the stomach as gastric emptying is largely due to the energy density of the solution or meal consumed (Vist and Maughan, 1994). McHugh and Moran (1979) describes the energy density of a solution to have an inverse relationship with the speed of gastric emptying of liquids. The half-emptying time of milk, for example, exhibits an approximately 14% slower emptying time than a carbohydrate-electrolyte drink (Calbet and Maclean 1997). Subjective perceptions questionnaires further affirm a greater sensation of stomach fullness after the consumption of milk compared to the same time points during a carbohydrate-electrolyte trial (Shirreffs et al. 2007). In reports, when different macronutrients are added to a rehydration drink, there is an increase in serum/ plasma osmolality (Evans et al. 2009; Osterberg et al. 2009; Seifert et al. 2006; Watson et al. 2008). This delay in gastric emptying may therefore decrease uptake of fluid into the circulation and limit signaling of volume and osmolality in the vascular space (Shirreffs et al. 2007).

Water Balance and Appetite

At present, relatively little is known about how deviations in water balance and water intake impact upon appetite and energy balance. What is more, the effect of hydration status on energy intake and appetite is not fully understood. Hypohydration is common after exercise (Sawka et al. 2007) and in the elderly (Ferry, 2005; Warren et al. 1991). Two population groups where reductions in energy intake are apparent (Mowe, Bohmer and Kindt, 1994). Research in animals has consistently reported water intake as being a major determinant of the amount of food consumed (Callahan and Rinaman, 1998; Senn, Gross-Luem, Kaufmann and Langhans, 1996; Silanikove, 1992 Watts, 1999). The same has not been shown in human studies, although to induce hypohydration to a similar extent to that seen in animal studies would not be ethically viable. A few studies have reported reduced energy intakes when fluid intake was manipulated, however the mechanism is not fully understood (Engell, 1988; Shirreffs et al. 2004).

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Water Balance and Subjective Appetite

The current commonly held view is that hunger signaling prompts eating to ensure energy needs are met while thirst is initiated to address hydration needs (McKiernan, Houchins and Mattes, 2008). However, the increased consumption of energy-yielding beverages has changed this relationship and poses new challenges to presumed homeostatic energy regulatory mechanisms. Observational research by McKiernan et al. (2009) collected hourly appetite perceptions and dietary recalls from 50 adults for seven consecutive days and revealed that the majority of fluid intake (75%) occurs peri-prandially and is derived mainly from energy-yielding beverages. Furthermore, a distinct absence of the associations between hunger and thirst were reported. Nevertheless, more in-depth methods of dietary and appetite analysis may be required to determine further hedonic perceptions (Lowe and Butryn, 2007). With this in mind, a hypohydrated state after exercise or after a prolonged period of insufficient fluid ingestion may drive differences in energy intake and appetite. Furthermore, many drinks ingested in the post-exercise period may contribute to the energy consumed. Rolls et al. (1998) reported a subjective hunger decrease and subjective fullness increase after an isogenic milk containing 600 mL of fluid compared to 300 mL of fluid. This response has also been shown after a bolus of water consumed before an *ad-libitum* meal where energy intake was decreased in older adults. Subjective fullness was reported to be increased significantly compare to the no fluid bolus trial and an accompanying decrease in energy intake was reported. Shirreffs et al. (2004) presented a decrease in energy intake of 28% when subjects were restricted of fluid for 48 h, although hunger perceptions were not different between trials.

Water Balance and Energy Intake

Although the effects of water balance on energy intake are largely uninvestigated, it remains an important aspect of appetite research, as hypohydration can affect many elements which could impact upon energy intake (McKiernan et al. 2009). For example, if fluid is insufficient during exercise (Sawka et al. 2007) or in the elderly (Ferry, 2005; Warren et al. 1991) hypohydration can occur which could affect energy intake (Mowe et al. 1994; Clarkston et al. 1997). Research in animals has shown that when fluid is restricted, voluntary energy intake is decreased (Senn et al. 1996; Silanikove, 1992; Watts et al. 1999). Senn et al. (1996) investigated the eating behaviour of lactating cows after 48 h of water

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deprivation. Water deprivation was found to reduce grass and corn pellet feed significantly with the size of the meals over the 48 h reducing significantly. The investigators concluded that ruminal fluid osmolality contributes to the hypohydration-induced hypophagia. When Silanikove, (1992) reviewed the effects of infrequent drinking, water restriction and heat load on feed intake and utilisation in ruminants similar findings were reported. In animals (sheep, goat and cattle) mild hypohydration, water restriction and heat stress were found to cause typically a 10-30% reduction in energy intake Silanikove 1985, Silanikov, 1992). Also, in rats hypohydrated by drinking 2.5% of hypertonic saline for 4 days, a reduction in nocturnal food intake was reported (Watts, 1999). In human studies Shirreffs et al. (2004) reported a reduction in energy intake when subjects restricted fluid intake for 37 h compared to when fluids were provided *ad-libitum*. Although not the aim of the study, subjects reduced their overall energy intake by 28% when asked to completely restrict fluid intake. This may be explained by an increased feeling of tiredness and feeling less inclined to exercise, thus not feeling they required the energy. Alternatively, the fact that subjects were asked to restrict their food choices to 'dry foods' in the hypohydrated trial might have limited their food choices or altered eating behavior such that their energy intake was reduced. When Engell (1988) reduced fluid intake by 40% of *ad-libitum* fluid intake (measured in the previous trial), energy consumption at meal times was voluntarily reduced by 37%. In contrast, Kelly et al. 2012) observed no difference in *ad-libitum* energy intake between a hypohydrated (2-3% body mass loss) and a euhydrated trial, with hydration status manipulated using a combination of exercise-induced sweating and fluid restriction. Collectively these studies may suggest that hypohydration effects voluntary food intake when fluid is restricted (Engell, 1988; Shirreffs et al. 2004) but may not when fluid is provided during feeding (Kelly et al. 2012) and warrants further investigation.

Water Balance, Stomach Fill and Appetite/ Energy intake

Stomach fullness can be defined as the enlargement of the stomach due to increased volume (Geliebter, 1988). This can have a profound effect on an individual's energy intake due to a decrease in the palatability of foods once a person is full (Rolls et al. 1980).

A study by Share et al. (1952) found that intragastric feeding into a balloon produced an inhibition of oral feeding whether this was with non-caloric or caloric value in dogs. It was concluded that the mechanical distention of the stomach was the main stimulus to decrease

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oral food intake. Similarly, Paintal, (1954) inserted a gastric balloon into the stomach and then filled it gradually to monitor gastric afferent fibres. Interestingly a linear relationship was found between the degree of stomach distension and the number of impulses/ sec in the fibres. In humans, gastric distention has been investigated by a gastric balloon being filled with varying volumes of water (0, 200, 400, 600 and 800 mL). As seen in the animals, food intake was significantly reduced as the balloon volume reached 400 mL. (Rolls et al. 1998). This mechanistic response is displayed when obese individuals have gastric band or Roux en Y surgery to make the stomach pouch smaller. The result of this surgery is increased stomach distention, leading to smaller portion sizes through increased perceptions of fullness (Kerrigan et al. 2011). Kelly et al. (2012) provided a large fluid bolus to rehydrate hypohydrated subjects in the rehydration trial but did not in the hypohydrated trial. This may have affected stomach fullness and therefore may explain a decrease in energy intake in the rehydration trial. Other findings presented by Van Walleghen (2007) and Davy et al. (2008) have found that when providing a 500 mL preload 30 minutes before an *ad-libitum* meal, energy intake is decreased in normal weight (Van Walleghen et al. 2007) and obese older adults (Davy et al. 2008). Rolls et al. (1998) also found that changing the volume of an isoenergetic milk-based drink preload (300mL, 450 mL and 600 mL) to an *ad-libitum* meal resulted in a reduced energy intake when the 600 mL drink was consumed compared to the 300 mL drink.

Water Balance and Salivary Flow

Salivary flow rate has been reported as an important factor for appetite in animals (Silanikov, 1992) and humans (Oliver et al. 2008; Walsh et al. 2004). Silanikov, (1992) reported restricted water availability to once every 24, 48 and 72 h and subsequently a reduction in salivary flow rate of 40%, 60% and 80% respectively was recorded. This was accompanied by a parallel reduction in voluntary consumption of feed intake of 40%, 60% and 80% respectively. This may account for a reduction in energy intake (28% reduction) observed by Shirreffs et al. (2004), where subjects completely restricted their fluid intake for 37 h and were instructed to ingest foods with a low water content. Hypohydration appears to alter gastrointestinal function (Walsh et al. 2004; Oliver et al. 2008) and these changes might influence the speed of digestion of a meal and consequently influence peri-prandial perceptions of satiety and food intake due to reduced gastric secretions (Neufer et al. 1989; Rehrer et al. 1990; Silanikov, 1992).

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Recovery and Nutrition

The impact of the supply of nutrients in the proximity to an exercise session is an important consideration for effective recovery. Some of the most important issues include restoration of liver and muscle glycogen stores, protein synthesis (Phillips and Van Loon, 2011) and the replenishment of electrolytes lost in sweat (Burke, 1997). The replenishment of carbohydrate, protein and fat have been widely researched and different strategies exist to maximise the utilisation of foods and drinks (Burke, Kiens and Ivy, 2004; Phillips, 2012). Sufficient carbohydrate and protein will be necessary in the hours after exercise to facilitate glycogen resynthesis (Burke et al. 2011) and protein synthesis (Phillips and Van Loon, 2011), respectively. Practical considerations following exercise may include the gastrointestinal comfort and the appetite appeal of food and drinks. Therefore, it is recommended that the supply of palatable drinks is available, along with the correct levels of nutrients for the individual (Burke, 1997), dependent on their goal

Macronutrients and Appetite

Research has focused on energy containing beverages (Anderson and Woodend, 2003; Stewart et al. 1997) around mealtimes due to their impact on overweight and obesity (Mattes et al. 2011) although additional calories from drinks can confuse findings.

Due to the need for an effective method to control energy intake and body weight, there is growing body of research examining the effect of different macronutrients on satiety/ appetite regulation (Poppitt et al. 2011). The overconsumption of sugar-sweetened beverages is suggested as one of the main contributory factors to overweight and obesity (Mattes et al. 2011). Glycemic index, proposed in 1981, is used as an alternate system for classifying carbohydrate-containing foods by their response on a person's blood glucose (Ludwig, 2002). High-glycemic index carbohydrates are associated with reduced appetite and energy intake within a short time period (Van Amelsvoort and Wesstrate, 1992) whereas lower-glycemic index carbohydrates exert their effects on satiety over a longer time period (2-6 h) (Andeson, Catherine, Woodend and Wolever, 2002; Anderson and Woodend, 2003; Van Amelsvoort and Wesstrate, 1992). Complex carbohydrates are broken down by intestinal enzymes into simple sugars. The focus is mainly on glucose, whereby incretin hormones will stimulate the production of insulin to reduce blood glucose by assisting

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glucose transportation into cells (Diakogiannaki, Gribble and Reimann, 2011). Mayer (1953) has suggested that low blood glucose concentrations trigger the onset of eating and high blood glucose will indicate satiety and meal cessation. Research affirms brief drops in blood glucose are associated with food intake in animals and humans (Campfield and Smith, 1990; Campfield et al. 1996). It is therefore clear that simple sugars will suppress food intake if the time period between consumption and energy intake is brief, however as they are digested quickly they may bypass regulatory systems and increase transient energy intake. Stewart et al. (1997) found that when consuming isovolumetric (400 mL) preloads of 75 g polycose, sucrose, glucose and glucose/ fructose mixture, the higher the glycemic response, the greater the decrease in appetite sensations and energy intake after 1 h.

Low-glycemic index foods will take longer to digest and assimilate allowing a prolonged contact time with receptors in the gastrointestinal tract to induce satiety signals (Andersen and Woodend, 2003). Contact of nutrients with the small intestine is associated with post-gastric satiety (Holt et al. 1992; Rayner et al. 2000; Spitzer and Rodin, 1987) and slow digesting, low-glycemic index carbohydrates, would expect to prolong this process (Leathwood and Pollet, 1988). After carbohydrates (glucose) are subjected to the intestines, specifically the L-cells, hormonal responses are triggered. GLP-1 is a putative satiety hormone involved in carbohydrate-related satiety (Chapman et al. 1998; Lavin et al. 1998). It is likely that appetitive responses are depicted by their absorptive characteristics. Low-glycemic index meals are therefore associated with greater satiety for up to 6 h after consumption (Raben et al. 1994). It therefore seems preferable to consume slowly absorbed carbohydrates if the goal is to control food intake.

Protein and Appetite

The consumption of dietary protein is promoted to produce a greater satiety and reduced energy intake compared with carbohydrates (Bowen et al. 2006). The final steps of protein digestion and absorption take place in the small intestine, although the role of protein in hormone release is unclear (Diakogiannaki et al. 2011). A number of mechanisms have been suggested to explain the effects of a high protein diet on appetite and energy intake (Skov et al. 1999; Weigle et al. 2005). Energy expenditure seems to depend on the types of protein consumed as the prolonged digestive process of proteins can increase diet-induced

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thermogenesis after consumption compared to iso-caloric amounts of carbohydrate or fat (Kissileff et al. 2004; Martini et al. 1994; Mela et al. 2001). Although this may contribute to satiety, the energy expended when consuming proteins will largely depend on the type consumed (animal vs. Vegetable). This remains only a small contribution to the energy expended throughout the day, so may not be significant enough for weight loss alone. Weigle et al. (2005) found that RMR, the major component of energy expenditure, did not increase with the high protein diet and that weight loss was explained by a reduction in energy intake. Therefore, a more meaningful mechanism for findings in weight loss interventions may be proteins ability to induce an increased post-prandial satiety (Weigle et al. 2005). Research has found a marked increase in satiety after an iso-caloric high protein diet compared to carbohydrate and fat (Skov et al. 1999; Weigle et al. 2005; (Raben, 2002; Raben et al. 2003). After consuming a high protein diet, hunger ratings are suppressed and ghrelin is decreased and CCK increased (Blom et al. 2006; Latner and Scwartz, 1999; Tannous et al. 2006). In addition, a high protein preload decreases hunger and food intake, via increased fullness compared with an isoenergetic preload of either high fat or sucrose based (Rolls, Hetherington and Burley, 1988). Therefore, protein may decrease appetite through a mechanical mechanism.

Milk Consumption and Appetite/ Energy Intake

Research, where whey or casein (both dairy proteins) was provided as a preload to an *ad-libitum* meal, reported an appetite-suppressing effect (Hall et al. 2003; Veldhorst et al. 2009; Astbury et al. 2010). Milk is naturally high in protein ($36g \cdot L^{-1}$), of which the majority (~80%) is casein protein, with the remaining mainly being whey protein (Billeaud et al. 1990). Milk also contains carbohydrate and electrolytes (sodium and potassium), and as such is a viable post-exercise option for an effective way to rehydrate (Shirreffs et al. 2007), improve protein balance (Elliot et al. 2006) and replenish glycogen stores (James, 2012; Josse et al. 2010). The different protein fractions present in milk may be a key factor when considering appetite and energy intake responses. Research suggests that a viscous drink reduces appetite (Mattes and Rothacker, 2001) and energy intake (Zijlstra et al. 2008) compared to a less viscous drink matched for energy density and macronutrient content (Bertenshaw et al. 2013; McCrickerd et al. 2012). Furthermore, in the presence of gastric acid, the casein fraction of milk protein clots, forming small fragments that slow emptying from the stomach (Miranda and Pelissier, 1983; Veldhorst et al. 2009). This might

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contribute to stomach distension and result in a subsequent reduction in voluntary energy intake (Gelibter, 1988). Whey protein may also contribute as the amino acid response (BCAA; leucine, isoleucine and valine) may have a unique metabolic role interacting with insulin to suppress appetite (Layman and Baum, 2004), as insulin responses are positively correlated with BCAA and carbohydrates (Van Loon et al. 2000).

Water Intake and Appetite/ Energy Intake

Many research studies have focused on the effects of an energy containing preload with different macronutrients on energy intake and appetite (Bertenshaw, Lluch and Yeomans, 2008; Bowen et al. 2006; Poppitt et al. 2011). Milk contains the majority of slow emptying proteins (casein), which contribute to the appetite suppressing effect of milk. This effect may be due stomach distention, which seems to be a key contributing factor to the satiation at a meal (Share et al. 1952), A systematic review of subjects dieting for weight loss or maintenance concluded that increased water consumption had a weight reducing effect (Muckelbauer et al. 2013). Also, ingesting 500 ml of water 30 minutes before a meal decreases energy intake in normal weight and obese older adults (Davy et al. 2008; Van Walleghen et al. 2007), although this was not the case in younger adults (Van Walleghen et al. 2007). As there was a difference found in older adults compared to younger adults gastric emptying may explain the difference, where older adults have a slower gastric emptying rate (Clarkston et al. 1997). After 30 minutes in younger subjects 500 ml of water would have emptied from the stomach leaving a decreased stomach distention and satiety (Vist and Maughan, 1994; Wang et al. 2008). Dennis et al. (2010) investigated the consumption of water intake 30 min before a meal combined with a hypocaloric diet over 12 weeks in older subjects. There was a significant difference compared to the hypocaloric diet with no water trial, whereby energy intake was lower at *ad-libitum* meals and greater weight loss (~2kg) was recoded. Previous research by Rolls et al. (1998) has shown that when the macronutrients of a preload is the same and the water volume is changed (300 ml, 450 ml and 600 ml) the energy intake at a subsequent meal is reduced as the energy density of the preload is decreased. This has also been reported when water is incorporated into a food to increase the volume of a food (Rolls et al. 1990; Rolls et al. 1999). In these studies the volume of water consumed as part of the meal (Rolls et al. 1990; Rolls et al. 1999) or as an isocaloric drink with different water amounts (Rolls et al, 1998) displayed an increased fullness and decreased hunger as the energy density was decreased, representing an

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increased satiety.

The mechanism by which water ingestion before a meal may reduce acute energy intake is not fully understood but might be related to increased gastric distention, producing increased perceptions of satiety and decreased hunger. Data from animal studies suggests that increased gastric distension reduces energy intake (Share et al. 1952), possibly via activation of gastric stretch receptors (Paintal, 1954). Therefore, as ensuring energy intake is controlled in younger adults either overweight and obese or normal weight trying to maintain their body weight, it is important that an effective intervention is found.

Thesis Aims and Hypotheses

Aims

- To determine the effect of hypohydration and/ or fluid availability on appetite and energy intake.
- To determine appetite and energy intake responses of different drinks, consumed after endurance-type exercise.
- To investigate the effects of immediate pre-meal water ingestion on appetite and energy intake in lean and overweight populations.

Alternative Hypotheses

- Hypohydration would increase *ad-libitum* fluid intake and decrease *ad-libitum* energy intake.
- Consumption of milk after intense exercise would decrease subsequent energy intake, possibly due to an increased viscosity compared to a carbohydrate or water preload.
- Ingestion of water immediately pre-meal would increase perceptions of fullness and decrease hunger and subsequently reduce *ad-libitum* energy intake in both lean and overweight individuals.

Chapter 2
General Methods

General Methods

This chapter describes the experimental methods used in the studies presented within this thesis as certain aspects of the methodology were common between studies.

Ethical Approval

Prior to the start of all experiments, ethical approval was granted by the Loughborough University Ethical Advisory Committee. Both written and verbal explanations of the nature of all experimental procedures involved in each experiment were provided to subjects and they were informed of their right to withdraw at any time (Appendix A). After any questions they had related to the experiment had been answered, each subject gave their full written and verbal consent to participate.

Subjects

For the studies reported in this thesis, subjects were recruited by word of mouth, poster and email advertisement. Subjects were given a subject information sheet describing the demands of the study and the associated risks and discomforts. Subjects provided written informed consent (Appendix B) and completed a health screen questionnaire (Appendix C) before any experimental procedures began. Most of the subjects recruited were students completing their studies at Nottingham Trent University for the first study (Chapter 3) and for the remaining four studies (Chapters 4-7), subjects were recruited from Loughborough University and the Leicester area. The following inclusion criteria were also specified for all studies:

- Non-smokers.
- Not currently dieting or on any extreme weight loss diets.
- Weight stable within the past 3-months i.e <2.3 kg change in body mass (St Jeor et al. 1997).
- Not taking any medication known to influence appetite regulation.
- No history of cardiovascular disease, metabolic disease or dyslipidaemia.
- Sufficient ability to complete the study demands i.e. The exercise protocols and fluid restraints.

There were no unusual eating behaviours as measured by the Three-Factor Eating Questionnaire (Appendix L)

Standardisation Prior to and During Experimental Trials

The energy and nutrient content of meals consumed on the evening prior to experimental trials affects appetite perceptions and gut hormone levels on the subsequent morning (Chandarana et al, 2009). Chapters 4, 5, 6, and 7 reported in this thesis commenced in the morning following an overnight fast (from 10pm) (Chapters 4, 6, 7) or a standardised breakfast (Chapter 5), whereas Chapter 3 commenced in the afternoon. Subjects were required to avoid alcohol intake 48 h before and during the trials and record their diet and physical activity patterns for a period of 24 h prior to the first experimental trial of each study. Physical activity levels were determined by a self-reported questionnaire completed by pen and paper (Appendix E), (Sylvia et al. 2014). This was adjusted based on the exercise performed in the studies (Chapter 3 and 7). Also, subjects were asked to only perform light activity (walking) before each trial. Distance from the laboratory was established by asking the subjects how far they had to travel and then a lift to the laboratory was offered. If the subjects did not get a lift to the laboratory they were asked to walk only and this was repeated in each subsequent trial. Subjects were given home-weighing scales to complete a weighed food record of all items consumed during this period and then replicated their intake during the 24 h before subsequent trials (Appendix D).

Anthropometry

During all the experiments described in this thesis, body mass was measured bare foot and wearing minimal clothing to the nearest 10 g (CFW150 digital scale; Adam, Milton Keynes, UK). Body mass loss (BML) (kg) was calculated as the change in nude body mass from baseline nude body mass for each trial. Percentage BML was also calculated relative to baseline nude body mass for each trial. Height was taken (International Standards for Anthropometric Assessment) from the subject with the base of their back on the stadiometer (Seca Ltd, Germany), feet together and their head in the Frankfort plane, measured to the nearest 1 mm. Subjects' body mass index was calculated as weight in kilograms divided by the square of their height in metres.

Measurements of subcutaneous fat were taken to estimate total body fatness by a trained ISAK qualified practitioner. Skinfold thickness (ISAK) was measured using skinfold calipers (John Bull, British Indicators, West Sussex, UK) at four anatomical locations (biceps, triceps, subscapular, suprailiac) and body density was calculated using the predictive equations of Durnin and Womersley (1974). Percentage body fat was then estimated using the Siri equation (Siri, 1956). All measurements were made in duplicate on the right side of the body with the subject standing and a mean score determined. If skinfold measurements were not within a technical error of measurement of 10% then the sites were repeated. Measurements were made by rotating through the anatomical sites to allow the skin time to regain normal texture and thickness.

Heart Rate Measurement

Heart rate was measured during preliminary exercise tests and main trials using short-range telemetry (Polar F4, Polar Electro, Kempele, Finland).

Rating of Perceived Exertion and Thermal Sensations

The Borg scale was used to ascertain subjective perceptions of exercise intensity during preliminary exercise testing and main trials (Appendix F; Borg, 1973). This scale ranges from six (no exertion) to 20 (maximal exertion). In Chapter 3 subjects reported their thermal sensations on a questionnaire (Appendix G; Gagge, Stolwijk and Hardy, 1967).

Peak Oxygen Uptake (VO$_2$ peak)

In Chapter 3, peak oxygen uptake (VO$_2$ peak) was determined using a continuous incremental exercise test on an electronically braked cycle ergometer (LODE Excalibur Sport; Cranlea, Birmingham, UK). Subjects began cycling at 105 W, with increments of 35 W every 3 min and continued until volitional exhaustion. Expired gas was collected into a Douglas bag during the final minute of each 3 min stage and during the last minute of exercise to represent maximal expired gas, also heart rate (Polar Electro, Finland) and rating of perceived exertion (RPE) were recorded at the end of each stage as well as during the last minute of exercise (Morgan and Borg, 1976). For each expired air sample, O$_2$ and CO$_2$ concentration was collected into Douglas bags (Plysu Protection Systems, Milton Keynes,

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UK) and determined using a paramagnetic oxygen analyser and an infra-red carbon dioxide analyser (Series 1400, Servomex, Crowborough, East Sussex, UK), before volume and temperature were measured using a dry gas meter (Harvard Apparatus, Edenbridge, Kent, UK) and thermistor (Edale, type 2984, Model C, Cambridge, UK).

In Chapter 7, a discontinuous incremental exercise test was performed on a electrically braked cycle ergometer (LODE Corival; Cranlea, Birmingham, UK) to volitional exhaustion to determine VO_2 peak. Subjects began cycling at 100 W, with increments of 50 W every 4 min with 1 min rest between increments. During the last minute of each stage and at exhaustion, expired air, heart rate (Sport tester PE Polar Electro, Finland) and Rating of Perceived Exertion (RPE), (Morgan and Borg, 1976)**,** were collected.

Assessment of Appetite

In each of the studies described in this thesis, subjective appetite perceptions (thirst, mouth pleasantness, fullness, prospective intake, hunger, head soreness, satisfaction and desire to eat) were assessed periodically using previously validated visual analogue scales (Flint et al. 2000) (Appendix H). Also, in Chapter 7 drink perceptions were recorded for differences in drink perceptions (Appendix I). The scales were analysed by measuring the horizontal distance in mm from the left hand side to the point on the line indicated by the subject to determine a score out of 100. The subjective perceptions questionnaire was printed and subjects rated their appetite perceptions by placing a vertical mark on a 100 cm line using a pen between the verbal anchors at either end (e.g. "not at all" and "extremely"). These were measured and recorded by the principal investigator using a 100 cm ruler to determine the subjective score. Questions asked were "How hungry do you feel?" and "How full do you feel?" Subjects could not refer to their previous ratings when completing the appetite scales.

The Three-Factor Eating Questionnaire

The Three-Factor Eating Questionnaire was completed at the start of the familiarisation trials during Chapters 5, 6 and 7 to measure three dimensions of human eating behaviour. These factors were to monitor extremes of cognitive restraint of eating, disinhibition and hunger using a 51-item questionnaire (Appendix L; Stunkard and Messick, 1985).

Ad-libitum Meals

During Chapter 3 a multi-item breakfast was provided to subjects and they were free to select and consume food and drink *ad-libitum*. The *ad-libitum* breakfast was set up identically before each trial with food being presented in excess of expected consumption. The items available were three varieties of cereal, cereal bars, white bread, brown bread, margarine, spreads (peanut butter, chocolate spread and strawberry jam), fresh fruit (apples, oranges, and bananas), semi skimmed milk, orange juice, apple juice, tea, coffee and water. Prior to main trials containing buffet type *ad-libitum* meals subjects completed a food preference questionnaire, selecting any foods they disliked (Chapter 3; Appendix J).

During all other *ad-libitum* meals single item meals were provided. For Chapter 4, 5,and 6 subjects were presented with a bowl of porridge (90 g dry porridge mix: 434 g semi-skimmed milk: 11g of granulated sugar), (Appendix K) and 500 mL water and 500 mL of squash. Once ½ to ¾ of the bowl had been consumed, it was replaced with a fresh bowl of porridge and this process continued until subjects indicated satiety. Similarly, the drinks provided were replaced when required.

For Chapter 7 subjects consumed a homogenous pasta meal *ad-libitum* (fusilli pasta, bolognese sauce, cheese and olive oil, Tesco Stores Ltd, Cheshunt UK), (100 g of food: pro: 19.5%, cho: 48.1%, fat: 32.4%). The meal was divided into 4 bowls, which were exchanged intermittently over the 30 min and weighed (Kern Top Pan Balance PFB 3000-2, Progen Scientific, London, UK) before and after the subject had eaten. Subjects were initially provided with ~ 400 g in a bowl and the bowl was replaced at intervals (determined during familiarisation) to ensure warm food was continuously available to the subject and that the subject did not completely finish a bowl. Water (600 mL) and sugar-free squash (600 mL) were also provided and consumed *ad-libitum* and weighed before and after the meal.

Subjects were instructed to "eat until comfortably full and satisfied" and indicate satiety by leaving the feeding booth and sitting in the adjoining laboratory. The feeding booth was set up to minimalise interaction with the researcher to ensure there was no effect on the food or drink intake. Interaction was also minimised by keeping verbal communication to a minimum with the subjects by only speaking to them when required. The feeding booth had 3 sides with the subject seating forwards, which enabled food and drink to be easily passed to the subjects. Consumption was ascertained by weighing food and drink items on digital

kitchen scales before and after eating, with the energy and macronutrient content of the items consumed determined using manufacturer values.

Urine Osmolality and Volume

Chapter 3 and 4 described in this thesis involved the collection of morning urine samples (Chapter 3) or urine collected over 24 h outside the laboratory (Chapter 4). For each urine sample, subjects completely emptied their bladder and collected the entire volume. For Chapter 4 subjects also collected all urine produced between 0 h and 24 h in a container provided. The volume of all urine samples was measured and a 5 mL sample retained for analysis of osmolality.

All urine samples were analysed for osmolality by freezing point depression (Gonotec Osmomat 030 Cryoscopic Osmometer; Gonotec, Berlin, Germany). All analysis was performed in duplicate, with mean values taken.

Blood Sampling and Analysis

Blood samples were taken during the studies described in Chapters 3, 4 and 7. The methods sections of each experimental chapter gives specific details of the nature, timing and volume of blood samples taken. All blood samples were collected by venipuncture with a 21-gauge butterfly needle and drawn into a plastic syringe, before being dispensed, as described in the methods section of each experimental chapter. Prior to each blood sample, subjects assumed an upright-seated position for at least 10 min to avoid any postural changes in blood and plasma volume (Shirreffs and Maughan, 1994).

Haemoglobin Concentration and Haematocrit

Whole blood was dispensed into tubes containing K_2EDTA (1.75 mg·mL-1) and used for the determination of haematocrit by microcentrifugation (Hawksley micro-haematocrit centrifuge; Hawksley and Sons Ltd., Lancing, UK) and haemoglobin concentration by the cyanmethaemoglobin method. Haematocrit was performed in triplicate, whilst haemoglobin concentrations were determined in duplicate. In Chapters 3 and 4 haematocrit and haemoglobin concentrations were used to estimate changes in blood, plasma and red cell

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volume relative to the 0 h blood sample (Dill and Costill, 1974).

Serum Osmolality

Whole blood was dispensed into serum tubes and allowed to clot at room temperature for 15 minutes before being centrifuged at 4°C and ~1750 g for 10 min (GS-15R Centrifuge, Beckman Coulter, Fullerton, USA). The serum was then separated and stored at 4°C before analysis. Serum osmolality were determined by the same methods described for the urine analysis. All measurements were made in duplicate.

Environmental Temperatures

For the experimental trials in chapter 4, daily environmental temperatures were recorded at the Met Office weather station at Sutton Bonnington, Loughborough, Leicestershire and this information was collated to determine humidity and temperature for the times outside of the laboratory. In each of the other studies described, environmental temperature and relative humidity were assessed periodically throughout main trials using a hand-held hygrometer and thermometer**.**

Ghrelin Analysis

For the determination of acylated ghrelin, 5 mL of blood was dispensed into a tube containing K_2EDTA (1.75 mg·mL^{-1}), as well as a solution (10 µl·mL^{-1} blood) of potassium phosphate buffering saline (0.05 M), p-hydroxymercuribenzoic acid (0.05 M) and sodium hydroxide, (0.06 M). The tube was then centrifuged at ~1750 g for 10 min at 4°C, after which the plasma was transferred to a plain tube containing 1 M hydrochloric acid (100 µl·mL^{-1} plasma) and centrifuged for a further 5 min. The resultant plasma was stored at -20°C for 24 h and then at -80°C until analysis for acylated ghrelin concentration using a commercially available ELIZA kit (SPI BIO, Montigny le Bretonneux, France).

Statistical Analysis

All data were first tested for normality of distribution using a Shapiro-Wilk test. After this, all data containing two independent variables were analysed using a two-way repeated

measures analysis of variance (ANOVA). If the assumption of sphericity was violated, the degrees of freedom where corrected using the Greenhouse-Geisser estimate. Where two-way ANOVA indicated significant main effects, *post-hoc* Bonferroni-adjusted t-tests for normally distributed data or post-hoc Bonferroni-adjusted Wilcoxon signed rank tests for non-normally distributed data were used to identify the location of differences. Normally distributed data containing one independent variable was analysed using a t-test or one-way repeated measures ANOVA with *post-hoc* Bonferroni-adjusted t-tests. Non-normally distributed data containing one independent variable was analysed using Wilcoxon signed rank test or Friedmans ANOVA with *post-hoc* Bonferroni-adjusted Wilcoxon signed rank tests. Normally distributed data are presented as mean (SD), whilst non-normally distributed data are presented as median (range). Data were accepted as being significantly different when *P*<0.05.

Coefficient of Variation

Table 2.1 Presents the coefficient of variation (CV) for the analytical procedures detailed in this thesis. The CV was determined from calculating the standard deviation of the difference between duplicates and then expressed as a percentage of the mean value of samples analysed throughout this thesis.

Table 2.1. Coefficient of variation (%) obtained for analytical procedures conducted throughout this thesis.

Assay	n	CV (%)
Haemoglobin (mmol·l^{-1})	30	0.4
Haematocrit (%)	30	0.4
Serum osmolality (mosmol·kg^{-1})	30	0.2
Urine osmolality (mosmol·kg^{-1})	30	0.3
Ghrelin (pg·mL^{-1})	30	6.8

Chapter 3
Effect of Hypohydration after exercise in the heat on Appetite and *ad-libitum* Energy Intake

Abstract

Little is known about the effects of hydration status on human appetite and the effect of hypohydration caused by suboptimal post-exercise rehydration is unknown. Ten males lost ~2% body mass through exercise in the heat (50% peak power output with 10 min blocks of cycling separated by 5 min rest until BM loss reached) in the afternoon/ evening. Over the next 13 h, subjects consumed two evening meals (1 snack and 1 dinner) and were either rehydrated (RE: water equal to 175% of their body mass loss) or remained hypohydrated (HYPO: 200 mL water), returning to the laboratory the following morning overnight fasted. Urine samples, blood samples, and subjective perceptions were collected pre-exercise, post-exercise and 13 h post-exercise, with an *ad-libitum* breakfast provided 13 h post-exercise. Total exercise time was not different for RE (53 (7) min) and HYPO (54 (9) min) (P=0.642), with total heat exposure including rest periods lasting 75 (10) min and 77 (14) min for RE and HYPO trials, respectively (P=0.767). During exercise, mean heart rate (RE: 157 (13), HYPO: 155 (15), P=0.227), RPE (RE: 14 (3), HYPO: 14 (3), P=0.215) and thermal sensation (RE: 6 (1), HYPO: 6 (1), P=0.32) were not different between trials. Total body mass loss at 13 h post-exercise was greater during HYPO (2.7 ± 0.5%) than during RE (0.5 ± 0.5%). Total energy intake at the *ad-libitum* breakfast was not different between trials (RE: 4237 ± 1459 kJ; HYPO: 4612 ± 1487 kJ; *P*=0.436). Furthermore, there was no difference in energy consumed in foods (*P*=0.600) or drinks (*P*=0.147) when data was separated. Total water ingestion at the *ad-libitum* breakfast meal was greater for HYPO (1641 ± 367 mL) than RE (797 ± 275 mL) (*P*<0.001), with this being explained by a greater water intake through fluids (*P*<0.001), but not through foods (*P*=0.429). Thirteen hours post-exercise, subjects reported greater thirst (*P*<0.001) and lower fullness (*P*<0.01) during HYPO than RE. These results demonstrate that hypohydration induced by inadequate post-exercise rehydration does not influence energy intake from an *ad-libitum* breakfast consumed 13 h post-exercise.

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Introduction

During prolonged exercise sweat losses generally exceed fluid intake meaning that hypohydration is commonly present immediately post-exercise, particularly if exercise is undertaken in a warm/ hot environment (Sawka et al. 2007). Athletes are also commonly reported to start exercise hypohydrated, as evidenced by high urine specific gravity (Volpe et al. 2009), indicating that in some situations rehydration does not appear to be optimal. To optimise recovery from prolonged exercise adequate water will be required to facilitate rehydration (Shirreffs et al. 2004). Sufficient carbohydrate and protein will also be necessary in the hours after exercise to facilitate glycogen resynthesis (Burke et al. 2011) and protein synthesis (Phillips and Van Loon, 2011), respectively. Currently, little is known about how hypohydration induced by exercise and/ or suboptimal rehydration influences appetite. If hydration status and particularly hypohydration affects appetite or food intake this might impact on the intake of other nutrients that might affect post-exercise recovery or adaptation to exercise training.

In animal models, experimental dehydration has been shown to reduce salivary flow rate and the rate of gastric emptying (Callahan and Rinaman, 1998; Silankove, 1992), as well as consistently reducing voluntary energy intake (Senn et al. 1996; Watts, 1999). In humans, complete restriction of fluid for 37 h (Shirreffs et al. 2004) inducing a body mass loss (hypohydration) of 2.7% has been reported to reduce energy intake by 28% compared to a euhydrated trial where fluids were ingested *ad-libitum*. In contrast, Kelly et al. (2012) reported that exercise-induced hypohydration of 2.3% did not affect energy intake at an *ad-libitum* meal compared to a euhydrated exercise trial.

Whilst these two human studies (Kelly et al. 2012; Shirreffs et al. 2004) report disparate findings, there are methodological factors that complicate the interpretation of the data. Shirreffs et al. (2004) required subjects to consume only dry foods during the fluid restriction trial, but allowed a free choice of foods during the euhydrated trial. This might have limited food choices during the fluid restriction trial and might account for the reduced energy intake in this trial. Furthermore, as subjects could consume fluids *ad-libitum* in the euhydrated trial energy consumed in fluids might account for some of the difference in energy intake between trials. During research performed by Kelly et al. (2012), subjects ingested a large bolus of water (1.5 x body mass loss during exercise) in the 30 min before

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the *ad-libitum* meal of the euhydrated trial but not in the hypohydration trial. Davy et al. (2008) have reported that consumption of just 500 mL water 30 min before an *ad-libitum* meal reduced voluntary energy intake, at least in older subjects. As such, the rehydration strategy used by Kelly et al. (2012) might have attenuated energy intake in the euhydrated trial.

The present study aimed to investigate subjective appetite and voluntary energy intake in response to hypohydration caused by inadequate rehydration after exercise-induced dehydration and to compare this to a euhydrated trial, involving appropriate post-exercise rehydration. It was hypothesised that hypohydration would reduce voluntary energy intake.

Methods

Subjects

Ten healthy males (age: 24 (12) y, body mass: 74.2 (9.6) kg, height: 1.79 (0.06) m, BMI: 22.9 (2.7), peak oxygen uptake: 49.6 (3.6) $mL \cdot kg^{-1} min^{-1}$) volunteered for this study. At the time of the study all subjects were physically active, but were not accustomed to training in a warm environment. Subjects were regular breakfast consumers, and did not have any problems with sleep, which were determined by asking them on the familiarisation trial. Also, when completing the food diary for standardisation subjects reported sleep amount and quality, although sleep was only monitored verbally after day 1. Experimental trials were performed in a randomised counterbalanced order and separated by ≥ 7 days.

Preliminary and Experimental Trials

During the first preliminary trial subjects height (stadiometer, Seca Ltd, Germany) and body mass (Adam CFW 150 scale; Adam Equipment Co Ltd, Milton Keynes, UK) were measured, and subjects were familiarised with all procedures (urine sampling, blood sampling and kitchen scales) before their peak oxygen uptake (VO_2 peak) was determined using a continuous incremental exercise test on an electronically braked cycle ergometer (LODE Excalibur Sport; Cranlea, Birmingham, UK). During the second preliminary trial subjects were familiarised with the experimental protocol by performing the rehydrated

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(RE) trial described below.

All experimental trials commenced in the late afternoon after a 4 h fast, with the exception of 500 mL water consumed 90 min before arrival at the laboratory. Water bottles were provided to subjects containing 500 mL and subjects were asked to bring the empty bottles with them up on arrival to the laboratory on day 1. Subjects completed 2 two-day experimental trials in a randomised counterbalanced order. On day one, subjects exercised in the afternoon/ evening to induce a body mass loss of ~2% pre-exercise body mass. Subjects were then either rehydrated (RE trial) or kept hypohydrated (HYPO trial) over night and returned to the laboratory the following morning (day two) when they were provided with an *ad-libitum* breakfast.

On arrival at the laboratory, subjects provided a urine sample and their body mass was measured (in underwear only), before they completed a questionnaire related to their subjective perceptions (SFQ) and a blood sample was collected. Subjects then performed cycling exercise in an environmental chamber (Design Environmental Limited, Ebbw Vale, UK), (Temperature: 35 (0.3) ^{o}C, Relative humidity: 77.6 (2.8)%) until they had lost 1.8% of their pre-exercise body mass. Target body mass loss (BML) was 2% and it was estimated subjects would lose the remaining 0.2% between exercise and the final body mass measurement (Maughan, Owen, Shirreffs and Leiper, 1996). Exercise commenced at 50% peak power output and subjects performed 10 min blocks of cycling, separated by 5 min rest in the chamber, during which they towel dried and were weighed (in underwear only) to determine BML. Workload was reduced if subjects could not maintain the selected workload. Heart rate, RPE and thermal sensation were recorded at the end of each block of exercise. At the end of exercise, subjects sat in a comfortable environment for 15 min, completed an SFQ and a blood sample was collected. Subjects then towel dried and their body mass was measured (in underwear only) to determine their total BML, before they provided a urine sample. Subjects then consumed their post-exercise food (HYPO and RE trials) and water (RE trial) and were provided with their food and drink to consume later in the evening (pizza and cereal bars).

Subjects slept at home and reported a good night sleep on the following day. Subjects returned to the laboratory the following morning 13 h post-exercise in an overnight fasted

state, with the exception of the water consumed 2 h before arrival in the RE trial. They provided a urine sample, completed an SFQ, their body mass was measured and a blood sample was drawn. A multi-item breakfast was then provided to subjects and they were free to select and consume food and drink *ad-libitum*. The *ad-libitum* breakfast was set up identically before each trial with food being presented in excess of expected consumption (as monitored during the familiarisation trial). The items available were three varieties of cereal, cereal bars, white bread, brown bread, margarine, spreads (peanut butter, chocolate spread and strawberry jam), fresh fruit (apples, oranges, and bananas), semi skimmed milk, orange juice, apple juice, tea, coffee and water. Subjects were instructed to "eat until comfortably full and satisfied" and that extra food and drink was available on request. Meals were consumed in isolation and subjects remained in the feeding area for the entire 30 min. Consumption was ascertained by weighing food and drink items before and after feeding, with the energy and macronutrient content of the items consumed determined using manufacturer values.

During the 13 h between exercise and the *ad-libitum* breakfast, subjects where provided with ~63 kJ·kg^{-1} body mass^{-1}, of which ~30% was consumed immediately after the post-exercise measures (cereal bars) and ~70% was consumed 2.5 h post-exercise (pizza). Subjects were asked to bring with them all empty packaging the next day and reported that all food was satisfactory and consumed in full as required. Food intake during post-exercise recovery was identical for both trials and amounted to 4570 (594) kJ, 160 (21) g carbohydrate, 34 (4) g protein, 35 (5) g fat, 1 (0.1) g sodium. During the RE trial, subjects ingested water equal to 175% of their total BML during exercise in four aliquots over the 13 h. These four aliquots were ingested immediately after post-exercise measures, 2.5 h post-exercise, 4 h post-exercise and 11 h post-exercise, with 761 (109) mL (30%), 634 (90) mL (25%), 507 (72) mL (20%) and 634 (90) mL (25%) of the total water volume ingested at these times, respectively. During the HYPO trial, subjects were provided 200 mL water to consume *ad-libitum* during the evening.
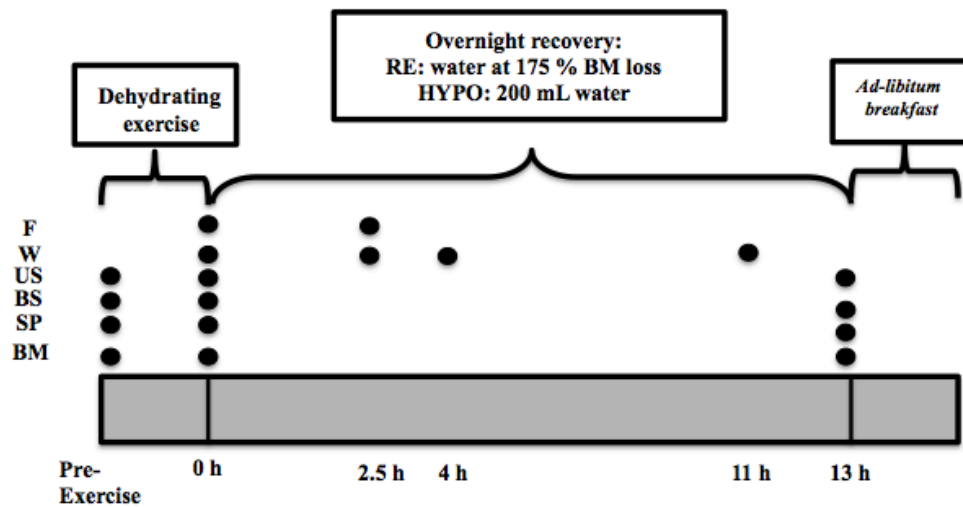
Figure 3.1. Study protocol. F: food, W: water, US: urine sample, BS: blood sample, SP: subjective perceptions, BM: body mass.

Sample Handling and Analysis

Venous blood samples (10 mL) were drawn from an antecubital vein before exercise, after exercise and 13 h post-exercise (before the *ad-libitum* breakfast) by venipuncture after subjects had rested for 15 min in an upright, seated position. Five mL of blood was mixed with K_2EDTA (1.75 mg·mL^{-1}) and used for the determination of haemoglobin concentration and haematocrit. Five mL of blood was used for the determination of acylated ghrelin.

Results

Pre-trial Measurements, Exercise and Post-exercise Nutrition

The subject's pre-exercise body mass, ($P=0.815$), urine osmolality ($P=0.491$) and subjective perceptions of thirst ($P=0.474$), fullness ($P=0.904$) and hunger ($P=0.646$) were not different between trials indicating subjects began trials in a similar state of hydration. Total exercise time was not different for RE (53 (7) min) and HYPO (54 (9) min) ($P=0.642$), with total heat exposure including rest periods lasting 75 (10) min and 77 (14) min for RE and HYPO trials, respectively ($P=0.767$). Mean heart rate (RE: 157 (13), HYPO: 155 (15), $P=0.227$),

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RPE (RE: 14 (3), HYPO: 14 (3), P=0.215) and thermal sensation (RE: 6 (1), HYPO: 6 (1), P=0.32) were not different between trials.

Hydration Measures

There was a main effect of time (P<0.001) and an interaction effect (P<0.001) for change in body mass (Figure 3.2), but no main effect of trial (P=0.128). Compared to pre-exercise, body mass was decreased post-exercise and 13 h post-exercise during RE (P<0.05) and HYPO (P<0.001). At 13 h post-exercise body mass loss was greater during HYPO than RE (P<0.001).

For change in plasma volume there was a main effect of time (P<0.001) and trial (P<0.01), as well as an interaction effect (P<0.001). Compared to pre-exercise, there was a decrease in plasma volume at post-exercise in RE and HYPO (P<0.001), as well as at 13 h post-exercise during HYPO (P<0.001), but not RE (P=0.846). Furthermore, plasma volume was reduced during HYPO compared to RE at 13 h post-exercise (P<0.01) (Figure 3.3).

There was a main effect of time (P<0.001), trial (P<0.01) and an interaction effect (P<0.001) for urine osmolality. Compared to pre-exercise, there was an increase in urine osmolality at post-exercise during RE and HYPO trials (P<0.01) and 13 h post-exercise during HYPO (P<0.001) but not during RE (P=0.147). Furthermore, urine osmolality was greater during HYPO than RE at 13 h post-exercise (P<0.001) (Figure 3.3)

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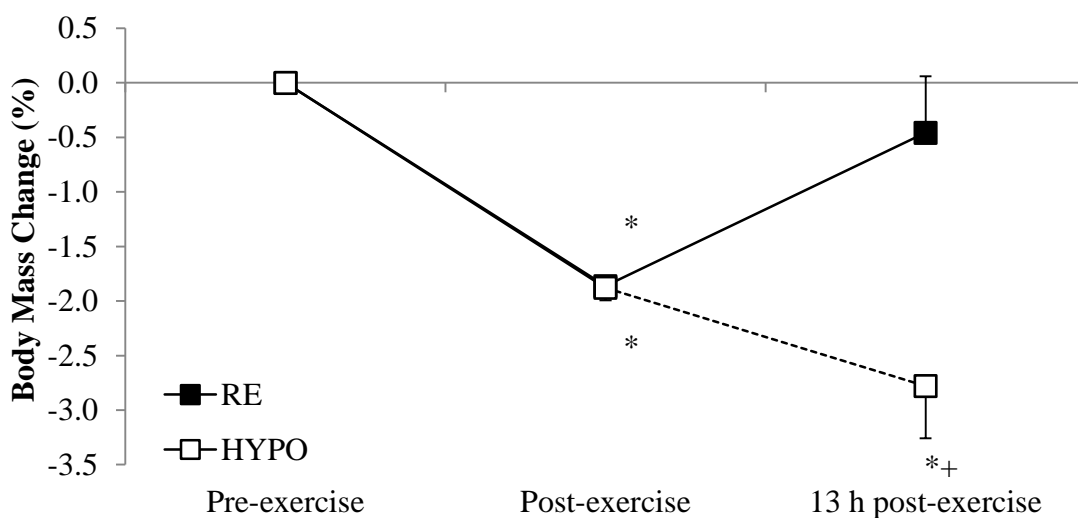
Figure 3.2. Body mass change (%) from pre-exercise. + Significantly different from RE trial. * Significantly different from pre exercise
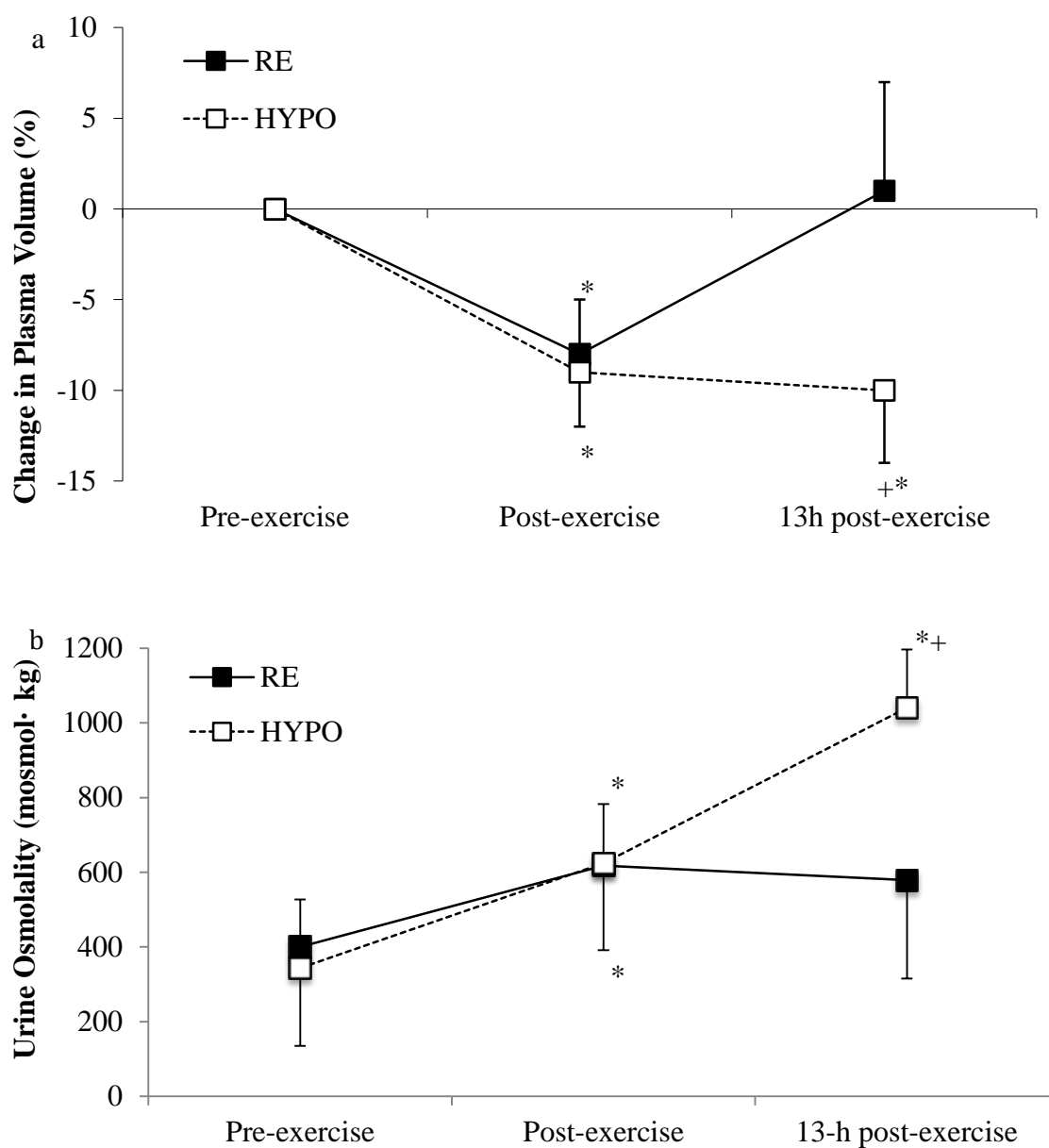
Figure 3.3. Change in plasma volume (%) from pre-exercise (a) and urine osmolality (mosmol·kg) (b). + Significantly different from RE trial. * Significantly different from pre exercise.

Appetite Measures

The total energy consumed at the *ad-libitum* breakfast was not different between trials ($P=0.436$) and there was also no difference between trials for energy consumed in foods

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(*P*=0.600) or drinks (*P*=0.147; Figure 3.4). Furthermore, protein (RE: 25 (15), HYPO 22 (11), *P*=0.415), carbohydrate (RE: 175 (54), HYPO 208 (68), *P*=0.185) and fat (RE: 32 (16), HYPO 27 (14), *P*=0.294) intake at the *ad-libitum* breakfast were not different between trials. Water intake from food was not different between trials (*P*=0.429), but a greater amount of water was ingested through drinks (*P*<0.001) as well as in total (*P*<0.001) during the HYPO trial (Figure 3.4).
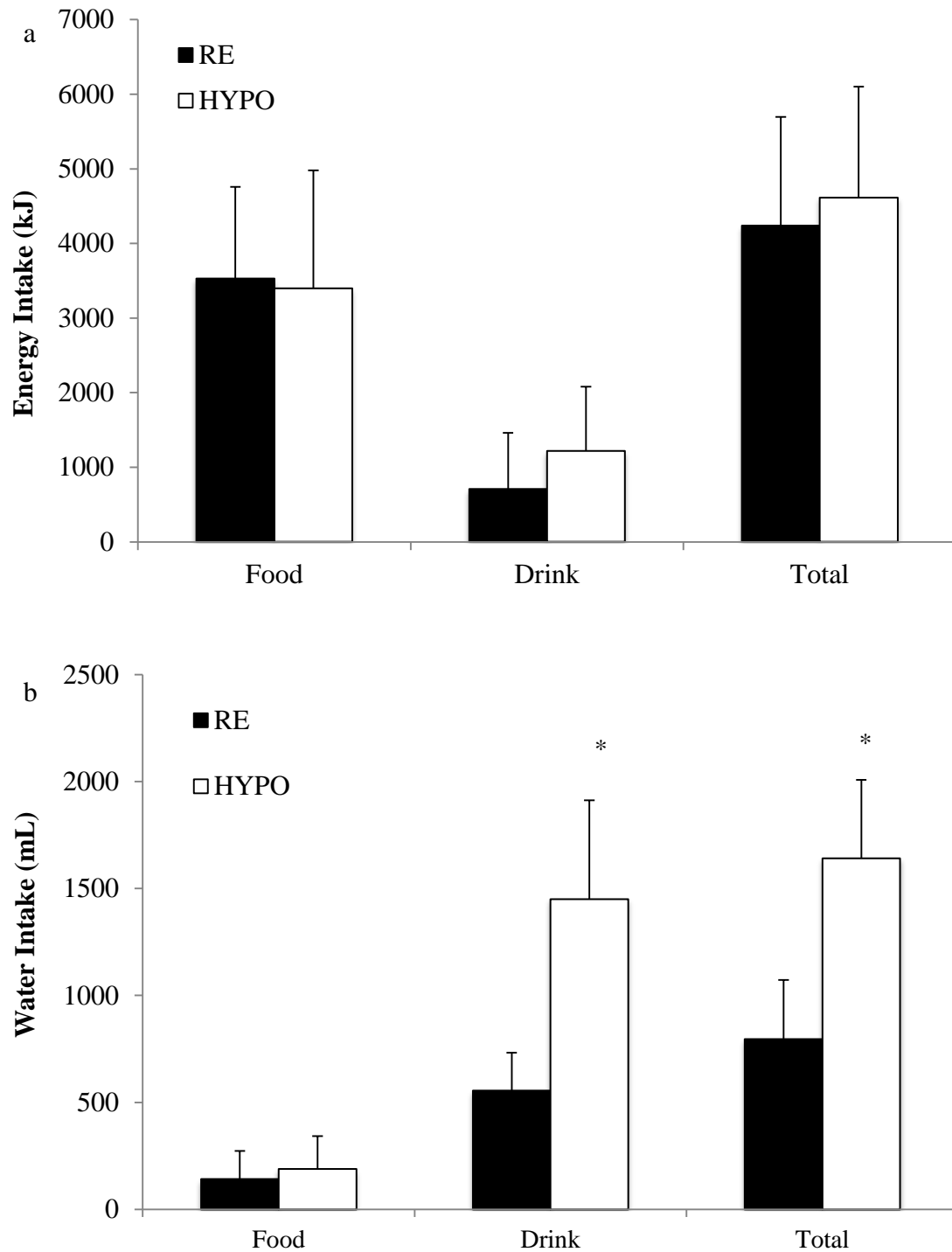
Figure 3.4. Energy (kJ) (a) and water (mL) (b) intake from food, drink and total ingested (food and drink) during the *ad-libitum* breakfast. * Significantly different from pre-exercise.
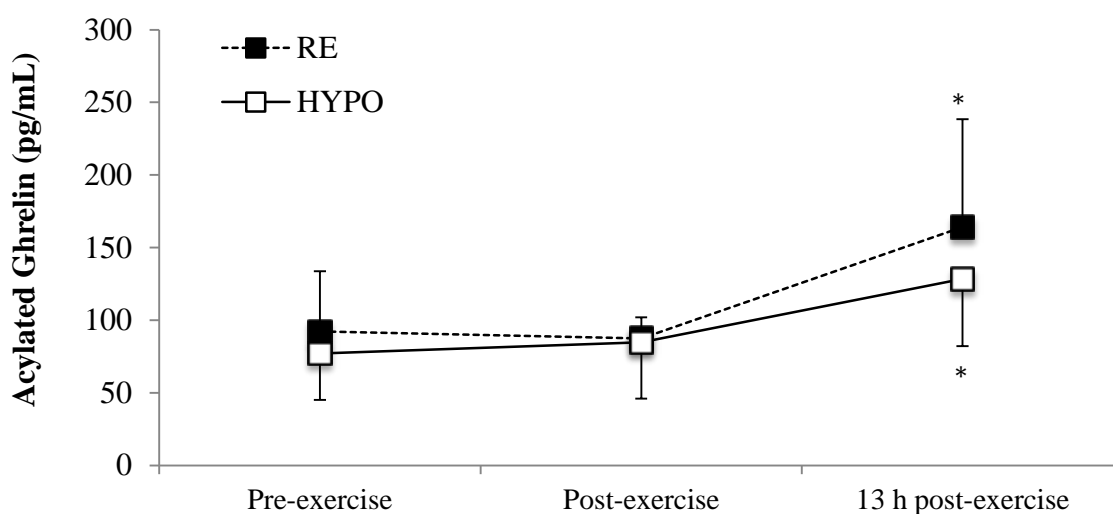
Figure 3.5 Acylated ghrelin (pg/mL) change from pre-exercise. * Significantly different from pre exercise.

There was a main effect of time ($P<0.001$), but no main effect of trial ($P=0.124$) or interaction effect ($P=0.318$) for acylated ghrelin (Figure 3.5). Compared to pre-exercise, acylated ghrelin was increased at 13 h post-exercise during both trials ($P<0.01$).

Subjective Perceptions

Subjective perceptions are presented in Table 3.1. There was a main effect of time ($P<0.001$) and trial ($P<0.05$), as well as an interaction effect ($P<0.001$) for perceptions of thirst and compared to pre-exercise, thirst was increased at post-exercise during both trials ($P<0.01$) and at 13 h post-ex during HYPO ($P<0.01$). Furthermore, perceptions of thirst were greater at 13 h post-exercise during HYPO compared to RE ($P<0.01$). There was a main effect of time ($P<0.05$) and trial ($P<0.001$), as well as an interaction effect ($P<0.001$) for perceptions of fullness. Compared to pre-exercise fullness was reduced post-exercise during both trials ($P<0.001$) and at 13 h post-exercise during HYPO ($P<0.01$). Additionally, perceptions of fullness were greater at 13 h post-exercise for RE compared to HYPO ($P<0.01$). For perceptions of hunger there was a there was an interaction effect ($P<0.019$), but no main effect of trial ($P=0.105$) or time ($P=0.838$). Compared to pre-exercise,

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perceptions of hunger were increased at 13 h post-ex during HYPO only ($P<0.01$).

Table 3.1. Subjective perceptions of thirst, fullness and hunger (mm).

	Pre-exercise	Post-exercise	13 h post-exercise
Thirst			
RE	27 (3-77)	89 (42-95)*	26 (6-85)
HYPO	16 (2-81)	86 (9-100)*	95 (62-97)*+
Fullness			
RE	59 (22-79)	24 (1-76)*	27 (4-53)*
HYPO	58 (22-80)	16 (6-65)*	8 (2-26)+
Hunger			
RE	31 (10-83)	61 (2-96)	61 (28-95)
HYPO	39 (3-75)	60 (15-87)	80 (62-94)+

+ Significantly different to RE trial (P< 0.05). * Significantly different from pre-exercise.

Discussion

The current study demonstrates that hypohydration equivalent to 2.7% body mass, produced by exercise-induced dehydration and inadequate rehydration does not affect voluntary energy intake at an *ad-libitum* breakfast or acylated ghrelin compared to a euhydrated control trial (0.5% body mass loss). These findings are contrary to our hypothesis, but are in agreement with the findings of Kelly et al. (2012).

Kelly et al. (2012) investigated the effects of hypohydration on energy intake at an *ad-libitum* meal 30 minutes after exercise, with hypohydration induced by a combination of fluid restriction and exercise. Kelly et al. (2012) manipulated hydration status by controlling fluid intake from 5pm onwards on the day before, as well as during/ after a 45 min treadmill run. During the euhydrated exercise trial, Kelly et al. (2012) provided water to subjects in a volume equivalent to 1.5 times their body mass loss during exercise, in the 30 min between

exercise and feeding, which might have attenuated food intake during the euhydrated trial (Davy et al. 2008). The aim of the current study was to examine the effect of hypohydration induced by inadequate post-exercise rehydration on appetite and energy intake. Consequently, the design of the current study meant that we eliminated any effects of immediate pre-prandial fluid intake on appetite/ energy intake as the last bolus of water in RE was provided 2 h before the *ad-libitum* breakfast. It therefore seems that moderate hypohydration 2.7% body mass loss) produced by exercise (Kelly et al. 2012) or inadequate post-exercise rehydration (present study) does not influence acute *ad-libitum* energy intake.

The notion that hypohydration does not affect appetite or voluntary energy intake is further supported by our acylated ghrelin data. Previous research has demonstrated that acylated ghrelin concentrations are reduced in response to ~60 min cycling exercise (Wasse et al. 2013). We observed no change in acylated ghrelin concentrations in response to exercise, which might be because the post-exercise blood sample was taken ~20 min post-exercise or because of the low intensity nature of the exercise protocol used. However, the finding that there was no change in acylated ghrelin in response to exercise-induced dehydration (both trials) and no difference in response between trials during the 13 h post-exercise period suggests that hypohydration has little effect on appetite regulation. Acylated ghrelin was increased at 13 h post-exercise in both trials, which is likely explained by the pre-exercise sample taken ~4 h fasting, whilst the 13 h post-exercise sample was taken after ~10.5 h fasting.

The present study and that of Kelly et al. (2012) are the only studies to investigate the effects of hypohydration induced by exercise on a voluntary energy intake. Previous research has reported in both animals (Senn et al. 1996; Watts, 1999) and humans (Engell, 1988; Shirreffs et al. 2004) that voluntary energy intake is attenuated with hypohydration or alterations in fluid balance produced by methods other than exercise. Shirreffs et al. (2004) observed that complete fluid restriction induced perceptions of thirst and dry mouth that were apparent within 13 h, but no change in hunger. Despite no difference in hunger, fluid restriction reduced energy intake by 28% over the 37 h trial compared to the euhydrated trial, a finding that has also been reported in animal studies (Senn et al. 1996; Watts, 1999). There are a number of physiological and behavioural mechanisms that might underpin these previously reported effects. Hypohydration appears to alter gastrointestinal function and

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these changes might influence the speed of digestion of a meal and consequently influence peri-prandial perceptions of satiety and food intake. For example, hypohydration decreases salivary flow rate (Walsh et al. 2004; Oliver et al. 2008), as well as slowing gastric emptying and reducing gastric secretions (Neufer, Young and Sawka, 1989; Rehrer et al. 1990). Although not reported in humans directly, these effects on gastrointestinal function have been reported in animals in parallel with reductions in food intake (Silankove, 1992; Callahan and Rinaman 1998).

Engell, (1988) has previously shown that peri-prandial fluid restriction to ~40% of *ad-libitum* intake reduces voluntary energy intake. This may offer a plausible explanation as to why previous studies have reported reductions in energy intake with hypohydration, but our research and Kelly et al. (2012) did not. In the present study and that of Kelly et al. (2012) fluids were provided during the *ad-libitum* meal and consequently subjects were likely to ingest adequate fluid to alleviate sensations of thirst. Previous studies that restricted fluid during feeding (Engell, 1988), as well as restricting foods to those with low moisture content (Silankove, 1992; Senn et al. 1996; Shirreffs et al. 2004 Watts, 1999) may have reduced the palatability of foods or caused a requirement for extra chewing due to reduced salivary flow rate (Oliver et al. 2007; Walsh et al. 2004). Behavioural factors might also explain the findings of previous studies, as eating and drinking are episodic behaviours that occur concurrent to each other (Engell, 1988; McKiernan et al. 2008). It therefore seems likely that mild hypohydration does not influence voluntary energy intake, but that inadequate fluid availability during meals might reduce food intake by altering the palatability of foods or affecting feeding behaviour.

Interestingly, we observed that perceptions of fullness were lower during the HYPO trial than during the RE trial 13 h post-exercise, whilst perceptions of hunger were increased at the same time point during the HYPO trial only. As subjects were provided with identical meals to consume after exercise, it seems likely that these sensations were caused by the 2536 (361) mL water consumed over the 13 h between exercise and the *ad-libitum* meal in the RE trial. The present study was designed to ensure that no water was ingested in the immediate pre-meal period. The water was ingested in four aliquots, three in the evening after exercise (13 h, 10.5 h and 9 h before the *ad-libitum* breakfast), with the final aliquot ingested in the morning, 2 h before the *ad-libitum* breakfast. We allowed 2 h between the

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final bolus of water and the *ad-libitum* breakfast to ensure all the water had emptied from the stomach (Vist and Maughan, 1994) and to avoid any effects of acute water intake on subjective appetite or food intake (Davy et al. 2008) that might occur due to increases in gastric distension (Vrang et al. 2003). Despite the divergent responses for fullness and hunger sensations between trials, there was no difference in food intake. This is not surprising as there is often a mismatch between subjective appetite responses and actual food intake (McKiernan et al. 2008). However, an alternative explanation might be that the increase in thirst sensations 13 h post-exercise during HYPO meant that a large proportion of the extra 896 (284) mL water ingested through drinks was ingested at the beginning of the 30 min feeding period before any food had been consumed. Although speculative, this might have reversed the subjective fullness/ hunger responses observed during the HYPO trial and attenuated food intake in this trial.

During the present study a variety of fluids were provided during the meal for subjects to ingest *ad-libitum*. Subjects ratings of thirst were greatly increased immediately before the meal in the HYPO trial and consequently they ingested 798 (284) mL more water through foods and drinks compared to the RE trial. Despite this increased thirst and fluid intake during the HYPO trial, total water intake was only sufficient to replace 69 (31) % of the total water loss (estimated from body mass changes over the trial). The American College of Sports Medicine (Sawka et al. 2007) recommends that a volume of fluid equivalent to ~150% of an individual's body mass loss should be ingested to fully and rapidly restore fluid balance. Whilst normal food and fluid intake is likely to restore fluid balance if sufficient time is available, the present study demonstrates that if a subsequent bout of exercise is to be performed in close proximity to an episode of hypohydration, then allowing an individual to ingest food and fluid *ad-libitum* will not fully restore fluid balance. In such situations, a structured rehydration plan should be implemented to ensure complete rehydration (Sawka et al. 2007). This might be of particular relevance to athletes competing in weight category sports who have just made weight or athletes undertaking more than one training session a day.

These findings have important implications for recovery from exercise, where athletes, coaches and support staff should ensure sufficient fluid is available during meal times so that food (and nutrient) intake in recovery is not compromised. It is well known that

adequate carbohydrate (Burke et al. 2011) and protein (Phillips and Van Loon, 2011) intake is required in the post-exercise period to optimise post-exercise recovery, as well as subsequent exercise performance. Previous research suggests that if inadequate fluid is available at meal times then food intake might be reduced (Engell 1988; Silankove, 1992; Senn et al. 1996; Shirreffs et al. 2004; Watts, 1999). This will have the effect of reducing the intake of specific macronutrients and micronutrients of importance for post-exercise recovery (e.g. glycogen resynthesis, protein synthesis etc.) and consequently this inadequate intake might impair subsequent exercise performance or adaptation to exercise training.

Conclusion

In conclusion, the findings of the present study suggest that whilst hypohydration might influence subjective perceptions of hunger and fullness it does not affect voluntary energy intake when fluid is readily available with a meal. Whilst hypohydration increased thirst sensations and *ad-libitum* fluid intake, the level of fluid intake was below that required to fully restore fluid balance. Future research should seek to examine the interaction between hydration status and fluid availability on appetite and energy intake.

Chapter 4
Effect of Hydration Status and Fluid Availability on *Ad-Libitum* Energy Intake of a Semi-Solid Breakfast

Abstract

The present study investigated the interaction between hypohydration and fluid availability on voluntary energy intake. Sixteen healthy males (age: 25 (4) y, body mass: 72.6 (8.6) kg, height: 1.78 (0.07) m, BMI: 22.9 (1.7), body fat %: 15.1 (4.4)%) completed four 24 h trials (euhydrated with (EU-F), or without fluid (EU-NF) at the *ad-libitum* meal or hypohydrated with (HYPO-F) or without fluid (HYPO-NF) at the *ad-libitum* meal. Food and water ($40 \cdot mL \cdot kg^{-1}$ body mass; EU trials only) was provided. Body mass, urine and blood samples were collected and subjective perceptions completed (0 h, 24 h and 24.5 h). Subjects consumed an *ad-libitum* breakfast at 24 h to satiety. Body mass decreased during HYPO-F (-1.78 (0.53)%) and HYPO-NF (-1.89 (0.45)%) ($P<0.001$), but not during EU-F (-0.28 (0.59)%) of EU-NF (-0.35 (0.51)%) ($P>0.05$). Urine volume was greater during EU-F (2262 (494) mL) and EU-NF (2478 (494) mL) than HYPO-F (724 (272) mL) and HYPO-NF (806 (201) mL) ($P<0.001$). Compared to 0 h, serum and urine osmolality increased and plasma volume decreased during HYPO-F and HYPO-NF ($P<0.001$) but not during EU-F and EU-NF ($P>0.05$). There was no change in energy intake at the *ad-libitum* meal 2658 (938) kJ (EU-F), 2353 (643) kJ (EU-NF), 2295 (529) kJ (HYPO-F), 2414 (954) kJ (HYPO-NF), ($P=0.188$), but fluid intake was greater at HYPO-F (618 (251) mL) than EU-F (400 (247) mL) ($P<0.01$). Apart from thirst ($P<0.05$), there were no difference in subjective perceptions (hunger and fullness; $P>0.05$). There was no difference in energy intake of hypohydration or fluid availability on energy intake at an *ad-libitum* semi-solid breakfast.

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Introduction

Deviations in energy balance (positive and negative) can have a profound effect on health (Bawadi et al. 2016), thus a better understanding of the physiological systems affecting energy balance is required. Alterations in appetite influence energy intake and consequently potentially impact on energy balance. Whilst much research has focused on the effects of the energy containing macronutrients on appetite, (Anderson et al. 2002; Clegg and Shaftat, 2010; Metges and Barth, 2000; Rodin, Reed and Jamner, 1988), relatively little is known about how deviations in water balance and water intake impact upon appetite and energy balance.

Currently, the effect hydration status has on appetite regulation and voluntary food intake in humans is not fully understood, but there are a number of situations where hydration status might impact appetite regulation and thus health or performance of an individual. Hypohydration is commonly a cause of morbidity and mortality in the elderly (Ferry, 2005), a population group where both chronic hypohydration (Ferry, 2005; Warren et al. 1991;) and a reduction in energy intake are apparent (Mowe, Bohmer and Kindt, 1994). Additionally, both competitive and recreational exercisers commonly finish exercise hypohydrated (Sawka et al. 2007) and if hydration status affects appetite regulation, optimal post-exercise nutritional intake might be compromised.

Research in animal models has consistently reported water intake being a major determinant of the amount of food consumed (Senn et al. 1996; Silanikove, 1992 Watts, 1999). For example, Senn et al. (1996) investigated water deprivation on eating behaviour over 48 h in lactating cows. Water deprivation was shown to reduce grass and corn pellet intake significantly, with the size of the first meal being reduced by ~30%. Watts (1999) induced dehydration differently, by providing 2.5% saline (w/v) for 4 days. By the fourth day, food intake was reduced to less than 20% compared to the normal water-drinking day. Silanikove (1992) observed similar findings in cows whereby when water availability was reduced in ruminants there was a parallel reduction in voluntary feed intake. These findings may be explained by the dry feed intake provided and the subsequent reduction in palatability when hypohydration occurs (Oliver et al. 2007). Silanikove, (1992) further explains that the restriction of water availability to once every 24, 48 and 72 h caused a reduction in resting salivary flow rate of 40%, 60% and 80 % respectfully.

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In humans, only a limited number of studies have investigated the impact of water balance on appetite regulation or food intake. Shirreffs et al. (2004) reported a reduction in energy intake with 37 h of complete fluid restriction compared to when fluids were provided *ad-libitum*, whilst Engell (1988) reported a reduction in food intake at 6 meals over 48 h when fluid was restricted at meal times. In contrast, two recent studies (Kelly et al. 2012; Chapter 3) observed no difference in *ad-libitum* energy intake between a hypohydrated (2-3% body mass loss) and euhydrated trial, with hydration status manipulated using a combination of exercise-induced sweating and fluid restriction. Both these studies (Kelly et al. 2012; Chapter 3) provided fluids at the *ad-libitum* meal. Taken together, these studies suggest that hypohydration effects voluntary food intake when fluid is restricted (Engell, 1988; Shirreffs et al. 2004), but not when fluid is provided during feeding (Kelly et al. 2012; Chapter 3).

Therefore, the purpose of the current study was to examine the interaction between pre-meal hydration status and fluid availability during feeding on voluntary food intake. It was hypothesised that fluid restriction during feeding would reduce food intake in both hypohydrated and euhydrated conditions, but that hypohydration would not affect food intake when fluid was available.

Methods

Subjects

Sixteen healthy male subjects aged: (25 (4) years, height: 1.78 (0.07) m, body mass: 72.6 (8.6) kg, body fat %: 15.1 (4.4)%, BMI: 22.9 (1.7) volunteered to participate in the present study. Subjects were all regular exercisers and breakfast consumers. There were 2 dropouts from this study due to the subjects not being able to fulfill the time commitments.

Preliminary and Experimental Trials

All subjects completed a familiarisation trial followed by 4 experimental trials, which were completed in a randomised, counterbalanced fashion and separated by at least 7 days.

The familiarisation trial was the same as the experimental trials as explained below. Subjects had the opportunity during the familiarisation trial to taste test the porridge to

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determine their preferred flavour and become familiarised with with all procedures (urine sampling, blood sampling and kitchen scales). Each subject completed 4 experimental trials in a randomised counterbalanced, crossover manner: euhydrated with fluid at the *ad-libitum* meal (EU-F), euhydrated with no fluid at the *ad-libitum* meal (EU-NF), hypohydrated with fluid at the *ad-libitum* meal (HYPO-F), hypohydrated with no fluid at the *ad-libitum* meal (HYPO-NF). After an overnight fast, subjects arrived at a time typical for them to consume breakfast (between 7 and 10 am). Each trial consisted of a two-day experimental protocol. On day 1, subjects emptied their bladder and bowels and their body mass (nude) was measured to the nearest 10 g. Following 15 min seated rest, a baseline blood sample (15 mL) was collected from an antecubital vein. During the 15 min seated, subjects rated their subject perceptions of thirst, fullness and hunger using 100 mm visual analogue scale questionnaires (Flint et al. 2000).

For all trials, food was provided to subjects on Day 1 (pizza, crisps, cereal bars, chocolate bars, sandwiches), along with water in the euhydrated trials (EU-F and EU-NF). Amounts were determined by using a predictive equation for resting energy expenditure (Mifflin et al. 1990), which was then multiplied by a physical activity level of 1.6. Total energy intake for the 24 h was 2554 (206) kJ; 68 (11) g of protein; 327 (35) g of carbohydrate; 108 (12) g of fat. Total water provided during the euhydrated trials was $40 \cdot mL \cdot kg^{-1} \cdot BM$ and provided in 6 equal aliquots consumed at set times during each trial (i.e. at 0 h, 4 h, 6 h, 8 h 10 h and 13 h of each trial). No water was provided in the hypohydrated trials (HYPO-F and HYPO-NF).

Subjects then returned 24 h later (day 2) again after an overnight fast and all measurements previously made on day 1 were repeated. After blood sampling, subjects were provided an *ad-libitum* breakfast meal consisting of porridge oats (Ready Brek, Weetabix, Kettering, UK) and semi-skimmed milk (Tesco Stores Ltd., Chestnut, UK) in a ratio of 100 g porridge oats to 423 mL milk. Each bowl of porridge received identical heating and cooling before being served. Once porridge was mixed it was placed in the microwave for 2 minutes at full power, it was then taken out stirred with a spoon and then placed in the microwave for a further 30 seconds. The porridge then rested for 3 minutes before being weighed and served. Although temperature was not measured, tasting was performed by investigators and subjects during the familiarisation trial and deemed ideal for consumption. The porridge was replaced every ~3min to ensure subjects always had warm porridge (time determined by

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familiarisation) (Appendix K).

Warm porridge was continually available for subjects in the feeding booth. During EU-F and HYPO-F, 500 mL of water and 500 mL of low sugar cordial were provided for subjects to drink *ad-libitum*, whilst during EU-NF and HYPO-NF no fluid was provided. At the end of the 30 min feeding period, subjects completed a final visual analogue scale questionnaire.

A urine sample was collected upon arrival to the laboratory at 0 h and 24 h and then all subsequent urine produced between 0 h and 24 h in a container provided. The volume of all urine samples was measured and a sample retained for analysis of osmolality.
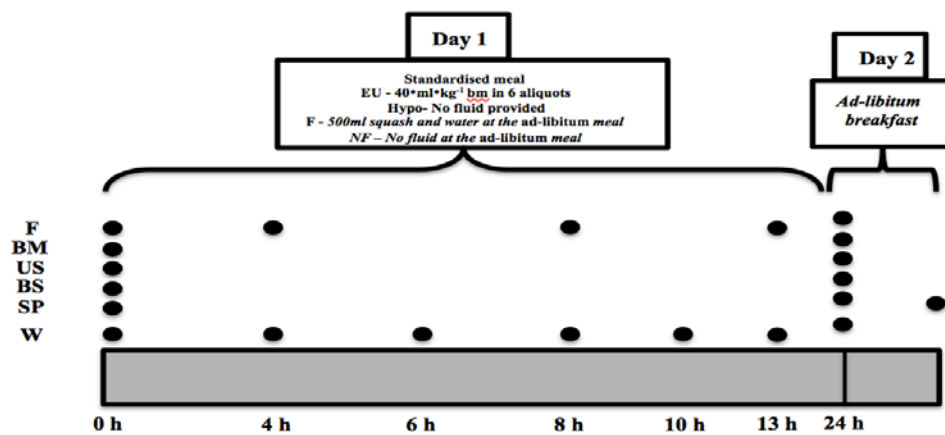
Figure 4.1. Study protocol. F: food, W: water, US: urine sample, BM: body mass, BS: blood sample SP: subjective perceptions.

Results

Pre-trial Measurements

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

Hydration Variables

There was a main effect of trial ($P<0.001$) and time ($P<0.001$), as well as an interaction effect ($P<0.001$) for body mass. Body mass was similar at 0 h, but at 24 h was lower during

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HYPO-F and HYPO-NF compared with EU-F and EU-NF ($P<0.001$). Over the 24 h, body mass decreased during HYPO-F (-1.78 (0.53)% ($P<0.001$) and during HYPO-NF (-1.89 (0.45)% ($P<0.001$) and remained unchanged during EU-F (-0.28 (0.59)%) ($P=0.07$) and decreased during EU-NF (-0.35 (0.51)% ($P<0.05$). Total 24 h urine volume was greater for EU-F (2262 (494) mL) and EU-NF (2478 (294) mL) compared to HYPO-F (724 (272) mL) and HYPO-NF (806 (201) mL) ($P<0.001$) (Table 4.1).

Table 4.1. Body mass (kg) change from 0 h.

	0 h	24 h
EU-F	72.49 (8.55)	72.30 (8.61)
EU-NF	72.46 (8.45)	72.22 (8.48)*
HYPO-F	72.46 (8.56)	71.16 (8.33)*+
HYPO-NF	72.61 (8.58)	71.24 (8.48)*+

+ Significantly different from EU trial. * Significantly different from 0 h.

For both urine and serum (Figure 4.2) osmolality, there were main effects of trial ($P<0.001$) and time ($P<0.001$), as well as an interaction effect ($P<0.001$). Compared to 0 h, urine osmolality at 24 h did not change during EU-F ($P=0.446$) and EU-NF ($P=0.163$), but increased during osmolality during HYPO-F ($P<0.001$) and HYPO-NF ($P<0.001$). Similarly, compared to 0 h, serum osmolality at 24 h did not change during EU-F ($P=0.524$) and EU-NF ($P=0.492$), but increased during HYPO-F ($P<0.001$) and HYPO-NF ($P<0.001$). Whilst there was no difference in urine or serum osmolality at 0 h, both were greater during HYPO-F and HYPO-NF compared to EU-F and EU-NF ($P<0.001$) at 24 h.

There was a main effect of trial ($P<0.01$) and time ($P<0.01$), as well as an interaction effect ($P<0.01$) for plasma volume. Over the 24 h, there was no change in plasma volume during EU-F (-1 (3) %: $P=0.640$) and EU-NF (-2 (3) %: $P=0.130$), but a decrease during HYPO-F (-3 (2) %: $P<0.01$) and HYPO-NF (-3 (2) %: $P<0.01$). At 24 h plasma volume was reduced during HYPO-F and HYPO-NF compared to EU-F and EU-NF ($P<0.01$).
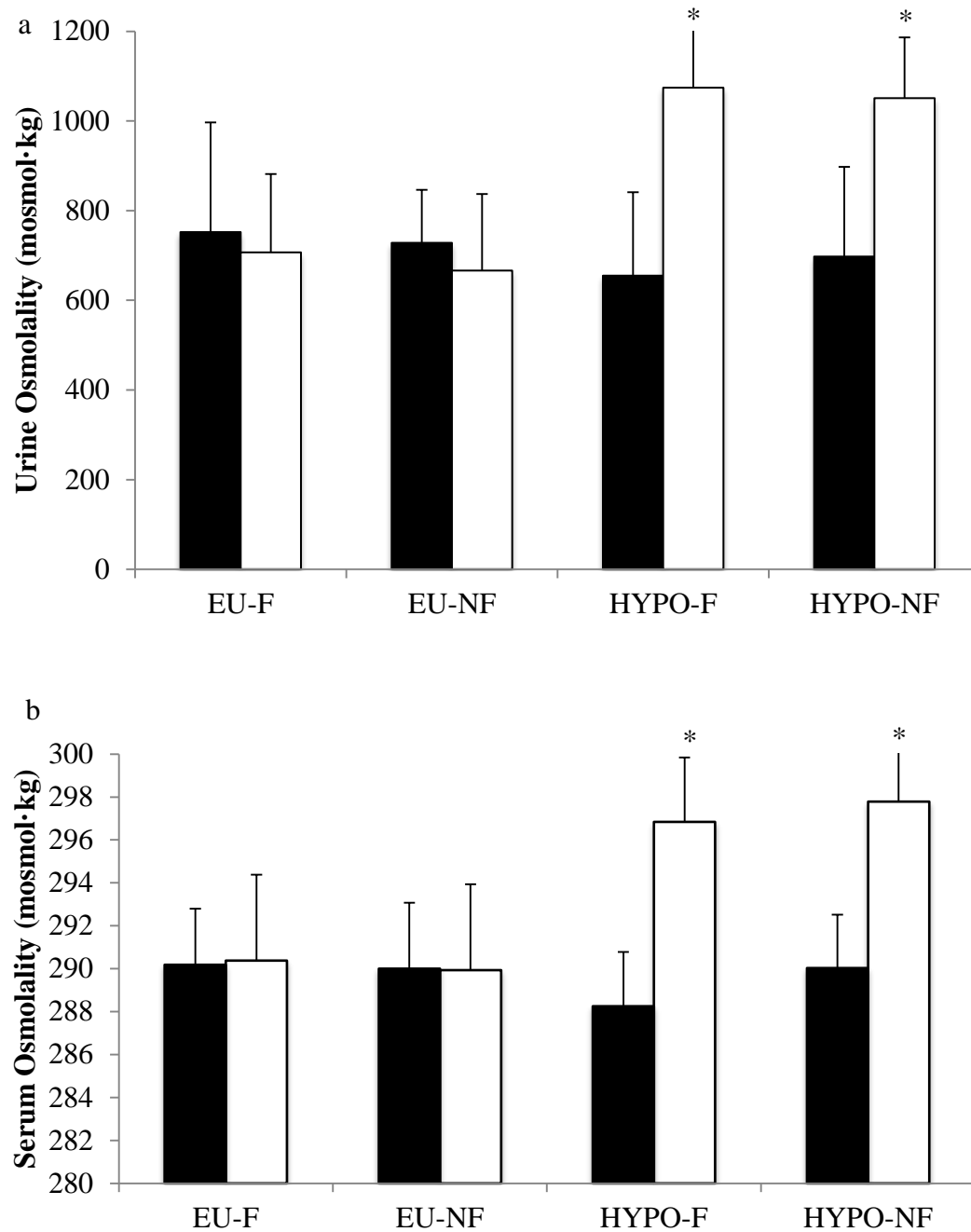
Figure 4.2. Urine osmolality (mosmol·kg^{-1}) (a) and serum osmolality (mosmol·kg^{-1}) (b) at 0 h (black) and 24 h (white).

Appetite Responses

There was no difference between trials for *ad-libitum* energy intake (P=0.205) (Figure 4.3). Furthermore, there was no difference in energy intake when data was grouped according to hydration status, (EU trials (2491 (796) kJ; HYPO trials (2313 (737) kJ; P=0.120) or fluid

availability (F trials (2460 (761) kJ; NF trials 2344 (780) kJ; P=0.410). More fluid was consumed in the HYPO-F trial, (618 (251) mL) compared to the EU-F trial (400 (247) mL) (P<0.05).
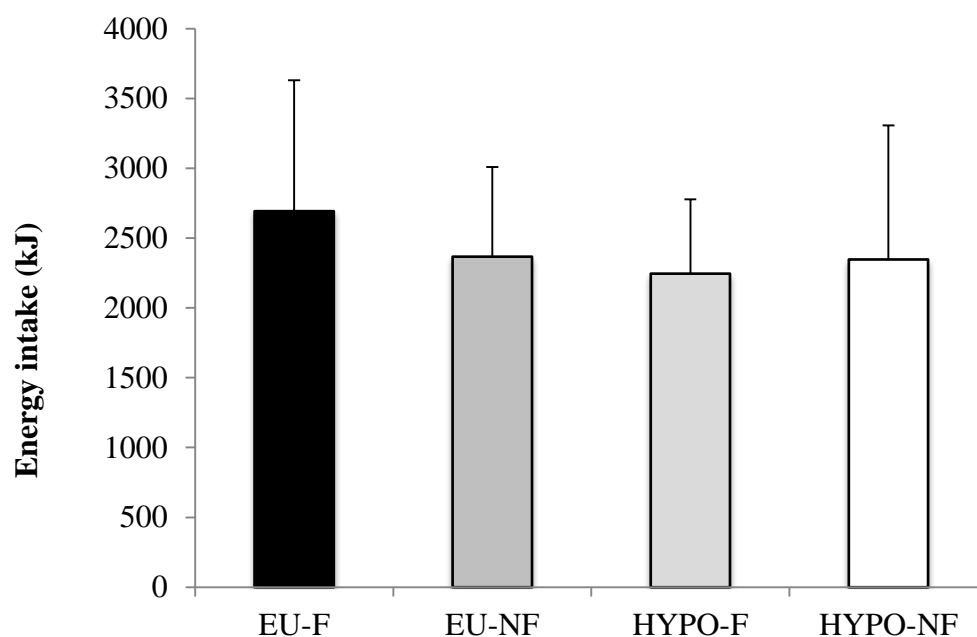
Figure 4.3. Energy (kJ) consumed at the breakfast *ad-libitum* meal.

For acylated ghrelin, there was a main effect of time ($P<0.01$), but no main effect of trial ($P=0.089$) or interaction effect ($P=0.985$). Mean values decreased over the 24 h for all trials, but this only reached significance during HYPO-F ($P<0.05$) and tended to decrease during EU-F ($P=0.052$) (Table 4.2).

Table 4.2. Acylated ghrelin (pg/mL). * Significantly different from 0 h. Values are mean and SD.

	0 h	24 h
EU-F	112 (78)	97 (65)
EU-NF	129 (97)	114 (80)
HYPO-F	161 (137)	144 (122)*
HYPO-NF	150 (110)	133 (96)

There was a main effect of time ($P<0.001$) and trial ($P<0.001$), as well as an interaction

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effect ($P<0.001$) for subjective perceptions of thirst (Table 4.3). Compared to 0 h, thirst was increased at 24 h during HYPO-F ($P<0.001$) and HYPO-NF ($P<0.05$) and reduced at 24.5 h during EU-F ($P<0.01$) and HYPO-F ($P<0.01$). Thirst was greater at 24 h during HYPO-F and HYPO-NF compared to EU-F and EU-NF ($P<0.001$) as well as at 24.5 h during EU-NF and HYPO-NF compared to during EU-F and HYPO-F ($P<0.001$). For both fullness and hunger (Table 4.3) there was a main effect of time ($P<0.001$), but no main effect of trial ($P=0.294$) or interaction effect ($P=0.069$). Compared to 0 h, fullness was increased at 24 h during EU-NF ($P<0.05$) and at 24.5 h during all trials ($P<0.001$). Conversely, compared to 0 h hunger was decreased at 24.5 h during all trials ($P<0.001$).

Table 4.3 Subjective perceptions reported using 100 mm visual analogue scales for thirst, fullness and hunger. ^ Significantly different from EU-F and EU-NF. # Significantly different from EU-NF and HYPO-NF. * Significantly different from 0 h

		0 h	24 h	24.5 h
Thirst	EU-F	52 (30)	49 (18)	14 (26)*#
	EU-NF	54 (26)	54 (26)	61 (30)
	HYPO-F	52 (17)	90 (7)^*	20 (31)*#
	HYPO-NF	62 (22)	85 (28)^*	66 (28)
Fullness	EU-F	25 (22)	26 (15)	80 (12)*
	EU-NF	23 (15)	33 (12)*	81 (9)*
	HYPO-F	28 (18)	25 (22)	83 (9)*
	HYPO-NF	30 (15)	28 (28)	75 (13) *
Hunger	EU-F	56 (31)	63 (21)	9 (6)*
	EU-NF	65 (26)	58 (26)	15 (19)*
	HYPO-F	66 (21)	65 (27)	9 (10)*
	HYPO-NF	65 (15)	66 (35)	15 (20)*

Discussion

This study compared food intake, acylated ghrelin and subjective appetite sensations in response to alterations in hydration status and fluid availability. The main findings indicated that voluntary food intake, acylated ghrelin and appetite sensations were not different between trials, although thirst was increased with hypohydration. These findings suggest that human appetite is not affected by moderate levels of hypohydration, which contrasts with previous research in animals (Senn *et al*. 1996; Silanikove, 1994; Watts, 1999) and humans (Engell, 1988; Shirreffs *et al*. 2004) as well as our main hypothesis.

Shirreffs, *et al*. (2004) reported that complete fluid restriction over a 37 h period reduced voluntary energy intake by ~28% compared to a euhydrated control trial where subjects were free to consume fluids *ad-libitum*, despite no difference in subjective appetite sensations. Shirreffs et al. (2004) limited subjects to the consumption of "dry foods" during the fluid restriction trial, but could consume any foods during the control trial. It is possible that these changes in feeding behaviour or the consumption of energy containing fluids in the control trial might explain the difference in energy intake between the trials. Engell (1988) investigated energy consumption during six consecutive meals over 48 h and found that when fluid was limited to 43% of *ad-libitum* fluid intake, voluntary food intake was reduced by ~37% compared to a trial where fluids were available *ad-libitum*. Although fluid was restricted, hydration status was not measured and therefore the findings have been attributed to a close relationship between eating and drinking patterns (Engell, 1988). This is further described by McKiernan et al. (2008), whereby drinking independently of eating is reportedly rare and approximately 75% of fluid intake is consumed with meals.

Although it appears there is a strong behavioural link between food and drink, it has been suggested that there may be certain physiological mechanisms that might explain changes in food intake in response to fluid restriction and/ or hypohydration. Walsh et al. (2004) and Oliver et al. (2008), have reported that hypohydration decreases salivary flow rate, as well others reporting decreases in gastric emptying and reducing gastric secretions in a hypohydrated state (Neufer et al. 1989; Rehrer et al. 1990). Decreased salivary flow rates and symptoms of dry mouth have also been presented and in the elderly (Lovat, 1996). This reduction in energy intake may be due to peri-prandial perceptions of satiety from the lack of palatability and fluid availability to appropriately assimilate foods. Silanikove (1992) has

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reported in ruminants, during a period of water restriction of up to a 72 h period, parallel reduction salivary secretions and feed intake. Previous studies that restricted fluid during feeding (Engell, 1988), as well as restricting foods to those with low moisture content (Silanikove, 1992; Shirreffs *et al*. 2004) may have reduced the palatability of foods or caused a requirement for extra chewing due to a reduced salivary flow rate (Lovat, 1996; Oliver *et al*. 2008; Silanikove, 1992; Walsh *et al*. 2004;).

Although previous research reported a behavioural link between food and drink (Engell, 1988; McKiernan et al. 2008) and hydration status can be linked to gastrointestinal functions which may affect energy intake (Neufer et al., 1989; Rehrer et al. 1990) we observed no change in energy intake between the trials, regardless of whether water was provided or not during the *ad-libitum* meal. The type of *ad-libitum* meal provided to the subjects may explain this lack of difference due to the semi-solid nature of the porridge. As the reported reductions in energy intake may be associated with reductions in salivary flow rate and palatability (Backtrom et al. 1995; Lovat, 1996; Silanikove, 1992; Shirreffs et al. 2004), the choice of a semi-solid porridge breakfast may have been enough to restored satiety during the meal. It may therefore be speculated that due to previous findings (Shirreffs et al. 2004) that if the breakfast was provided of a dry nature, there may have been decreases in energy intake due to palatability. However, it may also be highlighted that we only observed a single *ad-libitum* meal and if fluid was restricted during several meal opportunities, then a pattern may have emerged similar to findings observed previously (Engell, 1988).

During a study by Kelly et al. (2012) subjects did not differ in their energy intake at an *ad-libitum* breakfast buffet when hypohydrated by (2-3% body mass loss). Kelly et al. (2012) controlled from fluid intake 5pm onwards on the first day and then during/ after a 45 min treadmill run on the second day. Water was then provided during the Euhydrated trial only (1.5 times their body mass loss) in the 30 min between the cessation of exercise and the *ad-libitum* meal to rehydrate subjects to pre-trial levels. Consequently, the design of this study may have resulted in an attenuated food intake during the euhydrated trial due to the large bolus of water provided (Davy et al. 2008). Thus, we extended the post exercise rehydration period to eliminate any effects of immediate pre-prandial fluid intake. However, there were still no differences found between the trials when subjects were either rehydrated or hypohydrated. It could be therefore suggested that moderate hypohydration (2-3% body

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mass loss) produced by exercise (Kelly et al. 2012) or inadequate post-exercise rehydration (Chapter 3) does not influence acute *ad-libitum* energy intake. Interestingly, both of these research studies provided fluid during the *ad-libitum* meal and therefore may suggest that once the sensations of thirst for fluid is attenuated, satiety returns. Although not reported in humans, rats have been shown to rapidly restore normal eating patterns when water is provided again after 5 days of dehydration induced anorexia (Watts, 1999) and may help to explain the findings reported by Kelly et al. (2012) and (Chapter 3).

Therefore, the current findings suggest that when fluid intake is restricted and/or hypohydration induced during feeding, food intake is not different, which is further supported by our acylated ghrelin data and subjective perceptions for fullness and hunger. We observed no change in acylated ghrelin concentrations between trials in response to hypohydration, which we have also found previously (Chapter 3). During our previous study (Chapter 3) subjects were hypohydrated by exercise in the heat and the lack of difference in acylated ghrelin concentrations were accompanied by no differences in energy intake or perceptions of hunger and fullness at the *ad-libitum* meal. Unfortunately, research findings associated with energy intake decreases and hypohydration have not measured acylated ghrelin concentrations and therefore cannot be compared. Although, a study by Kelly et al. (2012) did find that when subjects were hypohydrated acylated ghrelin was decreased in relation to the euhydrated and control trials. Although interesting, this is the only study to represent this suppression in acylated ghrelin when hypohydrated during exercise, and although acylated ghrelin was changed, energy intake was still not different between the euhydrated and hypohydrated trials.

In the current study there was no effect of pre-meal hydration status or fluid availability at the meal suggesting that neither of these affect voluntary breakfast intake. Although there was no main effect for voluntary food intake at the *ad-libitum* meal in our study, individual comparison of the EU-F and HYPO-F trials revealed food intake was greater during EU-F than during HYPO-F ($P<0.05$), whilst fluid intake was greater during HYPO-F (618 (281) mL than EU-F (400 (247) mL, ($P<0.01$). Given the greater thirst perception pre-meal during HYPO-F, it seems logical that when presented with fluid, subjects would immediately ingest a bolus of fluid to satiate their thirst. What effect an immediate pre-meal bolus of water might have on food intake is not currently known, but data from animal studies suggests that

increasing gastric wall tension can have a profound effect on food intake (Paintal, 1954). Two studies (Van Walleghen et al. 2007; Davy et al. 2008) have investigated the effect of ingestion of a water preload on *ad-libitum* food intake. Both these studies provided water 30 min prior to an *ad-libitum* meal and whilst it appears that ingestion of a water preload 30 min prior to a meal might reduce food intake in an elderly population (Van Walleghen et al. 2007), there does not appear to be any effect in healthy young males. If ingestion of a water bolus attenuates food intake it is likely this will be due to a mechanical effect (i.e. increase gastric wall tension) as opposed to any physiological effect (Paintal, 1954). With the rapid gastric emptying rate of water, the majority of the water in these studies would be emptied from the stomach (Vist and Mauaghn, 1994) and thus there would be little gastric tension remaining, at least in healthy young subjects. The ingestion of a bolus of water immediately prior to food ingestion might however provide a novel method of acutely reducing food intake and warrants further investigation. The appetite perceptions, as with the acylated ghrelin data were unchanged for hunger and fullness, although subjective thirst was increased during the HYPO trials. Interestingly, although more fluid was consumed during the HYPO-F trial (618 (251) mL compared to the EU-F trial (400 (247) mL and throughout the EU trials, there were no differences in fullness and hunger perceptions.

Conclusion

In conclusion, these results demonstrate that in a laboratory setting there appears to be little effect of hypohydration or fluid availability on voluntary intake of a typical semi-solid breakfast meal. This suggests that the results of previous studies might therefore be related to the provision of dry foods during fluid restriction (Shirreffs et al. 2004) or behavioural links between food and fluid (Engell, 1988; McKiernan et al. 2008).

Chapter 5
Immediate Pre-Meal Water Ingestion Decreases Voluntary Food Intake in Lean Young Males

Abstract

Consuming 375-500 mL of water 30 min before a meal has been shown to reduce energy intake in older, but not younger adults (Davy et al. 2008; Van Walleghen et al. 2007). As the time interval to the meal may be the determining factor in younger adults, this study investigated the effect of immediate pre-meal water preload on *ad-libitum* energy intake in non-obese young adults. Fourteen healthy males (age 27 (3) y, Height 1.83 (0.05) m, body mass 80.47 (9.89) kg, body fat 17.5 (4.0) %, body mass index 24.0 (2.5) kg·m^2) completed two experimental trials in randomised counterbalanced, order. Subjects arrived at the laboratory overnight fasted and consumed an *ad-libitum* porridge breakfast. Immediately prior to the meal, subjects consumed either a 568 mL (1 pint) water preload (P) or no preload (NP). Visual analogue scale questionnaires to assess hunger, fullness and satisfaction were completed before and after the meal in both trials, as well as after the water preload during P. During the *ad-libitum* meal, more food (NP: 2551 (562) kJ, P: 1967 (454) kJ, $P<0.001$) and water (NP: 318 (226-975) mL, P: 116 (0-581) mL, ($P<0.001$)) was consumed during NP. The water preload increased fullness and satisfaction and decreased hunger compared to pre-trial ($P<0.001$). This study demonstrates that consumption of 568 mL water preload immediately before a meal reduces energy intake in non-obese young males. This is therefore an effective strategy to suppress energy intake in this population and assist with weight management.

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Introduction

The global prevalence of obesity and its co-morbidities means that there is a growing need to identify strategies to facilitate appropriate food intake (World Health Organisation, 2012). In overweight and obese populations, strategies that promote a negative energy balance and subsequent weight loss are required. To prevent the continued rise in obesity prevalence, interventions that attenuate or prevent a positive energy balance and weight gain among healthy weight individuals are vital (Health & Social Care Information Centre, 2014). Whilst much research has focused on the effects of the energy containing macronutrients on energy balance (Anderson and Moore, 2004; Bertenshaw et al. 2008; Bowen et al. 2006) more recent evidence suggests water might play an important role in facilitating weight management (Dennis et al. 2010; Muckellbauer et al. 2013; Stookey et al. 2008). After 12 months Stookey et al. (2008) found increases in water intake (absolute an relative) were associated with a significant loss in body weight and fat. Consistent with this, a more recent systematic review (Muckellbauer et al. 2013) concluded that increased water consumption in combination with a programme for weight maintenance/ weight loss, reduced body weight to a greater extent than the programme alone.

A number of acute studies have investigated the effects of ingesting water either as part of preload (milk-based) or as a preload 30 min before a meal on subsequent voluntary food intake (Rolls et al. 1998; Van Walleghen et al. 2007; Davy et al. 2008). Rolls et al. (1998) studied the effects of an isogenic milk based drink with different volumes of water (300mL, 450 mL and 600 mL). Less energy was consumed at the *ad-libitum* lunch when subjects consumed the 600 mL drink compare to the 300 mL as well as a subjective hunger decrease and subjective fullness increase. This study represents the additional 300 mL volume of the 600 mL milk preload consumed acted as an important determinant of satiety to decrease energy intake. When considering water intake directly as a preload Van Walleghen et al. (2007) provided 500 mL to men and 375 mL to women of two different age groups (young (n = 29; age 21-35 y) and older (n=21; age, 60-80 y). Although there was no change in energy intake in the young group, the older group significantly decreased their energy intake at an when consuming the water preload 30 min before the *ad-libitum* lunch. In follow-up studies providing a water preload 30 min before an *ad-libitum* meal (s), Davy et al. (2008) acutely found a reduction in energy intake in older obese subjects and Dennis et al. (2010)

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found a reduction in energy intake and body mass after 12 weeks on an accompanying hypocaloric diet.

The mechanism by which water ingestion before a meal reduces acute energy intake is not fully understood, but might be related to increased gastric distention, producing increased perceptions of satiety and decreased hunger. Data from animal studies suggests that increased gastric distension reduces energy intake (Share et al. 1952), possibly via activation of gastric stretch receptors (Paintal, 1954). The provision of water 30 minutes before a meal allows time for the water to empty from the stomach, which will reduce the amount of gastric distension present at the onset of feeding (Vist and Maughan, 1995). Given that the rate of gastric emptying for liquid meals is slower in older vs. younger adults (Clarkston et al. 1997), reducing the time between preload ingestion and feeding in young adults might increase gastric distention during feeding and reduce *ad-libitum* food intake.

Therefore, the purpose of the current study was to investigate the effects of a water preload consumed immediately before an *ad-libitum* meal on appetite and energy intake. The preload was consumed immediately pre-meal to maximise its impact on gastric distension. It was hypothesised that pre-meal water ingestion would suppress appetite and energy intake compared to a no preload control trial.

Methods

Subjects

Fourteen healthy, lean males (age 27 (3) y, height 1.83 (0.05) m, body mass 80.47 (9.89) kg, body fat 17.5 (4.0) %, body mass index 24.0 (2.5)) volunteered.

Preliminary and Experimental Trials

Each subject completed a familiarisation trial followed by two experimental trials in a randomised, counterbalanced fashion, separated by ≥7 days. The familiarisation trial was the same as the main trials as explained below. However subjects had the opportunity to taste test the porridge to determine their preferred flavour, complete the Three-Factor Eating Questionnaire (Appendix L) and become familiarised with the study procedures (weighing scales and fluid ingestion).

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Experimental trials began overnight fasted (~10 h) and at a time typical for each subject to consume breakfast (7-10 am). Subjects voided before body mass (in underwear) was measured. Subjects then entered a custom built feeding booth within an isolated laboratory and ingested either 568 mL water preload (P) or no water preload (NP), before being provided with the *ad-libitum* breakfast meal. The time it took for subjects to consume the water preload was recorded, with subjects consuming the water in 13 (5) s (range 7-21 s). Subjects were provided with a bowl of porridge (90 g dry porridge oats: 420 mL semi-skimmed milk) and a glass of water. Once ½ to ¾ of the bowl had been consumed, it was replaced with a fresh bowl of porridge and this process continued until subjects indicated satiety. Similarly, the drinking water was replaced when required. The porridge and the fluid replacement timings were observed and times recorded during the familiarisation and then repeated during the main trials.
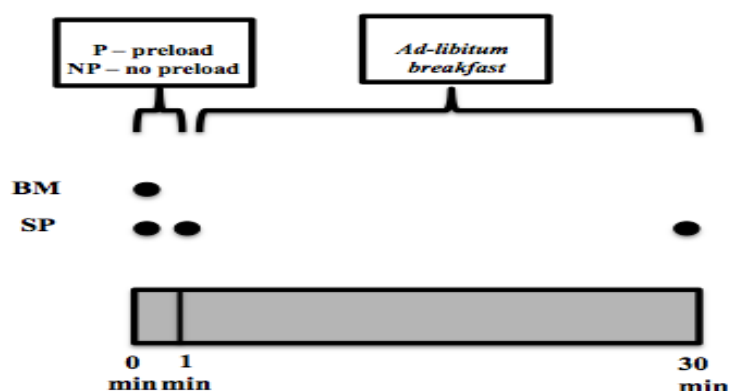
Figure 5.1. Study protocol. BM: body mass, SP: subjective perceptions.

Results

Pre-trial

Pre-trial body mass (P: 81.08 (9.69) kg; NP: 81.33 (9.49) kg; $P=0.117$) and subjective appetite perceptions of fullness ($P=0.103$), hunger ($P=0.168$) and satisfaction ($P=0.965$) were not different between trials.

Ad-libitum Meal

Energy intake at the *ad-libitum* meal was 22 (14) % lower ($P<0.001$) during P compared to NP (Figure 5.2). Water ingestion during the *ad-libitum* meal was also reduced during P (116 (0-581) mL) compared to NP (318 (226-975) mL) ($P<0.001$), although including the 568 mL water consumed immediately pre-meal during P, total water intake was greater during P (684 (568-1149) mL) ($P<0.001$).
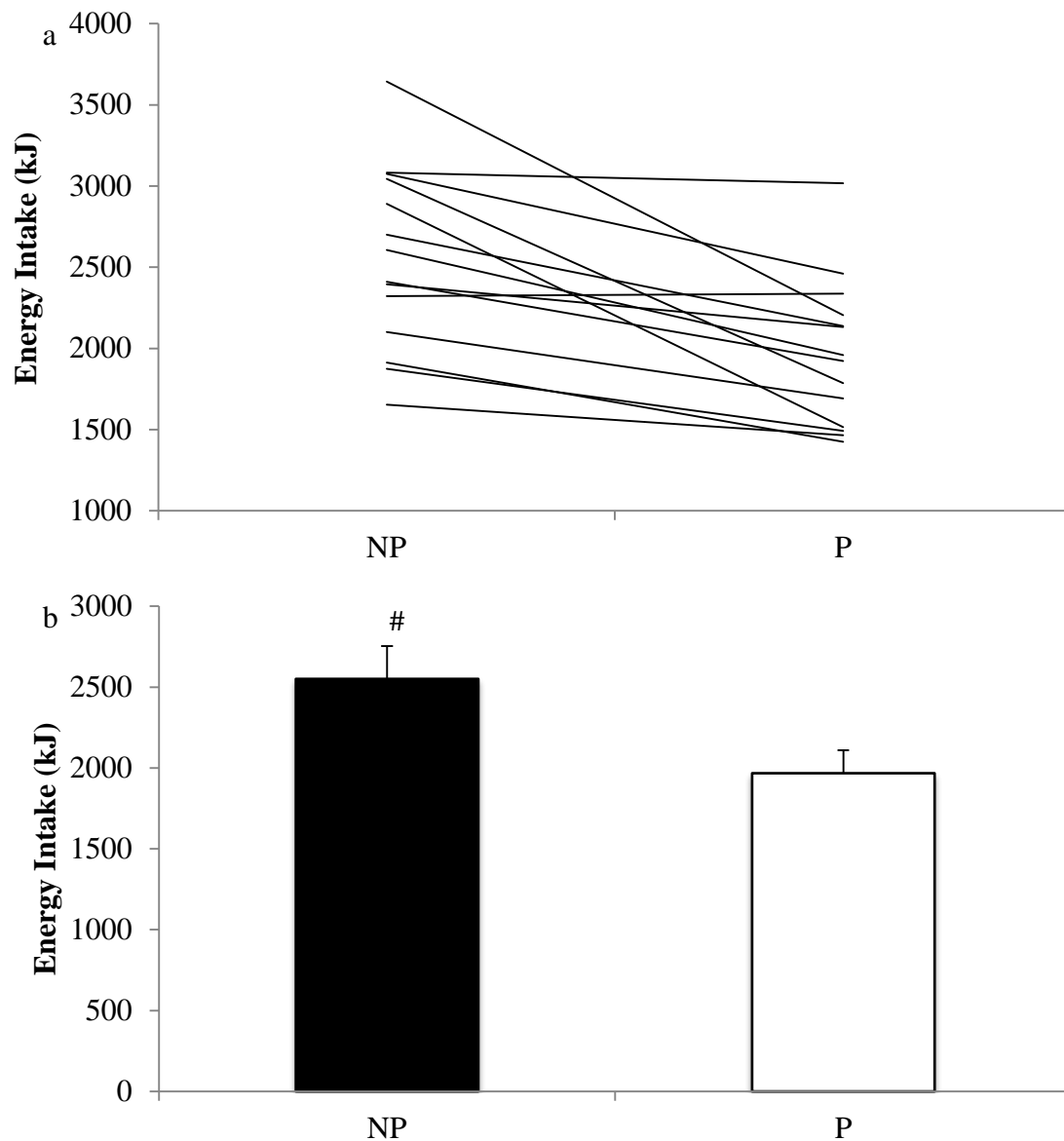
Figure 5.2. Energy intake (kJ) consumed during the *ad-libitum* breakfast for each subject (a) and for the group (b). # Significantly different from P.

Subjective Appetite Perceptions

There were interaction effects for fullness, hunger and satisfaction ($P<0.001$) (Table 5.1). Compared to pre-trial, fullness and satisfaction were increased and hunger decreased at post-trial during both trials ($P<0.001$), as well as at post-drink during P ($P<0.001$). Furthermore, at post-drink fullness and satisfaction were greater and hunger lower during P than during NP ($P<0.001$).

Table 5.1. Subjective perceptions of fullness, hunger and satisfaction. Values are median (range).

	Trial	**Pre-trial** Mean	**Post-drink** Mean	**Post-trial** Mean
Fullness	NP	16 (1-43)	16 (1-43)	91 (74-100)*
	P	16 (1-45)	55 (2-75)*#	88 (67-100)*
Hunger	NP	74 (23-98)	74 (23-98)	5 (1-17)*
	P	77 (59-97)	63 (27-91)*#	7 (2-19)*
Satisfaction	NP	21 (4-46)	21(4-46)	86 (54-96) *
	P	22 (5-47)	31 (12-67)*#	84 (62-99) *

\# Significantly different from NP. * Significantly different from pre-trial.

Discussion

This study examined the effect of immediate pre-meal water ingestion on acute *ad-libitum* energy intake. The main finding was that consumption of 568 mL (1 pint) of water immediately before breakfast reduced *ad-libitum* energy intake at the breakfast by 22%. Furthermore, subjective perceptions of hunger were decreased, whilst fullness and satisfaction were increased immediately after the water preload.

Previous studies have investigated the effect of water ingestion in the 60 minutes before feeding on *ad-libitum* energy intake at a single meal (Davy et al. 2008; Rolls et al. 1990 Van Walleghen et al. 2007). Studies of obese (Davy et al. 2008) and non-obese Van Walleghen et al. 2007) older adults (~60 y of age) have reported that a water preload ingested 30 minutes before a meal, reduces *ad-libitum* energy intake at the meal compared to a no

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preload trial. In contrast, studies of young adults have observed no effect on *ad-libitum* energy intake of water preloads provided 30 minutes and 60 minutes before a meal, or during a meal (Rolls et al. 1990).

What accounts for the divergent responses of young and old to a water preload ingested 30 min before feeding is not clear, but Van Walleghen et al. 2007) suggest it might be related to differences in gastric emptying rates. Indeed, (Clarkston et al. 1997) demonstrated that gastric emptying of both liquids and solids is delayed in old, compared to young subjects. The gastric emptying rate of a liquid is linearly related to the energy density of the liquid (Calbert and MacLean, 1997), with more energy dense liquids emptying from the stomach more slowly. Consequently, water empties from the stomach very rapidly and (Vist and Maughan, 1994) reported that 30 min after ingesting 600 mL water, only 44 mL remained in the stomach of a group of young (21-44 y) males. Therefore, a water preload ingested 30 min prior to an *ad-libitum* meal will be almost completely emptied from the stomach by the time of the meal, at least in healthy young populations, and thus is unlikely to influence *ad-libitum* energy intake.

The current study was designed to maximise perceptions of gastric distention during feeding, and as such the water preload was provided immediately before the *ad-libitum* meal. The results of this study contrast with these previous studies in young populations (Rolls et al. 1990; Van Walleghen et al. 2007) and suggest that the preload-to-meal time interval might be an important consideration for the efficacy of a water preload in reducing acute energy intake. Research on energy containing preloads suggests that a preload-to-meal time interval of 30 minutes is optimal for maximising the impact of a preload on acute *ad-libitum* energy intake (Rolls et al. 1990). However, the mechanism by which an energy containing preload impacts *ad-libitum* energy intake is unlikely to be the same as for water. Therefore, the optimal time interval between preload ingestion and feeding is unlikely to be the same for energy-containing and energy-free preloads. The present study only tested one volume of preload (i.e. 568 mL) and one preload-to-meal time interval (i.e. immediately pre-meal). Future studies should manipulate the preload volume and preload-to-meal time interval to maximise the impact on energy intake.

Whilst the mechanisms underpinning these findings are not fully elucidated by the present

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study, the most plausible explanation by which pre-meal water intake reduced *ad-libitum* energy intake is likely to be gastric distension caused by increased gastric fill. Consistent with this hypothesis, animal studies have demonstrated that increased gastric distension, via an intra-gastric balloon, reduced *ad-libitum* energy intake (Share et al. 1952), likely via vagal nerve stimulation (Paintal, 1954). The water preload ingested in the present study increased perceptions of stomach fullness from 16 (1-45) mm to 55 (2-75) mm, suggesting the volume was sufficient to produce, at least some, gastric distension. Water empties rapidly from the stomach, with a half emptying time of approximately 10 min (Vist and Maughan, 1994). Most subjects remained in the feeding booth for <10 min and thus a relatively large volume of water would be present in the stomach throughout feeding, resulting in increased perceptions of gastric distention during feeding. Whilst *ad-libitum* energy intake appears to be suppressed at a single meal, future studies should extend these findings by examining whether this reduction in energy intake during feeding influences appetite perceptions in the post-meal period or energy intake at subsequent feeding opportunities. Furthermore, water intake was reduced during feeding following the water preload. Whilst we only provided water with the *ad-libitum* meal, in free living situations energy containing drinks might be consumed with a meal and if peri-meal fluid intake is reduced, energy intake through energy containing drinks might also be reduced.

Cross-sectional data suggests a beneficial role for water consumption in weight management and water consumers have been shown to have lower daily energy intake (~200 kcal/ day) compared to those that don't consume water (Popkin et al. 2005). Stookey et al. (2008) conducted secondary analyses of a weight loss intervention trial and demonstrated that among dieters with a low baseline water intake, increasing water to >1 litre per day was associated with increased weight loss. These studies suggest that daily water intake appears to be important for weight management, but only one study has tested the chronic effects of pre-meal water preloads on weight loss (Dennis et al. 2010). Dennis et al. (2007) examined the effect of ingesting a 500 mL water preload 30 min before breakfast, lunch and dinner in overweight and obese middle aged and older adults consuming a prescribed hypocaloric diet for 12 weeks. Compared to a no preload control group, the water preload group lost 44% more weight (~2 kg). Overeating has been shown to be more prevalent among younger populations (Rolls and McDermott, 1991) and the results of the present study suggest that immediate pre-meal water ingestion might be a strategy that could help facilitate weight

management among younger adults.

Conclusion

In conclusion, the results of the present study demonstrate that consuming a 568 mL water preload immediately before a meal reduced perceptions of hunger, increased perceptions of fullness and satisfaction and resulted in a 22% reduction *in ad-libitum* energy intake in young lean males. Future studies should investigate the acute energy intake effects of immediate pre-meal water ingestion in lean young females and overweight/ obese young males and females, as well as examining the chronic weight loss effects of such a dietary strategy.

Chapter 6
Immediate Water Ingestion Reduces Voluntary Energy Intake in Overweight/ Obese Adults

Abstract

Immediate pre-meal consumption of ~500 mL water in lean young males has been shown to reduce energy intake, but it is not known whether this effect persists in overweight/ obese adults. Eight (6 male, 2 female) overweight/ obese young adults (age 27 (5) y, height 1.76 (0.11) m, body mass 103.1 (21.6) kg, body fat 29 (2) %, body mass index 34.0 (5.2) kg·m^2) completed a familiarisation trial and two experimental trials in randomised counterbalanced order. Subjects arrived overnight fasted and consumed an *ad-libitum* porridge breakfast. Immediately before the breakfast, subjects consumed either 500 mL water preload (P) or no preload (NP). Subjective appetite questionnaires, to assess hunger, fullness and satisfaction, were completed before and after the meal in both trials, and after the water preload. During the *ad-libitum* breakfast there was a greater energy intake during NP (2255 (1036) kJ vs. P (1791 (1004) kJ; *P*=0.003). Water intake from drinks during the breakfast was greater during NP (518 (277) mL vs. 338(146) mL; P=0.045). There was a trend for the water preload to increase fullness and satisfaction (*P*=0.056; *P*=0.053, respectively). These finding suggest that a 500 mL water preload consumed immediately before a meal reduces energy intake in obese young adults and is an effective strategy to assist with weight management.

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Introduction

The prevalence of obesity has increased sharply over the last three decades with currently 65% of adults classified as overweight or obese (Health and Social Care Information Centre, 2013). With these statistics reported, a need to identify strategies to induce a negative energy balance for resultant weight loss is required. Drinking a lot of water has been reported to be an effective way to facilitate weight management and weight loss (Dennis et al. 2010; Muckelbauer et al. 2013; Stookey et al. 2008). Specifically, systemic reviews conclude that there may be a beneficial effect of water consumption on weight control (Daniels and Popkin, 2010; Muckelbauer et al. 2013).

Rolls et al. (1998) has found that increasing the volume of a isocaloric milk based drink by 300 mL significantly decreased energy intake when consumed 30 min before an *ad-libitum* meal. However, when this has been investigated as a water preload there was no difference in energy intake found in young subjects (Rolls et al. 1990; Van Walleghen et al. 2007). Interestingly, following the same protocol, a water preload provided 30 min before an *ad-libitum* meal in normal weight (Van Walleghen et al. 2007) and obese (Davy et al. 2008; Dennis et al. 2010) older subjects found a reduction in energy intake and hunger and an increased fullness.

Vist and Maughan (2005) has shown that nearly all water consumed will be emptied from the stomach after 30 min leaving a small volume remaining in young subjects. Gastric emptying rates are slower in older individuals, (Clarkston et al. 1997) also caloric content (Calbet and MacLean, 1997) and the milk protein casein (Miranda and Pelissier, (1983) are key attribute to gastric emptying, which may explain disparate findings between studies (Rolls et al. 1990; Rolls et al. 1998; Van Walleghen et al. 2007; Davy et al. 2008; Dennis et al. 2010).

Recently, our research (Chapter 5) has displayed that providing a water preload immediately before an *ad-libitum* meal reduces energy intake. This can be explained by the reduced time allowed for gastric emptying and subsequent increased gastric distension before the meal inducing perceptions of increased satiety and decreased hunger (Chapter 5). Research in dogs has allowed an insight into the mechanisms involved, whereby stomach distension induced by an inflated balloon reduces energy intake (Share et al. 1952). Furthermore,
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Paintal (1954) reported the activation of gastric stretch receptor sites at the stomach as a balloon was filled to reduce hunger to induce satiety in cats.

Therefore, in light of previous findings in young non-obese subjects (Chapter 5) and the importance of the application to overweight and obese subjects, the aim of the current study is to investigate the effects of a 500 mL preload of water directly before an *ad-libitum* meal on appetite and energy intake in overweight and obese subjects. It is hypothesised that water ingestion will suppress energy intake compared to a control trial.

Methods

Subjects

Eight (6 male, 2 female) overweight/ obese young adults (age 27 (5) y, height 1.76 (0.11) m, body mass 103.1 (21.6) kg, body fat 29 (2) %, body mass index 34.0 (5.2) $kg \cdot m^2$) volunteered for the present study.

Preliminary and Experimental Trials

Subjects completed a familiarisation trial followed by two experimental trials, which were completed in a randomised, counterbalanced fashion and separated by ≥7 days. The familiarisation trial was the same as the main trials as explained below. However subjects had the opportunity to taste test the porridge to determine their preferred flavour, complete the Three-Factor Eating Questionnaire (Appendix L) and become familiarised with the study procedures (weighing scales and fluid ingestion). Female subjects trials were separated by one menstrual cycle.

The two experimental trials were a breakfast *ad-libitum* meal with no preload (NP) or 500 mL tap water immediately before the *ad-libitum* meal (P). During the familiarisation trials subjects were provided with plain commercially available porridge (Ready Brek, Weetabix, Kettering, UK) made up with semi-skimmed milk (Tesco Stores Ltd, Cheshunt UK) and added sugar (Weston Centre, 10 Grosvenor Street, London, UK) to taste before being served during the *ad libitum* breakfast during each trial.

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During the experimental trials subjects arrived at the laboratory at a time typical for them to consume breakfast (7-10 am), were weighed and then entered a custom built feeding booth and completed a baseline subjective perceptions questionnaire (SPQ) (pre-trial). During P a second SPQ was complete immediately after the water ingestion and the time it took was recorded 32 (50) s (range 7-154 s). Subjects were then presented with a bowl of porridge (90 g dry porridge mix: 434 g semi-skimmed milk: 11g of granulated sugar) and 500 mL water and 500 mL of squash. A final SPQ was then completed at the end of the 30 min feeding period (post-trial).
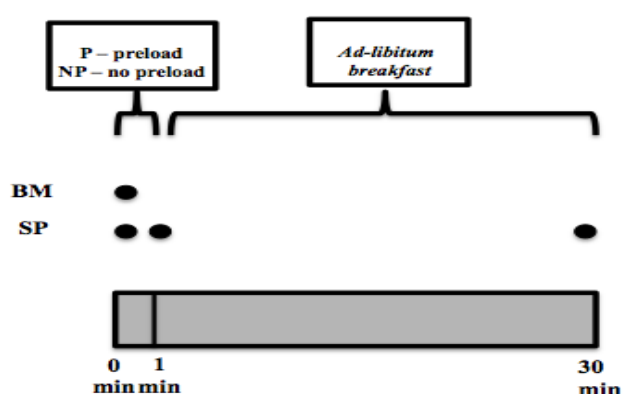
Figure 6.1. Study protocol. BM: body mass, SP: subjective perceptions.

Results

Ad-libitum Meal

During the *ad-libitum* meal, more energy (*P*<0.001) (Figure 6.2) was consumed during NP compared to P. Including the preload, more drink was consumed during trial P (808 (192) mL) compared to NP (518 (277) mL; *P*<0.001).
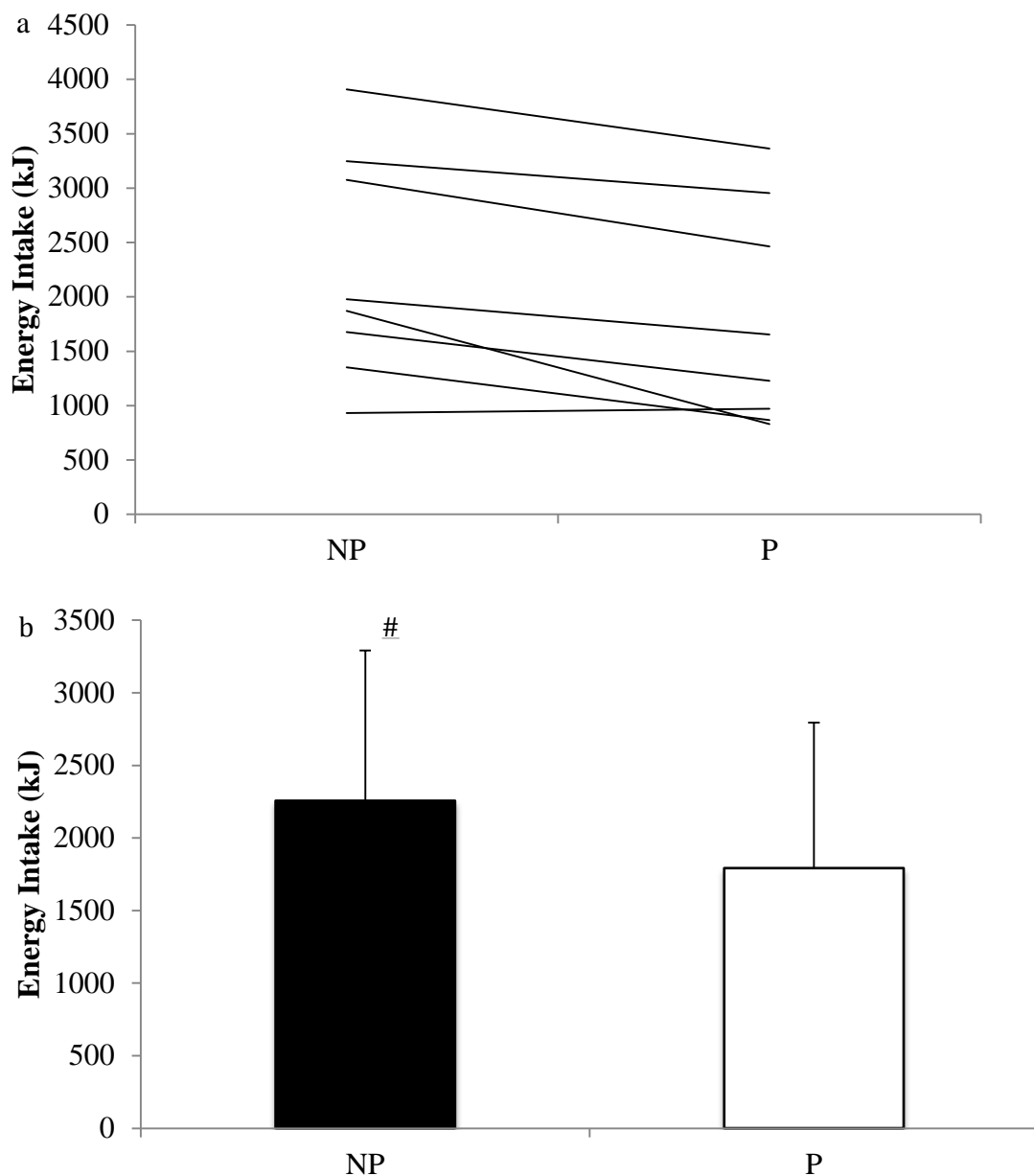
Figure 6.2 Energy intake (kJ) consumed during the *ad-libitum* breakfast for each subject (a) and for the group (b). # Significantly different from P.

Subjective Perceptions

Pre-trial subjective perceptions were not different (Table 6.1). There was a main effect of trial ($P<0.005$), time ($P<0.001$) and an interaction effect ($P<0.001$) for subjective perceptions of fullness. Compared to pre-meal, subjective fullness was increased at post meal during NP and the P ($P<0.001$) and after the water preload during P ($P<0.001$). For trial differences, there was an increased subjective fullness for P compared to NP at post-drink ($P<0.001$).

There was a main effect for time ($P<0.001$) and an interaction effect ($P<0.01$) but no main effect of trial ($P=0.844$) for subjective perceptions of hunger (Table 6.1). During post-meal, subjective hunger was less during NP and P compare to pre-meal ($P<0.005$) and after the water preload during P ($P<0.005$). For trial differences, there was a decreased subjective hunger for W compared to P at post-drink ($P<0.001$).

Table 6.1. Subjective perceptions of fullness and hunger.

		Pre-trial	Post-drink	Post-trial
Fullness	NP	22 (18)	23 (18)	89 (9)*
	P	20 (9)	46 (19)*	89 (12)*
Hunger	NP	73 (25)	74 (25)	9 (6)*
	P	71 (11)	62 (13)*	6 (5)*

* Significantly different from pre-trial.

Discussion

The results demonstrate clearly that when 500 mL of water is consumed immediately before an *ad-libitum* breakfast meal, energy intake is significantly decreased along with subjective perceptions of hunger and increased fullness in young overweight and obese subjects. The findings confirm previous research found in Chapter 6 in young non-obese subjects and is in agreement with our hypothesis.

The effect of water ingestion 30 minutes before an *ad-libitum* meal has been investigated on a number of occasions with various outcomes in different subject groups (Davy et al. 2008[Rolls et al. 1990; Van Walleghen et al. 2007). Specifically, research in older (55-80 y) obese (Davy et al. 2008) and non-obese (Van Walleghen et al. 2007) have described reductions in energy *ad-libitum* intake when a water preload was consumed 30 minutes before the meal compared to a no pre-load trial. However, when the same (Van Walleghen et al. 2007) or similar (Rolls et al. 1990) protocol was investigated in young subjects, observations presented no effects of the preload 30 min before the *ad-libitum* meal on energy intake.

The main differences found in previous research between young (Van Walleghen et al.

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2007) and older (Davy et al. 2008; Van Walleghen et al. 2007) subjects is unclear, however gastric emptying rates may play a key role. Clarkston et al. (1997) reported postprandial gastric emptying times were slower in elderly (70-84 y) compared to young (23-50 y) subjects. Furthermore, in young subjects water empties from the stomach at a rapid rate, whereby after 30 min, only 44 mL remained after a water bolus of 600 mL was consumed in young subjects (21-44 y) (Vist and Maughan, 1994). This may explain the lack of findings in previous research conducted in young subjects, whereby a water preload was provided 30 minutes before an *ad-libitum* meal (Rolls et al. 1990; Van Walleghen et al. 2007) as by the time of the *ad-libitum* meal it may be almost completely emptied. Whereas others (Chapter 6) have found reductions in energy intake in young subjects (22-31 y) when a water preload was consumed immediately before an *ad-libitum* meal.

Van Walleghen et al. (2007) investigated the effects of a water preload 30 minutes before an *ad-libitum* lunch meal in non-obese young and older subjects. Interestingly, a decrease in energy intake was observed at the *ad-libitum* meal in the older subjects, although there was no difference observed for the younger subjects. The same research design by Davy et al. (2008) also reported a decrease in energy intake when a water preload was consumed 30 minutes before an *ad-libitum* breakfast meal observed in overweight and obese older subjects. Therefore these two studies suggest that there is a decrease in energy intake at an *ad-libitum* meal when water is provided 30 minutes before an *ad-libitum* meal with older subjects but not in older subjects. The current study investigated whether providing a water preload directly before an *ad-libitum* meal would make a difference in young healthy subjects. We found energy intake after the *ad-libitum* meal to be lower when water was provided directly before the *ad-libitum* meal compared to no-preload provided. The water preload time delay was the only main difference compared to the previous acute studies investigated by Van Walleghen et al. (2007) and Davy et al. (2008) and therefore the gastric emptying of the water preload can be suggested as the reason for our study reporting a decrease at the *ad-libitum* meal where others have failed.

The current study design was specifically chosen to induce a greater satiety through a large bolus of water close to the *ad-libitum* meal. The reason why the current study design has shown a decrease at the *ad-libitum* meal where others have failed in younger subjects (Van Walleghen et al. 2007) could be due to the differences in gastrointestinal function reported

between younger and older subjects. Clarkston et al. (1997) observed postprandially that elderly subjects had less desire to eat and less hunger, which also accompanied a significantly slower gastric emptying time compared to young subjects when solid and liquid meal components were consumed. Furthermore, it has been observed by Vist and Maughan (1995) that when 600 mL of water was provided via a nasogastric tube, only a small amount of the consumed water was remaining in the stomach after 30 minutes. Therefore, in light of previous findings and the current observations, 30 minutes may be too long for a water preload to have an effect on energy consumption at an *ad-libitum* meal in young subjects.

Due to large bolus of fluid consumed and the small time delay provided before the *ad-libitum* meal, it can be proposed that distension of the stomach plays a key role in the satiety induced in the current study and the subsequent decrease in energy intake. Paintal (1954) investigated gastric stretch receptors and their role in satiation of hunger in cats and found that stomach stretch receptors exerted increased nerve impulses as the stomach was distended by a balloon constituting a peripheral mechanism for satiation of hunger. More recently, Rolls and Roe, (2002) provided a liquid preload via a nasogastric tube to bypass sensory cues containing either a 200mL/200kcal, 400mL/200kcal and 400mL/400kcal volume. There was a decrease in energy intake at the lunch after the 400mL/ 200kcal preload compared with the 200mL/ 200kcal preload, which suggests that gastric and post-gastric mechanisms are important in the satiety.

The notion that a preload of water provided directly before an *ad-libitum* breakfast suppresses energy intake is further supported by our subjective perceptions data. Previous research has demonstrated that subjective hunger is lower and fullness higher after a water preload (Van Walleghen et al. 2007) or a volume adjusted isoenergetic preload (Rolls et al. 1998). We observed similar findings whereby after the 500 mL preload fullness and satisfaction were increased and hunger decreased, although it must be noted that comparisons were made from pre-trial subjective perceptions in the NP. A number of possibilities exist to explain these changes in subjective perceptions after the water preload, although the most plausible seems to be a mechanical effect, whereby the stomach is more full and gastric fill induces a greater perception of satiety (Paintal, 1954). Interestingly, Van Walleghen et al. (2007) reported subjective ratings of hunger were lower and fullness

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ratings higher in older subjects compared with younger subjects, which further demonstrates our point. In our study we ensured that gastric emptying times were not a limitation to energy intake by providing the *ad-libitum* meal directly after the preload was consumed. Some subjects were quicker to drink than others, although all subjects consumed the drink in less than 10 s, which was not long enough for gastric emptying to effect appetite, which was further confirmed by lower energy intake and hunger reported in the P trial.

Popkin et al. (2005) reported improved dietary patterns (reduced soda and lower energy intakes) in water drinkers. Also, a systemic review by Muckelbauer, (2013) reported increased water consumption was associated with increased weight loss. Stookey et al. (2008) found that drinking >1 litre of water per day has been shown to result in weight loss of ~2 kg over 12 months. Although convincing, these investigations rely on self-reported data and thus to make conclusive evidence-based recommendations is difficult. Therefore, there is a need to utilise acute based study designs such as the present study and extend the investigation to see if the findings continue at a number of meals or in an overweight or obese population. Dennis et al. (2010) has extended findings in overweight and obese older adults to a 12-week randomised control trial, whereby 500 mL of water was provided at each meal in addition to a hypocaloric diet. The outcome was a reduction in body weight of ~2 kg and that body weight correlated with an increase in water consumption compared to the control trial. Although interesting, the same result may not be found in young subjects and preventing obesity as an intervention is important in a young population whereby overeating is more prevalent (Stice et al. 2006).

The current findings have important implications for preventing overeating in young adults. It would be interesting to see if the same results exist in an overweight or obese population for weight management or loss. Also the current study is a short design and energy compensation at a second meal may be a possibility. It is therefore important that a follow-up study investigates overweight or obese subjects and also proceeds a second or third feeding opportunity to assess whether a compensatory effect occurs. Due to the simplicity of the study design and accessibility of water, the application of a water preload before a meal may be an important intervention to prevent overeating at a meal in young adults. Furthermore soft drinks have been suggested as one of the greatest contributors of energy in the Western diet (Dennis et al. 2009) and thus water provided before a meal may not only

suppress hunger through gastrointestinal fill but may replace energy usually consumed from soft drinks.

Conclusion

In conclusion, the findings suggest that consuming a 500 mL water preload suppresses energy intake by 22% and also decreased subjective hunger and increases subjective fullness at an *ad-libitum* breakfast meal in overweight/ obese subjects. Future research should extend the current findings in young and/ or overweight/ obese young adults by providing a second *ad-libitum* meal to assess whether the reduction in energy consumption found in the current study is compensated for.

Chapter 7
Effect of Post-Exercise Skimmed Milk or Sucrose Drink Ingestion on Subsequent Appetite and Energy Intake

Abstract

The present study investigated the effects of manipulating a post-exercise recovery drink on subsequent appetite and *ad-libitum* energy intake (EI). Ten lean males: (age 21.3 (1.2) y, height 1.79 (0.65) m, body mass 79.2 (9.6) kg, body fat 16.5 (3.9)%) completed three randomised experimental trials. Two hours after a standard breakfast, subjects performed 30 min exercise at 60% VO_2peak and five 4 min intervals at 85% VO_2peak separated by 2 min rest. Post-exercise, a 615 mL of a placebo (PLA: 32 kJ), skimmed milk (MILK: 912 kJ) or sucrose (CHO: 918 kJ) drink was consumed, each containing 15 mL vanilla essence and 2 g aspartame. Sixty min after drink ingestion, subjects consumed an *ad-libitum* lunch. Acylated ghrelin was determined in blood samples collected pre-exercise, post-exercise and pre-meal, whilst subjective appetite was measured throughout the trial and flavour characteristics of drinks were rated. *Ad-libitum* EI was lower in MILK (6746 (2035) kJ) than CHO (7762 (1921) kJ) ($P<0.05$) and similar in MILK and PLA (7672 (2005) kJ) ($P=0.116$). When energy consumed in the post-exercise drink was considered, total EI was greater in CHO than PLA or MILK ($P<0.05$). There were no trial differences for acylated ghrelin concentration or subjective appetite ($P>0.05$). MILK was rated thicker and creamier than CHO and PLA, with MILK and CHO rated sweeter than PLA ($P<0.05$). These results demonstrate that consumption of skimmed milk, but not a sucrose drink ~75 min post-exercise suppresses subsequent EI at an *ad-libitum* meal.

Introduction

As the prevalence of overweight and obesity continues to rise globally, there is a growing need to identify effective strategies to facilitate weight management (World Health Organisation, 2012). Weight increases can occur when there is an imbalance in energy, therefore efforts should be made to maximise energy expenditure and minimise energy intake to induce a negative energy balance (Halton and Hu, 2004).

Exercise increases energy expenditure, where acute exercise may alter perceptions of hunger and satiety and fluctuate appetite hormones in the hours following, inducing a reduction in relative energy intake (King et al. 2012). Although the recovery of nutrients after exercise is key (Burke et al. 2004), the selection of drink (carbohydrate or protein) could alter the appetite response and possibly undo any energy deficit induced by exercise and result in a positive energy balance.

Milk is high in protein ($36g.L^{-1}$) that is easily attainable, cheap and naturally contains similar amounts of electrolytes (sodium and potassium) and carbohydrates as found in sports drinks (James, 2012). Due to these attributes, milk consumption after exercise is an effective way to rehydrate (Shirreffs et al. 2007), improve protein balance and replenish glycogen stores (Josse et al. 2010). Also, with protein being more satiating than carbohydrate (Astbury et al. 2010), milk may also provide benefits to appetite regulation after exercise compared to a carbohydrate drink.

Milk is associated with improvements in body composition (Josse et al. 2010) and in some studies suppresses energy intake at a subsequent *ad-libitum* meal (Douglas et al. 2013; Dove et al. 2009). Josse et al. (2010) demonstrated that over a 12-week period, the consumption of 500 mL of milk compared to a carbohydrate drink immediately and 1 h post-exercise, 5 times a week, exhibited a greater fat loss and lean mass gains in women. Although, the researchers did not directly assess appetite (*ad-libitum* meal) and only limited dietary analysis was performed, the finding show that milk was effective in suppressing appetite compared to carbohydrate over 12 weeks. These findings could be explained by milk containing majority the slow emptying protein (casein) (Miranda and Pelissier, 1983). This slow emptying of contents from the gastrointestinal tract contributes to satiety by causing distension of the stomach. This has been reported in dogs whereby stomach distension

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induced by an inflated balloon reduces energy intake (Share et al. 1952) and cats as activation of gastric stretch receptors are monitored as balloon was filled in the stomach to induce satiety (Paintal, 1954). Recently, our research has displayed that providing a water preload immediately before an *ad-libitum* meal in young healthy (Chapter 5) and overweight/ obese subjects (Chapter 6) reduces energy intake. This study was specifically designed to induce stomach distension without a caloric or nutrient load to limit other satiety prompts and in each study energy intake was reduced by 22%.

Dove et al. (2009) has provided an isoenergetic 600 mL of milk or fruit drink with breakfast followed by an *ad-libitum* meal 4 hours later. Energy intake was suppressed and satiety perceptions were rated higher during the milk trial compared to the fruit drink. Another study by Douglas et al. (2013) provided different dairy snacks 90 min before an *ad-libitum* lunch and reported all dairy snacks suppressed appetite compared with water, with milk suppressing energy intake the most. Recently, Rumbold et al. (2015) provided 600 mL of milk or orange juice 60 min after 30 min's of vigorous exercise. Although there were no differences in subjective feeling of hunger or fullness, energy intake was decreased at the *ad-libitum* lunch meal.

These findings have not been shown throughout all milk based preload studies (Almiron-Roig and Drewnowski, 2003; Harper et al. 2007). For example, energy intake was similar when a test meal was provided 30 min after an energy-matched preload of chocolate-flavoured milk or cola (Harper et al. 2007) and 50 min after an energy-matched preload of milk or drinks sweetened with sucrose or high-fructose corn syrup (Soenen and Westerterp-Plantenga, 2007). Also, energy intake at a test meal was similar between energy-matched drinks of skimmed milk, cola or orange juice when the drinks were provided with the test meal (DellaValle and Rolls, 2005) or as a preload 2 h and 15 min before the test meal (Almiron-Roig and Drewnowski, 2003).

With intense exercise having a appetite suppressing effect and the health benefits of milk after exercise compared to a carbohydrate based drink, the present study aims to determine the effects of an isocaloric post-exercise recovery drink (Carbohydrate or Milk) followed by an *ad-libitum* meal 75 minutes later.

Methods

Subjects

Ten healthy male subjects (age 21.3 (1.2) years, height 1.79 (0.65) m, body mass 79.2 (9.6) kg, body mass index 23.9 (2.1), peak oxygen uptake (VO_2 peak), 60.5 (10.1) $mL \cdot kg^{-1}min^{-1}$, body fat % 16.5 (3.9)%) volunteered to participate in the present study.

Preliminary and Experimental trials

Subjects completed two preliminary trials followed by three experimental trials, with a different post-exercise recovery drink ingested during each experimental trial. During the first preliminary trial subjects completed an incremental discontinuous VO_2 peak test. In the second preliminary trial subjects completed a trial identical to the experimental trials (described below) with the exception that only water was ingested after exercise. Subjects had the opportunity to try the pasta meal to ensure they liked it and complete the Three-Factor Eating Questionnaire (Appendix L) Subjects were also able to become familiarised with the study procedures (weighing scales, exercise, drink ingestion and blood sampling).

Subjects arrived in the morning 2 h after a standardised breakfast (2693 (216) kJ) providing 25% of energy requirements calculated by determining their resting metabolic rate (Mifflin et al. 1990) multiplied by a physical activity level of 1.7 They voided their bladder and were weighed (in underwear only). Subjects then rested for 15 min supine, completed a subjective perception questionnaire (SPQ) and a blood sample was obtained from an antecubital vein using the venipuncture technique by a qualified practitioner.

Subjects completed 60 min exercise in a climatic chamber in a controlled environment (21 ^{o}C temperature, 50% humidity). Subjects completed 30 min steady state exercise at ~ 60% VO2 peak, before resting for 2 min and then completed five 4 min high intensity intervals ~ 85% VO_2 peak separated by 2 min rest (Talanian, Galloway, Heigenhauser, Bonen and Spriet, 2010). Expired air, heart rate and RPE were collected between 14-15 min and 29-30 min of the steady state exercise and during the last min of intervals 1, 3 and 5. Subjects then consumed 615 mL of a post exercise recovery drink (Table 7.1) within 5 min and then a subjective drink questionnaire (SDQ) was completed immediately after. All drinks

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contained 15 mL of vanilla essence and 5 g of sweetener to blind subjects to which drink they were given. The SDQ was recorded on a 0-100 mm scale containing questions related to thickness, creaminess, stickiness, sweetness, sourness and fruitiness for perceptions of the drink consumed (Flint et al. 2000). Upon completion of exercise, subjects were immediately weighed again (in underwear only) and lay supine for 10 min, in which time they completed another SPQ before another blood sample was obtained. Subjects then had 55 min, in which time they were able to have a shower, watch TV (with no food prompts) or rest before resting supine for 10 min, completing a SPQ and then a final blood sample was obtained.
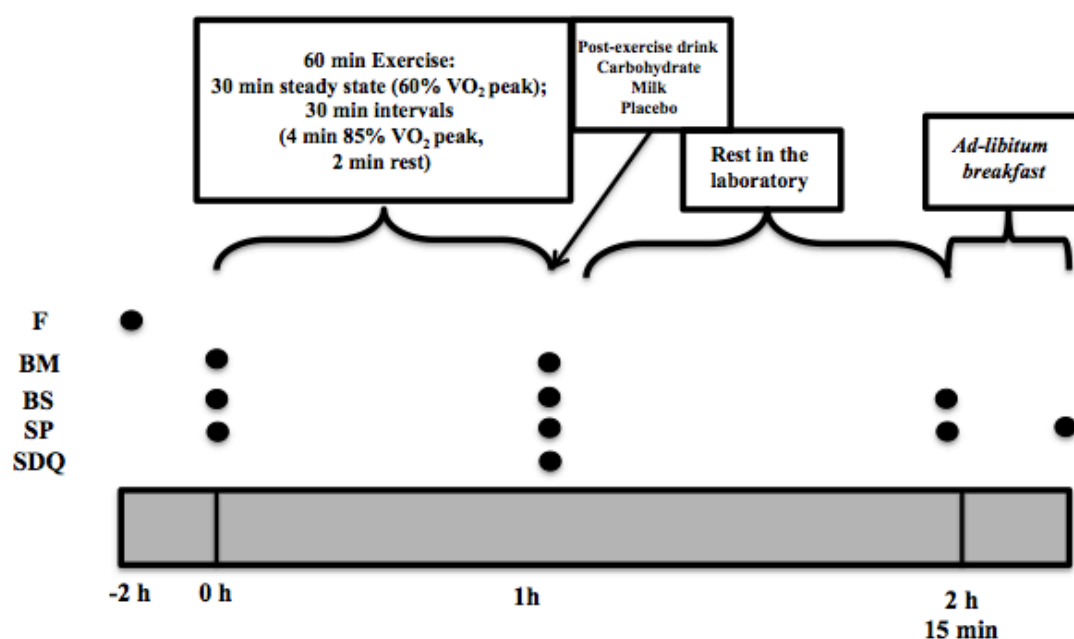
Figure 7.1. Study protocol. F: food, BM: body mass, BS: blood sample, SP: subjective perceptions, SDQ: subjective drink questionnaire.

Table 7.1. Composition of drinks provided as a preload to the *ad-libitum* meal

Drink	kJ	PRO (g)	CHO (g)	FAT (g)
PLA	32	0	2	0
MILK	912	21	33	1
CHO	918	0	55	0

Subjects then entered an isolated feeding booth for 30 min where they consumed a homogenous pasta meal *ad-libitum* (fusilli pasta, bolognese sauce, cheese and olive oil,

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Tesco Stores Ltd, Cheshunt UK), (100 g of food: protein: 19.5%, carbohydrate: 48.1%, fat: 32.4%) until 'comfortably full and satisfied'. The food was continually replaced over the 30 min and weighed (Kern Top Pan Balance PFB 3000-2, Progen Scientific, London, UK) before and after the subject had eaten. Subjects were initially provided with ~ 400 g in each bowl and the bowl was replaced at ~3 min intervals (determined during familiarisation) to ensure warm food was continuously available to the subject and that they did not completely finish a bowl. Water (500 mL) and sugar-free squash (500 mL) were also provided and consumed *ad-libitum* and weighed before and after the meal. Subjects completed a post meal SPQ. During the trial conditions in the laboratory were recorded as temperature: $21.1 \pm 0.3^{o}C$, relative humidity: $34.7 \pm 10.6\%$.

Results

Exercise

Mean work rate for the first 30 min cycle was 142(39) W, which elicited a mean O_2 consumption, equivalent ~ 58.3 (4.5)% VO_2 peak. Mean cycling work rate at the 30 min high intensity interval exercise (HIIE) was 212(59) W, which elicited a mean O_2 consumption, equivalent to ~ 84% of VO_2peak. Heart rate, RPE and $L^.min^{-1}$ O_2 consumption were not different between trials ($P>0.05$; Table 7.2).

Table 7.2. Exercise variables.

	PLA		MILK		CHO	
	SS	HIIE	SS	HIIE	SS	HIIE
HR	151 (16)	177(12)	148 (11)	178(7)	149 (16)	178 (10)
RPE	13 (1)	16(2)	13 (2)	17 (2)	13 (2)	17 (2)
VO$_2$	2.65 (0.35)	3.92 (0.37)	2.60 (0.38)	3.78 (0.46)	2.59 (0.38)	3.88 (0.40)

HR: Heart rate (Bpm). RPE: Rating of Perceived Exertion. SS: Steady state. HIIE: High intensity interval exercise. VO_2: O_2 consumption $(L^.min^{-1})$. W: Work rate

Food Intake and Acylated Ghrelin

Absolute energy intake was the energy consumed during the *ad-libitum* meal, whereas

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cumulative was the energy consumed during the *ad-libitum* meal plus the preload energy.

For energy intake at the *ad-libitum* meal there was a main effect for trial ($P<0.05$) (Figure 7.2). Compared to PLA, there was no change in absolute energy intake (kJ) during the MILK and CHO trial ($P>0.05$). Although, compared to the MILK trial, there was an increased absolute energy intake during the CHO trial ($P<0.05$).

For cumulative energy intake (energy intake at the *ad-libitum* meal plus energy intake through the post-exercise drink) there was a main effect for trial ($P<0.01$) (Figure 7.2). Compared to PLA, there was no change in cumulative energy intake (kJ) compared to the MILK trial ($P=0.990$). Although, compared to PLA and MILK trials there was an increase in cumulative energy intake in the CHO trial ($P<0.05$).
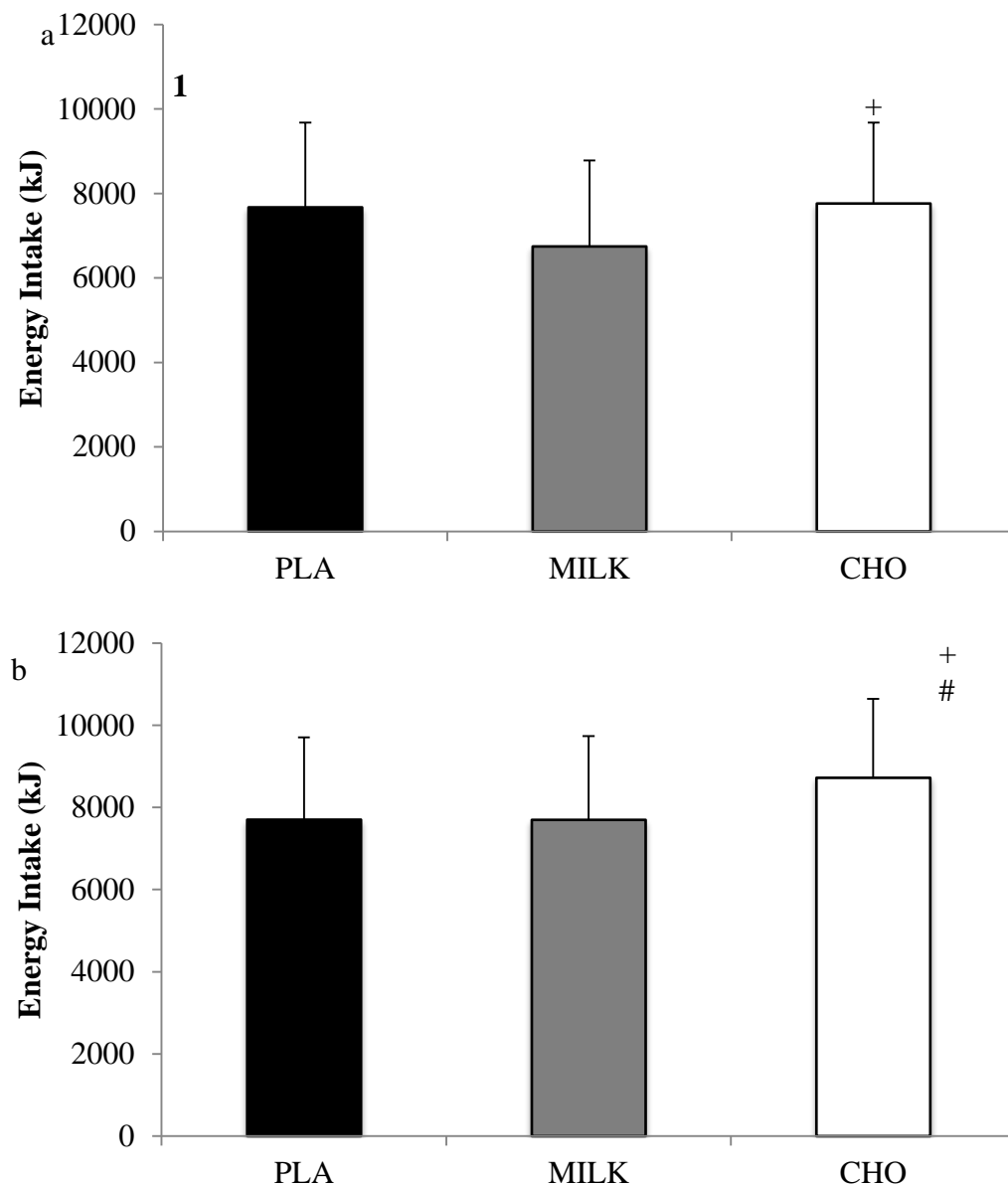
Figure 7.2. Energy intake (kJ) (a), consumed at the *ad-libitum* meal and energy intake (kJ) (b) consumed from preload and *ad-libitum* meal during PLA, MILK and CHO trials. + Significantly different from the MILK. # Significantly different from PLA

For acylated ghrelin concentration, there was a main effect for time ($P<0.05$), but no main effect for trial ($P=0.780$) or interaction effect ($P=0.157$). Acylated ghrelin was higher at pre-meal during the PLA compared to pre-ex and post-ex ($P<0.05$). There were no other changes in acylated ghrelin across time during the MILK and CHO trials ($P>0.05$) (Table 7.3).

Table 7.3. Acylated ghrelin (pg/mL).

	Pre-exercise	Post-exercise	Pre-meal
PLA	69 (28-279)	50 (10-192)	146 (33-357)+
MILK	97 (36-294)	97 (36-382)	105 (27-288)
CHO	90 (34-385)	73 (6-411)	135 (30-350)

+ Significantly different from pre-exercise

Subjective Appetite and Drink Perceptions

There were no differences between trials (PLA, MILK and CHO) at baseline appetite ratings for fullness, hunger, and desire to eat ($P>0.05$) (Table 7.4).

For subjective perceptions of fullness, there was a main effect of time ($P<0.001$), but no effect of trial ($P=0.296$) or interaction ($P=0.260$) (Table 7.4). Subjects perceived fullness to be less at post-ex, pre-meal and more at post-meal during MILK ($P<0.05$) compared to pre-ex. Fullness was less at pre-meal during CHO ($P<0.05$) and more at post-meal during PLA ($P<0.05$) compared to pre-ex. Compared to post-ex, fullness was perceived to be less at pre-meal during PLA only ($P<0.05$), although subjects were fuller at post-meal during MILK ($P<0.05$). At pre-meal, subjects were less full during PLA, MILK and CHO compared to post meal ($P<0.05$).

For subjective perceptions of hunger, there was a main effect of trial ($P<0.01$), time

($P<0.001$) and an interaction effect ($P<0.05$) (Table 7.4). Hunger was perceived to be more at pre-meal during PLA, MILK and CHO ($P<0.05$) and less at post-meal during PLA and MILK ($P<0.05$) compared to pre-ex. Hunger was also rated higher at pre-meal during PLA ($P<0.05$) and less at post-meal during CHO ($P<0.01$) compared to post-ex. Compared to pre-meal hunger was also less at post meal during PLA, MILK and CHO ($P<0.05$). Hunger was subjectively less during the PLA trial at post-ex compared to CHO ($P<0.05$), although once bonferoni adjusted, there was no significant difference.

Subjects perceived the MILK drink to be thicker than the CHO drink ($P<0.01$) and more creamy than the PLA drink ($P<0.05$), whilst both the MILK and the CHO drinks were perceived as sweeter than the PLA drink ($P<0.01$). There were no differences in the perceived stickiness ($P=0.516$), sourness ($P=0.747$) or fruitiness ($P=0.587$) of drinks (Table 7.5).

Table 7.4. Subjective perceptions questionnaire (0-100 mm) for fullness and hunger.

		Pre exercise	Post exercise	Pre-meal	Post-meal
Fullness	PLA	63 (12-84)	61 (22-82)	18 (0-61)^	87 (60-97)+#
	MILK	61 (31-83)	32 (13-86)+	19 (13-44)+	89 (57-98)+^#
	CHO	62 (25-90)	38 (14-69)	17 (11-52)+	91 (9-97)+#
Hunger	PLA	33 (13-61)	41 (8-79)	86 (74-98)+^	8 (0-28)+#
	MILK	28 (13-63)	60 (2-82)	83 (53-90)+	7 (1-32)+#
	CHO	38 (9-74)	69 (50-80)*	82 (70-96)+	9 (0-34)+^#

+ Significantly different from pre-ex ($P<0.05$). ^ Significantly different from post-ex ($P<0.05$). # Significantly different from pre-meal ($P<0.05$) .* Significantly different from PLA ($P<0.05$)

Table 7.5. Subjective drink perceptions (0-100 mm).

	PLA	MILK	CHO
Thickness	16 (6-58)	40 (17-69)	20 (2-44) #
Creaminess	19 (3-58)	56 (25-85)*	40 (3-86)
Stickiness	29 (9-67)	27 (11-66)	33 (9-66)
Sweetness	55 (19-71)	67 (35-94)*	85 (35-98)*
Sourness	9 (0-28)	3 (0-35)	6 (0-45)
Fruitiness	24 (0-69)	26 (0-84)	16 (0-71)

 * Significantly different from PLA trial (P<0.05). # Significantly different from MILK trial (P<0.05)

Discussion

After a 60 min bout of aerobic exercise, skimmed milk as a post exercise recovery drink led to a decrease in energy intake at an *ad-libitum* lunchtime pasta meal, compared to a carbohydrate based drink. These findings emphasise the importance of the type of drink consumed during the recovery period after exercise and consequently implies that 615 mL of skimmed milk may potentiate a greater negative energy balance to optimise weight loss.

Previous findings suggest that milk is associated with improvements in body composition (Josse et al. 2010). Also, several recent studies have found that compared to water (Douglas et al. 2013) or a fruit drink (Dove et al. 2009), the consumption of milk followed by an *ad-libitum* meal, has resulted in a reduced appetite and energy intake. However, these findings are not universal in milk based preload studies (Almiron-Roig and Drewnowski, 2003; Harper et al. 2007; Soenen and Westerterp-Plantenga, 2007).

The 615 mL of skimmed milk that was consumed after exercise during the present study contained ~22 grams of protein. There is evidence to suggest that an isoenergetic high protein drink compared to a high carbohydrate drink showed less energy consumed following the high protein drink (Astbury et al. 2010). 615 mL of milk was used in the current study to present an acceptable amount of protein (~ 20 g) after exercise to maximally

stimulate protein synthesis (Josse et al. 2010). Also, as evidence suggests decreases in body fat mass and energy intake with milk at approximately 20 g protein intake (Josse et al. 2010), it seemed viable to provide this amount of protein. Dove et al. (2009) compared milk to an isoenergetic fruit drink consumed with a fixed-energy breakfast and found that 4 hours later, energy intake was suppressed in the milk trial compared to the fruit drink trial. Interestingly, the milk contained 25 g of protein, similar to the current study and is the only study to demonstrate a significant reduction in energy intake an *ad-libitum* meal following milk consumption compared to an isoenergetic drink. Whereas other investigations have found no difference when milk was provided as a preload to a subsequent meal when protein amounts have been below 20 g (DellaValle et al. 2005; Harper et al. 2007).

The different components of milk protein may also be a factor to consider when comparing a milk drink to a carbohydrate drink. Research suggests that the viscosity of a drink reduces appetite (Mattes and Rothacker, 2001) and energy intake (Zijlstra et al. 2008) compared to a thin drink matched for energy density and macronutrient content. Milk protein contains predominantly 80% casein fractions, with the remaining being soluble whey protein (Billeaud et al. 1990). Casein exhibits slower emptying rates than whey protein, due to the fact that in the presence of gastric acid, casein clots and solidifies in the stomach forming small fragments that slow emptying from the stomach (Miranda and Pelissier, 1983). Due to distension of the stomach being a determinant to reducing voluntary energy intake (Gelibter, 1988), the slowed emptying rate of milk protein, particularly casein, may have contributed to stomach fill and thus a decreased energy intake shown in the milk trial.

Although casein empties slower than whey protein from the stomach, whey protein has been shown to suppress energy intake at a subsequent meal compared to casein (Veldhorst et al. 2009) and compared to glucose and lactose (Bowen et al. 2006). It has been suggested that the amino acid response (branced chain amino acids; leucine, isoleucine and valine) may have a unique metabolic role interacting with insulin to suppress appetite (Layman and Baum, 2004). Furthermore, insulin responses are positively correlated with BCAA and carbohydrates and can be amplified post exercise (Van Loon et al. 2000). As milk contains whey protein and carbohydrate (49 g.L^{-1}), a spike in amino acids and may have occurred after exercis and contributed to appetite suppression. Douglas et al. (2013) found that yogurt had the greatest suppressive effect on appetite 90 minutes prior to a hot pasta *ad-libitum*

meal being consumed, compared to milk and cheese. Yogurt was also found to have the greatest amino acid response compared to the other trials, which may further suggest elevated concentrations of amino acids playing a role in suppressing appetite. Unfortunately, we did not measure amino acids in our study.

Fischer et al. (2004) has reported protein to have a greater response on satiety over several hours compared to fat and carbohydrate. In comparison to other research with no effect of milk on appetite, the current study, similar to Dove et al. (2009) had a greater drink-meal interval before the *ad-libitum* meal (~75 min). Harper et al. (2007) didn't find a difference in energy intake between milk and an iso-energetic carbohydrate drink when there was 30 minutes before the *ad-libitum* meal. Also, Soenen and Westerp-Plantenga (2007) found no significant changes when milk, sugar or a high fructose corn syrup drink was consumed 50 minutes before an *ad-libitum* meal. As milk has majority slower emptying proteins (casein), the effect of a milk preload on appetite maybe more prolonged (Miranda and Pelissier, 1983). Dove et al. (2009) provided a large breakfast (2985 kJ), with a preload 4 h before an *ad-libitum* meal provided and found a decrease in energy intake. This could be a factor in prolonging satiety due to a larger gastric fill and thus the decreased emptying times before the *ad-libitum* meal. Gastric distension has been shown to be a key attribute to satiety, where dogs limit energy intake when a gastric balloon is inflated (Share et al. 1952). Also, when water is provided immediately before an *ad-libitum* meal, energy intake is reduced by 22% in healthy (Chapter 5) and overweight/ obese (Chapter 6) young adults.

A recent study by Rumbold et al. (2015) added the addition of vigorous exercise before a preload of either 600 mL of milk or orange juice. Although there was no change in fullness or hunger perceptions, energy intake was significantly reduced in the milk trial. The addition of exercise and milk consumption after exercise may cause a greater stimulation of satiety opposed to milk alone. Intense exercise has been shown to decrease gastric emptying rates (Murray, 1987) and suppress also suppress relative energy intake and ghrelin (King et al. 2011). Although not directly investigating appetite, Josse et al. (2010) demonstrated that over a 12-week period, the consumption of 500 mL of milk compared to a carbohydrate drink immediately and 1 h post exercise, 5 times a week, exhibited a greater fat loss and lean mass gains in women. These results, similar to the present findings may suggest that milk after exercise may acutely suppress energy intake, although limited dietary analysis

was performed in this study to confirm this.

The milk drink compared to the carbohydrate and placebo drink was subjectively rated as being thicker and creamier. These ratings may suggest a pre-existing expectation of satiety and thus affect the subsequent intake at an *ad-libitum* meal. McCrickerd, et al. (2012) confirmed that subtle manipulations of texture and creamy flavour can increase expectations of a dairy drink to be filling and suppress hunger, irrespective of the drinks energy content. Also it is suggested that the palatability of a drink may alter satiety (Yeomans and Chambers, 2011) and subsequent energy intake at a meal. Tsuchiya et al. (2006) found that when consuming either a yogurt based drink or a fruit based drink, lower hunger and higher fullness ratings were indicated after the yogurt drink. Moreover, Yeomans and Chambers (2011) investigated the effects of 6 different preloads to an *ad-libitum* meal, ranging from low-sensory, low energy to high sensory and high energy. The degree to which the beverage generated satiety was dependent on the nutrient content and sensory characteristics (high energy, high sensory), with subjects consuming less food when the thicker and creamier and higher energy preload was consumed. This may suggest an increased sensory satiety occurring with the milk based drink and although warrants further investigation, could be a contributing factor to the decrease in energy intake found. Although we tried to mask the flavouring of the drink with vanilla and artificial sweeteners, the masking of milk was difficult and subjective rating suggest that the drinks were distinguishable. Differences were found in drink thickness and creaminess but perceptions of fullness, hunger and desire to eat were no different between trials. Nevertheless the merit of a study must not be placed solely on subjective perceptions (Veldhorst et al. 2008).

Interestingly, we did not find that our ghrelin data represented the findings of our energy intake data. Ghrelin has been shown to increase appetite and energy intake when intravenous infusions were increased to 5.0 pmol/kg/min (Wren et al. 2001). While the data are limited and conflicting, ghrelin appears to be affected by macronutrient composition, declining with ingestion of carbohydrates and dietary protein (Bowen et al. 2006; Foster-Schubert et al. 2008). However, the half-life of ghrelin is short (9-13 min) (Akamizu et al. 2004) and levels peak immediately before meals (Cummings et al. 2001). Due to the 75 minutes between the preload and the *ad-libitum* meal this may have been too long to see a difference in acylated ghrelin levels. In contrast, cholecystokinin (CCK) is released in

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response to dietary fat, amino acids, and small peptides and is known to decrease hunger and inhibit food intake acutely (Konturek et al. 1973; Belinger and Degen, 2004). Taken together, the multiple elements to milk may have induced secretions of other appetite hormones and therefore it would have been interesting to see a variance of appetite hormones such as insulin, CCK and GLP-1.

With the current research findings, it could be recommended that milk should be favoured as a post-exercise drink to potentiate a negative energy balance. Milk is a cheap and easily attainable drink and naturally contains nutrients found similar to a carbohydrate-electrolyte sports drink. As a post-exercise drink, it has been found that there are other benefits with milk such as rehydration (Shirreffs et al. 2007), muscle recovery and weight loss (Josse, et al. 2010).

Conclusion

To conclude, these results demonstrate that the consumption of 615 mL of skimmed milk consumed after exercise decreased energy intake compared to a carbohydrate drink at an *ad-libitum* meal served 1 h after exercise. These findings suggest that milk may be a more favourable post-exercise drink to consume after exercise to decrease energy intake at a meal and as a strategy as part of a long-term weight maintenance/weight loss programme.

Chapter 8 General Discussion

General Discussion

Water represents a critical nutrient of which the importance to health has long been established (Popkin, Anci and Rosenberg, 2010). Recently, interest has been expressed on the effects of fluid regulation (sometimes in varying forms) on appetite and energy intake. The following research contributes to current research and aims to establish a clearer understanding of the effects of fluid manipulation on energy consumption. The studies presented in this thesis have attempted to extend knowledge within each of these related areas of enquiry by examining the effects of hydration status, fluid consumption, different macronutrient drinks and water intake before a meal on appetite and energy intake. The purpose of this chapter is to reflect upon this research collectively and discuss the findings presented in the experimental chapters of this thesis.

Water Balance, Appetite and Energy Intake

The starting aims for Chapter 3 and 4 were to determine the effects of hypohydration on appetite and energy intake. This area is largely under-researched in humans, although it poses an important question for individuals who do not adequately replenish their fluid losses either after exercise (Chapter 3) or throughout a prolonged period (Chapter 4). Recovery after exercise is largely dependent upon the amount and type of nutrients consumed in the hours following an exercise bout and hydration status may affect this (Burke et al. 2011). Furthermore, daily fluid fluctuations leading to hypohydration over a prolonged period may affect a person's health through reductions in energy intake, especially in the elderly (Ferry, 2005).

Although previous findings have reported reductions in energy intake when fluid was restricted (Shirreffs et al. 2004) or reduced (Engell, 1988), our current findings did not show any reductions in energy intake or appetite in young normal weight adults when hypohydrated to < 3% (Chapter 3 and 4). During, Chapter 3 we followed a similar protocol to a previous study by Kelly et al. (2012) where a large bolus of fluid (1.5 x body mass) before the *ad-libitum* meal may have affected the energy consumed during the rehydration trial. We addressed this issue by prolonging the rehydration period (13 h) and providing sufficient time (2 h) between the water consumption and the *ad-libitum* meal (Vist and Maughan, 1994). Regardless of this methodological change, energy intake, ghrelin and

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subjective perceptions of hunger and fullness remained similar (Chapter 3). During the *ad-libitum* meal, fluid intakes were ~55% higher during the hypohydrated trial compared to the euhydrated trial and thus it could be suggested that most of this fluid consumption would be ingested during the initial period of the buffet meal to alleviate sensations of thirst, but conversely affecting satiety (Paintal, 1954). Furthermore, the types of fluids available means that when subjects were hypohydrated they would consume these fluids first. We provided a buffet meal with a varying fluid selection to provide choice, however the overconsumption of energy containing fluids (orange, milk, coffee and tea) and fluid containing foods (cereals and fruits) would have been likely in the hypohydrated trial (Chapter 3). This initial ingestion of fluids may explain the lack of finding in Chapter 3 as distention of the stomach before a meal can affect energy intakes (Chapter 5 and 6). Interestingly, Kelly et al. (2012) found a decrease in ghrelin levels in the hypohydration trial, whereas we did not. Ghrelin is released when the stomach is empty and has a direct effect on a persons hunger levels (Murphy and Bloom, 2006). We observed no change in acylated ghrelin concentration in response to exercise, which may be explained by the collection of the post-exercise blood sample taken ~20 min after exercise or due to the low intensity nature of the exercise used. Nonetheless, the finding that there was no change in acylated ghrelin in response to exercise-induced dehydration (both trials) and no difference in response between trials during the 13 h post-exercise period, suggests that hypohydration might not effect the physiological regulation of energy intake. It cannot be discounted that hypohydration affects other hormones that influence appetite regulation and energy balance and future experiments should seek to document these responses. Acylated ghrelin was increased at 13 h post-exercise in both trials, which is likely because the pre-exercise sample was ~4 h post-prandial, whilst the 13 h post-exercise sample was ~10.5 h post-prandial (Borer, et al. 2009).

To further investigate fluid ingestion during the *ad-libitum* meal and separate the effects of hydration status, Chapter 4 aimed at investigating a euhydrated and hypohydrated state before a meal with either fluid or no fluid provided at an *ad-libitum* single item meal. There was no difference in energy intake in Chapter 4 regardless of being euhydrated or hypohydrated. Interestingly, this further confirms our finding in Chapter 3 but contradicts previous research restricting fluid intake (Engell, 1988; Shirreffs et al. 2004).

There are some physiological and behavioural mechanisms that may explain our findings

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(Chapter 3 and 4) and previous research (Engell, 1988; Shirreffs et al. 2004). Hypohydration has been reported as the predominant factor causing distress, along with subjective increases in malaise and tiredness (Shirreffs et al. 2004; Chapter 4). Although subjective hunger and fullness were not different between trials, subsequent acute distress following hypohydration may have had an effect on energy intake (Begg and Woods, 2013). During the trial subjects reported feeling increased tiredness and the hypohydration induced was quite extreme leading to low energy and in some cases headaches. Other non-homeostatic factors include palatability (mouth dryness) and gastrointestinal changes (speed of digestion) may have played a part in the findings in Chapter 4. During the hypohydration trial, subjects had a significantly greater thirst at 24 h compared to the 0 h and also reported a dry mouth (Chapter 4). Hypohydration has been reported to decrease salivary flow rate in animals (Silanikov, 1991) and humans (Walsh et al. 2004; Oliver et al. 2008) and increase time in gastric retention (Neufer et al. 1989; Rehrer et al. 1990; Silanikov, 1991). Although subjects did report increased thirst and a dry mouth, there was no difference in energy intake, ghrelin and subjective hunger and fullness between the hypohydrated and euhydrated trials. As previous research found decreases in energy intake when fluid was restricted (Shirreffs et al. 2004), it can be suggested that the choice of breakfast provided would be the difference. During the study by Shirreffs et al. (2004) subjects were required to only consume low moisture food. During our study (Chapter 4) we provided a semi-solid breakfast (porridge), which may have been enough to restore satiety and palatability of food (Watts, 1999). In Chapter 3 different fluids (milk, orange juice, coffee) were all energy containing and could have had the same effect to rehydrate and restore appetite and this is something that should be considered in future research investigations.

During Chapter 4, we also only provided one meal opportunity whereby other research extending fluid restriction and meals frequency have represented decreases in voluntary food intake (Engell, 1988). Therefore, we may have found differences if meal frequency and the fluid restriction time period was extended, however the real life application must be considered. As the consumption of food and drink are intricately linked (Engell, 1988; McKiernan et al. 2008), behavioural implications may apply and thus removing fluid intake during the meal may reduce energy intake at numerous meals (Engell, 1988).

Consequently, Chapters 3 and 4 indicate mild hypohydration does not affect energy intake

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or appetite, although dry food provision in conjunction with fluid restriction may affect energy intake. Interestingly in both Chapter 3 and 4, there was no change in energy consumption when subjects were hypohydrated, whether induced by exercise or by fluid restriction. Chapter 3 used a multi-item *ad-libitum* buffet meal whereas Chapter 4 used a single-item porridge meal. Although energy intake is reported to be greater during a buffet *ad-libitum* meal compared to a single-item *ad-libitum* meal (Tepper et al. 2011) values are still repeatable and is deemed a valid tool for assessing energy intake (Allirot et al. 2012).

Correct fluid is important for post-exercise recovery Burke et al. (2011) and the health of individuals, especially the elderly (Ferry, 2005). Thirst is an important indicator for fluid intake, although Chapters 3 and 4 provide evidence to suggest that when thirst subjective perceptions are increased after a period of fluid restriction, the implication of thirst may not be enough for fluid recovery. During Chapter 3, after a 13 h post-exercise period of hypohydration, subjects did not consume sufficient amounts of fluid to replace fluid losses (69 (31) %). Also, a 24 h period of hypohydration in Chapter 4 resulted in only 12 (1) % fluid replacement. These studies represent insufficient fluid restoration after a period of hypohydration and it seems a concerted effort in the form of specific recommendations are required.

Water Balance and Stomach Fill

Chapters 5 and 6 demonstrate clearly that when 568 mL of water is consumed immediately before an *ad-libitum* breakfast meal, energy intake is significantly decreased in normal and overweight/ obese subjects. These findings reported in Chapters 5 and 6 reported a decrease in energy intake of ~21% after an immediate water preload.

As food and fluid enter the stomach and subsequently empties into the small intestines, a variety of factors including gastric distension, nutrient stimulation, intestinal mechanoreceptors and chemoreceptor and gut peptides contribute to satiety (Horner et al. 2011; Powley et al. 2004). Therefore, the decrease in energy intake found in Chapters 5,6 and 7 are likely to be due to factors including stomach distension, gastric emptying and the subsequent subjective decrease in hunger and increase in fullness. Chapters 5 and 6 were designed specifically to maximise perceptions of gastric distension thus, it could be proposed that the large bolus of fluid consumed immediately before the *ad-libitum* meal-

induced sufficient distension of the stomach to decrease energy intakes. Animal studies have demonstrated that increased gastric tension, via an intra-gastric balloon, reduced *ad-libitum* energy intake (Share et al. 1952), possibly explained by vagal nerve stimulation (Paintal, 1954). Recent research also suggests the importance of gastric and post-gastric feedback in satiety when a liquid preload (via a nasogastric tube) containing 400mL/ 200kcal compared with 200mL/ 200kcal resulted in a decreased energy intake (Rolls and Roe, 2002).

Findings where large volumes of fluid are consumed after subjects are either hypohydrated (Chapter 4) or when provided with a water preload (Chapter 5 and 6) affect voluntary energy intakes and subjective fullness and hunger after an *ad-libitum* meal. During Chapter 4 there were no main effect for voluntary food intake at the *ad-libitum* meal, although individual comparison of the EU-F and HYPO-F trials revealed food intake was greater during EU-F than during HYPO-F (*P*<0.05), whilst fluid intake was greater during HYPO-F (618 (281) mL than EU-F (400 (247) mL, (*P*<0.01). Given the greater thirst perception pre-meal during HYPO-F, it seems logical that when presented with fluid, subjects would immediately ingest a bolus of fluid to satiate their thirst. This is confirmed by increased perceptions of stomach fullness from 16 (1-45) mm to 55 (2-75) mm when the water preload was ingested during Chapter 4 and suggests the volume was sufficient to produce, at least some, gastric distension. Similar findings are presented in younger lean (Chapter 5) and overweight/ obese adults (Chapter 6).

Although studies on older adults have reported decreased in energy intake after a water preload (Davy et al. 2008; Van Walleghen et al. 2007), research investigating younger subjects with the same study design have not (Van Walleghen et al. 2007). There is convincing evidence to suggest that gastric emptying influences appetite and energy intake (Bergman et al. 1992; Delgado-Aros et al. 2004; Nair et al. 2009; Sepple and Read, 1989). Gastric emptying may explain our findings (Chapter 5 and 6) compared to previous (Van Walleghen et al. 2007) as any water consumed before 30 minutes may empty from the stomach (Vist and Mauaghn, 1994) and thus, there would be little gastric tension remaining. This effect would be different in older subjects (Clarkston et al. 1997) and when the water preload is provided immediately before the *ad-libitum* meal (Chapter 5 and 6). Clarkston et al. (1997) reported postprandial gastric emptying times were slower in elderly (70-84 y) compared to young (23-50 y) subjects. Water empties from the stomach in younger subjects

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at a rapid rate, whereby after 30 min, only 44 mL remained after a water bolus of 600 mL was consumed in young subjects (21-44 y) (Vist and Maughan, 1994). Water empties rapidly from the stomach, with a half emptying time of approximately 10 min. During our study (Chapter 5 and 6) most subjects remained in the feeding booth for <10 min and thus, a relatively large volume of water would be present in the stomach throughout the feeding period, resulting in increased perceptions of gastric distension during feeding.

There was no difference in ghrelin levels when water intakes were 55% higher in the hypohydrated trial in Chapter 3 and when energy intake was reduced in the HYPO-F trial compared to the EU-F trial (Chapter 4). We did not collect appetite hormones for Chapters 5 and 6, although gastric distension by a balloon has reported reductions in ghrelin levels (Mion et al. 2005). Furthermore, CCK Moran and McHugh, 1982), PYY and GLP-1 have been reported to decrease motility in contributing to in post absorptive satiety (Gryback et al. 1997). Also, CCK (Lal et al. 2012) and GLP-1 (Vrang et al. 2002) have been reported to modulate sensations of gastric distension.

Drinking water is publically promoted for weight loss and weight maintenance (Muckelbauer et al. 2013) along with epidemiological research suggesting a beneficial role for water consumption to reduce energy intake and promoting weight maintenance and weight loss (Dennis et al. 2009). Popkin et al. (2005) reported improved dietary patterns (reduced soda and lower energy intakes) in water drinkers in the US over two years. Also, replacing sugar-sweetened beverages with non-caloric diet beverages can help to lower energy intake over 12 months (Stookey et al. 2008). Acute research studies represent promising data, where energy intake is reduced after a ~500 mL water preload in older adults (Davy et al. 2008; Van Walleghen et al. 2007) and younger adults (Chapter 5 and 6). Although, the need to extend these research findings over an increased period and meal frequency are evident. Dennis et al. (2010) have extended findings in overweight and obese older adults to a 12-week randomised control trial, where 500 mL of water was provided at each meal in addition to a hypocaloric diet. The outcome was a reduction in body weight of ~2 kg and that body weight correlated with an increase in water consumption compared to the control trial. Though interesting, it is important to investigate a young population whereby overeating is more prevalent (Stice et al.2006) and the results of the present study suggest that immediate pre-meal water ingestion might be a strategy that could help

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facilitate weight management among younger adults.

Milk Consumption and Appetite/ Energy Intake

The replenishment of carbohydrates, protein and fats have been widely researched and different strategies exist to maximise the recovery after exercise (Burke et al. 2012). However, sugar-sweetened beverage intake has been associated with the trends in overweight and obesity (Ludwig et al. 2001). Therefore, the selection of drink (carbohydrate or protein) after exercise could be important in determining the appetite response and any energy deficit induced by exercise. It was clear in Chapter 7 that milk was more beneficial for controlling energy intake as well as being a cheap, easily attainable recovery drink (James, 2012). Therefore, with milk providing a satiating effect in some studies (Astbury et al. 2010; Rumbold et al. 2015, Chapter 7), the provision of milk around mealtimes and as well as an effective post-exercise drink may provide a beneficial alternative to sugar-sweetened beverages.

As mentioned above, post-exercise nutrition is an important part of effective recovery after exercise (Burke et al. 2011). With increases in commercial post-exercise drink consumption for the athletic and general population, interest lies in the effects of such drinks on appetite and energy intake (Josse et al. 2010; Rumbold et al. 2015). A 60 min bout of aerobic exercise followed by skimmed milk, led to a decrease in energy intake at an *ad-libitum* lunchtime pasta meal, compared to a carbohydrate based drink (Chapter 7). These findings emphasis the importance of the type of drink consumed during a recovery period after exercise and consequently implies that 600 mL of skimmed milk may potentiate a greater negative energy balance to optimise weight loss.

Previous findings suggest that milk is associated with improvements in body composition (Josse et al. 2010). Also, several recent studies have found that compared to water (Douglas et al. 2013), fruit drink (Dove et al. 2009) or orange juice after exercise (Rumbold et al. 2015) the consumption of milk followed by an *ad-libitum* meal decreased energy intake.

There are some elements to skimmed milk attributable to the findings, with one of the most explored being dietary protein. The protein amount provided (~22g) in Chapter 7 is

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sufficient to induced satiety as reported with a high protein meal (Wycherley et al. 2010) and dose response preloads (Astbury et al. 2010). Up to 20 g of protein is also reported to maximally stimulate protein synthesis after exercise (Josse et al. 2010) with the key attribute being whey protein. Whey protein makes up ~20% of milk and drives increases in amino acids (particularly leucine) which has a unique metabolic role interacting with insulin to suppress appetite (Layman and Baum, 2004; Douglas et al. 2013) possibly amplified post-exercise (Van Loon et al. 2000). Another fast-acting feature of milk is carbohydrate (49 g.L-1) of which is the disaccharide lactose (glucose and galactose enzymatically linked). Lactose has been reported to reduce energy intake by 11% compared to glucose (Bowen et al. 2006) along with galactose suppressing hunger (Duckworth et al. 2016).

Research suggests that the viscosity of a drink reduces appetite (Mattes and Rothacker, 2001) and energy intake (Zijlstra et al. 2008) compared to a thin drink matched for energy density and macronutrient content. During Chapter 7 subjects rated the milk preload to be subjectively thicker and creamier than the carbohydrate or control. The texture and creamy flavour can increase fullness and suppress hunger (McCrickerd, et al. 2012) along with palatability altering satiety (Yeomans and Chambers, 2011). The content of fat in the milk may have contributed to this, and further increased palatability (Hill et al. 2000).

Casein, makes up ~80% mass and constitutes the viscosity of milk (Billeaud et al. 1990), exhibits slower emptying rates than whey possibly due to coagulating in the presence of gastric acid (Veldhorst et al. 2009). Slower gastric emptying increases gastric distension and results in reductions in voluntary energy intake (Gelibter, 1988). Appetite responses to faster emptying (whey and lactose) and slower emptying constitutes (Casein), may be the difference in a drink-meal interval to provide a plausible explanation. With protein based isocaloric cream reporting a greater response on satiety over several hours compared to fat and carbohydrate (Fischer et al. 2004; Douglas et al. 2013).

Only one other study to Chapter 7 has the inclusion of exercise to a preload research study (Rumbold et al. 2015). Exercise has been shown to induce hormonal fluctuations, such as suppressing ghrelin (a hormone which stimulates appetite) (King et al. 2012). Other appetite hormonal responses reported coincide with attributes of milk intake, such as protein, gastric distension (Cholecystokinin (CCK) (Bowen et al. 2006) and carbohydrate (lactose) intake

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(Glucagon-like peptide-1 (GLP-1) and insulin) causing a appetite suppressive effect.

Milk is a cheap and easily attainable drink and naturally contains nutrients found similar to a carbohydrate-electrolyte sports drink. As a post-exercise drink, it has been found that there are other benefits with milk such as rehydration (Shirreffs et al. 2007), muscle recovery (Phillips and Van Loon, 2011) and weight loss (Josse, et al. 2010). These findings suggest that milk may be a more favorable post-exercise drink to consume after exercise to decrease energy intake at a meal and as a strategy as part of a long-term weight maintenance/weight loss programme (Chapter 7).

Limitations

In Chapter 3 we aimed to decrease the water ingestion before the *ad-libitum* meal by making the rehydration fluid into smaller aliquots compared to the study design by Kelly et al. (2012) where a large fluid bolus was provided before the *ad-libitum* meal in the rehydration trial. The aim of our study design was to limit stomach distension before the *ad-libitum* meal to ensure that the last water bolus consumed would empty from the stomach (Vist and Maughan, 1994) not confounding the *ad-libitum* meal (Geliebter, 1988). However, we found that subjects reported feeling subjectively more thirsty during the hypohydration trial. As we provided a variety of drinks during the *ad-libitum* buffet meal, subjects were able to firstly consumed drinks to rehydrate and then started on the food. During the hypohydration trial subjects consumed an extra 508 ± 112 kJ in energy from drink and Subjects ratings of thirst were greatly increased immediately before the meal in the HYPO trial and consequently they ingested 798 ± 284 mL more water through foods and drinks compared to the RE trial of fluid. In results of the findings in Chapter 4, 5 and 6, this extra fluid ingested during the *ad-libitum* meal would affect the energy intake consumed.

At the *ad-libitum* meal a variety of food and drink was provided to the subjects in an *ad-libitum* buffet. This enabled a good choice for all of the subjects, however providing this choice meant that all subjects had the opportunity to over consume the foods provided (King et al. 2011). The energy density of the foods provided at the *ad-libitum* meal also could not be controlled with the *ad-libitum* buffet. Research by Rolls et al. (2005) has shown that when adjusting for the volume of water in a food or drink energy consumption can be affected. Therefore, in light of the above a single-item *ad-libitum* meal would have

controlled better for fluid and energy density allowing for clarity on findings between the trials.

In Chapter 4, we decided to control for some of the limitations in Chapter 3. We hypohydrated subjects in a similar way to Shirreffs et al. (2004), whereby fluid was restricted for 24 h by only consuming dry foods. During the *ad-libitum* meal we only provided a single-item *ad-libitum* meal and fluid was limited to either water or sugar-free squash. Shirreffs et al. (2004) found that there was a decrease in energy consumed by 28%, when subjects restricted fluid for 48 h, however we did not find a difference at the *ad-libitum* meal between the hypohydrated and the rehydrated trials. The main difference may have been the meal provided as we decided to provide a semi-solid porridge breakfast as it was simple to mix ingredients and to cook and control (Appendix K). Although this was a well-received choice (everybody enjoyed the meal) and it is a typical breakfast for the subjects, it must be considered that the semi-solid nature of the meal may have been the reason that we did not find a difference between the trials. During studies in animals (Silanikov, 1992) found that salivary flow rate was decreased in parallel with energy consumption and in humans hypohydration is shown to affect gastrointestinal function (Oliver et al. 2007; Walsh et al. 2004).

During Chapter 5 and 6 this was a simple study design that provided some interesting results when a bolus of fluid (500-568 mL) was provided immediately before an *ad-libitum* meal. Although, energy intake was significantly decreased when subjects consumed the bolus of fluid compared to no fluid, we are not able to establish whether this decrease in energy would be compensated for. Therefore future studies should look to lengthen this study design to determine whether any compensatory responses are seen at the next *ad-libitum* meal and whether these finding will account for a decrease in body mass. Also, we unfortunately did not have the budget to investigate the effects of appetite hormones in this study design. Studies have shown CCK and GLP-1 changes to be associated with gastric distention (Kissileff et al. 2003; Vrang et al. 2003), therefore monitoring these inline with the current findings would have been interesting and further clarified findings.

Chapter 7 found that a milk preload of 615 mL decreased energy intake compared to a carbohydrate and water preload. During this study a single-item pasta *ad-libitum* meal was provided, however we found that this was a very energy dense meal, which resulted in high-

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energy intakes during all trials. The use of cheese and olive oil, as two very high-energy dense foods due their high fat and low water content, would have increased the caloric load of the meal. This therefore may have inflated the actual energy intake and in future the use of this meal without the cheese and olive oil may have been more realistic for a lunch time meal.

Strengths

The strengths of this thesis are the in-depth investigation in to hydration status and appetite and energy intake. In chapters 3 and 4 we were able to utilise different hypohydration and rehydration methods to ensure that we could determine the effects of hypohydration specifically on appetite rather than the ingestion of fluid. In the study design of Chapter 3 we followed a similar protocol investigated by Kelly et al. (2012), however rather than 1 large bolus of water before the *ad-libitum* meal to rehydrate, we provided water in 4 aliquots, consuming the last drink at 11 h post-exercise (2 h before the breakfast meal). The four aliquots were ingested as follows, immediately after post-exercise measures, 2.5 h post-exercise, 4 h post-exercise and 11 h post-exercise, with 761 (109) mL (30%), 634 (90) mL (25%), 507 (72) mL (20%) and 634 (90) mL (25%) of the total water volume ingested at these times, respectively. During the HYPO trial, subjects were provided 200 mL water to consume *ad-libitum* during the evening. Using this study design has allowed for us to account for any distention affects which maybe induced by a large rehydration amount of fluid. Chapter 4 we further accounted for fluid intake by having 4 different trials (hypohydrated with fluid at the *ad-libitum* meal, hypohydrated without fluid at the *ad-libitum* meal, rehydrated with fluid at the *ad-libitum* meal, rehydrated without fluid at the *ad-libitum* meal). This design enabled us to further distinguish between the trials and the main effects of fluid provided at the *ad-libitum* meal.

Chapters 5 and 6 followed on from Chapter 4 by providing fluid immediately before a porridge *ad-libitum* meal. Previous research has not found a difference in younger subjects when fluid is provided 30 minutes before an *ad-libitum* meal (Van Walleghen et al. 2007). However, we decided to provide fluid immediately before the *ad-libitum* meal to limit gastric emptying (Vist and Maughan, 1994). There was a decrease in energy intake when we investigated this protocol in young normal weight (Chapter 5) and overweight and obese (Chapter 6) subjects. As controlling energy intake in a young population is important for

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health, these are important studies in the contribution of controlling overweight and obesity.

Chapter 7 investigated the effects of different post-exercise drinks and found that milk intake 75 min after exercise reduces *ad-libitum* energy intake. This is an important finding as milk has many benefits to including being high in protein ($36g.L^{-1}$), easily attainable, cheap and naturally similar amounts of electrolytes (sodium and potassium) and carbohydrates as found in sports drinks (James, 2012). Due to these attributes, milk consumption after exercise is an effective way to rehydrate (Shirreffs et al. 2007), improve protein balance and replenish glycogen stores (Josse et al. 2010). In light of these findings, this is an important study to encourage milk consumption after exercise compared to a sports drink to benefit from milks attributes and also decrease after exercise energy intake to the same level as a water based drink.

Future Research Directions

The future directions for this research would be to add more detail and lengthen the findings in Chapter 5, 6 and 7. Currently it is unknown to whether subjects would compensate for the decrease in energy intake found when a bolus of water was provided immediately before an *ad-libitum* meal (Chapter 5 and 6). Therefore, providing a bolus of water over 3 meals in a day or over a number of weeks where subjects are instructed to consume 500 mL of water before each meal may prove to be a strategy for repeated decreases in energy intake at meals and lead to body mass losses. Furthermore the use of different drinks such as calorie free cola, sugar-free squash or carbonated water would be interesting to research. The carbonated element to a drink may further lead to increased fullness. Also, future research should look at decreasing the amount of fluid consumed immediately before a meal to determine the ideal amount to create an effect. A few subjects reported difficulty drinking the 500-568 mL of water in such a short period of time, therefore a smaller volume of fluid may enable a more applicable way to reduce energy intake.

Chapter 9
Conclusions and Practical Applications

Conclusions

The main conclusions from the work on fluid manipulation and energy and appetite effects can be summarised as follows:

While hypohydration might influence subjective perceptions of hunger and fullness; it does not affect voluntary energy intake when fluid is either available or not available with a meal. Furthermore, increased thirst sensations did not induce sufficient *ad-libitum* fluid intake to fully restore fluid balance after exercise or 24 h of fluid restriction (Chapter 3 and 4).

In a laboratory setting there appears to be little effect of hypohydration or fluid availability on the voluntary intake when consuming a typical semi-solid breakfast meal (Chapter 4). This suggests that the results of previous studies might, therefore, be related to the provision of dry foods during fluid restriction (Shirreffs et al. 2004) or behavioural links between food and fluid (Engell, 1988; McKiernan et al. 2008).

Consuming a bolus of fluid above ~500 mL suppresses subjective hunger and increases subjective fullness as well reduces *ad-libitum* energy intake in young healthy and overweight and obese subjects. (Chapter 5 and 6).

Consumption of 600 mL of skimmed milk after exercise compared to a carbohydrate drink reduces *ad-libitum* energy intake and suggests that milk may be more favorable after exercise as a strategy as part of a long-term weight maintenance/weight loss programme (Chapter 7).

Practical Applications

The results of Chapter 3 and 4 suggest that individuals consuming food and fluid *ad-libitum* will not fully restore fluid balance. In such situations, a structured rehydration plan should be implemented to ensure complete rehydration (Sawka et al. 2007). This might be of particular relevance to athletes competing in weight category sports who have just made weight or athletes undertaking more than one training session a day.

The results of Chapter 3 and 4 represent hypohydration either after exercise or after 24 h of fluid restriction will not alter energy intake if fluid is provided with the meal. However,

providing only dry foods may result in a decrease in energy intake and subsequent deficient in nutrient replenishment.

Results from Chapter 4, 5, 6 represent consuming a ~500 mL fluid bolus may be an effective intervention for managing energy intake in normal weight and overweight/obese subjects, future studies should extend these findings by examining whether this reduction in energy intake during feeding influences appetite perceptions in the post-meal period or energy intake at subsequent feeding opportunities.

The results reported in Chapter 7 show it could be recommended that milk should be favored as a post-exercise drink to potentiate a negative energy balance and to encourage a decrease in fat mass. Milk is a cheap and easily attainable drink and naturally contains nutrients found similar to a carbohydrate-electrolyte sports drink.

Although the Chapters 3 and 4 did not find a difference in energy intake between hypohydration and euhydrated trials, the findings suggest that the level of fluid intake was below that required to fully restore fluid balance, regardless of increased thirst sensations reported by subjects. Future research or practical applications, should seek to examine the interaction between hydration status and fluid availability on appetite and energy intake. With the increased thirst sensations, there was an initial bolus of fluid, which impacted energy intake between EU-F and HYPO-F trials in Chapter 4. This finding was further elaborated in Chapter 5 and 6 and suggests that pre-meal water intake might be an easy practical method to suppress energy intake and optimise energy balance. Furthermore milk intake after exercise and 75 min before a meal reduces energy intake compared to a carbohydrate or water drink of 615 mL. These findings suggest milk would be a preferred drink to consume after exercise compared to a carbohydrate drink for people aiming to decrease their energy balance to manage their body mass.

Currently, this PhD research has not changed practice in the field, although further research with investigating rehydration strategies (Chapter 3 and 4) or increasing the size of the acute finding in Chapters 5, 6 and 7 would provide some applicable strategies for change of practice in the field. Specifically focus on the intake of fluid (water or milk) ingestion before a meal would be an important strategy for weight management or weight loss.

The next steps for this research would be to increase the length of the findings in Chapters 5,6 and 7 to determine whether there would be any commentary effects of the decrease in energy intake found during these studies. Also, different drink utilisation is important to determine whether these findings can be repeatable with commonly consumed drinks. This research has enabled many more questions to be investigated and it is important that these are further explored in future research.

These findings suggest that either water intake (immediately) or milk (75 min) before a meal is an effective strategy to decrease energy intake at a meal and as part of a long-term weight maintenance/weight loss programme.

In conclusion, these results demonstrate that in a laboratory setting there appears to be little effect of hypohydration or fluid availability on voluntary intake of a typical semi-solid breakfast meal. This suggests that the results of previous studies might therefore be related to the provision of dry foods during fluid restriction (Shirreffs et al. 2004) or behavioural links between food and fluid (Engell, 1988; McKiernan et al. 2008).

Chapter 10
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Appendices

Appendix A

Subject Information Sheets

Chapter 3

Study Requirements

You will be a healthy male volunteer aged 18 or over. You will complete a preliminary trial, lasting approximately 45-60 minutes, a familiarisation trial lasting approximately 19 hours (5 hours of which will be spent in the laboratory), followed by 2 experimental trials lasting approximately 24 hours (6 hours of which will be spent in the laboratory).

Location

Human Performance Laboratory and Physiology laboratory in the School of Science and Technology, Erasmus Darwin Building, Clifton Campus, Nottingham Trent University.

Restrictions During Testing

Food and drink intake will be controlled at various times of the study and analysed by the investigators accordingly.

Preliminary Procedures

During the first visit we will:
- Explain the objectives of the study
- Familiarise you with testing procedures and equipment
- Familiarise you with dietary recording
- Answer any questions you may have

Maximal Cycle Ergometer Test

You will cycle to volitional fatigue (VO_2 peak) on a cycle ergometer. The speed will be kept constant which will be pre-selected by you. The intensity of the cycle will be increased every 3 minutes and during the last minute of each stage you will be asked to breathe through a mouth piece while wearing a nose clip.

Main Trials

After this you will complete a familiarisation trial and 2 experimental trials (of which are randomly assigned).

During each trial you will arrive at the laboratory at 15:30 after at least a 4 hour fast and 2 hours after consuming 500 ml plain water. You will provide a urine and blood sample (20ml) and complete an intermittent moderate intensity exercise session in a temperature (~35°C) and humidity (~70% relative humidity) controlled environment, designed to dehydrate you by approximately 2% body mass (1.4 kg for a 70 kg individual). After this, you will have a shower before providing urine and blood samples (20ml) and being provided with food and drink to consume that evening and will be free to leave the laboratory. You will return to the laboratory the following morning and again provide urine and blood samples (20ml). You will then be given free access to a variety of foods and drink before being free to leave the laboratory. You will then be required to weigh and record all food and drink consumed over following 24 hours.

Preparations for Testing

Recording your Diet

You will be asked to weigh and record everything you eat and drink when you leave the laboratory for two days. This is very important in order to control for diet, this will be discussed with you prior to the main trials. No alcohol should be consumed on the days when you are recording your diet. Also it is crucial to the success of the experiment that you should undertake exactly the same type and amount of food and water for the 2 days before the start of each main trial.

Controlling for Physical Activity

It is crucial to the success of the experiment that you should undertake exactly the same type and amount of activity the 2 days before the start of each main trial.

Fasting Before the Trial

You will finish eating by 11pm on the evenings before the main trials and 12pm on the afternoon trials. You will report to the laboratory at 3:30am on the day of the main trial and 8:30am the following morning of each of the main trials without eating breakfast.

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How Much Time Will It Take?

Urine samples will be collected throughout the study, which will be given in private. Blood samples will be collected before and after exercise (20ml), as well as the following morning. Your body mass will be measured in boxer shorts (need to bring spares for post exercise), but in private before and after exercise, as well as the following morning. You will also complete a short questionnaire to determine your subjective feelings before and after exercise, as well as the following morning.

Potential Benefits to You

You will be provided with information about your sweat rate and composition along with basic information regarding diet and VO_2 peak results. We will also provide you with feedback on your own results and will be happy to discuss these with you.

Potential Risks to You

Occasionally, individuals might feel faint, dizzy or unwell whilst exercising in the hot, humid environment. Venipuncture risks associated with having blood drawn are slight but may include, excessive bleeding, fainting or feeling light-headed. However, the amount collected is small and the person collecting blood is fully qualified. You will experience all aspects of the study during the familiarisation trial and if you are not comfortable with any parts of the study you are of course free to withdraw at any point without giving a reason.

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Chapter 4

In the course of the present study we will assess your individual hydration status and the physiological responses of your body. These investigations will give us new insights in the complex mechanisms of hydration status and the possible contribution appetite and weight control.

Subjects Should:

- be male
- be aged between 18 and 45 years
- have no personal history of gastric, digestive, cardiovascular or renal disease, and resting blood pressure < 140/90 mmHg
- not be dieting or having extreme dietary habits
- be non-smoking and not be taking drugs
- no lactose/ dairy or wheat/ gluten intolerance

Preliminary and Main Trials

Each subject will complete an initial familiarisation trial and 4 four experimental trials dehydrated; buffet with fluid, dehydrated; buffet without fluid, hydrated; buffet with fluid hydrated; buffet without fluid which are to be completed in a randomised manner.

Prior to the actual trials subjects are asked to perform a 48 h standardisation period, where we will ask you to record your all food and drink consumed over 48 h (you will be provided with scales and diary). We also ask you to avoid alcohol, strenuous physical activity and supplementation over those 2 days. We will ask you to repeat this food record prior to each trial.

At the first visit in the laboratory we will explain the whole procedure and equipment to you and you will complete a health questionnaire and a consent form. We will start the familiarisation period on this day and take you through all proceedings as described below.

Day 1

After the standardisation period we ask you to report to the laboratory in the morning after an overnight fast (you should stop eating and drinking at 10pm). Then we will take a urine sample, body weight and then after 15 min rest (in which subjective feelings questionnaire will be completed) a blood sample (this will take approximately between 30 and 40 minutes). After those measurements you will be provided with the food and
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drinks for the next 24h, adjusted to your energy requirements for the day. During the dehydrated trials, you will completely restrict your fluid intake for 24h, whilst in the hydrated trials, 40 ml/kg body mass of water will be provided (approx. 2-3 litres). Subjects are able to leave the laboratory and continue with their normal daily activities (except any physical activity), returning 8am the next day. We ask you to collect all urine in this period and you will be provided with all equipment you need (scales etc). You will come back to the lab after those 24h and we will repeat the measurements and provide you with a buffet-type breakfast. This procedure would be repeated 4 times after the familiarisation trial (5 total visits).

Day 2:

After the arrival at the laboratory at the same day, you will undergo the same procedures as on Day 1. Subsequently you will be offered a buffet meal for 30 minutes, with either fluid or no fluid provided. You will be asked to eat until you are satisfied and are free to leave the lab after that.

Any medication you are currently on or any allergies to foods or food groups should be mentioned at the start of the testing.

Taking part in this study will be kept confidential and all the data will be stored anonymously and confidentially.

Main trials and familiarisation will take place at Loughborough University, Clyde Williams Building (beside the Sports Hall and opposite Martin Hall) in the laboratory.

You are able to withdraw from the study at any time and also can request withdrawal of any information from the study.

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Chapter 5 and 6

Introduction

There is a growing interest in identifying factors to manage or prevent the epidemic scale of obesity. A number of recent investigations have indicated that manipulating fluid intake can affect energy balance. Similarly, timing of fluid intake have been documented and may effect energy intake but little is currently known about this.

Who is doing this research and why?

Rob Corney is investigating research for his PhD and results will be used for a PhD thesis.

Lewis James (Lecturer in Sports Nutrition) is supervising him in conducting this research.

Are there any exclusion criteria?

Aged between 18 and 40 years, no immune deficiencies, non-smokers, not currently on a weight gain/weight loss diet and have no known history of gastric, digestive, cardiovascular or renal disease, resting blood pressure <140/90 mmHg, no lactose, dairy, gluten intolerances or allergies to used food groups.

Once I take part, can I change my mind?

Yes. After you have read this information and asked any questions you may have we will ask you to complete an Informed Consent Form, however if at any time before, during or after the sessions you wish to withdraw from the study please just contact the main investigator. You can withdraw at any time, for any reason and you will not be asked to explain your reasons for withdrawing.

Will I be required to attend any sessions and where will these be?

Yes. All trials will all be held at Loughborough University in the Clyde Williams building in the laboratory.

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How long will it take?

Familiarisation trial 45 min, Experimental trials 45 min (2 trials to be completed).

Is there anything I need to do before the sessions?

You will need to complete a food diary for 48 hours before the start of each trial. This will require you to standardise your diet and exercise (no alcohol, record all foods, no strenuous exercise, no supplementation).

Is there anything I need to bring with me?

Any medication you are currently on.

Preliminary and Main Trials

You will be asked to complete a familiarisation trial and 2 experimental trials (water before meal (WBM) and water during meal (WDM).

During the preliminary trial, you will visit the laboratory for measurement of height, weight and body fat. You will then complete a familiarisation trial similar to the NW trial. For the familiarisation and experimental trials, you will arrive at the laboratory in the morning fasted (~7am). You will then complete a questionnaire as to your subjective feelings at that time and be provided with either 600 ml of plain water immediately before or during breakfast meal. The breakfast meal will then be provided at ~7:30am (porridge, choice of three flavours). You will then complete a further SFQ immediately after the meal, 30min after and 60min after and be free to leave.

What Personal Information Will be Required from Me?

You will be asked to provide your weight, height, age and then any information on diet will be subsequently recorded.

Are There Any Risks in Taking Part?

If you are allergic to certain food or intolerant to lactose or dairy, you will need to let the investigators know, which will be established before you begin the study.

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Will My Taking Part in This Study be Kept Confidential?

All names of subjects and trials will be encoded and anonymity maintained.

The only person to hold a list of subjects will be the primary investigator and this list will be destroyed once the study is over. Subjects' will be recorded to as a number not a name.

What Will Happen to the Results of the Study?

The results of the study will be used in conferences and published in a journal and in a PhD thesis.

What do I Get for Taking Part?

You will gain information from the study once all subjects have completed the experimental trials. This will include a report of diet and general dietary recommendations.

If I Have Some More Questions Who Should I Contact?

Rob Corney:email: R.corney@lboro.ac.uk Tel:07912355835

Lewis James: email: L.james@lboro.ac.uk Tel: 01509 226352

What if I am Not Happy with How the Research was Conducted?

You are able to withdraw from the study at any time and also can request withdrawal of any information from the study.

Chapter 7

What is the Purpose of the Study?

There is a growing interest in identifying factors to manage or prevent the epidemic scale of obesity. A number of recent investigations have indicate that manipulating nutritional intake can affect energy balance. Similarly, the effects of exercise on energy balance have been documented and exercise appears to acutely effect energy intake in the hours after exercise, but little is known about the combined effects of nutrition and exercise on energy balance.

Who is Doing this Research and Why?

Rob Corney is investigating research for his PhD and results will be used for a PhD thesis.

Lewis James (Lecturer in Sports Nutrition) is supervising him in conducting this research.

Are There any Exclusion Criteria?

Aged between 18 and 40 years, fit and healthy, no immune deficiencies, non-smokers, not currently on a weight gain/weight loss diet and have maintained a stable weight for the previous 6 months (self reported), have no known history of gastric, digestive, cardiovascular or renal disease, resting blood pressure <140/90 mmHg, no lactose, dairy, gluten intolerances or allergies to used food groups.

Once I Take Part, Can I Change My Mind?

Yes! After you have read this information and asked any questions you may have we will ask you to complete an Informed Consent Form, however if at any time before, during or after the sessions you wish to withdraw from the study please just contact the main investigator. You can withdraw at any time, for any reason and you will not be asked to explain your reasons for withdrawing.

Location

All trials will all be held at Loughborough University in the Clyde Williams building in the laboratory.

How Long Will it Take?

Preliminary trial 1 h in the lab, Familiarisation trial 3 h (to be completed once) Experimental trials 5 h (3 trials to be completed) Within the time you are seated you can read or watch TV.

Is There Anything I Need to do Before the Sessions?

You will need to complete a food diary for 48 hours before the start of each trial. This will require you to standardise your diet and exercise (no alcohol, record all foods, no strenuous exercise, no supplementation).

Is There Anything I Need to Bring with Me?

Any Medication you are Currently on.
Loose fitting clothing e.g. shorts, t-shirt, vest, training shoes

Preliminary and Main Trials

You will be asked to complete a preliminary, familiarisation and 3 experimental trials. During the preliminary trial, you will visit the laboratory for measurement of height, weight and body fat. You will then complete an incremental discontinuous exercise test to fatigue that will start at a low intensity and finish at your maximum to determine peak oxygen uptake.

For the familiarisation and experimental trials, you will arrive at the laboratory in the morning (10am) after a standardised breakfast. You will void your bladder and then body mass (in boxers only) will be recorded. You will then be lying for 25 min and a blood sample will be collected via a vein in the arm and a resting breathing air sample will be collected. You will then exercise on a bike at an intensity equivalent to ~60% peak oxygen output for 30 minutes. Then after 2 min rest you will perform a HIIT (high intensity interval training) protocol involving 4 min exercise at a speed equivalent to

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~85% VO$_2$ peak and 2 min complete rest, which will be repeated 5 times (60 min total time). Oxygen samples and heart rate will be collected throughout the both exercise sessions. Immediately after this a further oxygen and blood sample will be collected (~12:00pm) and then you will drink a post-exercise recovery drink (provided by the investigators). A blood samples will be collected before the lunch. You will then be provided with a lunch (~1pm) (pasta, tomato sauce, cheese and olive oil) in a booth with no distractions and in silence and will eat until you feel satisfied (30 min time provided). Upon finishing the meal (~1:30pm) you will remain in the laboratory for a further 60 min, with oxygen taken at 60 min (~2:30pm) after meal consumption. You will then be free to leave the laboratory.

All blood samples will be kept in a -80 freezer for storage until analysis, then destroyed after 6 months.

What Personal Information will be Required from Me?

You will be asked to provide your weight, height, age and then any information on diet will be subsequently recorded.

Are there any Risks in Taking Part?

If you are allergic to certain food or intolerant to lactose or dairy, you will need to let the investigators know, which will be established before you begin the study. The aerobic exercise will involve high intensity bouts as described above.

Will my Taking Part in this Study be Kept Confidential?

All names of subjects and trials will be encoded and anonymity maintained.
The only person to hold a list of subjects will be the primary investigator and this list will be destroyed once the study is over. Subjects' will be recorded to as a number not a name.

What Will Happen to the Results of the Study?

The results of the study will be used in conferences and published in a journal and in a PhD thesis.

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What do I get for Taking Part?

You will gain information from the study once all subjects have completed the experimental trials. This will include a report of diet and general dietary recommendations.

If I Have Some More Questions Who Should I Contact?

Rob Corney:email: R.corney@lboro.ac.uk Tel:07912355835

Lewis James: email: L.james@lboro.ac.uk Tel: 01509 226352

What if I am not Happy with How the Research was Conducted?

You are able to withdraw from the study at any time and also can request withdrawal of any information from the study.

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Appendix B

Informed Consent Form

(to be completed after Subject Information Sheet has been read)

The purpose and details of this study have been explained to me.

I understand that this study is designed to further scientific knowledge and that all procedures have been approved by the Loughborough University Ethical Advisory Committee.

I have read and understood the information sheet and this consent form.
I have had an opportunity to ask questions about my participation.
I understand that I am under no obligation to take part in the study.
I understand that I have the right to withdraw from this study at any stage for any reason, and that I will not be required to explain my reasons for withdrawing.

I understand that all the information I provide will be treated in strict confidence and will be kept anonymous and confidential to the researchers unless (under the statutory obligations of the agencies which the researchers are working with), it is judged that confidentiality will have to be breached for the safety of the subject or others.
I agree to participate in this study.

Your name

_____

Your signature

_____

Signature of investigator

_____

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Appendix C

Name/Number   ....…….

Health Screen Questionnaire

Note to Investigators: This HSQ can be used in its entirety but you can also remove some of the questions if you know they are not relevant to your study.

As a volunteer participating in a research study, it is important that you are currently in good health and have had no significant medical problems in the past. This is (i) to ensure your own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

If you have a blood-borne virus, or think that you may have one, please do not take part in this research *[only include for projects involving invasive procedures].*

Please complete this brief questionnaire to confirm your fitness to participate:

1.At present, do you have any health problem for which you are:

(a)	on medication, prescribed or otherwise	Yes ☐	No ☐
(b)	attending your general practitioner	Yes ☐	No ☐
(c)	on a hospital waiting list	Yes ☐	No ☐

2.In the past two years, have you had any illness which required you to:

(a)	consult your GP	Yes ☐	No ☐
(b)	attend a hospital outpatient department	Yes ☐	No ☐
(c)	be admitted to hospital	Yes ☐	No ☐

3.Have you ever had any of the following:

(a)	Convulsions/epilepsy	Yes ☐	No ☐
(b)	Asthma	Yes ☐	No ☐
(c)	Eczema	Yes ☐	No ☐
(d)	Diabetes	Yes ☐	No ☐
(e)	A blood disorder	Yes ☐	No ☐
(f)	Head injury	Yes ☐	No ☐

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(g)	Digestive problems	Yes		No	
(h)	Heart problems	Yes		No	
(i)	Problems with bones or joints	Yes		No	
(j)	Disturbance of balance/coordination	Yes		No	
(k)	Numbness in hands or feet	Yes		No	
(l)	Disturbance of vision	Yes		No	
(m)	Ear / hearing problems	Yes		No	
(n)	Thyroid problems	Yes		No	
(o)	Kidney or liver problems	Yes		No	
(p)	Allergy to nuts	Yes		No	

4.Has any, otherwise healthy, member of your family under the age of 35 died suddenly during or soon after exercise?   Yes [ ]   No [ ]

If YES to any question, please describe briefly if you wish (eg to confirm problem was/is short-lived, insignificant or well controlled.)

....

....

5.Allergy Information

(a)	are you allergic to any food products?	Yes		No	
(b)	are you allergic to any medicines?	Yes		No	
(c)	are you allergic to plasters?	Yes		No	

If YES to any of the above, please provide additional information on the allergy

…………………………………………………………………………………………

…………………………………..

5.Additional questions for female subjects

(a)	are your periods normal/regular?	Yes		No	
(b)	are you on "the pill"?	Yes		No	
(c)	could you be pregnant?	Yes		No	
(d)	are you taking hormone replacement therapy	Yes		No	

Please provide contact details of a suitable person for us to contact in the event of any

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incident or emergency.

Name:

……………………………………………………………………………………………

Telephone   Number:

……………………………………………………………………………………………

Work ☐ Home ☐ Mobile ☐

Relationship   to

Subject:…………………………………………………………………………………

Are you currently involved in any other research studies at the University?

Yes ☐   No ☐

If yes, please provide details of the study

…………………………………………………………………………………………

…………………………………………………………………………………………

…………………………

Appendix D

Food, Activity and Sleep Record

Please describe each item of food and drink as fully as possible – type of food, method of cooking etc.

Please also weigh and list all drinks (including water that you consume in the 48 hours.

You should not consume any food or drink in the from 10pm in each laboritory visit

Subject number: _____ Trial: _____

Date: ____/_____/___

Guidelines for use of food record book

Please weigh all foods on the scales provided by placing your plate on the scales, pressing the pad, and then loading on the item of food. If the meal consists of several items then zero the scales before each of these and record the weights i.e. Put plate on scales, zero scales, load on first item eg meat – 125g shown, record in booklet, zero scales, load on another item eg potatoes – 150g shown, record in booklet, repeat stages 5 to 7 until you have made up your meal.

If eating out you will have to either (a) ask for an empty plate so that you can transfer each item to the new plate while you record the weight or (b) weigh the complete meal and eat each item separately and reweigh the plate after each stage so that you can work out the weight by difference ie

Some foods come in standard sizes and the weight will be printed on the label eg packet of crisps, pot of yoghurt, Mars bar, can of drink, and so these are easy to record.

Record only one food item on each line of the food record book.

Describe each item as fully as possible, giving the type and make of food. Eg.

Bread –brown, hovis, wholemeal, white

Milk –whole, skimmed, semi-skimmed

Biscuit –shortcake, digestive, chocolate covered

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Cheese –Kraft processed slices, Scottish cheddar, St.Ivel cottage cheese

Fish –mackerel, tuna, haddock

Cider –sweet or vintage

Tinned fruit –in heavy syrup or fruit juice

Describe the method of cooking – boiled, roast, fried, grilled.

Indicate whether skins are eaten.

If using weights, make it clear whether they are in ounces or grams. Grams are preferred.

Remember to record cups of tea and coffee together with any milk and sugar added.

To record total intake per day of commonly eaten foods (eg milk and sugar in tea/coffee, or butter/margarine on bread) weigh the tub of margarine, packet of sugar at the beginning of each day and again at the end of the day.

Make sure nobody else uses your tub of margarine, bag or sugar etc.

If ingredients are too light to weigh, please use household measures (½ teaspoon of salt, sugar, coffee, herbs etc)

You may weigh ingredients raw or cooked eg rice, but please indicate what you have done.

Some examples of the kind of details needed about each food or drink eaten are given on the first page of the diary

Operation of the scales

The scales give a digital reading in grams. They can be zeroed simply by pressing the "ON.OFF.TARE" button on the front. The scales will switch on as soon as this button is pressed and will switch off when they have been left unused for a short period of time. You should have plenty of time to record the weight on the balance before this happens but beware of delays!

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Example food sheet

Time	Description of food	Weight of food	Weight left over
8.30AM	Kellogs Cornflakes	40g	0g
	Semi-skimmed milk	200g	0g
	1 slice toast, Hovis granary bread	50g	0g
	Margarine, St Ivel gold with no added salt	10g	0g
11.00AM	Coffee, Nescafe decaffeinated granules	180g	0g
	Whole milk	17g	0g
	Kit-Kat biscuit, 2 fingers	35g	0g
1.15PM	Marks & Spencer sandwich: White bread, 2 medium slices	100g	0g
	Margarine on bread	20g	0g
	Grated cheddar cheese	40g	0g
	3 slices tomato	10g	0g
	1 can diet coke	330g	50g
6.00PM	Grilled lean lamb chop	150g	20g
	Boiled new potatoes in their skins	250g	43g
	Processed peas, Cross and Blackwell	100g	22g
	1 banana	30g	0g
	1 glass fresh orange juice	148g	0g
10.00PM	Tea	40g	0g
	Semi-skimmed milk	200g	0g
	4 biscuits, McVities chocolate digestive	50g	0g

Day 1 Food Record

Time	Description of Food	Weight of Food	Weight Left over

Physical Activity Diary

Time	Activity	Intensity	Duration
09:00	Cycle to university	Low	20 minutes
18:00	Walk the dog	Low	30 minutes

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Please record your physical activity over the 24 hour experimental period in the table above. We also ask you to refrain from participating in any strenuous activity during this time.

Day 2 Food Record

Time	Description of Food	Weight of Food	Weight Left over

Physical Activity Diary

Time	Activity	Intensity	Duration
09:00	Cycle to university	Low	20 minutes
18:00	Walk the dog	Low	30 minutes

Please record your physical activity over the 24 hour experimental period in the table above. We also ask you to refrain from participating in any strenuous activity during this time.

To be completed at the end of the record period.

Have all your meals been typical of your normal eating habits?

……………………………………………………………………………………………
……………

If not, please explain (social functions, illness etc).

……………………………………………………………………………………………
……………………………………………………………………………………………
…………………………

Has your pattern of activities been fairly typical?

……………………………………………………………………………………………

If not, please explain.

………………………………………………………………………………………………

………………………………………………………………………………………………

Any other comments or information you would like to give ?

………………………………………………………………………………………………

………………………………………………………………………………………………

Sleep Questionnaire

What time did you go to bed?

………………………………………………………………………………………………
…

Approximately what time did you go to sleep?

………………………………………………………………………………………………

What time did you wake?

………………………………………………………………………………………………

How hard was it to get to sleep?

Not at all hard   Very hard

_____

How comfortable was your sleep?

Not at all comfortable   Very comfortable

_____

Physical Activity Questionnaire

During one week, how many times on average do you do the following kinds of exercise for more than 15 minutes?

Strenuous exercise (heart beats rapidly)

For example; running, jogging, squash, hockey, football, volleyball, vigorous swimming, vigorous long distance cycling.

___times per week.

Moderate exercise (not exhausting)

For example; fast walking, tennis, easy cycling, badminton, easy swimming, dancing.

___ times per week.

Mild exercise (minimal effort)

For example; yoga, archery, fishing, bowling, golf, easy walking.

___ times per week.

Appendix F

Rating of Perceived Exertion

6

7 Very very light

8

9 Very light

10

11 Fairly Light

12

13 Fairly hard

14

15 Hard

16

17 Very hard

18

19 Extremely hard

20 Maximum

Appendix G

Thermal Comfort Scale

-10 Cold impossible to bear

-9

-8 Very cold, shivering hard

-7

-6 Cold, light shivering

-5

-4 Most areas of the body feel cold

-3

-2 Some areas of the body feel cold

-1

0 Neutral 1

2 Some areas of the body feel warm

3

4 Most areas of the body feel hot 5

6 Very hot, uncomfortable 7

8 Extremely hot, close to limit 9

10 Heat impossible to bear

Appetite Perceptions Questionnaire

Subject:_____Trial:_____Time: _____Date:_____

Answer the following questions related to how you feel now:

How thirsty do you feel now?

Not at all thirsty   Very thirsty

How does your mouth taste now?

Not at all pleasant   Very pleasant

How full does your stomach feel now?

Not at all full   Very full

How much food do you think you could eat?

Nothing at all   Alot

How hungry do you feel now?

Not at all hungry   Very hungry

How does your head feel now?

Not at all sore   Very sore

How satisfied do you feel?

Not at all satisfied   Very satisfied

How strong is your desire to eat?

Not strong at all   Very strong

Appendix I

Drink Perception Questionnaire

Subject:_____Trial:_____Time: _____Date:_____ Trial: _____

Answer the following questions related to how you feel now:

How thick do you think the drink was?

Not at all thick   Very thick

How creamy do you think the drink was?

Not at all creamy   Very creamy

How was the stickiness?

Not at all sticky   Very sticky

How sweet do you think the drink was?

Nothing at all sweet   Very sweet

How sour do you think the drink was?

Not at all sour   Very sour

How fruity do you think the drink was?

Not at all fruity   Very fruity

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Appendix J

Buffet Items Available at the Cold Buffet Meal. (Used in Chapter 1)

Subjects were asked to circle foods they disliked

<div align="center">

Cereal

Cereal bars

White bread

Brown bread

Margarine

Peanut butter

Chocolate spread

Strawberry jam

Apples

Oranges

Bananas

Semi skimmed milk

Orange juice

Apple juice

Tea

Coffee

Water

</div>

Appendix K

Porridge Meal Preparation

Day before trial

Prepare porridge oats the day before

Ensure subjects are recording all food, fluid and exercise for 24 h before the trial

The day of the trial

Measure 420 mL of milk

Measure 500 mL of water in 2 separate glasses

Add milk 420 mL to the 90 g of oats and mix until homogenous

Weigh the porridge on the scales with spoon included and record weight (g)

Put into the microwave for 2 min

Take out and stir then place in microwave again for a following 30 seconds

Place second bowl into the microwave, following the above guidelines

Let the first porridge rest for 3 minutes then weigh on scales and record weight (g) with spoon, before serving

Serve with 500 mL of water and 500 mL of squash

Record post weight

Three-Factor Eating Questionnaire

Part 1: Please answer true or false

When I smell a sizzling steak or see a juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal.

True □ False □

I usually eat too much at social occasions, like parties and picnics.

True □ False □

I am usually so hungry that I eat more than three times per day.

True □ False □

When I have eaten my quota of calories, I am usually good about not eating any more.

True □ False □

Dieting is so hard for me because I just get too hungry

True □ False □

I deliberately take small helpings as a means of controlling my weight.

True □ False □

Sometimes things just taste so good that I keep eating even when I am no longer hungry.

True □ False □

Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat.

True □ False □

When I feel anxious, I find myself eating.

True □ False □

Life is too short to worry about dieting.

True □ False □

Since my weight goes up and down, I have gone on reducing diets more than once.

True ☐ False ☐

I often feel so hungry that I just have to eat something

True ☐ False ☐

When I am with someone who is overeating, I usually overeat too.

True ☐ False ☐

I have a pretty good idea of the number of calories in common food.

True ☐False ☐

Sometimes when I start eating, I just can't seem to stop.

True ☐ False ☐

It is not difficult for me to leave something on my plate.

True ☐ False ☐

At certain times of the day, I get hungry because I have gotten used to eating then.

True ☐False ☐

While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it.

True ☐ False ☐

Being with someone who is eating often makes me hungry enough to eat also.

True ☐ False ☐

When I feel blue, I often overeat.

True ☐False ☐

I enjoy eating too much to spoil it by counting calories or watching my weight.

True ☐ False ☐

When I see a real delicacy, I often get so hungry that I have to eat right away.

True ☐ False ☐

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I often stop eating when I am not really full as a conscious means of limiting the amount that I eat.

True ☐ False ☐

I get so hungry that my stomach often seems like a bottomless pit.

True ☐ False ☐

My weight has hardly changed at all in the last ten years.

True ☐ False ☐

I am always hungry so it is hard for me to stop eating before I finish the food on my plate.

True ☐False ☐

When I feel lonely, I console myself by eating.

True ☐ False ☐

I consciously hold back at meals in order not to gain weight.

True ☐ False ☐

I sometimes get very hungry late in the evening or at night.

True ☐ False ☐

I eat anything I want, anytime I want.

True ☐ False ☐

Without even thinking about it, I take a long time to eat.

True ☐ False ☐

I count calories as a conscious means of controlling my weight.

True ☐ False ☐

I do not eat some foods because they make me fat.

True ☐ False ☐

I am always hungry enough to eat at any time

True ☐ False ☐

I pay a great deal of attention to changes in my figure.

True ☐ False ☐

While on a diet, if I eat food that is not allowed, I often then splurge and eat other high calorie food.

True ☐ False ☐

Part 2: Please answer the following questions by circling the number with the response that is appropriate to you.

How often are you dieting in a conscious effort to control your weight?
1 (rarely) 2 (sometimes) 3 (usually) 4 (always)

Would a weight fluctuation of 5 lbs. affect the way you live your life?
1 (not at all) 2 (slightly) 3 (moderately) 4 (very much)

How often do you feel hungry?
1 2 3 4
(only at meal times) (sometimes between meals) (often between meals)   (almost always)

Do your feelings of guilt about overeating help you control your food intake?
1 (never)2 (rarely)3 (often)4 (always)

How difficult would it be for you to stop eating halfway through dinner and not eat for the next few hours?
1 (easy)2 (slightly difficult)3 (moderately difficult)4 (very difficult)

How conscious are you of what you are eating?
1 (not at all)2 (slightly)3 (moderately)4 (extremely)

How frequently do you avoid 'stocking up' on tempting foods?
1 (almost never)2 (seldom)3 (usually)4 (almost always)

How likely are you to shop for low calorie foods?
1 (unlikely) 2 (slightly unlikely) 3 (moderately likely) 4 (very likely)

Do you eat sensibly in front of others and splurge alone?
1 (never) 2 (rarely) 3 (often) 4 (always)

How likely are you to consciously eat slowly in order to cut down on how much you eat?
1 (unlikely)2 (slightly likely)3 (moderately likely)4 (very likely)

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How frequently do you skip desert because you are no longer hungry?

1 (unlikely)2 (seldom)3 (at least once a week)4 (almost every day)

How likely are you to consciously eat less than you want?

1 (unlikely)2 (slightly likely)3 (moderately likely)4 (very likely)

Do you go on eating binges though you are not hungry?

1 (never)2 (rarely)3 (sometimes)4 (at least once a week)

On a scale of 0 to 5, where 0 means no restraint in eating (eating whatever you want, whenever you want it) and 5 means total restraint (constantly limiting food intake and never 'giving in'), what number would you give yourself?

0

Eat whatever you want, whenever you want it

1

Usually eat whatever you want, whenever you want it

2

Often eat whatever you want, whenever you want it

3

Often limit food intake, but often 'give in'

4

Usually limit food intake, rarely 'give in'

5

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Constantly limiting food intake, never 'give in'

To what extent does this statement describe your eating behaviour? 'I start dieting in the morning, but because of any number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow.'

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(not like me) (little like me) (pretty good description of me) (describes me perfectly)

Scoring

One point is given for each item in Part 1 and for each item (numbered question) in Part 2. The correct answer for the true/false items is described below. In part 1, an 'incorrect' response results in zero point being added to that factor. 'Correct' answers receive one

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point. The direction of the question in Part 2 is determined by splitting the responses at the middle. If the item is labelled '+', those responses above the middle are given a zero. Vice versa for those with a '-'. For example, scoring 3 or 4 on the first item of Part 2 (no. 37) would receive one point. Anyone scoring 1 or 2 would receive a zero.

Scoring

Question number	Correct Answer	Score	Factor concerning
1	True		DH
2	True		DH
3	True		H
4	True		DR
5	True		H
6	True		DR
7	True		DH
8	True		H
9	True		DH
10	False		DR
11	True		DH
12	True		H
13	True		DH
14	True		DR
15	True		DH
16	False		DH
17	True		H
18	True		DR
19	True		H
20	True		DH
21	False		DR
22	True		H
23	True		DR
24	True		H
25	False		DH
26	True		H
27	True		DH
28	True		DR

29	True		H
30	False		DR
31	False		DH
32	True		DR
33	True		DR
34	True		H
35	True		DR
36	True		DH
37	+		DR
38	+		DR
39	+		H
40	+		DR
41	+		H
42	+		DR
43	+		DR
44	+		DR
45	+		DH
46	+		DR
47	-		H
48	+		DR
49	+		DH
50	+		DR
51	+		DH

	Tally	Score	Boundaries
Dietary restraint (DR)			0-10 low 11-13 high 14-21 clinical
Dietary disinhibition (DH)			0-8 low 9-11 high 12-16 clinical
Hunger (H)			0-7 low 8-10 high 11-14 clinical