University of Sussex

A University of Sussex DPhil thesis

Available online via Sussex Research Online:

http://sro.sussex.ac.uk/

This thesis is protected by copyright which belongs to the author.

This thesis cannot be reproduced or quoted extensively from without first obtaining permission in writing from the Author

The content must not be changed in any way or sold commercially in any format or medium without the formal permission of the Author

When referring to this work, full bibliographic details including the author, title, awarding institution and date of the thesis must be given

Please visit Sussex Research Online for more information and further details

Understanding the role of Umami in appetite control: A protein-specific effect?

Una Masic

Submitted for the degree of

Doctor of Philosophy in Psychology

School of Psychology

University of Sussex

December 2013

Declaration

I hereby declare that this thesis has not been and will not be, submitted in whole or in part to another University for the award of any other degree. The research submitted for this studentship was supported by Ajinomoto Co., Inc. There were no conflicts of interest throughout the duration of the studentship between the two parties.

Signature:

Portions of the thesis have also been published and submitted to the journals listed below:

Reported in Chapter 6: Masic, U., & Yeomans, M.R. (2013). Does monosodium glutamate interact with macronutrient composition to influence subsequent appetite? *Physiology & Behavior*, *116-117,* 23-29. http://dx.doi.org/10.1016/j.physbeh.2013.03.017

Reported in Chapter 7: Masic, U., & Yeomans, M.R. (2014). Monosodium glutamate delivered in a protein-rich soup improves subsequent energy compensation. *Journal of Nutritional Sciences*, *in press*.

Reported in Chapter 8: Masic, U., & Yeomans, M.R. (2014). Umami flavour enhances appetite but also enhances satiety. *American Journal of Clinical Nutrition*, *under review*.

<u>Acknowledgements</u>

UNIVERSITY OF SUSSEX

Una Masic

Doctor of Philosophy in Psychology

Understanding the role of Umami in appetite control: A protein-specific effect?

Summary

The fifth basic taste, 'umami', is the flavour function elicited by amino acids like monosodium glutamate (MSG) in foods. This taste is recognized for its flavour enhancing properties but little is known about its effects on appetite and intake. Thus the experiments in this thesis aimed to understand how umami influences pleasantness, appetite stimulation, satiation and satiety using MSG, with some additional focus on its associated ribonucleotide inosine 5'-monophosphate (IMP).

Chapter 2 established a bland, low glutamate control soup which was used throughout all subsequent experiments to test the effects of MSG on palatability using commercially-relevant concentrations. Chapters 3 and 4 assessed the influence of increasing palatability on rated appetite and intake of this soup with either added MSG (Chapter 3) or added sucrose (Chapter 4). No increase in hunger or intake was found after the more palatable conditions. Chapter 5 explored the relationship between MSG taste and protein regulation, assessing acute and habitual protein intake with findings indicating that high protein consumers liked high MSG concentrations more after an acute protein deprivation than sweet, salty or control flavours. Chapter 6 examined the time course of rated MSG satiety alone and in combination with either protein or carbohydrate in a preload soup and found enhanced rated satiety in MSG protein conditions. This design was extended in Chapter 7 to include an intake test after a pre-specified time of consuming the preload soup. The results indicated better compensation after MSG protein conditions but no differences in intake were found across carbohydrate or control conditions. Chapter 8 assessed MSG and IMP with or without added protein using the same design as Chapter 7 and found reductions in intake in MSG/IMP conditions. This suggests that the flavour of umami plays an important role in the regulation of appetite and intake.

Table of Contents

Chapter 4:

Does sweetness generate an appetizer effect in a main meal? – Paper 3.......125 - 145

Chapter 8:

Umami flavour enhances appetite but also increases satiety – Paper 7........265 - 287

List of Tables

List of Figures

Figure 3.1. Mean Experiment 1 (Panel A) and Experiment 2 (Panel B) sensory and hedonic assessments (mm) of three soups differing in MSG concentration (no MSG (□), 0.6% added MSG (⊡) and 1% added MSG (▤). Data are mean ±SE. Significant differences between added and no MSG conditions are indicated: * P<0.05.

Figure 3.2. Mean (± SEM) Experiment 2 VAS ratings (mm) for hunger (Panel A), fullness (Panel B) and pleasantness (Panel C) at interrupt points (g) before, during and after ingestion of soup varying in MSG concentration (no MSG (▬●▬), 0.6% MSG (.....◆..) or 1% MSG (▬ ▲ ▬).

Figure 3.3. Mean (±SEM) a*d-libitum* energy intake (Kcal) of three soups differing in MSG concentration (no MSG, 0.6% MSG and 1% MSG).

Figure 3.4. Mean (±SEM) experience of satiation (satiation quotient score) in relation to the volume consumed at specified interrupts (Panel A) and eating rate (g/min) at mean time interrupts during ingestion of soup varying in MSG concentration (no MSG (▬●▬), 0.6% MSG (....◆...) or 1% MSG (▬ ▲▬)). Statistically significant protected contrasts between control and 1% MSG are indicated at the end of the meal (Panel A) and over the meal (Panel B) are indicated: * P<0.05.

Figure 4.1. Mean (±SEM) VAS ratings (mm) for hunger (Panel A), fullness (Panel B), pleasantness (Panel C) and desire (Panel D) at interrupt points (g) before, during and after ingestion of soup with (▬●▬) or without (▬●▬) added sweetness.

Figure 4.2. Mean (±SEM) eating rate (g/min) at mean time interrupts (Panel A) and rate of satiation in relation to volume consumed at specified interrupts (Panel B) during ingestion of a soup with (▬●▬) and without (▬●▬) added sweetness. Statistically significant differences between no and added sweet conditions are indicated: ** P<0.001.

Figure 5.1. Change from baseline pleasantness assessments (mm) of three flavours (Monosodium glutamate; MSG, Panel A, sodium chloride; NaCl, Panel B and Acesulphame K; Ace K, Panel C) of a soup delivered in weak (▪) or strong (⊡) flavour concentrations and compared to a bland control (□) across high and low protein breakfast exposure days. Data are mean±SEM. Statistically significant difference between low and high protein test day (main effect of day) is indicated: * P<0.05.

Figure 5.2. Pleasantness assessments (mm) of weak (Panel A) and strong (Panel B) concentrations of MSG soup samples after protein manipulations (baseline no manipulation (▬▬), low protein breakfast (▬▬) or high protein breakfast (▬▬)) as a function of habitual protein consumption (Protein Status Score) using linear regression coefficients.

Significant t-test difference on high concentration day between manipulation conditions P=0.05.

Figure 6.1. Sensory and hedonic assessments (mm) of three levels of soup (low energy control (■), high energy protein (□) and high energy carbohydrate (▤) with (Panel A) and without (Panel B) added MSG. Data are mean ±SE. Significant differences between no and added MSG are indicated: ** P<0.01.

Figure 6.2. Change from baseline VAS hunger (Panel A) (mm) and fullness (Panel B) ratings across three levels of soup (low energy control, high energy carbohydrate and high energy protein) containing no (■) and added (▱) MSG. Data are mean ± SE.

Figure 6.3. Change in hunger ratings (mm) post-ingestion across three levels of soup (low energy control (Panel A) and high energy protein (Panel B) and high energy carbohydrate (Panel C)) with (_ _ _ .) and without (____) added MSG over time (minutes). Data are mean ± SEM. Significant protected contrasts (p = <.02) are denoted with *.

Figure 6.4. Change in fullness ratings (mm) post-ingestion across three levels of soup (low energy control (Panel A) and high energy protein (Panel B) and high energy carbohydrate (Panel C)) with (_ •_ .) and without (_•_) added MSG over time (minutes). Data are mean ± SEM.

Figure 7.1. *Ad-libitum* lunch intake after fixed preload consumption of three versions of soup (low energy control, high energy carbohydrate and high energy protein) with (▱) and without (■) added MSG. Data are mean ±SEM. Significant main effect of condition is indicated: * P<0.05.

Figure 7.2. Energy compensation overall at an *ad-libitum* test meal (Panel A) after fixed consumption of high energy carbohydrate and high energy protein soup preloads with and without added MSG. Different letters indicate statistically significant differences (p = <.05) between conditions (using within-subjects Bonferroni corrected contrasts); statistically significant contrast between no and added MSG carbohydrate conditions (a); no and added MSG protein conditions (b) and no MSG carbohydrate and all protein conditions (ab). Data are mean±SEM.

Figure 8.1. *Ad-libitum* lunch intake (A) after fixed preload consumption of a high energy protein or low energy control with (■) and without (□) added MSG/IMP and (B) compensation for the extra energy ingested in the high energy protein-rich condition across added and no MSG/IMP conditions. Data are mean ±SE. Significant differences between added and no MSG/IMP conditions are indicated: * P<.05.

List of Appendices

Appendix 1.1. Glossary of terms

Appendix 2.1. Graphical depiction of the experimental design employed in experiments 1 and 2.

Appendix 3.1. Graphical depiction of the experimental design carried out for experiments 1 and 2. Experiment 2 also included an additional training soup day.

Appendix 4.1. Graphical depiction of the experimental design carried out for the training day (Panel A) and on test days (Panel B).

Appendix5.1. Portion size estimation sheet

Appendix 5.2. Protein Food Frequency Questionnaire

Appendix 5.3. Graphical depiction of the study design employed.

Appendix 6.1. Graphical depiction of the study design employed.

Appendix 7.1. Graphical depiction of the study design employed.

Appendix 8.1. Graphical depiction of the study design employed.

List of Abbreviations

Ace K – Acesulphame K

BMI – Body mass index (kg/m²)

CCK – Cholecystokinin

CHO – Carbohydrate

CMF – Cow's milk formula

COMPX – Energy compensation

DE – Dextrose equivalent

ED – Energy density (Kcal/g)

EI – Energy intake

ENaC – Epithelial sodium channel

ePHF – Extensive protein hydrolysate formula

FFQ – Food frequency questionnaire

fMRI – Functional magnetic resonance imaging

FSA –Food Standards Agency

GLP-1 – Glucagon-like peptide 1

GMP – Guanosine 5'-monophosphate

h - hours

IBU – Ingestive Behaviour Unit

IMP – Inosine 5'-monophosphate

Kcal – kilocalorie

M – Men

min - minutes

MSG – Monosodium glutamate

MSG+ - Added MSG

MSG- - No added MSG

NaCl – Sodium chloride

NCI – National Cancer Institute

NHANES – National health and nutrition examination survey

OFC –Orbitofrontal cortex

PRO –Protein

PYY – Peptide YY

SE or SEM – Standard error of the mean

SIPM – Sussex Ingestion Pattern Monitor

SQ – Satiety quotient

TFEQ – Three factor eating questionnaire

UEM – Universal Eating Monitor

VAS – Visual analogue scale

W – Women

CHAPTER 1:

UNDERSTANDING THE ROLE OF UMAMI IN APPETITE CONTROL: A
PROTEIN-SPECIFIC EFFECT?

1.1 Brief Background

1.1.1 Why should we assess the effects of umami on appetite and intake?

The flavour of umami is one generally enjoyed across the world and can be described as
a unique taste quality elicited by certain amino acids and related compounds, most
notably monosodium glutamate (MSG). MSG is a sodium salt of glutamic acid which is
an amino acid found in protein (Loliger, 2000). MSG is particularly abundant in a wide
array of foods such as animal protein, vegetables and cheeses (Garcia-Bailo, Toguri,
Eny, & El-Sohemy, 2009; Jinap & Hajeb, 2010; Luscombe-Marsh, Smeets, &
Westerterp-Plantenga, 2009; Yamaguchi & Ninomiya, 2000). The umami experience
generated by MSG has consequently shaped cooking practices across centuries dating
back to the Romans' use of fermented fish sauce (Beauchamp, 2009; Curtis, 2009) to
more modern day cooking with inventions such as 'umami paste' and the 'umami
burger' increasing in popularity. Indeed, the simple combination of foods used to make
soups and stocks may have evolved as a means of blending foods high in glutamic acid,
such as MSG containing foods, with complementary ribonucleotides to further boost the
umami taste (Uneyama, Uematsu, Iwatsuki, & Nakamura, 2012; Yamaguchi &
Ninomiya, 2000). These umami compounds are extracted from natural sources, for
instance MSG was originally isolated from dried kelp (Ikeda, 1908) whilst the umami
associated ribonucleotides inosine 5'-monophosphate (IMP) was first isolated from
dried skipjack tuna (Kodama, 1913) and guanosine 5'-monophosphate (GMP) was
isolated from dried shiitake mushrooms (Kuninaka, 1960). These flavour enhancers act
to produce the hedonic responses experienced when tasting umami in dietary sources
(Prescott, 1998) with IMP and GMP acting synergistically with MSG to enhance the
fifth basic taste of umami that MSG may also elicit alone.

Further understanding of the role of this basic taste in food selection and intake is vital
as the basic tastes play a fundamental part in the regulation of suitable and unsuitable
foods to eat and provide us with the necessary information as to why these foods are

selected based on their flavour. As the history of research surrounding umami has been controversial, our understanding of how umami compounds such as MSG and IMP influence appetite and intake is inadequate. Kwok (1968) initially suggested that MSG was linked to Chinese restaurant syndrome, characterised by numbness, weakness and headaches resulting in the branding of MSG as detrimental to health (Prescott & Young, 2002). However, numerous placebo-controlled studies have failed to support the role of MSG as the cause of such adverse reactions (Geha et al., 2000; Tanphaichitr, Leelahagul, & Suwan, 2000). Indeed, Bazzano and colleagues (1970) gave their participants doses of up to 147g MSG per day over a 30 day period noting no ill effects. Similarly, MSG has not been linked to any long-term negative health consequences (Walker & Lupien, 2000), yet the opinion that flavour enhancers are in some way detrimental to health still prevails (Shi et al., 2010).

1.1.2 MSG and the risk for weight gain

The reputation of MSG as damaging to health has not only been unsubstantiated in relation to immediate harmful effects, but has also been unsupported with regards to long-term negative health outcomes such as the potential for weight gain and obesity (Shi, et al., 2010). This relationship between MSG and weight gain was originally suggested after experimental work in animals showed increases in weight gain in animals injected with high concentrations of MSG during the weanling period (Hermanussen et al., 2005; Xue, Wong, & Leong, 1997). However, these studies often also involved lesions to certain brain areas or caused central nervous system pathologies related to appetite and body metabolism due to these extreme doses of injected MSG (Shi, et al., 2010). Such effects have not been found in a range of studies providing animals with free access to MSG concentrations (Ackroff & Sclafani, 2011; Kondoh & Torii, 2008; Ren, Ferreira, Yeckel, Kondoh, & de Araujo, 2011). In humans this relationship between MSG and weight gain has generated mixed findings. Due to the known differences in obese and healthy weight participants such as higher preferences for sweet and fatty flavours (Salbe, DelParigi, Pratley, Drewnowski, & Tataranni, 2004), possibly due to polymorphisms in T1R taste receptor genes which have been linked to differences in dietary patterns between healthy and overweight individuals (Garcia-Bailo, et al., 2009), it may be that MSG perpetuates higher weight maintenance because of its effects on enhancing flavour. Donaldson and colleagues (2009) found that

obese women rated MSG as more intense in flavour than did normal weight or overweight women. Thus it may be argued that the palatable element of MSG may be said to encourage intake (or maintain higher intake) of energy dense foods to which MSG has been added in these individuals (Cox, Perry, Moore, Vallis, & Mela, 1999). He and colleagues (2008) reported a positive correlation between MSG and BMI in a sample of Chinese adults, with a higher prevalence of overweight found in those reporting regular use of MSG when controlling for physical activity and total energy intake. However, from the mentioned research it is not clear whether MSG leads to obesity or whether obese individuals would choose to consume foods that are high in MSG. Similarly, many studies conducted on humans that appear to support the link between MSG and obesity do not take accurate measures of MSG ingestion into account (Shi, et al., 2010). For instance, He and colleagues did not assess dietary patterns and foods ingested (which include natural glutamic acid in the form of free glutamates found in foods instead of added to food in the form of MSG). Indeed, when natural free glutamates are taken into account, the relationships between MSG and weight gain were not substantiated (Shi, et al., 2010). This has also been found in mice offered free access to MSG (Ren, et al., 2011), with some researchers suggesting that mice fed high fat diets actually show reductions in weight gain after being given *ad-libitum* access to 1% MSG solutions (Kondoh & Torii, 2008).

The encouraging findings reported by Kondoh & Torii (2008) in mice instead highlight the potential for a different role of flavour enhancers generating the experience of umami such as MSG, one which may be more related to reducing energy intake. Indeed, umami flavour has been associated with producing a 'meaty' taste experience and has been related to the sensing of protein (Luscombe-Marsh, Smeets, & Westerterp-Plantenga, 2008; Luscombe-Marsh, et al., 2009). Upon discovering MSG, Ikeda claimed that this umami compound may be used by the body as a signal for the presence of protein in foods (Ikeda, 1908). Subsequently, this idea has been endorsed by many researchers (Kurihara, 2009; Naim, Ohara, Kare, & Levinson, 1991) and has led to a more concentrated effort to understand what role umami compounds may play in guiding intake, and whether these compounds influence intake when provided in different macronutrient combinations. As the effects of protein on reducing appetite and intake are well known (Bertenshaw, Lluch, & Yeomans, 2008; Lejeune, Kovacs, & Westerterp-Plantenga, 2005; Tome, Schwarz, Darcel, & Fromentin, 2009; Westerterp-

Plantenga, Nieuwenhuizen, Tomé, Soenen, & Westerterp, 2009), could combining MSG with protein act to further reduce feelings of hunger and intake (whilst maintaining appetitive satisfaction) due to the presence of an additional protein cue? Could this effect be magnified further by providing complementary ribonucleotides that are present in dietary protein sources (such as IMP)? Could this further improve adherence to high protein diets (which can be low; Brinkworth et al., 2004; St Jeor et al., 2001) by improving the flavour of meals supplemented with protein? Without investigating the effects of this important basic taste on rated appetite and intake, and in combination with other macronutrients, the scope for understanding how this taste may inform human feeding practices remains incomplete.

1.2 Motivational processes in the control of appetite

1.2.1 Defining Hunger

The traditional homeostatic approach of intake conceptualises hunger as a physiological cue which promotes feeding due to nutrient shortage. This is deemed as biologically useful because it reminds us of the body's need for sustenance (Blundell, Lawton, Cotton, & Macdiarmid, 1996). Hunger may also be characterised by the motivation to locate and consume food. Indeed, individuals state that it is this motivation to consume coupled with a physiological need state that dictates the onset of eating (Blundell et al., 2010). However, these definitions only encompass hunger as motivating eating behaviour based on the body's needs. Identifying hunger also relies on processes including how palatable the food may be, the availability of the food, previous experiences with the food, the social context in which the food is consumed, and the sensory information the food is providing (Blundell, et al., 2010) amongst others. All of these factors influence the way in which hunger is stimulated and expectations about how the food will affect hunger (Brunstrom, Shakeshaft, & Scott-Samuel, 2008) even before the eating occasion has begun. As a consequence, this multifaceted experience of hunger is important in determining the type, quantity and timing of food consumed. During food intake, thoughts relating to hunger relay information about the sensory experience of the food and how this interacts with the physiological cues of nutrient shortage (Booth, Toates, & Platt, 1976). This suggests that hunger is complex and not only dependent on a shortfall of nutrients. Thus hunger may be said to relate to a

combination of physiological and psychological factors, with the physiological messages integrated with the available information from the food source and the environment affecting the resulting behavioural response.

1.2.2 Defining Satiation

Satiation may be defined as an inhibitory process during an eating episode that brings an eating episode to an end and relies on the cues arising from ingestion to terminate the feeding episode (also termed intra-meal satiation). It is reliant on both the positive feedback (stimulating appetite) provided by strong hunger, low fullness and high palatability (see section 1.2.4) and the negative feedback (suppressing appetite) provided by low hunger and strong fullness and is an expression of the change in these appetite percepts. Satiation is also important for determining meal size and the rate of consumption (Blundell, Goodson, & Halford, 2001) and may be governed by a number of factors including the volume and weight of the meal (Blundell, et al., 1996), with feelings of fullness acting as a potent satiation signal to inhibit further meal intake (Halford & Harrold, 2012). As satiation is closely related to hunger, and particularly rated hunger in experimental situations (Blundell et al., 2009), the complex factors which influence hunger also apply to the rate of satiation. Indeed, just as the intensity of hunger may be determined by habitual eating patterns, social or cultural factors or other such periprandial circumstances (Blundell, et al., 1996), so too is satiation. Similarly, satiation may be influenced by palatability, with food pleasantness acting to stimulate hunger and thus delay intra-meal satiation (see section 1.2.4). Fullness also influences satiation in relation to the volume consumed. Indeed, rated fullness has been suggested to depend more on physiological cues such as volume and stomach distension rather than the more cognitive cues that apply to estimations of hunger (Allirot et al., 2013). Thus, satiation can be said to rely on interactions between physiological regulation, the external environment (such as cultural and social influences), sensory information, motivational constructs, and brain mechanisms (such as cognitions and attributions) (Blundell, et al., 1996).

1.2.3 Defining Satiety

Satiety (sometimes referred to as inter-meal satiety) follows the end of an eating episode and is the state in which further intake between eating episodes is inhibited, thus delaying the onset of the next meal (Blundell, et al., 2010; Blundell, et al., 1996). The strength of satiety may be measured by the duration of hunger suppression (using rated appetite measures or time elapsed until self-determined meal intake occurs) and by the quantity of subsequent meal intake (by provision of an *ad-libitum* meal; Blundell et al., 2009). Satiety may be influenced by the qualities of the meal consumed such as the chemical and physical meal components (including the energy density and macronutrient composition) but also may be influenced by other factors similar to satiation. Blundell and colleagues (Blundell, Rogers, & Hill, 1987) suggested that four mediating processes act to mediate the maintenance of hunger inhibition between meals. These include the sensory, cognitive, post-ingestive (pre-absorptive), and post-absorptive processes. This Satiety Cascade model (Figure 1; Blundell et al., 1987) suggests that various aspects of a food influence the mediating processes determining satiety (and satiation) differently therefore affect the experience of satiety (and satiation) differently (such as the effects of different macronutrients on satiating efficiency – see section 1.4). It also demonstrates how the sensory qualities of a meal may interact with the macronutrient profile and energy density to trigger the relevant regulatory processes and also takes the cognitive aspects of intake (rated appetite and thoughts about the meal consumed; Blundell et al., 2001) into account to determine satiety. Thus, the cascade allows for integration between the traditional biological systems underpinning appetite and the psychological behavioural indices that determine the rate of satiety (Halford & Harrold, 2012).
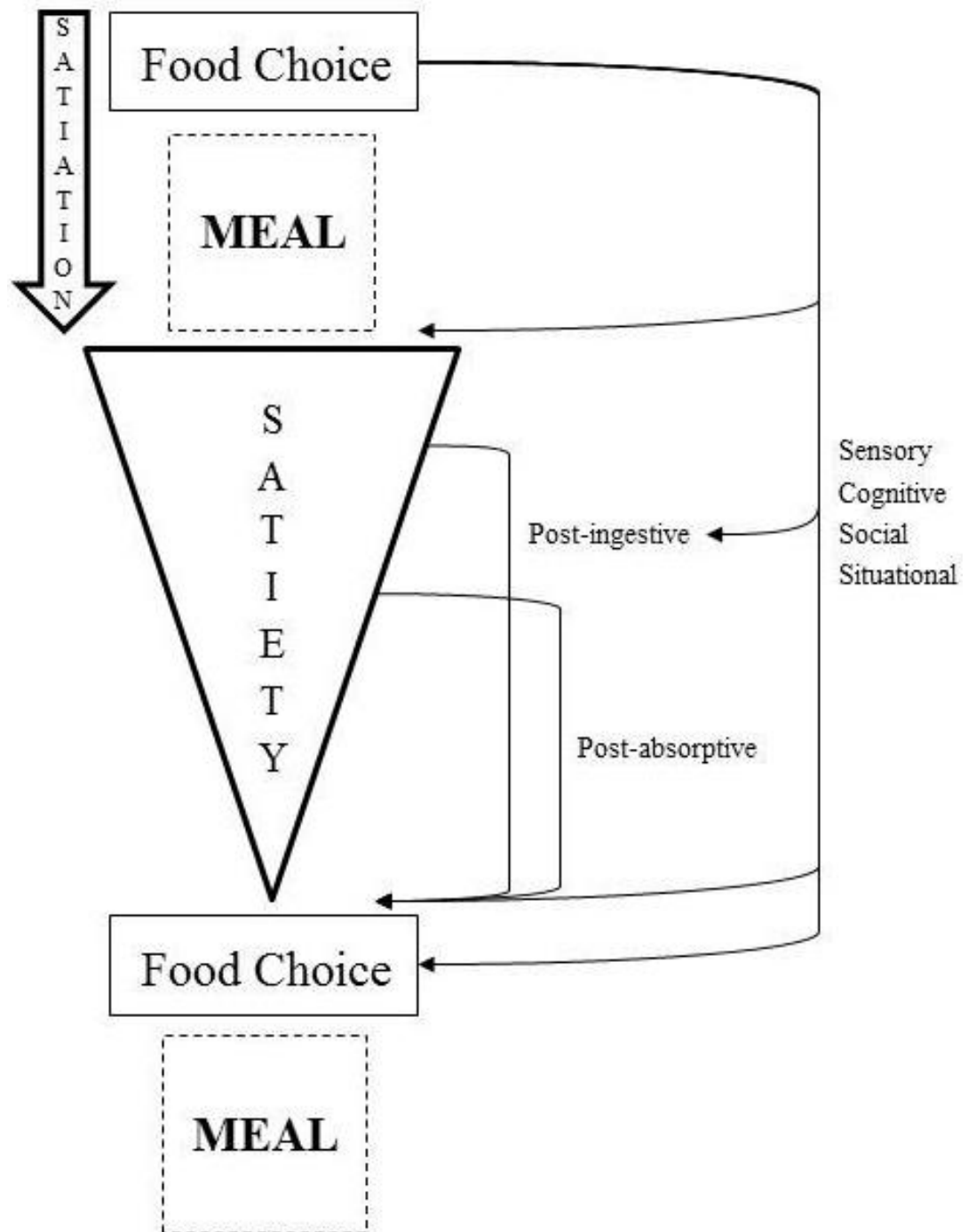
Figure 1. The Satiety Cascade (adapted from Blundell et al., 1987)

Developing a good understanding of the interplay between these biological and psychological processes is essential for understanding both satiation and satiety. For instance, familiarity with a food generates learned associations which trigger expectations about the likely palatability and satiating potential of that food which in turn may shape the quantity consumed. This then influences satiation through stomach distension, detected by nutrient receptors in the gut wall which also process energy load, further impacting on the experience of satiation. Additional post-ingestive signals activated by the initiation of digestion then generate hormone release which influences the rate of gastric emptying and, post-absorptively, circulating insulin levels as well as satiety relevant hormones (such as cholecystokinin; CCK, glucagon-like peptide 1; GLP-1 and peptide YY; PYY), in turn affecting satiety (Blundell, et al., 2010; Blundell, et al., 2001; Halford, Boyland, Blundell, Kirkham, & Harrold, 2010; Halford & Harrold, 2012; Moran, 2000). Understanding the interplay between these physiological and psychological factors further inform our knowledge of how basic tastes such as umami may influence satiation and satiety separately and how the relevant processes mentioned may interact with umami compounds to bring about a qualitatively different experience of satiation and satiety.

1.2.4 The role of flavour hedonics in appetite and intake

The perception of flavour depends on a multisensory process of inputs from olfactory, gustatory, and trigeminal systems and is influenced by food properties such as texture, temperature, fat and composition alongside others (Auvray & Spence, 2008; Loliger, 2000). All of these sensory inputs combine during an eating occasion to allow for learned associations between a flavour and its post-ingestive consequences (Gibson & Brunstrom, 2007) which can occur while eating to further monitor present intake (as has been found in rats; (Myers, Taddeo, & Richards, 2013; Zukerman, Ackroff, & Sclafani, 2011) and identify valuable nutrition sources (as is further discussed in section 1.4).

Flavour hedonics play a vital role in the learned associations made between relevant flavours and their biological consequences (see Yeomans, 2000) and can encourage conditioning of flavour preferences when the positive post-ingestive consequences of ingestion are associated with a flavour (flavour consequence learning) or the positive experience of one flavour is generalized to another flavour (flavour-flavour learning).

This model predicts that an appetitive state such as hunger would generate a preference for a flavour associated with a high energy source, with stronger hunger increasing sensitivity to the calories present in a food (Gibson & Wardle, 2001). In this way hunger can be said to influence palatability evaluations (Cabanac, 1971; Yeomans & Mobini, 2006) and increased palatability has been related to larger meal size in experimental sessions (Robinson, Gray, Yeomans, & French, 2005; Yeomans, 1996; Yeomans, Blundell, & Leshem, 2004; Yeomans & Gray, 1997; Yeomans, Weinberg, & James, 2005) and in natural environments using food diaries (de Castro, Bellisle, & Dalix, 2000; de Castro, Bellisle, Dalix, & Pearcey, 2000). Meal pleasantness has also been shown to affect rated appetite and in turn accurately determine meal size early during consumption. This 'appetizer effect' (Yeomans, 1996) states that rated hunger after tasting an optimally palatable meal will increase compared to hunger ratings made before tasting the meal and this increase can determine the amount consumed. For instance, participants provided with a well-seasoned main course not only preferred the flavour of this meal in comparison to a bland or over-seasoned condition, but also ate more and at a faster rate of the optimally seasoned course (Yeomans, 1996). Similarly, intake of a meal deemed palatable after tasting has been found to increase irrespective of previously received energy. Participants provided with high or low energy soup preloads and a main course meal either high or low in palatability 30 minutes after soup ingestion consumed significantly more of the palatable meal irrespective of the energy content of the soup previously received (Yeomans, Lee, Gray, & French, 2001). Likewise, reducing need state has not been shown to reduce palatability (Raynor & Epstein, 2000).

But how do such findings fit in with traditional approaches to appetite control? Originally, physiological cues were said to stimulate feeding due to energy and nutrient shortage (causing hunger) whilst gastric cues were claimed to end the eating episode when sufficient energy had been received (encouraging satiation). However, this approach does not account for the established effects of flavour pleasantness on intake. Consequently, it has been suggested that appetite and intake rely on the sensory information received as well as the cues arising from ingestion (Yeomans & Gray, 1997). Integrating these ideas may explain the situations in which sensory information may override the homeostatic regulation of appetite and intake. For instance, when consuming a palatable meal, the rewarding sensation (positive feedback) of palatability

may alter perceptions of hunger and drive further intake due to a desire to experience more reward at the expense of physiological need (Berridge, 2004; Berthoud & Morrison, 2008). Indeed, participants given a dose of naltrexone, an opioid antagonist implicated in orosensory reward (Bertino, Beauchamp, & Engelman, 1991; Fantino, Hosotte, & Apfelbaum, 1986), showed reduced liking, hunger, eating rate and intake of a pasta meal compared to baseline and placebo condition intake (Yeomans & Gray, 1997). This opioid effect has not been related to a change in perception with participants still rating saltiness or sweetness as the same across naltrexone and placebo conditions (Drewnowski, Krahn, Demitrack, Nairn, & Gosnell, 1992; Ścińska et al., 2000) suggesting that the reduction in intake was largely reward-driven (Yeomans & Gray, 2002).

However, palatability effects on appetite and intake have also generated mixed results with some research indicating an increase in consumption but no difference in rated hunger after tasting a palatable meal (Yeomans & Symes, 1999), others observing lower hunger ratings after fixed consumption of a palatable preload and no difference in subsequent test meal intake (De Graaf, De Jong, & Lambers, 1999; Warwick, Hall, Pappas, & Schiffman, 1993) and others still reporting no differences in appetite or intake immediately after a palatable meal but more rapid hunger recovery and desire to eat after the preload (Hill, Magson, & Blundell, 1984; Monneuse, Bellisle, & Louis-Sylvestre, 1991; Rogers & Blundell, 1990). Indeed, some evidence seems to suggest that palatability only influences energy intake with meals served as conventional courses (starter, main and dessert) as opposed to serving sweet and savoury sandwiches or semi-liquid meals (Guy-Grand, Lehnert, Doassans, & Bellisle, 1994). The inconsistencies found between studies may also relate to the palatability of the test meals used as some of the 'bland' control meals tested were rated as aversive (De Graaf, et al., 1999; Hill, et al., 1984) whilst other study designs using palatable preloads varied greatly in timings between the preload and test meal (De Graaf, et al., 1999; Hill, et al., 1984; Monneuse, et al., 1991; Rogers & Blundell, 1990; Warwick, et al., 1993) suggesting that palatability and hunger effects may be time dependent. Warwick and colleagues (1993) found no difference in energy intake in a test meal served 5 hours after a palatable test meal despite a greater decline in initial hunger ratings immediately after fixed palatable preload consumption. de Graaf and colleagues (1999) also observed no influence of preload palatability in a test meal delivered 2 hours after preload intake.

Likewise, highly preferred equicaloric meals enhanced hunger ratings during the intake of the meal but did not influence appetite after consumption (Sorensen, Moller, Flint, Martens, & Raben, 2003). This suggests that palatability effects are most pertinent to ensure food acceptability whilst information regarding the type of flavour experienced further informs physiological post-oral processes.

1.3 The fifth basic taste

1.3.1 Umami - the fifth basic taste

Monosodium glutamate (MSG) is a sodium salt of glutamic acid which was initially discovered by Kikunae Ikeda in 1908 as a unique taste component in dried kelp but is now made commercially from the fermentation of sugar sources (Sano, 2009). The flavour of MSG was coined 'umami' which translates to 'savoury deliciousness' (Chandrashekar, Hoon, Ryba, & Zuker, 2006; Kurihara, 2009). The umami taste is elicited by glutamate ions whilst the glutamate enantiomer contains flavour enhancing properties, boosting the palatability of foods (Rundlett & Armstrong, 1994). MSG mimics the non-essential amino acid glutamic acid (elicited by glutamate salts which are also termed 'glutamate'), which has been found to be one of the most plentiful naturally occurring amino acids found in foods of both plant and animal origin (Garcia-Bailo, et al., 2009; Jinap & Hajeb, 2010; Yamaguchi & Ninomiya, 2000). Higher concentrations of free glutamate have been related to the increased ripeness and flavour of natural foods (Ninomiya, 1998) as well as man-made foods such as contributing to the taste and texture of cheese (Ramos, Cáceres, Polo, Alonso, & Juarez, 1987) and cured meats (Cordoba et al., 1994). Bound glutamate on the other hand is bound with other peptides and proteins in foods and does not give rise to the flavour of umami unless it is converted to free glutamate which can occur by breaking down peptide bonds during chewing through the action of proteases. As glutamate is a non-essential amino acid, it is naturally produced by the body and aids metabolism (Appaiah, 2009; Bellisle, 1999; Fürst & Stehle, 2004), particularly acting as a major source of energy for the intestines (Young & Ajami, 2000). As MSG contains glutamate and provides a flavouring function similar to naturally occurring free glutamate (Yamaguchi & Ninomiya, 2000), it is a potent flavour enhancer typically added to commercial produce in similar levels to naturally occurring glutamate found in traditional dishes (Bellisle, 1999; Luscombe-

Marsh, et al., 2009). Indeed, the body cannot distinguish between glutamate ingested naturally in foods and glutamate added to foods (Appaiah, 2009).

The flavour of MSG is not very palatable (Beauchamp & Pearson, 1991; Rolls, 2000) and has been described as "sweet-saline" (Foster, 1955) when tasted alone. However, when added to foods MSG elicits a "savoury" or "brothy" flavour (Chandrashekar, et al., 2006; Jinap & Hajeb, 2010; Loliger, 2000). The distinctive taste quality of umami led to its establishment as the fifth basic taste. Umami is coded by the taste system as completely unique (Nelson et al., 2002; Rolls, 2000) from the four basic tastes (salty, sweet, sour and bitter) despite sharing taste receptors that code sweet and salty flavours (see section 1.3.2). Indeed, the addition of umami does not enhance the taste of sweetness, saltiness, sourness or bitterness (Yamaguchi, 1987) and the threshold for detecting the experience of these tastes on the tongue is not lower with added MSG as might be expected with an enhancement of flavour (Yamaguchi, 1967). The uniqueness of umami taste is clear in the human taste system. Participants provided with 21 taste stimuli varying in the four basic tastes and umami were asked to sort the stimuli based on taste quality and similarity. This was used to produce a similarity matrix using multidimensional modelling which gave rise to a 'taste tetrahedron' in which the established four basic tastes were located. However, MSG was found to be located outside of this tetrahedron, suggesting that its flavour was indeed perceived as qualitatively different from the other four basic tastes (Yamaguchi & Kimizuka, 1979). Umami detection is also present cross culturally (Prescott, 1998) and has been found in both naïve participants and panellists trained to identify the umami taste (Sinesio, Peparaio, Moneta, & Comendador, 2010).

1.3.2 The umami taste system

Since the discovery of umami, considerable research has explored the taste system underpinning this fifth basic taste. Information about bitter, salty, sweet and sour tastes are relayed to the primary and secondary cortex via receptor membranes in taste buds inducing impulses in the nerves related to gustatory sensitivity (Bellisle, 1999). The brain areas stimulated by these basic tastes to generate a response are independent of one another, establishing their status as basic tastes. The question remained as to

whether umami also operated a distinct taste receptor mechanism, consequently validating its status as a basic taste.

Glutamate has been shown to independently activate G-protein coupled receptors in specialized glutamate taste receptor cells (Chaudhari, Landin, & Roper, 2000; Chaudhari, Pereira, & Roper, 2009; Chaudhari et al., 1996; Gabriel, Maekawa, Uneyama, & Torii, 2009). These receptors have been found both in the posterior region of the tongue in metabotropic glutamate taste bud receptors (mGluRs) (Yasumatsu et al., 2009) and in the anterior region in T1R1/T1R3 taste receptors. T1R class receptors consist of specialized class C G-protein coupled taste receptor cells that relay sensations of umami and sweet taste. Deciphering which taste is being experienced relies on these taste receptors (T1R1, T1R2 and T1R3) acting together to communicate this information. Indeed T1R1/T1R3 have been established as umami-specific receptors in humans (Egan & Margolskee, 2008; Li et al., 2002; Yasumatsu, et al., 2009) whilst T1R2/T1R3 are related to 'sweet' taste sensing (Stone, Barrows, Finger, & Kinnamon, 2007). This understanding developed when assessing the expression patterns of taste molecules.

These taste receptors also map directly onto the genes that express them, Tas1r1 and Tas1r3 (Damak et al., 2003; Zhao et al., 2003). Indeed, glutamate taste receptors have been found to bear a pharmacological and structural resemblance to brain glutamate receptors (Brand, 2000). The presence of specific receptor sites for umami detection allow for a greater understanding of the experience of umami, the ability to discriminate umami stimuli and how sensitive the umami taste system is. Indeed, when T1R1 or T1R3 receptors are not expressed (in mouse genetic knock out models removing the Tas1r1 and/or Tas1r3 gene), umami taste preference is greatly reduced in animals (Damak et al., 2003; Zhao et al., 2003) and as human T1R1 and T1R3 are more selective for umami stimuli (Li, et al., 2002), this effect would be expected to present even more strongly in humans (Li, 2009). Identifying a taste also relies on other G-proteins working in combination with the T1R taste receptors. α-gustducin has been suggested to be fundamental in T1R transduction pathways, with mice lacking this protein showing defects in the ability to taste sweet and umami stimuli (Glendinning et al., 2005; Ruiz, Wray, Delay, Margolskee, & Kinnamon, 2003). Indeed, T1R2/T1R3 pathways have been shown to express α-gustducin but T1R1 receptors only express this

protein half of the time (Stone, et al., 2007). This may explain why α-gustducin has been implicated in the detection of MSG but not IMP (He et al., 2004; see section 1.3.3 for the synergism between MSG and IMP). Developing an understanding of the complex connections between the receptors and proteins implicated in umami taste also allows for a greater appreciation of the associations between umami and sweet taste as these two flavours are considerably linked and are clearly separable from other tastes such as bitter (which is expressed by T2R class receptors) or salty (mediated by epithelial sodium channel ENaC; Chandrashekar et al., 2010).

The evidence for pathways and proteins dedicated to umami taste discrimination further supports the role of the function of this basic taste in facilitating adequate nutrition (Yamaguchi & Ninomiya, 2000) despite the status of glutamate as a non-essential amino acid (Bellisle, 1999; Fürst & Stehle, 2004). This indicates that, just as saltiness informs of mineral content (Krause & Sakai, 2007; Richter, 1936) and sweetness indicates an energy source (Anderson, 1995; Sclafani, 1987), the evolution of the detection of umami may signal the availability of another food source such as savoury foods. In particular, the taste of glutamate has been related to savoury foods rich in protein due to the abundance of umami in these foods (Ikeda, 1908; Laska & Hernandez Salazar, 2004; Luscombe-Marsh, et al., 2008; Mori, Kawada, Ono, & Torii, 1991; Smriga & Torii, 2000).

1.3.3 Glutamate synergism with other flavour enhancers

The flavour enhancers that produce the experience of glutamate also include disodium inosinate and disodium guanylate which are derived from the purine nucleotides inosine 5'-monophosphate (IMP) and guanosine 5'-monophosphate (GMP). These nucleotides mimic naturally occurring umami found in animal products for IMP (Lindemann, 1996; Yamaguchi & Ninomiya, 2000) and plant-based foods for GMP (Ninomiya, 1998) and enhance the taste of umami when combined appropriately with MSG (Kurihara, 2009). Indeed, as natural and processed foods have distinct umami profiles, subtle differences in umami taste can be generated (Fuke & Konosu, 1991) and flavour can be improved when combined with appropriate concentrations of MSG, IMP and/or GMP. Hence MSG alone may improve sensory assessments more effectively in certain foods whilst additional IMP or GMP may enhance pleasantness more in others (Baryłko-Pikielna &

Kostyra, 2007). This is evident in the cooking practices seen cross culturally throughout the world, with the enhancement of meals with umami stocks made up of animal and plant based proteins to further improve flavour (Kurihara, 2009).

When MSG is combined with one or both nucleotides (IMP and/or GMP), a sensory synergism occurs enhancing the subjective experience of umami taste intensity more so than either component presented alone (de Araujo, Kringelbach, Rolls, & Hobden, 2003; Lindemann, 1996; Ninomiya, Tanimukai, Yoshida, & Funakoshi, 1991; Rifkin & Bartoshuk, 1980). This interaction effect has also been found at the receptor level, with glutamate-nucleotide synergisms apparent in T1R1/T1R3 receptors (Kurihara, 2009; Zhang et al., 2008; Zhao et al., 2003). Indeed, T1R3 damage has been shown to abolish the synergistic response to MSG and IMP (Damak, et al., 2003) and T1R1 has been proposed to be vital for GMP and IMP detection (Zhang, et al., 2008) providing a physiological basis for this synergism. The activity of the chorda tympani, which is vital for relaying taste information to the brain, has also been shown to be particularly sensitive to MSG-nucleotide synergisms (Kumazawa, Nakamura, & Kurihara, 1991).

Further support for a synergistic effect was found in the orbitofrontal cortex (OFC) which was more strongly activated by the additive influence of IMP to MSG than when either solution was provided in isolation (de Araujo, et al., 2003). This suggests that the OFC may combine the information from the channels detecting MSG/IMP to produce a larger response to MSG/IMP when in combination (de Araujo, et al., 2003). Indeed, it may be that IMP and GMP do not have an intrinsic umami taste and only act to enhance an effect by glutamate (Beauchamp, 2009). These nucleotides show very weak taste intensities when presented alone which may occur due to the small amounts of glutamate naturally present in saliva, thus they may instead enhance an intrinsic umami taste but instead enhance it (Yamaguchi & Kobori, 1991). Indeed, doses of IMP which do not elicit the umami taste themselves have been shown to increase sensitivity to glutamate detection (Li, et al., 2002). Such synergism has not been found with other tastes such as the addition of sweet (Zhang, et al., 2008), indicating that this is an umami-specific effect.

This synergism has also been related to further enhancing the pleasantness generated by umami as the OFC also provides information about the rewarding aspects of taste

(O'Doherty, Rolls, Francis, Bowtell, & McGlone, 2001); thus the stronger OFC activation may be indicative of a further improvement in flavour with umami synergism than when umami tastants were provided alone. However, this enhancement of pleasantness by synergism may depend on the balance between MSG and IMP, with higher concentrations of MSG to IMP reducing pleasantness assessments (Baryłko-Pikielna & Kostyra, 2007). Likewise, an enhancement of MSG/IMP was not found to increase intake in rats when compared to MSG alone conditions (Ackroff & Sclafani, 2011). This may indicate similar pleasantness across conditions or indeed may indicate that the effects of IMP occur primarily in the mouth to improve glutamate detection and thus the selection of glutamate containing foods.

1.3.4 MSG and food palatability

Foods rich in naturally occurring glutamate are rated as high in palatability (Yamaguchi & Ninomiya, 2000) and this positive affective reaction is evident from birth (Beauchamp & Mennella, 2009; Steiner, 1973). Thus, it is unsurprising to note that as MSG is primarily used for its flavour enhancing properties, the addition of this seasoning to meals can improve flavour pleasantness in a concentration-specific manner (Halpern, 2000; Prescott, 2004; Rogers & Blundell, 1990) and activates the OFC (McCabe & Rolls, 2007), but too high a dose is deemed aversive (Halpern, 2000). MSG also improves flavour pleasantness when combined with salty flavours, further enhancing palatability assessments than sodium chloride alone (Halpern, 2000). Likewise foods with added MSG are rated as more palatable, even when sodium is equivalent (Okiyama & Beauchamp, 1998) or when no sodium is present (as is found with calcium glutamate; Bellisle, Dartois, & Broyer, 1992). However MSG does not improve the flavour of sweetened foods (Yamaguchi, 1998) possibly because glutamate does not occur naturally in most sweet foods.

MSG may also be used to increase palatability for novel, congruent food flavours with repeated pairings (Prescott, 2004; Roininen, Lähteenmäki, & Tuorilla, 1996) as has been documented with highly palatable flavours such as sweet (Brunstrom & Fletcher, 2008; Mobini, Chambers, & Yeomans, 2007) and macronutrients such as fat (Johnson, McPhee, & Birch, 1991; Kern, McPhee, Fisher, Johnson, & Birch, 1993). Indeed, conditioned preferences for new food flavours have been shown with repeated pairings

of the food when ingested with MSG (Prescott, 2004; Yeomans, Gould, Mobini, & Prescott, 2008). This suggests that MSG may increase liking of initially disliked foods with high nutritional value by adding needed appeal and palatability (Loliger, 2000). Indeed, food selection preferences have also been found to be affected by the addition of MSG, increasing intake of MSG-containing products over foods containing no MSG (Bellisle et al., 1991). However, this selection may also be mediated by top-down processes alongside the sensory experience of MSG. Broths containing added MSG labelled as 'rich and delicious taste' are rated as significantly more pleasant and increase activation in the medial OFC more strongly than when described as 'boiled vegetable water' or 'MSG' (Grabenhorst, Rolls, & Bilderbeck, 2008). This indicates that cognitive expectations can override sensory experiences but it must be noted that such examinations may be heavily influenced by demand characteristics.

Hunger assessments are also influenced by MSG as a function of palatability. Yeomans and colleagues (2008) provided participants with a novel savoury soup (control) which was then paired with (or without) added MSG over four exposure sessions before providing participants with the control soup (without added MSG) once again. The addition of MSG in the training stage significantly increased rated hunger after tasting the control soup post-training compared to the no MSG trials as well as increasing liking and intake, indicating appetite stimulation by flavour. Similarly, MSG has been related to more rapid hunger recovery, possibly due to its appetizing effects (Rogers & Blundell, 1990). However, no research to date has specifically assessed the nature of MSG enhanced meals in relation to palatability, rated appetite and intake.

The first series of papers within this thesis are predominantly focussed on the experience of umami and its effect on stimulating appetite due to its palatability whereas the later papers discussed are more focussed on the effects of umami as a signal for protein and in the promotion of satiety. Consequently these experiments are summarized in turn, following on from the literature that gave rise to them. It was initially essential to establish a vehicle which was not only of neutral palatability but that could be consumed as either a preload or a main meal and to which the addition of MSG would improve flavour (Paper 1). This flavour enhancing effect was subsequently assessed for its effects on satiation and appetite stimulation (Paper 2), with an additional

experiment further testing the methodology used with an additional basic taste (Paper 3).

1.3.4.1 Summary of Paper 1

As the flavour of umami is so abundant in a range of foods, it was essential to limit the naturally occurring free glutamate present in the vehicle to be used for testing the effects of MSG. This was to ensure that any effect of MSG was not masked by a potential interaction between large quantities of available free glutamate and MSG. Indeed, too high a dose of free glutamate has been shown to be unpalatable (Jinap & Hajeb, 2010; Yamaguchi & Takahashi, 1984). Thus if baseline levels of glutamate are naturally high, any additional effect by MSG may taste unpleasant, affecting appetite and intake and not reflecting the use of MSG commercially (Loliger, 2000). Previous research assessing the influence of MSG on appetite and intake does not take natural free glutamate levels into account, potentially confounding the effects reported as was found by Shi et al (2010) in the study published by He and colleagues (He, et al., 2008).

Based on the relevant literature it was deemed essential to formulate a hedonically neutral control soup to which the addition of MSG would enhance palatability. Formulating a low glutamate soup would also allow for any effect by MSG to be attributed to MSG and not the potential interaction between natural glutamate present in the vehicle and MSG. Paper 1 is divided into two experiments. Both experiments utilized the same methodology, with participants rating the sensory and hedonic characteristics of samples of two different vegetable soups with no MSG, 0.6% added MSG or 0.8% added MSG. This resulted in a six sample tasting which was carried out twice in the same session. Experiment 1 assessed a pumpkin or butternut squash soup however these soups were rated as highly unpalatable with no significant increase in palatability evident with the addition of MSG. Thus Experiment 2 examined a carrot and spice soup with or without added cream. Both carrot soups were rated as hedonically neutral with the addition of MSG improving flavour palatability. However as the carrot soup without cream was lower in naturally occurring glutamate, it was preferred for further assessment. However, as the concentrations of MSG in the soups yielded more inconsistent results in terms of ratings of savouriness and strength of flavour, the concentrations of MSG to be used were further assessed in Paper 2.

1.3.4.2 Summary of Paper 2

As mentioned in section1.3.4, the appetizing nature of MSG has not previously been assessed. It was deemed important to examine the influence of MSG on indices of hunger and fullness during intake of the early stages of a meal to further understand whether the experience of umami was the same or qualitatively different from other appetizing seasonings (see Yeomans et al., 2000) or from that found previously with MSG conditioned flavour preferences (Yeomans, et al., 2008). As Yeomans and colleagues' (2008) study did not explicitly assess the impact of an MSG containing meal on rated appetite and intake, the aim of the experiment in Paper 2 was to investigate the palatable nature of MSG on the appetizer effect. This involved utilizing a similar study design to that used in the original appetizer experiment adopted by Yeomans (1996) and consisted of participants rating their appetite after tasting and at 25g intervals for 75g consumption and at the 200g re-fill stage during *ad-libitum* consumption of a soup varying in added MSG concentrations.

Paper 2 is divided into two experiments with the first establishing the concentrations to be used throughout testing. This was deemed vital as a clear enhancement of palatability was required to assess the role of meal hedonics on rated hunger and intake. The first experiment was also necessary to ensure the computer rating system used would remain sensitive with a semi-liquid vehicle as previous assessments have used predominantly solid meals (Yeomans, 1996; Yeomans, Gray, & Conyers, 1998). Rated meal pleasantness was found to increase upon tasting the MSG enhanced conditions relative to the control in a concentration-specific manner however, due to the speed of consumption influencing the sensitivity of the balance system used, further within-meal analyses were not possible in Experiment 1. The second experiment in Paper 2 adopted an additional training session with a separate, well accepted soup which improved analysis of within-meal ratings. However, no appetizing effect was evident across MSG conditions and no differences were noted in meal intake in Experiment 2. This may be due to the similar palatability ratings found across conditions. Nevertheless, rated hunger and eating rate was lower in added MSG conditions after 200g intake as compared to no added MSG conditions. Experiment 2 also revealed that the added MSG conditions were rated as more satiating than no added MSG soups, despite the similar

quantities consumed across conditions, indicating the potential for stronger satiety with added MSG conditions.

1.3.4.3 Implications of Paper 2 and summary of Paper 3

No clear appetizer effect was evident in Paper 2 and a high participant exclusion rate indicated that the methodology employed required revisions. Thus in Paper 3 the appetizer effect was assessed with a sweet taste, to determine whether the hedonic qualities of sweetness would influence appetite and increase intake relative to a bland control soup. Sweetness was examined due to its profile as inherently palatable (Sclafani, 1987), its presence in many energy dense foods (Bellisle & Drewnowski, 2007; Yamamoto & Ishimaru, 2013), and as it has not been assessed before in relation to the appetizer effect whereas other basic tastes such as saltiness have (Yeomans, 2000). Similarly, sweet taste shares a taste receptor with umami taste (the T1R3 receptor; Li, 2009; see section 1.3.2).

In addition to employing the same methodology as Paper 2, Paper 3 only differed in the inclusion of a training session using non-edible food items where Paper 2 utilized a different well-accepted soup. This was due to efforts to reduce the potential for a negative contrast effect (Flaherty, 1982; Flaherty, Coppotelli, Grigson, Mitchell, & Flaherty, 1995) as the lack of a perceived appetizer effect in Paper 2 may have been due to participants preferring the more familiar training soup to the relatively novel test soup provided. Additional ratings of motivation to consume the test soup were also included in Paper 3 to understand whether the influence of soup pleasantness during intake was more related to the liking or wanting indices of meal intake as previous research suggests a decline in liking results in a reduction of intake (Yeomans & Gray, 1997) but the willingness to work for sucrose rewards (which may be indicative of behaviours related to wanting an item) has been found to be a strong motivator for intake in rats (Uematsu et al., 2011).

No clear appetizer effect was evident upon tasting the sweetened soups as compared to the bland control and no differences were noted in appetite, pleasantness or motivation to consume the sucrose sweetened soup relative to the bland control. Similarly there were no differences in intake between conditions but eating rate was found to be slower

in the sweet condition relative to the control. The similar pleasantness ratings found suggested that the hedonic influence of the tasted food could not be meaningfully compared. However, the outcome of Paper 3 shed further light on the unexpected results of Paper 2, firstly due to the known effects of palatable substances on evaluations of hunger and consumption (Yeomans, 2000) and secondly due to the maintained palatability but marginally increased experience of satiation in added MSG conditions relative to control which was not evident in Paper 3. Despite similar intake across conditions across Papers 2 and 3, participants were only more satiated in the added MSG conditions relative to control. This was not seen in Paper 3 and indicated the potential for a bi-phasic effect of MSG on appetite and intake, maintaining palatability but potentially acting to enhance the experience of satiety. However, this finding could not be substantiated unless further ratings of appetite were assessed over the duration after meal consumption.

1.3.5 MSG and intake

Upon completing Papers 2 and 3 the question of how the effects of MSG influence hunger and intake remained unclear. However, although very subtle, the results were surprisingly more in line with those found in the literature. Although many studies show an initial enhancement of intake (Bellisle et al., 1996; Bellisle, et al., 1991; Bellisle, Tournier, & Louis-Sylvestre, 1989; Mathey, Siebelink, de Graaf, & Van Staveren, 2001; Schiffman, Sattely-Miller, Zimmerman, Graham, & Erickson, 1994), few have found maintenance of intake in MSG-added conditions as compared to control (Mathey, et al., 2001; Schiffman, et al., 1994), with intake more often returning to baseline levels (Bellisle, et al., 1996; Bellisle, et al., 1991; Bellisle, et al., 1989; Essed, van Staveren, Kok, & de Graaf, 2007). Mathey and colleagues (2001) and Essed and colleagues (2007) conducted 16 week interventions in nursing home elderly who were given meals with added or no added MSG sprinkled over the cooked component of the course. Intake over time was assessed as well as measures of weight at 8 (Mathey, et al., 2001) and 16 (Mathey et al., 2001; Essed et al., 2007) weeks. Mathey et al., reported an increase in intake and weight in the added MSG group compared to the control condition, which were found to lose weight during the period. Conversely, Essed et al. (2007) reported a reduction in energy intake in the MSG added group and no change in body weight in this condition compared to baseline. This was despite the low

concentrations of MSG (0.3% MSG) added to the meals. Why was there such a difference in energy intake and weight gain between these nursing home elderly? One suggestion may be related to the foods to which the MSG was added. Essed et al. (2007), sprinkled the MSG only onto the high protein components of the meal whilst Mathey and colleagues (2001) seasoned the entire meal, including the high carbohydrate foods with the flavour enhancer. Likewise, Bellisle and colleagues (Bellisle, 1999; Bellisle, et al., 1996; Bellisle, et al., 1991; Bellisle, et al., 1989) enhanced the low energy carbohydrate components of meals with added MSG, showing increased intake of these foods relative to the high protein foods with no added MSG in healthy (Bellisle, et al., 1989), elderly (Bellisle, et al., 1991) and diabetic elderly (Bellisle, et al., 1996) populations. When Bellisle and colleagues (see Bellisle et al., 1999) further assessed the intake of diabetic elderly individuals over a four week period, they reported increased intake of the starch components of meals with added MSG as compared to control. Thus it may be that the carbohydrate components of a meal enhance intake with added MSG as has been suggested elsewhere (Simpson & Raubenheimer, 2005).

However, despite participants increasing their consumption of the more palatable starch-based elements, Bellisle and colleagues repeatedly found no increases in calorie load or overall energy intake over time (Bellisle, 1999; Bellisle, et al., 1996; Bellisle, et al., 1991; Bellisle, et al., 1989). Support for these findings has also been reported by Rogers & Blundell (1990) who found that adding MSG to beef consommé soups increased ratings of pleasantness and hunger recovery compared to control but did not influence energy intake at a later course delivered 2 or 30mins after the soup preload. Equally, Carter and colleagues (Carter, Monsivais, Perrigue, & Drewnowski, 2011) provided participants with chicken broth preloads with added MSG, added MSG and nucleotides (IMP and GMP) or with added fat which were delivered in two exposures two hours apart then provided an *ad-libitum* lunch 35 minutes after the second preload exposure. They reported no differences in consumption between added MSG and control conditions with only the addition of fat decreasing *ad-libitum* intake. Although, unlike Rogers & Blundell (1990) they also found that added MSG reduced rated hunger and desire to snack before the meal, indicating a stronger experience of satiety in MSG conditions. These findings suggest that the increased palatability found with added MSG does not necessarily maintain heightened energy intake (Bellisle, 1999; Bellisle, et al., 1996; Bellisle, et al., 1991; Bellisle, et al., 1989; Essed, et al., 2007) or influence

subsequent energy intake (Carter, et al., 2011; Rogers & Blundell, 1990). Indeed, Bellisle (1999) suggests that the short-term enhancement of intake in meals with added MSG may be in response to the sudden increase in dietary palatability, with intake returning to baseline after a few days due to regulatory mechanisms intervening. Other important aspects such as the macronutrient compositions of the foods consumed may also be taken into account during energy regulation, consequently contributing to the experience of satiety.

The effects of high palatability meals enhanced with MSG on intake do not match those found with high palatability energy dense (ED) foods. Indeed, long-term MSG intake has been found to have a negative or no effect on weight gain in human and animal studies (Essed, et al., 2007; Kondoh & Torii, 2008; Shi, et al., 2010) whereas this is not the case for ED foods, with intake associated with high weight status (Ledikwe et al., 2006; Zizza, Siega-Riz, & Popkin, 2001) and body fat content (McCrory, Fuss, Saltzman, & Roberts, 2000). One suggestion for this may be related to 'liking' and 'wanting' of MSG enhanced foods as opposed to other basic tastes (such as sweetness which may predict palatability and energy). Hedonic 'liking' may be related to palatability assessments whilst 'wanting' judgements may be based on approach responses and the willingness to work for these rewards (Berridge, 1996) as may be demonstrated when MSG enhanced foods are selected over those foods containing no MSG (Bellisle, et al., 1996). These constructs are not only said to be separable but are postulated to rely on separate neural networks with 'liking' mediated by the opioid system in the rostro-dorsal medial shell region of the nucleus accumbens and the ventral pallidum (Smith & Berridge, 2007) whilst 'wanting' is associated with dopamine interactions with corticolimbic glutamate (Kelley, 2004) as found with sucrose. However, it may be that MSG is accessing different neural network systems to elicit varied behavioural responses. Some research has assessed this by providing rats with a range of concentrations of MSG (0-120mM) and sucrose (0-480mM) with fixed progressive ratio operant tasks to assess 'wanting' and gusto-facial responses and naloxone treatment to assess 'liking'. Positive hedonic reactions to MSG concentrations (30-120mM) were observed as was found for sucrose (10-480mM), with naloxone treatment decreasing these positive reactions for both MSG and sucrose, implicating the opioid system in MSG and sucrose taste processing (Uematsu, et al., 2011). The animals were also willing to work for lower concentrations of MSG (up to 60mM) but this

'wanting' (also termed incentive salience) of the MSG reward was further reduced with naloxone treatment. This was not seen with the sucrose concentrations, with incentive salience remaining high despite naloxone treatment. As MSG does not trigger dopamine release in oral pathways (Uematsu, Tsurugizawa, Kondoh, & Torii, 2009) and incentive salience was influenced by naloxone treatment, umami 'wanting' may be mediated by the opioid system instead. Thus MSG may influence approach behaviours differently to sugar sweetened foods, which have also been related to higher energy intake (Harnack, Stang, & Story, 1999). However, these effects have not been assessed in humans and thus selection and intake may be mediated by a variety of factors.

It may be that the incentive salience experienced with sucrose is inherently related not to palatability but to energy density. As sucrose is experienced largely in high ED foods and beverages and itself is a signal of energy (Bellisle & Drewnowski, 2007; Sclafani, 1987; Torii, Mimura, & Yugari, 1987; Yamamoto & Ishimaru, 2013) whilst MSG may be consumed in both low ED (such as umami broths, vegetable preparations and lean protein) and high ED foods, the learned association between sucrose and energy density, not palatability, may be driving and maintaining intake (Brunstrom & Rogers, 2009). Indeed, high ED foods are said to have a higher incentive salience as the capacity of these foods to satisfy hunger is greater per portion than low ED foods (Brunstrom & Shakeshaft, 2009; Rogers, 2011). However, these foods are also selected in larger calorie portions irrespective of palatability than low ED foods as the same weight of a high ED food and a low ED food would provide a different number of available calories, thus high ED foods are less satiating than low ED foods on a calorie-for-calorie basis (Brunstrom & Rogers, 2009; Rogers, 2011). It is not known whether selection of a flavour that is not a reliable predictor of high ED (such as MSG) may influence incentive salience but it is clear that MSG containing foods are both preferred and selected more than no MSG-foods (Bellisle, 1999; Bellisle, et al., 1996) and that these preferences are maintained over time (Bellisle, 1999; Bellisle, et al., 1996; Bellisle, et al., 1991) irrespective of the energy density of the food. So it may be that the incentive salience experienced with MSG may be more dependent on palatability or other aspects related to MSG flavour such as information about the macronutrients available in the food.

1.4 Macronutrients in the diet

The role of ED in energy intake regulation is well documented in the literature (de Castro, 2006; Rolls, 2009; Rolls, Roe, & Meengs, 2004). However, the essential macronutrients (carbohydrate, fat and protein) that comprise the human diet have been suggested to affect the regulation of energy intake independently from ED (Blundell & Stubbs, 1999). These nutrients are vital for normal biological functioning and serve as important energy sources. Our diets are recommended to be comprised of 50% carbohydrate, 35% fat and 15% protein (FSA, 2006) to meet the energy needs of the body. However, much research points towards an excess in fat and carbohydrate intake (Krebs, 2009; Simpson & Raubenheimer, 2005). These macronutrients require differing amounts of energy to convert to the fuel required by the body using glucostatic mechanisms (Van Itallie, 1989). Fat requires the largest proportion of energy to be expended (9 Kcal/g) whilst protein and carbohydrate require less (4 Kcal/g). Thus, gram for gram protein and carbohydrate provide less energy than fat however, per unit of energy consumed, carbohydrate (Stubbs, Mazlan, & Whybrow, 2001) and protein (Bertenshaw, et al., 2008; Rogers, 1990; Rolls, Hetherington, & Burley, 1988; Yeomans & Chambers, 2011) have been shown to have a higher satiating efficiency, that is to say, a smaller load of carbohydrate or protein increases the experience of satiation more strongly than fat (Kissileff, Gruss, Thornton, & Jordan, 1984; Tremblay, Plourde, Despres, & Bouchard, 1989). This is because energy from fat is more readily stored as it causes the smallest energy cost to store but the highest to expend (Rogers, 1990). Similarly, ingested fat tends to be low in fibre and bulk, thus reducing its satiating efficiency (Rogers, 1990). Indeed, high carbohydrate preloads have been shown to reduce feelings of rated hunger (Chambers & Yeomans, 2011; Cotton, Burley, Weststrate, & Bhmdell, 1994; Eller, Ainslie, Poulin, & Reimer, 2008; Poppitt, McCormack, & Buffenstein, 1998) and food intake (Robinson, et al., 2005) more than high fat preloads. Equally, due to the elevated palatability of most high fat foods, these items have been shown to stimulate intake and appetite of a subsequent course more strongly than a high carbohydrate food (Robinson, et al., 2005). It may also be argued that the differences in intake and appetite mentioned may be related to the different sensory experience of high fat as compared to high carbohydrate foods (Rogers, 1990). Indeed, the orosensory experience of a food may be important for evaluations about how satiating the food is. For instance, high energy preloads are rated as more satiating

than low energy preloads when consumed than when infused into the stomach (Cecil, Francis, & Read, 1999). The taste of fatty acids can be detected (Abumrad, 2005; Chalé-Rush, Burgess, & Mattes, 2007; Mattes, 2009) even when other sensory information such as smell is blocked (Chalé-Rush, et al., 2007), suggesting that this taste provides a different orosensory experience to carbohydrate. Similarly, specific carbohydrate taste has been discussed in animal models (see Sclafani, 1987). This suggests that the nutrient profile of a food is detected from the first taste, indicating that it is important to match the sensory experience of the food if macronutrient intake is being assessed.

Some researchers also report no differences in appetite (de Graaf, Schreurs, & Blauw, 1993; Raben, Agerholm-Larsen, Flint, Holst, & Astrup, 2003; Rolls et al., 1991) or intake (Chambers & Yeomans, 2011; Cotton, et al., 1994; Yeomans, et al., 2001) between high fat and high carbohydrate conditions. This may be due to individual differences in responsiveness to orosensory experiences of fat and fat sensing by the body as has been suggested elsewhere (Little & Feinle-Bisset, 2011; Stewart et al., 2010) and may be related to the impact of these macronutrients in the regulation of peripheral appetite peptides (Karhunen, Juvonen, Huotari, Purhonen, & Herzig, 2008), as well as the differences in the preload time intervals and energy densities used between studies (Almiron-Roig, Chen, & Drewnowski, 2003; Almiron-Roig et al., 2013).

1.4.1 The importance of protein

Dietary protein is vital for generating signals controlling gastric motility (Tome et al., 2009) with the quality and quantity of protein consumed during a meal essential for stimulating adequate digestion (Kondoh, Mori, Ono, & Torii, 2000). General protein intake in human diets has been shown to be the most stable as a percentage of energy in the diet (Simpson & Raubenheimer, 2005) and in terms of absolute amounts consumed (Westerterp-Plantenga, 1994) over time and across populations. It has been suggested to be the most tightly regulated macronutrient with protein imbalance monitored by the body over time (Kondoh, et al., 2000).

Indeed, when faced with unbalanced diets, protein has been shown to be prioritized and regulated at a more constant level than carbohydrate and fat (Simpson, Batley, &

Raubenheimer, 2003; Simpson & Raubenheimer, 2005). Participants tested over a 6 day period were given free access to buffet meals (breakfast, lunch and dinner) with varied food items for 2 days before one group was provided with a 2-day diet limited in protein but not carbohydrate or fat whilst the second group were limited in carbohydrate and fat but not protein. Participants were then once again given free access to all of the food items for the final 2 day period. The researchers reported that participants consuming the restricted protein diet increased energy intake by carbohydrate and fat compared to baseline during protein deprivation and increased their protein intake during the final 2 day period. Conversely, those consuming high protein diets did not alter energy intake before and after the diet manipulation (Simpson, et al., 2003). This led the researchers to propose the Protein Leverage Hypothesis (Simpson & Raubenheimer, 2005) which asserts that the need to regulate protein at a constant level is the primary driver of energy intake. Thus, when faced with imbalanced diets, the absolute intake of protein is prioritized by overconsumption of carbohydrate and fat sources until protein needs are met (Austin, Ogden, & Hill, 2011; Larsen et al., 2010; Simpson, et al., 2003). Whereas the regulation of excessive carbohydrate and fat intake is not as acute as that of protein (Simpson & Raubenheimer, 2000) and may be due to our limited evolutionary experience with carbohydrate and fat sources as they were not as readily available as they are in the developed world (Simpson & Raubenheimer, 2000).

One way in which this regulation may occur is related to the sensory experience of the ingested food (as previously mentioned with fat and carbohydrate taste; see section 1.4). Foods with sensory qualities predicting protein may increase intake of these items when in a protein deficient state (Booth, 1985) and can be demonstrated in the learning of protein-specific appetite in rats (Baker, Booth, Duggan, & Gibson, 1987; Booth & Baker, 1990; Gibson & Booth, 1986; Piquard, Schaefer, & Haberey, 1978) and humans (Gibson, Wainwright, & Booth, 1995). Participants were found to learn and modestly prefer a food flavour previously paired with the delivery of protein when in mild protein deprivation (Gibson et al., 1995). This suggests that the sensory nutrient interaction between the orosensory experience of protein and its post-ingestive effects may have conditioned a flavour preference, thus allowing for more efficient protein regulation. As this effect has not been found with carbohydrate in rats (DiBattista, 1991), it may be that the orosensory experience of protein is different to that of carbohydrate. Indeed, the sensory cues of thickness and creaminess have been proposed as potential sensory

components of protein detection and when these cues are provided with high carbohydrate sources participants have been shown to adjust intake in line with the expectation of receiving protein (Bertenshaw, Lluch, & Yeomans, 2013). This suggests that the immediate orosensory experience is a key mediator of protein sensing. However such sensing is not specific to protein. Restricted access to fat sources has also been shown to generate appetite for fat in non-energy deprived rats, with rats reducing standard chow intake to account for the extra energy consumed and thus maintaining stable weight (Corwin et al., 1998) and may be related to the similar creaminess and thickness experienced with fat. Indeed, this can also be seen in humans with restricted fat intake in low fat diets resulting in enhanced fat intake over time (McGuire, Wing, Klem, Lang, & Hill, 1999) however, human participants show poorer regulation of extra energy received as fat, resulting in faster weight re-gain (Mozaffarian, Hao, Rimm, Willett, & Hu, 2011) but this has not been shown to the same extent with protein (Layman et al., 2003; Lejeune, et al., 2005).

Protein has also been implicated in reducing fat mass (Layman, et al., 2003; Parker, Noakes, Luscombe, & Clifton, 2002; Skov, Toubro, Rønn, Holm, & Astrup, 1999), and increasing thermogenesis (Halton & Hu, 2004) and the maintenance of fat free mass (Lejeune, et al., 2005; Westerterp-Plantenga, Lejeune, Nijs, Van Ooijen, & Kovacs, 2004). Thus protein may have low energy efficiency, boosting energy expenditure as has been found during overfeeding (Stock, 1999) and may as a consequence encourage weight loss. Trials assessing this hypothesis have found an increase in weight loss in participants following high protein as compared to high carbohydrate diets (Arciero et al., 2008; Halton & Hu, 2004; Weigle et al., 2005), with maintained weight loss and improved body composition shown over time as was found 10 weeks after moderate (125g protein/day) as opposed to average (65g protein/day) protein diets (Layman et al., 2003) and 6 months after self-selected high protein compared to high carbohydrate diets (Skov et al., 1999). Indeed, participants were found to have slower weight re-gain at one year follow-up after high protein diets (1kg) as compared to low protein diets (3.9kg) (Lejeune et al., 2005) and the weight regained largely consisted of fat free mass as opposed to fat mass (Westerterp-Plantenga et al., 2004; Lejeune et al., 2005).

But what are the determinants of reductions in weight with high protein diets? The increases in energy intake and weight following low protein diets cannot account for the

weight loss found with heightened protein intake. Indeed, the processing of protein has been related to a stronger down regulation of orexigenic neuropeptide-Y and melanocortin pathways (Tome, 2009), and decreased post-prandial ghrelin as well as up regulation of anorexigenic CCK, GLP-1 and PYY (Batterham et al., 2006; Beglinger, Degen, Matzinger, D'Amato, & Drewe, 2001; Blom et al., 2006; Bowen, Noakes, Trenerry, & Clifton, 2006; Degen, Matzinger, Drewe, & Beglinger, 2001; Hall, Millward, Long, & Morgan, 2003) than carbohydrate and fat. This indicates that protein may improve satiety regulation (Tome et al., 2009) and suggests that not only is protein acutely regulated but that it may have a higher satiating efficiency than carbohydrate or fat. Indeed, protein has been related to increases in satiety despite similar or low energy intake (Arciero, et al., 2008; Lejeune, Westerterp, Adam, Luscombe-Marsh, & Westerterp-Plantenga, 2006; Westerterp-Plantenga, et al., 2009). Gosby and colleagues (2011) provided participants with *ad-libitum* breakfasts, lunches, dinners and snacks with 10%, 15% or 25% energy from protein over 4 non-consecutive test days. Additional food diaries were collected on test days documenting the previous 4 day's energy intake and appetite ratings were assessed throughout test days to examine energy intake and rated appetite. They reported a 12% increase in energy intake in 10% protein conditions, and similar intake in 15% and 25% conditions. Interestingly, Gosby et al. also found that this enhanced intake in the 10% condition was due to increased snacking and hunger ratings were found to be stronger in this condition relative to the 15% and 25% conditions, especially after breakfast consumption. This implies that compensation for low protein availability encouraged earlier hunger recovery and meal initiation (such as consuming snacks) as opposed to increasing the size of a low protein meal (Gosby et al., 2011). Thus satiety was affected by the lower protein load. Leidy and colleagues (Leidy, Tang, Armstrong, Martin, & Campbell, 2011) also reported greater feelings of fullness, lower late night desire to eat and lower food preoccupied thoughts during the day in overweight and obese participants provided with high (25% energy from protein) compared to average (14% energy from protein) protein diets. This effect on rated satiation and satiety is well documented within the literature (Johnson & Vickers, 1993; Johnstone, Horgan, Murison, Bremner, & Lobley, 2008; Lejeune, et al., 2006; Stubbs, Van Wyk, Johnstone, & Harbron, 1996) and has even been found in short-term preload manipulations. For instance, protein has been consistently shown to reduce subsequent test meal intake in comparison to isoenergetic carbohydrate preloads (Bertenshaw, et al., 2008; Bertenshaw, Lluch, & Yeomans, 2009), and isoenergetic carbohydrate, fat and

alcohol preloads (Poppitt et al., 1998) and has been found to increase the delay in hunger between the preload and test meal when protein, fat and carbohydrate preloads were compared (Marmonier, Chapelot, & Louis-Sylvestre, 2000; Poppitt, et al., 1998). Indeed, large volume consumption (1L) of high protein loads (40% and 72% energy from protein) were found to decrease feelings of hunger and subsequent intake 4 hours after preload intake when compared to carbohydrate preloads (Latner & Schwartz, 1999). Protein containing preloads have also been found to improve compensation for the energy received at a subsequent meal more effectively than isoenergetic carbohydrate conditions, reducing intake at the *ad-libitum* meal in line with the calories ingested in the preload (Bertenshaw, et al., 2009; Westerterp-Plantenga & Verwegen, 1999) further supporting the assertion that protein is processed differently to carbohydrate. This may be due to sensory information (increasing satiation) or the post-ingestive consequences of protein ingestion (maintained satiety and lower *ad-libitum* intake) or a combination of both of these factors.

Despite the abundance of research promoting the role of protein in encouraging maintained weight loss, not all studies show improved and maintained weight loss in high protein conditions. Indeed, Aldrich and colleagues (2011) found no differences in total weight or fat loss between participants provided with control, mixed protein or whey protein diets during an 8-week manipulation or over a 12-week follow up period. Similarly, participants provided with low carbohydrate, high protein diets achieved greater short-term weight loss than participants on low fat diets but this weight loss was not maintained at 12-month follow-up (Foster et al., 2003). This may be due to the poor compliance found with maintaining high protein diets (Brinkworth, et al., 2004; St Jeor, et al., 2001). Consuming meals higher in protein has also been found to have no impact on energy intake but increased satiation and satiety (Fischer, Colombani, & Wenk, 2004; Harper, James, Flint, & Astrup, 2007; Lejeune, et al., 2006), no influence on satiation or satiety but decreased energy intake (Bertenshaw et al., 2008) and no influence on energy intake or satiety (Blatt, Roe, & Rolls, 2011; Griffioen-Roose, Mars, Finlayson, Blundell, & de Graaf, 2011; Raben, et al., 2003) when compared to low protein conditions. The equivalent effects of high as compared to low protein diets and preloads on subsequent intake and satiety may be related to energy density. Some research suggests equivalent effects of high and low protein conditions when energy density is held constant (Bell, Castellanos, Pelkman, Thorwart, & Rolls, 1998; Blatt, et

al., 2011; Stubbs, et al., 1996) however, others show reduced intake (Rolls, et al., 1988) and stronger satiety (Lejeune et al., 2006) in protein conditions with matched energy density.

Another factor that may influence the discrepancies found across studies is related to the quality of protein ingested. For instance, whey protein has been found to increase satiation and satiety more effectively than casein or soy protein (Anderson, Tecimer, Shah, & Zafar, 2004; Veldhorst et al., 2009) and has been shown to reduce subsequent buffet lunch intake when compared to casein protein (Hall, et al., 2003). Similarly, mice consuming diets high in whey protein displayed a longer inter-meal interval than those consuming soy or gluten protein diets (Yu, South, & Huang, 2009). This effect of protein variety may be related to its digestibility as whey protein is highly digestible whilst proteins such as casein require a longer gastric emptying time (Boirie et al., 1997). Indeed, whey has been shown to increase postprandial protein synthesis and amino acid oxidation earlier and more strongly than slowly digested proteins such as casein (Dangin et al., 2001; Dangin, Boirie, Guillet, & Beaufrère, 2002). Some researchers also argue that many of the positive effects of protein reported are due to the use of extracted protein such as whey protein as opposed to dietary protein as these proteins are purer sources and thus may be used in quantities exceeding those found naturally in the diet (Blatt et al., 2011). However when control, dietary sources of protein, and dietary protein with mixed whey were compared, there were no differences found in weight loss across conditions (Aldrich et al., 2011) and a maintained effect of protein reducing energy intake has also been found (Weigle et al., 2005).

The inconsistencies encountered in short-term preload studies may also relate to the time interval employed. Some researchers report no influence of protein preloads on subsequent test meal intake after a 3 hour (Blom et al., 2006), or 30 minute (Chung Chun Lam, Moughan, Awati, & Morton, 2009) inter-meal interval as compared to control whilst others show reduced energy intake and hunger ratings after consuming protein as opposed to carbohydrate preloads 4 hours after intake (Latner & Schwartz, 1999). Others still report an effect of protein reducing intake irrespective of inter-meal interval (either 30, 60, or 120 minutes) as compared to control (Chung Chun Lam, Moughan, Henare, & Ganesh, 2012) as has been found elsewhere (Bellissimo et al., 2007). However, Fischer and colleagues (2004) assessed the impact of pure protein,

carbohydrate and fat gels on short-term satiety (including rated appetite, blood indices and indirect caliometry) over 3 hours after intake finding equivalent effects of carbohydrate and protein after 1 hour but stronger desire to eat and gastric emptying in carbohydrate and fat conditions as compared to protein from 2-3 hours. The wide array of conflicting findings reported is most likely related to the combined influence of protein variety, quantity, composition and the time interval used (Almiron-Roig, et al., 2003; Blundell, et al., 2010). Indeed, the satiating effects of protein have been stated to be most potent when timing of the meal interval is matched to timing of the amino acid profiles (Luhovyy, Akhavan, & Anderson, 2007), which is dependent on the type, volume and composition of protein used. Thus attempts to assess the rate of satiation and satiety of the type of protein under investigation may be important in further understanding appropriate inter-meal interval timings and the relative influence of protein on general appetite and intake. Similarly, focussing on one type and quantity of protein across study designs may be an effective means of maintaining consistency to understand the influence of this type of protein on appetite and intake.

1.4.2 Umami as a signal for protein in the diet

Despite research in the animal literature suggesting that the positive post-ingestive experience of protein determines protein satiation (L'Heureux-Bouron et al., 2004), there may also be a role for the sensory impact of protein on appetite indices as previously mentioned (Bertenshaw et al., 2013; see section 1.4.1). Indeed, the provision of creamy and thick high protein or high carbohydrate preloads was shown to reduce subsequent test meal intake and increase satiety more than high protein preloads without creamy and thick sensory cues. This suggests that these orosensory markers are acting as feedback of the macronutrient availability of the ingested foods, further informing satiety relevant processes. Indeed, when thicker high protein preloads were compared to less thick isoenergetic carbohydrate drinks, subsequent intake at a test meal was lower after the protein condition compared to the carbohydrate condition (Bertenshaw et al., 2008). However, many dietary protein sources, such as animal protein, are not inherently creamy or thick, thus it may be that 'creaminess' and 'thickness' are not the only markers of protein availability. It may instead be argued that the flavour of glutamate (umami) is a more potent signal for protein detection (Ikeda, 1908).

Typical dietary proteins have been shown to be tasteless when presented alone (excluding sweet proteins such as monellin; Kant, 2005). Similarly, glutamate availability is most reliably paired with the presence of protein in foods (Kurihara et al., 2000; Naim et al., 1991) but these dietary sources of glutamate are not required by the body as they are already synthesised in sufficient quantities required for metabolic processes (Reeds, Burrin, Stoll, & Jahoor, 2000). Consequently the taste of glutamate may function as the signal to adequately regulate protein intake (Laska & Hernandez Salazar, 2004; Mori, et al., 1991; Smriga & Torii, 2000) just as sodium chloride consumption guides mineral intake and sweet taste determines carbohydrate intake (Lindemann, 2000) and may explain how protein regulation has remained consistent over time as previously discussed (Simpson & Raubenheimer, 2005; see section1.4.1). Indeed, the experience of glutamate alone in the form of MSG and in combination with IMP has also been described as the taste of dietary protein in taste perception studies (Brand, Teeter, Kumazawa, Huque, & Bayley, 1991; Luscombe-Marsh, et al., 2008; Ninomiya, et al., 1991) and in fMRI research (de Araujo, et al., 2003). The stimulation of gut glutamate receptors by luminal glutamate and subsequent activation of vagal afferent fibres during protein digestion may be the mechanism by which protein ingestion by MSG is detected (Kondoh, Mallick, & Torii, 2009) whilst the potential site of an umami recognition centre (Smriga & Torii, 2000) has also been suggested in rat models with strong activation in the lateral hypothalamus during MSG intake (Torii, 1998) and compromised function in this area found in protein deficient rats (Kondoh et al., 2000; Smriga & Torii, 2000).

MSG taste detection, palatability, and preference have also been related to protein intake in the diet. Indeed, protein-deficient individuals have been found to prefer higher concentrations of MSG than well-nourished controls (Murphy, 1987). Equally, underweight, energy and protein malnourished infants were found to prefer higher concentrations of MSG in soups and consumed more than nutritionally replete children (Vazquez, Pearson, & Beauchamp, 1981) indicating heightened sensory responding to the available amino acids to relieve protein deficiency (Shi, et al., 2010). Similarly, the unanimous preference for umami taste in domestic ruminants has been related to the rewarding aspects of protein delivery (Ginane, Baumont, & Favreau-Peigné, 2011) and preference for MSG solutions have been found to differ according to dietary habits. For instance, spider monkeys, which consume low proportions of animal matter, have more

sensitive thresholds for MSG and higher taste preference thresholds compared to water than squirrel monkeys, which consume large amounts of animal matter and are less sensitive to MSG thresholds, with moderate preference of MSG sources as compared to sucrose and salt (NaCl; Laska & Hernandez Salazar, 2004). Indeed, Laska and Hernandez Salazar (2004) identified a significant negative correlation between taste threshold preference for MSG and proportion of animal matter consumption in the diet. This may be related to optimal foraging theory (Stephens & Krebs, 1986) as animals consuming low protein diets are more sensitive to the small amounts of protein available to them in their diet to meet their protein needs. However, others have found increases in preference and consumption of MSG-enriched soups in both malnourished and well-nourished infants (Beauchamp & Pearson, 1991) and rats fed protein-free and low-protein diets were not found to show a preference for umami containing solutions (Torii, 1987). However, as protein availability increased, so too did preference for umami sources (Naim et al., 1991). This has also been found in humans with the ability to detect lower concentrations of MSG (Luscombe-Marsh et al., 2009) and MSG and IMP (Luscombe-Marsh et al., 2008) stimuli predicted by greater self-reported protein liking. These inconsistencies may be related to the ways in which the body senses MSG as a consequence of previous experience and in relation to the body's protein stores (He et al., 2004).

Developing an understanding of the ways in which protein regulation occurs may be informative due to the known effects of protein on appetite control (see section 1.4.1). If the orosensory information provided by umami sources is important in maintaining this regulation, as thick and creamy cues have been shown to be (Bertenshaw et al., 2008), then it may be interesting to assess whether protein intake in the diet influences liking for MSG sources. From the research discussed it is not clear whether this effect of palatability for umami sources occurs as a result of chronic under-exposure to protein foods, whether it may be manipulated more acutely or whether it occurs at all under experimental conditions in humans. As palatability is so important in appetite control (see section 1.2.4), understanding the nature of the relationship between umami and protein in terms of palatability and liking, and the conditions under which altering one (such as protein) may change the hedonic qualities of the other (umami) is important to further explore whether protein is regulated in some way by sensory cues such as

umami. Thus, Paper 4 aimed to assess this relationship in relation to the palatable experience of umami flavour.

1.4.2.1 Summary of Paper 4

The variation in the literature with regards to the influence of protein status on preference for MSG sources was further explored in Paper 4. This paper examined the potential influence of acute mild protein deprivation and habitual protein intake in the diet on the potential for a change in flavour pleasantness of high and low concentrations of MSG in comparison to other basic tastes such as salty (NaCl) and sweet (Ace K). This was to further understand the ways in which protein regulation by MSG may occur and whether this is moderated by changes in liking. Participants completed a baseline taste test session (having consumed their regular breakfast) and were then provided with a high or low protein breakfast matched in energy density on the second and third test day for the acute protein manipulation. They returned at lunchtime sessions to complete taste tests with soup samples differing in flavour (control, umami, salty and sweet) and concentration (strong or weak). An additional protein food frequency questionnaire was completed to assess protein intake over the 6 month period prior to study participation and to assess whether long-term protein intake may predict liking of MSG sources as has been found previously (Luscombe-Marsh, et al., 2008, 2009) and whether sample flavour pleasantness differed by test day manipulation as a function of habitual protein intake. It was important to include additional basic tastes in Paper 4 to ensure that any influence of protein was specific to the umami flavour and not an artefact of liking for sodium which is present in MSG. Equally, as participants arrived for their taste test in a fasted state, the influence of sweetness (a predictor of energy) was also included to ensure pleasantness ratings were not affected by energy need.

Pleasantness was found to be higher on the low protein day as compared to the high protein day for all flavours and a stronger desire for savoury flavours was evident on the low as compared to high protein day. No clear predictive influence of long-term protein status on liking for MSG sources was found. Thus, participants generally consuming large amounts of protein did not differ in their liking of MSG to those consuming lower quantities of protein. However, similar to the findings reported by Laska and Hernandez Salazar (2004), individuals habitually consuming more protein were found to like the

flavour of high concentrations of MSG more when they had been subjected to an acute protein deprivation (low protein breakfast) than any other flavour. This subtle effect may suggest that protein regulation is moderated in some way by richer sources of umami flavour and that this may occur when high protein consumers are faced with short-term protein deprivation. Habitual high protein consumers exposed to an acute protein deficit also reduced their liking of high MSG sources after the high protein breakfast, further implicating umami flavour in protein regulation in these individuals. However, the questionnaire used may have been biased to only assessing high protein diets from animal sources instead of general macronutrient intake as a whole. Indeed, the effects found were subtle and the experiment suffered from being underpowered. Nevertheless, as the acute manipulation allowed for a more controlled assessment of the potential effects of protein regulation, assessing this alongside habitual protein intake provided a detailed representation of the interaction between the regulation of nutrients and how this may be manifested by palatability.

1.4.3 The role of umami in protein satiation and satiety

As the umami flavour generated by MSG and its associated nucleotides has been related to specific detection of protein sources (Naim et al., 1991; Kurihara et al., 2000; Luscombe-Marsh et al., 2008, 2009), it has been suggested to play some role in influencing liking depending on protein regulation (Paper 4), and as protein has been argued to be the most satiating macronutrient, it may be that the taste of umami in umami-rich foods is especially satiating. For instance, studies in infants report that protein hydrolysate formulas, which are rich in free glutamate, are more satiating than equivalent low glutamate milk-based formulas such as cow's milk formulas (CMF) (Mennella & Beauchamp, 1996; Mennella, Ventura, & Beauchamp, 2011). This has also been shown to influence weigh gain as infants assigned a formula high in protein and free glutamate (extensive protein hydrolysate formula; ePHF) or lower in protein but high in free glutamate (CMF with added glutamate to match levels in ePHF) consumed less formula to satiation, maintained prolonged satiety and gained less weight than those fed on isocaloric low glutamate CMF (Ventura, Beauchamp, & Mennella, 2012). This suggests that not only do infants self-regulate free glutamate intake and thus can adequately balance protein intake, but that the high glutamate formulas helped promote both satiation and satiety. The quantities of glutamate in ePHF also closely

match those found in breast milk and infants fed on mother's milk have been shown to be of a lower weight after their first year than those consuming CMF (Owen, Martin, Whincup, Smith, & Cook, 2005). This is most likely due to the glutamate signal cuing the presence of satiating protein. Indeed, both animal and human studies support the role of free glutamate as a key signal for satiation (Niijima, 2000; San Gabriel, Maekawa, Uneyama, Yoshie, & Torii, 2007; Viarouge, Caulliez, & Nicolaidis, 1992). For instance, rats given *ad-libitum* free access to MSG solutions from pre-weaning to adulthood were found to weigh less as adults than those not exposed to MSG solutions (Kondoh & Torii, 2008).

Thus it may be that early experiences of glutamate may become a learned cue for the satiating aspects of protein (Gibson & Brunstrom, 2007). Indeed, rat models have found that when oral glutamate is paired with protein it acts as a conditioned stimulus to encourage the cephalic phase of pancreatic secretion, inducing a concentration-dependent release of glucagon and thus maintaining satiety (Bertrand, Gross, Puech, Loubatières-Mariani, & Bockaert, 1993). Similarly, this may explain why Essed and colleagues saw a reduction in weight gain in added MSG conditions over their 16-week manipulation (Essed et al., 2007) whilst Mathey and colleagues did not (Mathey et al., 2001) as discussed previously (see section 1.3.5). Such results indicate that MSG may be giving rise to a biphasic effect on appetite, increasing flavour pleasantness and hunger during a meal but then acting to maintain feelings of satiety after meal intake. Lejeune and colleagues (Lejeune, Smeets, & Westerterp-Plantenga, 2007) also assessed the effects of MSG and IMP with added protein on indices of appetite. They provided participants with high-protein diets over 3 days with or without added MSG and IMP combinations in food or provided as a dietary supplement, revealing lower ratings of desire to eat in participants consuming high protein diets with added MSG/IMP in foods as compared to control high protein or high protein supplemented MSG/IMP diets. However, there were no differences across these conditions in rated satiation or prospective consumption but as only 12 participants were tested, the study may have been inadequately powered. Nevertheless, others have similarly reported no effect of MSG or MSG/IMP combinations on appetite and intake. For instance, Luscombe-Marsh and colleagues (2009) provided participants with high protein preloads either provided alone or with added MSG or added MSG and IMP 30 minutes before giving access to an *ad-libitum* buffet meal. They reported no differences in hunger or fullness ratings and

showed an increase in buffet meal intake after the MSG preload relative to all other conditions. Carter and colleagues also saw similar results when providing added MSG, or added MSG/IMP as compared to added fat broths before an *ad-libitum* meal (Carter et al., 2011). Zai and colleagues suggest that this may be due to increases in gastric emptying time and thus earlier hunger-recovery as when MSG was combined with high protein meals gastric emptying time increased but this was not found with isocaloric high carbohydrate meals (Zai et al., 2009). Alternatively, as intake also increased in the high protein condition, it may be that the buffet courses provided in the previously mentioned studies (Carter et al., 2011; Luscombe-Marsh et al., 2009) encouraged intake due to the variety of items on offer. It may also be that Luscombe-Marsh et al. (2009), delivered the test meal too soon before the post-ingestive influence of MSG or MSG/IMP could occur whilst the aversive flavour of the broths provided in Carter et al's (2011) study may have influenced subsequent intake.

It may additionally be argued that glutamate is equally abundant in many low protein foods such as the high concentrations found in tomatoes and mushrooms (Jinap & Hajeb, 2010; Ninomiya, 1998; Yamaguchi & Ninomiya, 2000), thus the total glutamate content of foods (including both free glutamate which can be tasted and protein bound glutamate which may be released with chewing; see section 1.3.1) may provide a more reliable index of protein ingestion (Kondoh et al., 2009) and was not controlled for in the previously mentioned studies (Carter et al., 2011; Luscombe-Marsh et al., 2009). Thus some effect of glutamate from control conditions cannot be discounted. Similarly, as the umami taste derived by glutamate is generated by MSG and is further expressed with the addition of GMP and/or IMP, some researchers argue that the effect of MSG on protein-based satiety can only occur when accompanied by the appropriate amino acids or flavours that reinforce this message (Beauchamp, 2009). Indeed, as previously mentioned (see sections 1.1.1 and 1.3.3), IMP is found in animal protein (Lindemann, 1996; Yamaguchi, 1998) whilst GMP is more associated with plant-based protein (Yamaguchi 1967). Thus, it may be that the strength of the MSG protein cue is more reliant on its associated ribonucleotides and thus has not been consistently found to decrease intake. Indeed, experiments conducted with MSG/IMP and protein are few, with those mentioned low in participant numbers (Lejeune et al., 2007) or not taking confounds regarding the inter-meal interval (Luscombe-Marsh et al., 2009) or the test foods used (Carter et al., 2011; Luscombe-Marsh et al., 2009) into account.

The research discussed in this section indicates mixed findings about the relationship between umami flavour and protein in relation to satiation and satiety. As the experiments mentioned varied in the length of the inter-meal interval used (which may be important for determining the satiating aspect of macronutrient effects; see section 1.4.1), the type of meal provided with umami (whether in the form of liquid soups, semi-liquids or solids), and the free glutamate content of the meal before adding umami sources (see section 1.3.4), it is important to control for these effects to understand the conditions under which umami and protein may interact to influence appetite control. Thus, the need to further explore the rate of satiety of umami sources such as MSG in different macronutrient combinations is important to understand whether MSG may slow hunger recovery in a protein context in comparison to a different macronutrient context such as carbohydrate (Paper 5). It is also essential to measure what effects, if any, combining MSG with specific macronutrients has on subsequent intake of a meal (Paper 6) and whether the synergism between umami sources that may be predictive of protein (such as MSG and IMP) influence satiety further (Paper 7). As these papers deal with the effects of umami and protein specifically on the experience of satiation and satiety, they have been consequently presented as a series of complementary studies.

1.4.3.1 Summary of Paper 5

The aim of Paper 5 was to assess the time course of MSG satiety when provided as a fixed volume preload in low energy control or high energy added protein or added carbohydrate conditions and to compare this to equivalent no added MSG conditions. This was to further elucidate the role of MSG on satiety and particularly to understand whether this changed as a function of the macronutrient content of the meal provided. Participants completed ratings of appetite before and after the preload to investigate satiation whilst additional ratings of appetite were then completed in 15 minute intervals for 120 minutes post-ingestion to understand the role of MSG on satiety.

The satiating effect of MSG decreasing hunger and increasing fullness immediately after intake was not repeated in Paper 5 as it had been found in Paper 2, especially when provided with added protein. This discrepancy may have been due to the methods employed as in Paper 2 participants ate *ad-libitum* and thus controlled their portion size

whilst in Paper 5 a fixed volume of the soup was provided based on previous research with a male sample (Yeomans et al., 1998). Also, Paper 2 included a female sample alongside males thus the quantities consumed may have been lower than would be expected with an all-male sample. Nevertheless, a significant linear contrast effect was evident at specific time points in satiety evaluations with added MSG protein soups maintaining reduced feelings of hunger more strongly from 30-60 minutes post-ingestion than no added MSG soups.

Paper 5 allowed for an accurate examination of the rate of satiety in specific combinations of MSG and macronutrient preloads and was established at the 45 minute mark. This provided more precise profiling of the generation of satiety with the specific type and variety of protein used as has not been accounted for in previous studies and may explain the variation in the results seen (see section 1.4.1). However, no main effects and no main interaction effects were evident in the results which may have been due to the variations present in appetite ratings across participants. Similarly, the results presented may suggest that satiety was stronger with added MSG protein preloads but cannot account for potential reductions in consumption at a subsequent course as appetite ratings may not be the most accurate predictor of subsequent intake (Blundell & Greenough, 1994; Crovetti, Porrini, Santangelo, & Testolin, 1998; Fischer, et al., 2004).

1.4.3.2 Summary of Paper 6

As a consequence of the results of Paper 5, Paper 6 wished to further assess the influence of MSG and macronutrient combinations on rated satiation and satiety but additionally looked at the influence of these preload combinations on intake at a subsequent meal. Thus the same fixed volume preload conditions (control, added carbohydrate and added protein with and without added MSG) were assessed with appetite ratings made before, after tasting and after intake of the soup course followed by a 45 minute inter-meal interval (in accordance with the results of Paper 5). Participants then returned for an *ad-libitum* two course meal (main course and dessert) and completed appetite ratings before, after tasting and at the end of each respective course. Intake at the *ad-libitum* course was recorded and assessed alongside appetite ratings at the relevant time points.

The added protein preloads were found to reduce intake at the *ad-libitum* meal more effectively than either carbohydrate or control preloads irrespective of added MSG. However, the addition of MSG to the protein preloads further improved compensation for the extra energy received in the high energy protein preload as compared to the high energy carbohydrate preload. Thus participants were able to adjust their intake more precisely to the calories ingested in the soup course with the added MSG cue in a protein context than when no cue was present. Similarly, the experience of satiation was strongest in the added MSG protein condition over the course of the *ad-libitum* meal despite the lowest energy intake in this condition. This further supports the potential role of MSG in prolonging protein satiety as suggested in Papers 2 and 5.

However, there was no clear difference in appetite across conditions at the 45 minute interval in contrast to what was found previously (Paper 5). This may be due to the differences in the methods employed as in Paper 6 participants may have been more insensitive to perceptions of hunger due to the knowledge that they would be receiving a two course meal after these ratings whilst in Paper 5 no additional meal was provided. Similarly, appetite ratings were not found to decrease during soup intake (Paper 2) or increase immediately after intake of the MSG added conditions (Paper 5) in Paper 6. This suggests that the immediate experience of a fixed portion of MSG and protein was less sensitive to rated appetite than if this combination was potentially consumed to satiation (Paper 2). However, the clear long lasting effect of maintained lower hunger ratings in MSG added protein conditions during the *ad-libitum* meal matched those found in Paper 5 and this stronger satiation during the meal relates to the findings of Paper 2, but the added protein acted to reduce further calorie intake in Paper 6 where intake remained similar in Paper 2.

Despite a clear indication that adding MSG to protein preloads improved energy compensation, intake at the *ad-libitum* meal was not found to differ in added and no MSG conditions with no significant main effect found and no significant interaction between condition and MSG, although the data did indicate trends in the right direction. It may be that this effect was not evident due to the influence of MSG on intake being very subtle and prone to more variation. However, it may also be that the MSG signal experienced was not salient enough to give rise to a robust bi-phasic effect. Just as

Paper 4 found stronger preferences for high concentrations of MSG in high protein consumers when in protein need, it may be that the umami signal was too weak to generate an effect or may have required complementary amino acids to reinforce the umami protein signal (as previously suggested by Beauchamp, 2009) as the umami generated by MSG is found in both low- and high-protein sources.

1.4.3.3 Summary of Paper 7

As a consequence of the results found in Paper 6, Paper 7 investigated the influence of a more robust umami signal on appetite and intake, taking not just the umami effects of MSG into account but the synergistic consequences of combining MSG and IMP. This allows for a better understanding of the effects of umami in a meal context as high dietary protein sources tend to include high levels of IMP alongside MSG flavour (Jinap & Hajeb, 2010; Ninomiya, 1998; Uneyama et al., 2012; Yamaguchi & Ninomiya, 2000). Thus, Paper 7 utilized a similar design to Paper 6 but only assessed the impact of MSG/IMP combinations in low energy control and high energy protein conditions compared to no added MSG/IMP conditions. Also, the *ad-libitum* meal provided 45 minutes after the fixed soup preload portion consisted of a main savoury course and no dessert. This was due to efforts to regulate the palatability of a dessert course impacting more strongly on hedonic hunger as was found in Paper 6. It is important to note that the differences between macronutrient and MSG conditions did not influence dessert intake in Paper 6, which tended to be similar across macronutrient and MSG soup courses, indicating a stronger influence of palatability on intake.

Unlike Paper 6, Paper 7 found an appetizer effect in added MSG/IMP conditions and an increase in hunger ratings after first tasting the umami-enhanced soups. However this initial rise in hunger declined to match no MSG/IMP conditions at the end of fixed preload consumption. A significant main effect of MSG/IMP on intake revealed that added MSG/IMP conditions reduced subsequent *ad-libitum* test meal intake when provided in a protein context and, more interestingly, also reduced *ad-libitum* intake of the main course in the low protein MSG/IMP condition. Thus the added MSG/IMP conditions effectively acted to reduce subsequent intake despite low availability of protein. This suggests that the more salient umami signal generated by the addition of IMP may act as an effective protein cue, subsequently reducing intake due to the satiety

generated by protein. Indeed, as this reduction in intake was also evident in the low energy protein MSG/IMP condition as well as the high energy protein MSG/IMP condition, it indicates the potential for improved weight maintenance without having to increase the energy content of the meal. Additionally, compensation at the *ad-libitum* main course for the extra energy received as protein was significantly better when MSG/IMP had been combined with protein than when protein was presented alone. This indicates that MSG/IMP may further allow for more accurate energy regulation than protein alone. Such results may also provide support for a bi-phasic effect of umami, maintaining hunger as a consequence of palatability within the meal but then acting to increase the experience of satiety over time.

Interestingly, no corresponding main effect of MSG/IMP conditions were seen in the main course when appetite ratings were assessed but a significant interaction between protein and MSG/IMP revealed that participants experienced lower hunger over the course of the meal in added MSG/IMP protein conditions than no MSG/IMP protein conditions suggesting that added MSG/IMP protein conditions had long lasting effects on the conscious experience of appetite at the *ad-libitum* meal whilst the impact of MSG/IMP alone may have influenced meal intake without affecting rated appetite. Thus, just as was found in Paper 6, participants experienced similar rated satiation across no added and added MSG/IMP conditions but because intake was lower in added MSG/IMP conditions, satiation was stronger due to consuming less, thus satisfaction was maintained despite lower energy intake. This may suggest that the combined effect of MSG and IMP may provide a valuable umami protein cue without the need for the addition of protein due to increasing the salience of this cue with complementary ribonucleotides in the form of IMP.

1.5 General conclusions

This thesis aimed to understand the role of the fifth basic taste, umami, in relation to appetite and intake, particularly when in combination with specific macronutrients such as protein. The importance of understanding the role of taste and how it may influence feeding practices is evident throughout the literature (Beauchamp & Mennella, 2009; Drewnowski, 1997; Krebs, 2009). However, the flavour of umami as experienced by MSG with some additional focus on its association with another umami substance,

namely inosine monophosphate (IMP) has not been researched in such detail and may allow for a greater understanding of how this taste guides appetite control. This may be of particular interest as the flavour of umami is attributed to the taste of protein in foods and as protein is important in energy regulation, may indicate a role for umami as the flavour function essential for this regulation.

The first paper in this thesis aimed to establish a low glutamate, hedonically neutral soup to use across all experiments. This was vital to determine the extent to which MSG influenced appetite and intake as opposed to the potential interaction between high free glutamate levels and MSG. Indeed, as too high a concentration of free glutamate is aversive (Jinap & Hajeb, 2010; Yamaguchi & Takashi, 1984) and previous studies have found inconsistent results when glutamate content was not measured (Shi et al., 2010), it was essential to formulate an appropriate vehicle to test the influence of umami by MSG. It was also additionally important to ensure that the soup itself was not aversive as this would not reflect true eating behaviour (as individuals do not generally pick and eat aversive foods) and to formulate a vehicle that could be consumed both as a meal and as a preload. Once the soup was determined, the following two papers in this thesis examined the effects of enhanced palatability on appetite and intake using MSG (Paper 2) and sucrose (Paper 3) as previous research indicates that palatability leads to increased intake and maintained hunger after tasting the palatable food (the appetizer effect; Yeomans et al., 1996). It was important to assess the effects of MSG in this way to understand whether its flavour enhancing elements increased appetite and intake when consumed to satiation relative to a bland control (Paper 2). This was also tested using another basic taste (Paper 3) to determine whether the effects found were specific to MSG and whether there were potential issues with the methodology used. Interestingly, both Papers 2 and 3 found no appetizer effect with the flavour enhanced conditions and there was no difference in meal intake. This may be due to the comparable pleasantness ratings found for supposedly 'bland' and flavour enhanced conditions. However, despite the similar quantities consumed across conditions in both Papers 2 and 3, only the MSG added soups (Paper 2) increased the experience of satiation more quickly during intake, indicating the potential for MSG to maintain satiety. This was not evident in the sucrose condition in Paper 3.

This potential for a bi-phasic effect of appetite, with added MSG increasing pleasantness during intake but possibly maintaining satiety over time was suggested to be related to the effects of MSG acting as a possible cue for protein in the diet. If this was the case and MSG was in some way related to protein, it was expected that manipulating the protein intake in the diet may have influenced liking of MSG sources. Paper 1 found high pleasantness ratings across MSG added conditions during intake, but this potential for MSG palatability influencing appetite was not apparent (as found in Paper 2). However, if MSG was a marker for protein (which, as mentioned, is tightly regulated) and protein availability was low, the question remained as to whether this would influence liking for MSG sources. Similarly, it was important to understand whether these effects were more sensitive to acute as opposed to long-term protein intake or whether a combination of both influenced this liking. Indeed, Paper 4 revealed a significant increase in the desire for savoury foods when in protein deficit than when given a high protein load at breakfast. However, the acute protein manipulation also showed a more general increase in liking of all flavours (bland, MSG, sweet and salty) when in protein deficit, rather than just those predicting protein (strong and weak MSG concentrations) when compared to the baseline (no manipulation) test day. Habitual protein consumption was also not directly related to MSG liking, but when generally high protein consuming participants were subjected to a low protein manipulation, their liking for high concentrations of MSG was stronger than low concentrations and all other flavour samples and this liking for MSG was lower on the high protein test day. This modest effect of high concentrations of MSG influencing liking in these individuals suggested that an aspect of protein regulation may be related to MSG sensing in habitual protein consumers but as the effects seen were so subtle due to issues of power, further investigation of the effects of MSG on appetite control were not restricted to habitually high protein consuming individuals.

The extent to which umami flavours and protein may interact to influence the experience of satiation, but also importantly, the generation of satiety was consequently assessed using rated appetite over time (Paper 5) and subsequent intake (Papers 6 and 7). Papers 5 and 6 looked at the influence of MSG when in combination with high energy protein and carbohydrate preloads as compared to equivalent low energy no MSG conditions. Paper 5 focussed on assessing the time course of MSG satiety over two hours after intake of a fixed preload across the six soup conditions whilst Paper 6

utilized the results from Paper 5 to determine when to provide a subsequent *ad-libitum* meal (established as 45 minutes after preload intake). Although no main effects were evident for rated hunger over time in Paper 5 due to variation across participants, contrast analysis indicated an effect of MSG added protein conditions showing slower hunger recovery as compared to all other conditions. As this was not evident in the carbohydrate condition, further evidence for a subtle interaction between MSG and protein was clear. Paper 6 additionally found an interaction between MSG and protein for energy compensation, indicating better compensation than in the MSG and carbohydrate condition. Satiety was also found to remain strongest during *ad-libitum* meal intake in the MSG added protein condition. However, no main effects were found for MSG and no interaction between MSG and protein was evident during *ad-libitum* intake, suggesting that this effect remained subtle and prone to variation.

As there was no clear effect of MSG reducing intake as protein had been found to in Paper 6, this suggested that MSG alone may not have been acting as a complete protein cue on its own, but only one that worked to amplify the satiating aspects of protein. Thus, Paper 7 aimed to determine whether an additional nucleotide present in high protein sources (IMP) could act to increase the salience of a potential umami protein cue and whether this would occur with an absence of protein. Following on from the design of Paper 6 but only assessing high protein and low energy control conditions, when MSG/IMP preloads were consumed 45 minutes before an *ad-libitum* single item meal, intake of the subsequent course reduced with the addition of MSG/IMP irrespective of the presence of protein. Similarly, the addition of MSG/IMP also acted to improve energy compensation more than when protein was provided alone (as was found with MSG in Paper 6). Nevertheless, rated satiety was improved when MSG/IMP was combined with protein, indicating that the more subtle effects seen with MSG alone (Papers 2, 4, 5 and 6) may have been due to a weaker umami signal that could only be effective with the addition of protein. However, when this cue is amplified it may further act to signal the presence of protein even when no protein is available, but this is not necessarily consciously perceived. Indeed, ratings of hunger and fullness were not lower in the MSG/IMP conditions and only when protein was added alongside MSG/IMP did appetite ratings indicate stronger satiety. This suggests that the palatable elements of the MSG/IMP control condition were maintained even during *ad-libitum*

intake, but also that intake was consistently lower in these conditions, despite the higher ratings of hunger.

Overall, these findings may further add to the literature regarding the importance of taste in guiding food preferences and intake. This thesis suggests that MSG does not reduce satiation by increasing *ad-libitum* intake despite maintaining palatability in the relevant food (Paper 2). MSG may also be useful in guiding protein regulation in those that regularly consume protein due to increased liking of MSG sources (although it should not be assumed that liking of these flavours translates to increased intake of these sources; Paper 4). MSG may also be said to subtly increase the experience of satiety when combined with protein (Papers 5 and 6). This effect is further amplified without the presence of protein when MSG is combined with complementary ribonucleotides such as IMP, indicating that MSG/IMP combinations may act as relevant satiety cues as found when consuming a high protein food (Paper 7). As a consequence such a cue may be exploited as a tool for maintaining adherence to higher protein diets which has been suggested to be difficult (Brinkworth et al., 2004; St Jeor et al., 2001) and may as a result also contribute to improved weight loss through maintained satiety but also reduced energy density (as protein increases energy density whilst MSG and IMP do not). However, these findings have also been shown to be subtle and prone to variation with MSG, whilst the consistent result of MSG/IMP reducing *ad-libitum* intake in Paper 7 requires replication.

1.5.1 Limitations

Despite every effort being taken to ensure all experiments presented in this thesis were of a high standard, there were a number of limitations that restricted the conclusions that could be drawn. Although these limitations are mentioned in the relevant chapters, more general issues are addressed here.

1.5.1.1 Participants

All experimental work detailed within the thesis was carried out on a young, Caucasian male and female sample who were non-obese, healthy and educated individuals. This was due to the studies being largely conducted on students at the University of Sussex.

The limitations of the sample therefore restrict the generalizability of the research to older individuals, those of a different ethnic background or obese individuals.

1.5.1.2 Methodology

A number of methodological issues must also be raised in the research carried out which limited the conclusions drawn. As all of the studies conducted were carried out in an experimental setting, it may be argued that the results reported are not indicative of eating behaviour in the natural environment. Indeed, Papers 2, 3 and 7 all included measures of within-meal interrupts to assess appetite responses whilst Papers 6 and 7 also included a 45 minute inter-meal interval between what may have been perceived as a 'starter' (the soup), 'main meal' (the pasta meal; Papers 6 and 7) and 'dessert' (Paper 6). These variations in eating may explain some of the differences found between Paper 5 and Papers 6 and 7. As mentioned in Papers 6 and 7, appetite was not found to differ 45 minutes after the preload in added protein and MSG or MSG/IMP meals whilst in Paper 5 this difference was clear. It may be argued that participants were acting in anticipation of the meal to be received in Papers 6 and 7 whereas this was not the case in Paper 5. Similarly, the discord apparent in VAS rated hunger and fullness and actual intake in Papers 6 and 7 may point to a difficulty in identifying perceptions of appetite during a meal and this relationship has been shown to be weak elsewhere (Blundell & Greenough, 1994; Blundell & Rogers, 1980; Crovetti, et al., 1998; de Graaf, et al., 1993; Fischer, et al., 2004). This suggests that a more unconscious process such as feelings of hunger and fullness during an eating occasion must be made consciously available in an experimental situation and may, as a result, be difficult to quantify.

Further limitations with regards to the 'savoury' label were evident throughout testing. It may be that participants were confused by this descriptor. Indeed, the 'savoury flavour' label may be applied to more specific taste experiences which vary according to each individual and meal. For instance, some may deem the taste of cheese as 'savoury' whilst others may attach savouriness to the flavour of a mushroom or broth. As these tastes differ to one another, individuals may have found it difficult to associate the umami taste in the vehicle used to the experience of savouriness. Research with trained panels have been successful (Ishii & O'Mahony, 1990; Sinesio, et al., 2010) but were not feasible in the presented research which relied on naïve participants due to the

potential influence of experience on VAS ratings (Kobayashi & Kennedy, 2002) and due to previous success with naïve participants (Sinesio et al., 2010). The detection of umami may instead have been related to a difference in the strength of flavour and saltiness of the soup. This relates to the appetite enhancing effects of glutamate and the sodium ion found in MSG. Indeed, these labels may have made flavour distinctions easier as strength of flavour is not related to specific taste but is more reliant on pungency whilst saltiness is a more universally agreed upon unitary taste experience. Similarly, the inconsistencies found in rated palatability between pilot tests of the relevant soup (Paper 1) and the main experiment (Papers 2, 5 and 6) may be a consequence of the VAS labels used or may be more related to the method of pilot testing used. Indeed, as participants were provided with tasting samples of the relevant flavour, the potential for comparison between flavours was high. Although efforts to regulate prospective negative contrast effects were made by employing counterbalancing across flavour conditions. The similar palatability ratings across added and no MSG soups during testing (Papers 2 and 6) but not found during soup formulation (Paper 1) or with the addition of IMP (Paper 7) may instead be related to individual variability between participants. It would also have been useful to include sensory ratings of thickness and creaminess throughout experiments to assess whether the soup formulated provided any additional sensory cues which may have influenced the experience of satiety, particularly as these cues have been related to the effects if protein on satiety (Bertenshaw et al., 2013). However, as Paper 1 found neutral VAS ratings for thickness, further assessments were not considered.

It may also be argued that the effects seen in Papers 6 and 7 were found only using a single item test meal which may be a more sensitive means of assessing satiety. Indeed, the use of a multi-item buffet may have been another means of assessing the effects of umami on appetite. However, buffet-style meals have been suggested as a more valuable means of determining subsequent macronutrient selection after a preload (Blundell et al., 2009) which was not the basis of much of the research presented. Multi-item meals have also been suggested to reduce the sensitivity of detecting changes in appetite, resulting in higher energy intake (Long, Griffiths, Rogers, & Morgan, 2000; Raynor & Epstein, 2001), although this has not been found elsewhere (Wiessing et al., 2012) and may not reflect general eating behaviours. Additionally, the influence of protein with no added flavour enhancer on intake in Papers 5, 6 and 7 could not

discount some baseline effects of glutamate despite efforts to regulate the quantity of glutamate in the control condition. Indeed, as whey protein isolate is derived from an umami containing source, the effects of glutamate could not be completely separated and could not be measured although levels in the protein soups were predicted to be low. It may also be stated that the effects seen across experiments remained subtle and require further substantiation.

1.5.2 Suggestions for future research

Given the limited scope of research assessing the influence of umami on indices of appetite control and the findings reported in this thesis, a number of directions may be taken to further understand the nature of the effects of umami. Taking the findings that habitual protein consumers like strong concentrations of MSG more when in acute protein deficit and less when given a high protein load (Paper 4) into account, a further assessment of intake after the relevant protein deprivation would be valuable to understand how these differences in liking translate to consumption. Assessing macronutrient selection by using a multi-item buffet style meal may also be of interest to assess whether nutrient selection is further influenced by umami sources. As it was the habitual protein consumers that displayed these differences in liking for MSG, it may also be of interest to assess the responses of these individuals and further explore where these differences between habitual and non-habitual protein consumers may lie. For instance, it may be that learning about the predictive value of umami as a protein source may be stronger in habitual protein consumers due to repeated exposure to high protein umami sources. One way in which this potential flavour nutrient learning may be investigated would be to assess whether brain opioid responses to MSG and MSG/IMP as compared to (and in combination with) protein sources using fMRI differs in these individuals as compared to low protein consumers as opioids have been implicated in the hedonic response to, and the incentive salience (or 'wanting' related behaviours) of MSG sources (Uematsu, et al., 2011). Similar effects of dopamine firing have been seen when assessing carbohydrate and fat sources in animal models (Oliveira-Maia et al., 2011). It may be that the rate of opioid signalling in habitual protein consumers in protein deficit is similar when subsequently receiving an MSG source (as opposed to another flavour) as when subsequently receiving a protein source

and this may differ in low protein consumers. Indeed, this type of research has not been conducted with protein and glutamate sources in animals or humans before.

The findings reported in Papers 5, 6 and 7 suggested that combining umami with protein may be an effective means of maintaining satiety by reducing feelings of hunger (Papers 5 and 7) and improving energy compensation (Papers 6 and 7). Paper 7 also found reduced intake in MSG/IMP conditions without added protein. Firstly it would be important to replicate the results found in Paper 7 to further corroborate the satiety generated by MSG/IMP, particularly as the effects found were independent of protein (which has not been found previously, Carter et al., 2011; Lejeune et al., 2005; Luscombe-Marsh et al., 2009). It would also be important to assess this influence of MSG/IMP in a high energy, high carbohydrate soup to understand whether the effect found was specific to a low energy item (the control) and whether carbohydrate preloads may differ to protein preloads. Additionally, the synergism of guanosine 5'-monophosphate (GMP) with MSG would be vital to assess further to understand whether the umami elicited by MSG and IMP (found in animal protein) is specifically acting as a marker for protein or whether the umami elicited by GMP (which is found predominantly in low protein plant-based foods) may also affect appetite control, and, if so, in what way. In particular it would be interesting to consider the synergism between MSG, IMP and GMP, to understand the effects of these flavour enhancers on appetite control. Would the added GMP influence appetite control as IMP was found to? Would this differ by macronutrient conditions?

As the effects found in Papers 5-7 all occurred post-orally either 45 minutes after intake or during a subsequent meal, the gastric impact of these flavour enhancers would be invaluable to assess. As glutamate receptors are found in the gut as well as the taste receptors on the tongue (Akiba, Watanabe, Mizumori, & Kaunitz, 2009; Bezençon, le Coutre, & Damak, 2007; Kondoh & Torii, 2008; Niijima, 2000; San Gabriel, et al., 2007), it may be that the effects of umami on satiety are acting on these gut receptors in a similar way to protein. To further gauge whether the impact of these flavour enhancers is purely physiological or whether the oral exposure of MSG and its related ribonucleotides is additionally important for learning about the satiating nature of the food consumed, it may be useful to compare the sensory experience of umami coupled with its post-ingestive effects to the post-ingestive effects of umami alone without

tasted umami flavour (using microencapsulated MSG and/or IMP) and the taste of umami without the post-ingestive consequences (using modified sham feeding). This would also be interesting to study with or without added protein to further shed light on the relevant mechanisms involved in the satiety enhancement found. Such research may also clarify whether learning about the orosensory experience of the flavour is important for determining its expected satiating capacity. Indeed, certain gut chemosensors that may generate these nutrient conditioning signals such as sweet (T1R3) and fatty acid (CD36) have been excluded (See Sclafani & Ackroff, 2012) thus it would be interesting to know whether MSG or MSG/IMP may promote flavour nutrient learning in this way. Such research would also further expand upon whether this differs when protein is provided alongside the relevant flavour enhancer or when it is tested in isolation. Likewise, it may be useful to further explore the oral and gut experience of umami using fMRI techniques to understand how umami is processed in the brain and whether this differs with or without a gut nutrient infusion, particularly one of protein, as this work has never been conducted in animal or human models (Sclafani & Ackroff, 2012).

If an orosensory basis for the satiety experienced by umami sources was found to be a relevant contributor of protein-based satiety, further understanding about the relevant sensory cues that may underline the satiating aspects of protein would be vital to explore. For instance, thickness and creaminess have been related to protein-based satiety (Bertenshaw et al., 2013) and as the preload developed for the research in this thesis was thicker than the more conventional broth-based soups tested previously (Carter et al., 2011), with the addition of protein potentially providing a creamier mouth feel, it may be that the combination of thickness, creaminess and umami acted to enhance the experience of satiety further than thickness and creaminess alone (although the soups tested were not rated as particularly thick in the one experiment in which this sensory percept was measured; Paper 1). Additional research may wish to separate thickness, creaminess and umami to assess which sensory cue may be the most potent for appetite control and whether these cues further reduce intake when presented together.

Appendix 1.1 Glossary of terms

Appetite – the effects of a food on hunger and fullness processes determined by subjective ratings (using VAS scales) or approach response (consumption).

Fullness – the feeling of having consumed enough based on stomach distension and volume consumed.

Hunger – feeding promoted by the desire to locate and consume food which may be influenced by physiological bodily need but also may change according to cognitive and contextual cues (such as the hedonic qualities of the food consumed).

Pleasantness/Palatability – the hedonic experience of a food as a good taste which may promote an approach response (the desire to consume more of the food).

Satiation – the interaction between the positive feedback cues of hunger (appetite stimulation), and the negative feedback cues of fullness (appetite suppression) within a meal that leads to the end of that meal.

Satiety – the time period after finishing one meal and before deciding to consume the next meal (also known as the inter-meal interval).

1.6 References

Abumrad, N. A. (2005). CD36 may determine our desire for dietary fats. *Journal of Clinical Investigation, 115*(11), 2965-2967.

Ackroff, K., & Sclafani, A. (2011). Flavor preferences conditioned by post-oral infusion of monosodium glutamate in rats. [doi: 10.1016/j.physbeh.2011.05.013]. *Physiology & Behavior, 104*(3), 488-494.

Akiba, Y., Watanabe, C., Mizumori, M., & Kaunitz, J. D. (2009). Luminal L-glutamate enhances duodenal mucosal defense mechanisms via multiple glutamate receptors in rats. *American Journal of Physiology-Gastrointestinal and Liver Physiology, 297*(4), G781-G791.

Aldrich, N. D., Reicks, M. M., Sibley, S. D., Redmon, J. B., Thomas, W., & Raatz, S. K. (2011). Varying protein source and quantity do not significantly improve weight loss, fat loss, or satiety in reduced energy diets among midlife adults. *Nutrition Research, 31*(2), 104-112.

Allirot, X., Saulais, L., Disse, E., Nazare, J. A., Cazal, C., & Laville, M. (2013). Integrating behavioral measurements in physiological approaches of satiety. *Food Quality and Preference, 31*(0), 181-189. doi: http://dx.doi.org/10.1016/j.foodqual.2011.05.005

Almiron-Roig, E., Chen, Y., & Drewnowski, A. (2003). Liquid calories and the failure of satiety: how good is the evidence? *Obesity Reviews, 4*(4), 201-212. doi: 10.1046/j.1467-789X.2003.00112.x

Almiron-Roig, E., Palla, L., Guest, K., Ricchiuti, C., Vint, N., Jebb, S. A., & Drewnowski, A. (2013). Factors that determine energy compensation: a systematic review of preload studies. *Nutrition Reviews*.

Anderson, G. H. (1995). Sugars, sweetness, and food intake. *The American Journal of Clinical Nutrition, 62*(1), 195S-201S.

Anderson, G. H., Tecimer, S. N., Shah, D., & Zafar, T. A. (2004). Protein source, quantity, and time of consumption determine the effect of proteins on short-term food intake in young men. *The Journal of Nutrition, 134*(11), 3011-3015.

Appaiah, K. M. (2009). Monosodium Glutamate in Foods and its Biological Effects. In C. Boisrobert, A. Stjepanovic, S. Oh & H. Lelieveld (Eds.), *Ensuring Global Food Safety: Exploring Global Harmonization* (pp. 217-227). London: Academic Press.

Arciero, P. J., Gentile, C. L., Pressman, R., Everett, M., Ormsbee, M. J., Martin, J., . . . Vukovich, M. D. (2008). Moderate protein intake improves total and regional body composition and insulin sensitivity in overweight adults. *Metabolism, 57*(6), 757-765.

Austin, G. L., Ogden, L. G., & Hill, J. O. (2011). Trends in carbohydrate, fat, and protein intakes and association with energy intake in normal-weight, overweight, and obese individuals: 1971–2006. *The American Journal of Clinical Nutrition, 93*(4), 836-843. doi: 10.3945/ajcn.110.000141

Auvray, M., & Spence, C. (2008). The multisensory perception of flavor. *Consciousness and Cognition, 17*(3), 1016-1031. doi: http://dx.doi.org/10.1016/j.concog.2007.06.005

Baker, B. J., Booth, D. A., Duggan, J. P., & Gibson, E. L. (1987). Protein appetite demonstrated: Learned specificity of protein-cue preference to protein need in adult rats. *Nutrition Research, 7*(5), 481-487. doi: http://dx.doi.org/10.1016/S0271-5317(87)80004-0

Baryłko-Pikielna, N., & Kostyra, E. (2007). Sensory interaction of umami substances with model food matrices and its hedonic effect. *Food Quality and Preference, 18*(5), 751-758. doi: http://dx.doi.org/10.1016/j.foodqual.2007.01.002

Batterham, R. L., Heffron, H., Kapoor, S., Chivers, J. E., Chandarana, K., Herzog, H., . . . Withers, D. J. (2006). Critical role for peptide YY in protein-mediated satiation and body-weight regulation. *Cell metabolism, 4*(3), 223-233.

Bazzano, G., D'Elia, J. A., & Olson, R. E. (1970). Monosodium glutamate: feeding of large amounts in man and gerbils. *Science, 169*, 1208-1209. doi: 10.1126/science.169.3951.1208

Beauchamp, G. K. (2009). Sensory and receptor responses to umami: an overview of pioneering work. *American Journal of Clinical Nutrition, 90*(3), 723S-727S. doi: 10.3945/ajcn.2009.27462E

Beauchamp, G. K., & Mennella, J. A. (2009). Early Flavor Learning and Its Impact on Later Feeding Behavior. *Journal of Pediatric Gastroenterology and Nutrition, 48*, S25-S30 10.1097/MPG.1090b1013e31819774a31819775.

Beauchamp, G. K., & Pearson, P. B. (1991). Human development and umami taste. *Physiology & Behavior, 49*, 1009–1012.

Beglinger, C., Degen, L., Matzinger, D., D'Amato, M., & Drewe, J. (2001). Loxiglumide, a CCK-A receptor antagonist, stimulates calorie intake and hunger

feelings in humans. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 280*(4), R1149-R1154.

Bell, E. A., Castellanos, V. H., Pelkman, C. L., Thorwart, M. L., & Rolls, B. J. (1998). Energy density of foods affects energy intake in normal-weight women. *The American Journal of Clinical Nutrition, 67*(3), 412-420.

Bellisle, F. (1999). Glutamate and the UMAMI taste: sensory, metabolic, nutritional and behavioural considerations. A review of the literature published in the last 10 years. *Neuroscience and Biobehavioral Reviews, 23*(3), 423-438.

Bellisle, F., Dalix, A. M., Chappuis, A. S., Rossi, F., Fiquet, P., Gaudin, V., . . . Slama, G. (1996). Monosodium Glutamate Affects Mealtime Food Selection in Diabetic Patients. *Appetite, 26*(3), 267-276.

Bellisle, F., Dartois, A., & Broyer, M. (1992). Two studies on the acceptability of calcium glutamate as a potassium-free sodium substitute in children. *J. Renal Nutr, 2*(Suppl 1), 42-26.

Bellisle, F., & Drewnowski, A. (2007). Intense sweeteners, energy intake and the control of body weight. *European Journal of Clinical Nutrition, 61*(6), 691-700.

Bellisle, F., Monneuse, M. O., Chabert, M., Larue-Achagiotis, C., Lanteaume, M. T., & Louis-Sylvestre, J. (1991). Monosodium glutamate as a palatability enhancer in the european diet. *Physiology & Behavior, 49*(5), 869-873.

Bellisle, F., Tournier, A., & Louis-Sylvestre, J. (1989). Monosodium glutamate and the acquisition of food preferences in a European context. *Food Quality and Preference, 1*, 103-108.

Bellissimo, N., Desantadina, M., Pencharz, P., Berall, G., Thomas, S., & Anderson, G. (2007). A comparison of short-term appetite and energy intakes in normal weight and obese boys following glucose and whey-protein drinks. *International Journal of Obesity, 32*(2), 362-371.

Berridge, K. C. (1996). Food reward: Brain substrates of wanting and liking. *Neuroscience & Biobehavioral Reviews, 20*(1), 1-25. doi: http://dx.doi.org/10.1016/0149-7634(95)00033-B

Berridge, K. C. (2004). Motivation concepts in behavioral neuroscience. *Physiology & Behavior, 81*(2), 179-209. doi: http://dx.doi.org/10.1016/j.physbeh.2004.02.004

Bertenshaw, E. J., Lluch, A., & Yeomans, M. R. (2008). Satiating effects of protein but not carbohydrate consumed in a between-meal beverage context. *Physiology & Behavior, 93*(3), 427-436. doi: 10.1016/j.physbeh.2007.09.014

Bertenshaw, E. J., Lluch, A., & Yeomans, M. R. (2009). Dose-dependent effects of beverage protein content upon short-term intake. *Appetite, 52*(3), 580-587. doi: http://dx.doi.org/10.1016/j.appet.2009.01.010

Bertenshaw, E. J., Lluch, A., & Yeomans, M. R. (2013). Perceived thickness and creaminess modulates the short-term satiating effects of high-protein drinks. *British Journal of Nutrition, FirstView*, 1-9. doi: doi:10.1017/S0007114512005375

Berthoud, H.-R., & Morrison, C. (2008). The Brain, Appetite, and Obesity. *Annual Review of Psychology, 59*(1), 55-92. doi: doi:10.1146/annurev.psych.59.103006.093551

Bertino, M., Beauchamp, G. K., & Engelman, K. (1991). Naltrexone, an opioid blocker, alters taste perception and nutrient intake in humans. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 261*(1), R59-R63.

Bertrand, G., Gross, R., Puech, R., Loubatières-Mariani, M.-M., & Bockaert, J. (1993). Glutamate stimulates glucagon secretion via an excitatory amino acid receptor of the AMPA subtype in rat pancreas. *European journal of pharmacology, 237*(1), 45-50.

Bezençon, C., le Coutre, J., & Damak, S. (2007). Taste-signaling proteins are coexpressed in solitary intestinal epithelial cells. *Chemical Senses, 32*(1), 41-49.

Blatt, A. D., Roe, L. S., & Rolls, B. J. (2011). Increasing the Protein Content of Meals and Its Effect on Daily Energy Intake. *Journal of the American Dietetic Association, 111*(2), 290-294.

Blom, W. A., Lluch, A., Stafleu, A., Vinoy, S., Holst, J. J., Schaafsma, G., & Hendriks, H. F. (2006). Effect of a high-protein breakfast on the postprandial ghrelin response. *The American Journal of Clinical Nutrition, 83*(2), 211-220.

Blundell, J., De Graaf, C., Hulshof, T., Jebb, S., Livingstone, B., Lluch, A., . . . Westerterp, M. (2010). Appetite control: methodological aspects of the evaluation of foods. *Obesity Reviews, 11*(3), 251-270. doi: 10.1111/j.1467-789X.2010.00714.x

Blundell, J., E., & Stubbs, R. J. (1999). High and low carbohydrate and fat intakes: limits imposed by appetite and palatability and their implications for energy balance. *European Journal of Clinical Nutrition, 53*, S148- S165.

Blundell, J. E., De Graaf, C., Finlayson, G., Halford, J. C. G., Hetherington, M. M., King, N., & Stubbs, R. J. (2009). Chapter 8: Measuring Food Intake, Hunger, Satiety and Satiation in the Laboratory. In D. B. Allison & L. B. Monica (Eds.), *Handbook of Assessment Methods for Eating Behaviors and Weight-Related Problems. Measures, Theory and Research* (pp. 283-326). California, United States of America: SAGE Publications, Inc. (Reprinted from: Second Edition).

Blundell, J. E., Goodson, S., & Halford, J. C. G. (2001). Regulation of appetite: role of leptin in signalling systems for drive and satiety. *International Journal of Obesity, 25*, s29-s34.

Blundell, J. E., & Greenough, A. (1994). Pharmacological aspects of appetite: implications for the treatment of obesity. *Biomedicine & Pharmacotherapy, 48*(3–4), 119-125. doi: http://dx.doi.org/10.1016/0753-3322(94)90100-7

Blundell, J. E., Lawton, C. L., Cotton, J. R., & Macdiarmid, J. I. (1996). Control of Human Appetite: Implications for The Intake of Dietary Fat. *Annual Review of Nutrition, 16*(1), 285-319. doi: doi:10.1146/annurev.nu.16.070196.001441

Blundell, J. E., & Rogers, P. J. (1980). Effects of anorexie drugs on food intake, food selection and preferences and hunger motivation and subjective experiences. *Appetite, 1*(2), 151-165.

Blundell, J. E., Rogers, P. J., & Hill, A. J. (1987). *Evaluating the satiety power of foods: Implications for acceptance and consumption*. London: Academic Press.

Boirie, Y., Dangin, M., Gachon, P., Vasson, M.-P., Maubois, J.-L., & Beaufrère, B. (1997). Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proceedings of the National Academy of Sciences, 94*(26), 14930-14935.

Booth, D. (1985). Food-conditioned Eating Preferences and Aversions with Interoceptive Elements: Conditioned Appetites and Satieties. *Annals of the New York Academy of Sciences, 443*(1), 22-41.

Booth, D. A., & Baker, B. J. (1990). dl-Fenfluramine challenge to nutrient-specific textural preference conditioned by concurrent presentation of two diets. *Behavioral Neuroscience, 104*(1), 226.

Booth, D. A., Toates, F. M., & Platt, S. V. (1976). Control system for hunger and its implications in animals and man. In D. Novin, W. Wyricka & G. Bray (Eds.), *Hunger: basic mechanisms and clinical implications*. New York: Raven Press.

Bowen, J., Noakes, M., Trenerry, C., & Clifton, P. M. (2006). Energy intake, ghrelin, and cholecystokinin after different carbohydrate and protein preloads in overweight men. *Journal of Clinical Endocrinology & Metabolism, 91*(4), 1477-1483.

Brand, J. G. (2000). Receptor and Transduction Processes for Umami Taste. *The Journal of Nutrition, 130*(4), 942.

Brand, J. G., Teeter, J. H., Kumazawa, T., Huque, T., & Bayley, D. L. (1991). Transduction mechanisms for the taste of amino acids. [doi: 10.1016/0031-9384(91)90201-X]. *Physiology & Behavior, 49*(5), 899-904.

Brinkworth, G. D., Noakes, M., Keogh, J. B., Luscombe, N. D., Wittert, G. A., & Clifton, P. M. (2004). Long-term effects of a high-protein, low-carbohydrate diet on weight control and cardiovascular risk markers in obese hyperinsulinemic subjects. *Int J Obes Relat Metab Disord, 28*(5), 661-670.

Brunstrom, J. M., & Fletcher, H. Z. (2008). Flavour–flavour learning occurs automatically and only in hungry participants. *Physiology & Behavior, 93*(1–2), 13-19. doi: http://dx.doi.org/10.1016/j.physbeh.2007.07.009

Brunstrom, J. M., & Rogers, P. J. (2009). How Many Calories Are on Our Plate? Expected Fullness, Not Liking, Determines Meal-size Selection. *Obesity, 17*(10), 1884-1890.

Brunstrom, J. M., & Shakeshaft, N. G. (2009). Measuring affective (liking) and non-affective (expected satiety) determinants of portion size and food reward. *Appetite, 52*, 108-114.

Brunstrom, J. M., Shakeshaft, N. G., & Scott-Samuel, N. E. (2008). Measuring 'expected satiety' in a range of common foods using a method of constant stimuli. *Appetite, 51*(3), 604-614. doi: http://dx.doi.org/10.1016/j.appet.2008.04.017

Cabanac, M. (1971). Physiological role of pleasure. . *Science 173*, 1103–1107.

Carter, B. E., Monsivais, P., Perrigue, M. M., & Drewnowski, A. (2011). Supplementing chicken broth with monosodium glutamate reduces hunger and desire to snack but does not affect energy intake in women. *British Journal of Nutrition, 106*(9), 1441.

Cecil, J. E., Francis, J., & Read, N. W. (1999). Comparison of the Effects of a High-Fat and High-Carbohydrate Soup Delivered Orally and Intragastrically on Gastric

Emptying, Appetite, and Eating Behaviour. [doi: 10.1016/S0031-9384(99)00069-4]. *Physiology & Behavior, 67*(2), 299-306.

Chalé-Rush, A., Burgess, J. R., & Mattes, R. D. (2007). Multiple routes of chemosensitivity to free fatty acids in humans. *American Journal of Physiology-Gastrointestinal and Liver Physiology, 292*(5), G1206-G1212.

Chambers, L., & Yeomans, M. R. (2011). Individual differences in satiety response to carbohydrate and fat. Predictions from the Three Factor Eating Questionnaire (TFEQ). *Appetite, 56*(2), 316-323. doi: 10.1016/j.appet.2011.01.003

Chandrashekar, J., Hoon, M. A., Ryba, N. J. P., & Zuker, C. S. (2006). The receptors and cells for mammalian taste. [10.1038/nature05401]. *Nature, 444*(7117), 288-294.

Chandrashekar, J., Kuhn, C., Oka, Y., Yarmolinsky, D. A., Hummler, E., Ryba, N. J. P., & Zuker, C. S. (2010). The cells and peripheral representation of sodium taste in mice. [10.1038/nature08783]. *Nature, 464*(7286), 297-301. doi: http://www.nature.com/nature/journal/v464/n7286/suppinfo/nature08783_S1.html

Chaudhari, N., Landin, A. M., & Roper, S. D. (2000). A metabotropic glutamate receptor variant functions as a taste receptor. *Nature Neuroscience 3*, 113-119.

Chaudhari, N., Pereira, E., & Roper, S. D. (2009). Taste receptors for umami: the case for multiple receptors. *American Journal of Clinical Nutrition, 90*(3), 738S-742S. doi: 10.3945/ajcn.2009.27462H

Chaudhari, N., Yang, H., Lamp, C., Delay, E., Cartford, C., Than, T., & Roper, S. (1996). The taste of monosodium glutamate: membrane receptors in taste buds. *Journal of Neuroscience, 16*, 3817–3826.

Chung Chun Lam, S., Moughan, P. J., Awati, A., & Morton, H. R. (2009). The influence of whey protein and glycomacropeptide on satiety in adult humans. *Physiology & Behavior, 96*(1), 162-168.

Chung Chun Lam, S., Moughan, P. J., Henare, S. J., & Ganesh, S. (2012). Effect of time of consumption of preloads on measures of satiety in healthy normal weight women. *Appetite, 59*(2), 281-288.

Cordoba, J. J., Antequera, T., Garcia, C., Ventanas, J., Lopez Bote, C., & Asensio, M. A. (1994). Evolution of free amino acids and amines during ripening of Iberian cured ham. *Journal of Agricultural and Food Chemistry, 42*(10), 2296-2301. doi: 10.1021/jf00046a040

Corwin, R. L., Wojnicki, F. H. E., Fisher, J. O., Dimitriou, S. G., Rice, H. B., & Young, M. A. (1998). Limited Access to a Dietary Fat Option Affects Ingestive Behavior But Not Body Composition in Male Rats. *Physiology & Behavior, 65*(3), 545-553. doi: http://dx.doi.org/10.1016/S0031-9384(98)00201-7

Cotton, J. R., Burley, V. J., Weststrate, J. A., & Bhmdell, J. E. (1994). Dietary fat and appetite: similarities and differences in the satiating effect of meals supplemented with either fat or carbohydrate*†. *Journal of Human Nutrition and Dietetics, 7*(1), 11-24. doi: 10.1111/j.1365-277X.1994.tb00402.x

Cox, D. N., Perry, L., Moore, P. B., Vallis, L., & Mela, D. J. (1999). Sensory and hedonic associations with macronutrient and energy intakes of lean and obese consumers. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity, 23*(4), 403-410.

Crovetti, R., Porrini, M., Santangelo, A., & Testolin, G. (1998). The influence of thermic effect of food on satiety. *European Journal of Clinical Nutrition, 52*, 482.

Curtis, R. I. (2009). Umami and the foods of classical antiquity. *American Journal of Clinical Nutrition, 90*(3), 712S-718S. doi: 10.3945/ajcn.2009.27462C

Damak, S., Rong, M., Yasumatsu, K., Kokrashvili, Z., Varadarajan, V., Zou, S., . . . Margolskee, R. F. (2003). Detection of Sweet and Umami Taste in the Absence of Taste Receptor T1r3. *Science, 301*(5634), 850-853. doi: 10.1126/science.1087155

Dangin, M., Boirie, Y., Garcia-Rodenas, C., Gachon, P., Fauquant, J., Callier, P., . . . Beaufrère, B. (2001). The digestion rate of protein is an independent regulating factor of postprandial protein retention. *American Journal of Physiology-Endocrinology And Metabolism, 280*(2), E340-E348.

Dangin, M., Boirie, Y., Guillet, C., & Beaufrère, B. (2002). Influence of the protein digestion rate on protein turnover in young and elderly subjects. *The Journal of Nutrition, 132*(10), 3228S-3233S.

de Araujo, I. E. T., Kringelbach, M. L., Rolls, E. T., & Hobden, P. (2003). Representation of umami taste in the human brain. [Article]. *Journal of Neurophysiology, 90*(1), 313-319. doi: 10.1152/jn.00669.2002

de Castro, J. M. (2006). Macronutrient and dietary energy density influences on the intake of free-living humans. *Appetite, 46*(1), 1-5. doi: http://dx.doi.org/10.1016/j.appet.2005.05.005

de Castro, J. M., Bellisle, F., & Dalix, A.-M. (2000). Palatability and intake relationships in free-living humans: measurement and characterization in the French. *Physiology & Behavior, 68*(3), 271-277. doi: http://dx.doi.org/10.1016/S0031-9384(99)00166-3

de Castro, J. M., Bellisle, F., Dalix, A.-M., & Pearcey, S. M. (2000). Palatability and intake relationships in free-living humans: characterization and independence of influence in North Americans. *Physiology & Behavior, 70*(3–4), 343-350. doi: http://dx.doi.org/10.1016/S0031-9384(00)00264-X

De Graaf, C., De Jong, L. S., & Lambers, A. C. (1999). Palatability Affects Satiation But Not Satiety. *Physiology & Behavior, 66*(4), 681-688. doi: http://dx.doi.org/10.1016/S0031-9384(98)00335-7

de Graaf, C., Schreurs, A., & Blauw, Y. H. (1993). Short-term effects of different amounts of sweet and nonsweet carbohydrates on satiety and energy intake. [doi: 10.1016/0031-9384(93)90290-V]. *Physiology & Behavior, 54*(5), 833-843.

Degen, L., Matzinger, D., Drewe, J., & Beglinger, C. (2001). The effect of cholecystokinin in controlling appetite and food intake in humans. *Peptides, 22*(8), 1265-1269.

DiBattista, D. (1991). Effects of time-restricted access to protein and to carbohydrate in adult mice and rats. *Physiology & Behavior, 49*(2), 263-269. doi: http://dx.doi.org/10.1016/0031-9384(91)90042-M

Donaldson, L. F., Bennett, L., Baic, S., & Melichar, J. K. (2009). Taste and weight: is there a link? *American Journal of Clinical Nutrition, 90*(3), 800S-803S. doi: 10.3945/ajcn.2009.27462Q

Drewnowski, A. (1997). Taste preferences and food intake. *Annual Review of Nutrition, 17*(1), 237-253. doi: 10.1146/annurev.nutr.17.1.237

Drewnowski, A., Krahn, D. D., Demitrack, M. A., Nairn, K., & Gosnell, B. A. (1992). Taste responses and preferences for sweet high-fat foods: Evidence for opioid involvement. *Physiology & Behavior, 51*(2), 371-379. doi: http://dx.doi.org/10.1016/0031-9384(92)90155-U

Egan, J. M., & Margolskee, R. F. (2008). Taste cells of the gut and gastrointestinal chemosensation. *Molecular Interventions, 8*, 78–81.

Eller, L. K., Ainslie, P. N., Poulin, M. J., & Reimer, R. A. (2008). Differential responses of circulating amylin to high-fat vs. high-carbohydrate meal in healthy men. *Clinical endocrinology, 68*(6), 890-897.

Essed, N. H., van Staveren, W. A., Kok, F. J., & de Graaf, C. (2007). No effect of 16 weeks flavor enhancement on dietary intake and nutritional status of nursing home elderly. [doi: 10.1016/j.appet.2006.06.002]. *Appetite, 48*(1), 29-36.

Fantino, M., Hosotte, J., & Apfelbaum, M. (1986). An opioid antagonist, naltrexone, reduces preference for sucrose in humans. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 251*(1), R91-R96.

Fischer, K., Colombani, P. C., & Wenk, C. (2004). Metabolic and cognitive coefficients in the development of hunger sensations after pure macronutrient ingestion in the morning. [doi: 10.1016/S0195-6663(03)00116-8]. *Appetite, 42*(1), 49-61.

Flaherty, C. (1982). Incentive contrast: A review of behavioral changes following shifts in reward. *Animal Learning & Behavior, 10*(4), 409-440. doi: 10.3758/bf03212282

Flaherty, C. F., Coppotelli, C., Grigson, P. S., Mitchell, C., & Flaherty, J. E. (1995). Investigation of the devaluation interpretation of anticipatory negative contrast. *Journal of Experimental Psychology: Animal Behavior Processes, 21*(3), 229.

Foster, D. (Ed.). (1955). *Taste testing methodology concerning monosodium glutamate.* (Vol. The Quarter Masters Food and Container Institute for the Armed Forces). Chicago, IL.

Foster, G. D., Wyatt, H. R., Hill, J. O., McGuckin, B. G., Brill, C., Mohammed, B. S., . . . Klein, S. (2003). A randomized trial of a low-carbohydrate diet for obesity. *New England Journal of Medicine, 348*(21), 2082-2090.

FSA. (2006). *FSA Nutrient and Food Based Guidelines for UK Institutions*. London.

Fuke, S., & Konosu, S. (1991). Taste-active components in some foods: A review of Japanese research. *Physiology & Behavior, 49*(5), 863-868. doi: http://dx.doi.org/10.1016/0031-9384(91)90195-T

Fürst, P., & Stehle, P. (2004). What Are the Essential Elements Needed for the Determination of Amino Acid Requirements in Humans? *The Journal of Nutrition, 134*(6), 1558S-1565S.

Gabriel, A. S., Maekawa, T., Uneyama, H., & Torii, K. (2009). Metabotropic glutamate receptor type 1 in taste tissue. *American Journal of Clinical Nutrition, 90*(3), 743S-746S. doi: 10.3945/ajcn.2009.27462I

Garcia-Bailo, B., Toguri, C., Eny, K. M., & El-Sohemy, A. (2009). Genetic Variation in Taste and Its Influence on Food Selection *OMICS: A Journal of Integrative Biology, 13*(1), 69-80. doi: 10.1089/omi.2008.0031

Geha, R. S., Beiser, A., Ren, C., Patterson, R., Greenberger, P. A., Grammer, L. C., . . . Saxon, A. (2000). Review of Alleged Reaction to Monosodium Glutamate and Outcome of a Multicenter Double-Blind Placebo-Controlled Study. *The Journal of Nutrition, 130*(4), 1058.

Gibson, E. L., & Booth, D. A. (1986). Acquired protein appetite in rats: Dependence on a protein-specific need state. *Experientia, 42*(9), 1003-1004. doi: 10.1007/bf01940706

Gibson, E. L., & Brunstrom, J. M. (Eds.). (2007). *Learned influences on appetite and food intake: Evidence in human beings.* London: Elsevier

Gibson, E. L., Wainwright, C. J., & Booth, D. A. (1995). Disguised protein in lunch after low-protein breakfast conditions food-flavor preferences dependent on recent lack of protein intake. [doi: 10.1016/0031-9384(95)00068-T]. *Physiology & Behavior, 58*(2), 363-371.

Gibson, E. L., & Wardle, J. (2001). Effect of contingent hunger state on development of appetite for a novel fruit snack. *Appetite, 37*(2), 91-101. doi: http://dx.doi.org/10.1006/appe.2001.0420

Ginane, C., Baumont, R., & Favreau-Peigné, A. (2011). Perception and hedonic value of basic tastes in domestic ruminants. [doi: 10.1016/j.physbeh.2011.07.011]. *Physiology & Behavior, 104*(5), 666-674.

Glendinning, J. I., Bloom, L. D., Onishi, M., Zheng, K. H., Damak, S., Margolskee, R. F., & Spector, A. C. (2005). Contribution of α-Gustducin to Taste-guided Licking Responses of Mice. *Chemical Senses, 30*(4), 299-316. doi: 10.1093/chemse/bji025

Gosby, A. K., Conigrave, A. D., Lau, N. S., Iglesias, M. A., Hall, R. M., Jebb, S. A., . . . Simpson, S. J. (2011). Testing Protein Leverage in Lean Humans: A Randomised Controlled Experimental Study. [doi:10.1371/journal.pone.0025929]. *PLoS ONE, 6*(10), e25929.

Grabenhorst, F., Rolls, E. T., & Bilderbeck, A. (2008). How Cognition Modulates Affective Responses to Taste and Flavor: Top-down Influences on the Orbitofrontal and Pregenual Cingulate Cortices. *Cerebral Cortex, 18*(7), 1549-1559. doi: 10.1093/cercor/bhm185

Griffioen-Roose, S., Mars, M., Finlayson, G., Blundell, J. E., & de Graaf, C. (2011). The effect of within-meal protein content and taste on subsequent food choice and satiety. *Br J Nutr, 106*(05), 779-788.

Guy-Grand, B., Lehnert, V., Doassans, M., & Bellisle, F. (1994). Type of Test-meal Affects Palatability and Eating Style in Humans. *Appetite, 22*(2), 125-134. doi: http://dx.doi.org/10.1006/appe.1994.1012

Halford, J. C. G., Boyland, E. J., Blundell, J. E., Kirkham, T. C., & Harrold, J. A. (2010). Pharmacological management of appetite expression in obesity. [10.1038/nrendo.2010.19]. *Nat Rev Endocrinol, 6*(5), 255-269.

Halford, J. C. G., & Harrold, J. A. (2012). Satiety-enhancing products for appetite control: science and regulation of functional foods for weight management. *Proceedings of the Nutrition Society, 71*(02), 350-362. doi: doi:10.1017/S0029665112000134

Hall, W., Millward, D., Long, S., & Morgan, L. (2003). Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. *British Journal of Nutrition, 89*(2), 239-248.

Halpern, B. P. (2000). Glutamate and the Flavor of Foods. *Journal of Nutrition, 130*(4), 910-.

Halton, T. L., & Hu, F. B. (2004). The Effects of High Protein Diets on Thermogenesis, Satiety and Weight Loss: A Critical Review. *Journal of the American College of Nutrition, 23*(5), 373-385.

Harnack, L., Stang, J., & Story, M. (1999). Soft Drink Consumption Among US Children and Adolescents: Nutritional Consequences. *Journal of the American Dietetic Association, 99*(4), 436-441. doi: http://dx.doi.org/10.1016/S0002-8223(99)00106-6

Harper, A., James, A., Flint, A., & Astrup, A. (2007). Increased satiety after intake of a chocolate milk drink compared with a carbonated beverage, but no difference in subsequent ad libitum lunch intake. *British Journal of Nutrition, 97*(03), 579-583. doi: doi:10.1017/S0007114507339846

He, K., Zhao, L., Daviglus, M. L., Dyer, A. R., Van Horn, L., Garside, D., . . . Group, I. C. R. (2008). Association of Monosodium Glutamate Intake With Overweight in Chinese Adults: The INTERMAP Study. *Obesity, 16*(8), 1875-1880. doi: 10.1038/oby.2008.274

He, W., Yasumatsu, K., Varadarajan, V., Yamada, A., Lem, J., Ninomiya, Y., . . . Damak, S. (2004). Umami Taste Responses Are Mediated by α-Transducin and α-Gustducin. *The Journal of Neuroscience, 24*(35), 7674-7680. doi: 10.1523/jneurosci.2441-04.2004

Hermanussen, M., Garcia, A. P., Sunder, M., Voigt, M., Salazar, V., & Tresguerres, J. A. F. (2005). Obesity, voracity, and short stature: the impact of glutamate on the regulation of appetite. *European Journal of Clinical Nutrition, 60*(1), 25-31.

Hill, A. J., Magson, L. D., & Blundell, J. E. (1984). Hunger and palatability: Tracking ratings of subjective experience before, during and after the consumption of preferred and less preferred food. *Appetite, 5*(4), 361-371. doi: http://dx.doi.org/10.1016/S0195-6663(84)80008-2

Ikeda, K. (1908). On a New Seasoning. *Journal of Tokyo Chemistry Society, 30*, 820-836.

Ishii, R., & O'Mahony, M. (1990). Group taste concept measurement, verbal and physical definition of the umami taste concept for Japanese and Americans. *Journal of Sensory Studies, 4*(4), 215-227. doi: 10.1111/j.1745-459X.1990.tb00473.x

Jinap, S., & Hajeb, P. (2010). Glutamate. Its applications in food and contribution to health. *Appetite, 55*(1), 1-10. doi: 10.1016/j.appet.2010.05.002

Johnson, J., & Vickers, Z. (1993). Effects of flavor and macronutrient composition of food servings on liking, hunger and subsequent intake. . *Appetite, 21*(1), 25–39.

Johnson, S. L., McPhee, L., & Birch, L. L. (1991). Conditioned preferences: Young children prefer flavors associated with high dietary fat. *Physiology & Behavior, 50*(6), 1245-1251. doi: http://dx.doi.org/10.1016/0031-9384(91)90590-K

Johnstone, A. M., Horgan, G. W., Murison, S. D., Bremner, D. M., & Lobley, G. E. (2008). Effects of a high-protein ketogenic diet on hunger, appetite, and weight loss in obese men feeding ad libitum. *The American Journal of Clinical Nutrition, 87*(1), 44-55.

Kant, R. (2005). Sweet proteins – Potential replacement for artificial low calorie sweeteners. *Nutrition Journal 4*, 5.

Karhunen, L., Juvonen, K., Huotari, A., Purhonen, A., & Herzig, K. (2008). Effect of protein, fat, carbohydrate and fibre on gastrointestinal peptide release in humans. *Regulatory peptides, 149*(1), 70-78.

Kelley, A. E. (2004). Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning. *Neuroscience & Biobehavioral Reviews, 27*(8), 765-776.

Kern, D. L., McPhee, L., Fisher, J., Johnson, S., & Birch, L. L. (1993). The postingestive consequences of fat condition preferences for flavors associated

with high dietary fat. *Physiology & Behavior, 54*(1), 71-76. doi: http://dx.doi.org/10.1016/0031-9384(93)90045-H

Kissileff, H. R., Gruss, L. P., Thornton, J., & Jordan, H. A. (1984). The satiating efficiency of foods. *Physiology & Behavior, 32*(2), 319-332.

Kobayashi, C., & Kennedy, L. M. (2002). Experience-induced changes in taste identification of monosodium glutamate. [doi: 10.1016/S0031-9384(01)00634-5]. *Physiology & Behavior, 75*(1–2), 57-63.

Kodama, S. (1913). On a procedure for separating inosinic acid. *Journal of the Tokyo Chemical Society, 34*, 751.

Kondoh, T., Mallick, H. N., & Torii, K. (2009). Activation of the gut-brain axis by dietary glutamate and physiologic significance in energy homeostasis. *American Journal of Clinical Nutrition, 90*(3), 832S-837S. doi: 10.3945/ajcn.2009.27462V

Kondoh, T., Mori, M., Ono, T., & Torii, K. (2000). Mechanisms of Umami Taste Preference and Aversion in Rats. *Journal of Nutrition, 130*(4), 966-.

Kondoh, T., & Torii, K. (2008). MSG intake suppresses weight gain, fat deposition, and plasma leptin levels in male Sprague-Dawley rats. . *Physiology & Behavior, 95*, 135–144.

Krause, E. G., & Sakai, R. R. (2007). Richter and sodium appetite: From adrenalectomy to molecular biology. *Appetite, 49*(2), 353-367. doi: http://dx.doi.org/10.1016/j.appet.2007.01.015

Krebs, J. R. (2009). The gourmet ape: evolution and human food preferences. [Proceedings Paper]. *American Journal of Clinical Nutrition, 90*(3), 707S-711S. doi: 10.3945/ajcn.2009.27462B

Kumazawa, T., Nakamura, M., & Kurihara, K. (1991). Canine taste nerve responses to umami substances. *Physiology & Behavior, 49*(5), 875-881. doi: http://dx.doi.org/10.1016/0031-9384(91)90197-V

Kuninaka, A. (1960). Studies on taste of ribonucleic acid derivatives. *Journal of the Agricultural Chemical Society of Japan, 34*, 487–492.

Kurihara, K. (2009). Glutamate: from discovery as a food flavor to role as a basic taste (umami). *American Journal of Clinical Nutrition, 90*(3), 719S-722S. doi: 10.3945/ajcn.2009.27462D

Kwok, R. H. M. (1968). Chinese restaurant syndrome. *New England Journal of Medicine, 18*(178), 796.

L'Heureux-Bouron, D., Tomé, D., Bensaid, A., Morens, C., Lacroix, M., Huneau, J. F., & Fromentin, G. (2004). Preabsorptive factors are not the main determinants of intake depression induced by a high-protein diet in the rat. *Physiology & Behavior, 81*(3), 499-504.

Larsen, T. M., Dalskov, S.-M., van Baak, M., Jebb, S. A., Papadaki, A., Pfeiffer, A. F. H., . . . Astrup, A. (2010). Diets with High or Low Protein Content and Glycemic Index for Weight-Loss Maintenance. *New England Journal of Medicine, 363*(22), 2102-2113. doi: doi:10.1056/NEJMoa1007137

Laska, M., & Hernandez Salazar, L. T. (2004). Gustatory responsiveness to monosodium glutamate and sodium chloride in four species of nonhuman primates. *Journal of Experimental Zoology Part A: Comparative Experimental Biology, 301A*(11), 898-905. doi: 10.1002/jez.a.118

Latner, J. D., & Schwartz, M. (1999). The Effects of a High-carbohydrate, High-protein or Balanced Lunch upon Later Food Intake and Hunger Ratings. *Appetite, 33*(1), 119-128. doi: http://dx.doi.org/10.1006/appe.1999.0237

Layman, D. K., Boileau, R. A., Erickson, D. J., Painter, J. E., Shiue, H., Sather, C., & Christou, D. D. (2003). A Reduced Ratio of Dietary Carbohydrate to Protein Improves Body Composition and Blood Lipid Profiles during Weight Loss in Adult Women. *The Journal of Nutrition, 133*(2), 411-417.

Ledikwe, J. H., Blanck, H. M., Kettel Khan, L., Serdula, M. K., Seymour, J. D., Tohill, B. C., & Rolls, B. J. (2006). Dietary energy density is associated with energy intake and weight status in US adults. *The American Journal of Clinical Nutrition, 83*(6), 1362-1368.

Leidy, H. J., Tang, M., Armstrong, C. L. H., Martin, C. B., & Campbell, W. W. (2011). The Effects of Consuming Frequent, Higher Protein Meals on Appetite and Satiety During Weight Loss in Overweight/Obese Men. *Obesity, 19*(4), 818-824.

Lejeune, M. P., Westerterp, K. R., Adam, T. C., Luscombe-Marsh, N. D., & Westerterp-Plantenga, M. S. (2006). Ghrelin and glucagon-like peptide 1 concentrations, 24-h satiety, and energy and substrate metabolism during a high-protein diet and measured in a respiration chamber. *The American Journal of Clinical Nutrition, 83*(1), 89-94.

Lejeune, M. P. G. M., Kovacs, E. M. R., & Westerterp-Plantenga, M. S. (2005). Additional protein intake limits weight regain after weight loss in humans. *British Journal of Nutrition, 93*(02), 281-289. doi: doi:10.1079/BJN20041305

Lejeune, M. P. G. M., Smeets, A. J. P. G., & Westerterp-Plantenga, M. S. (2007). Effects of a high-protein diet with or without monosodium-glutamate in combination with inosine-monophosphate-5 on 24-h energy metabolism and appetite profile. [doi: 10.1016/j.appet.2007.03.120]. *Appetite, 49*(1), 308.

Li, X., Staszewski, L., Xu, H., Durick, K., Zoller, M., & Adler, E. (2002). Human receptors for sweet and umami taste. *Proceedings of the National Academy of Sciences, 99*(7), 4692-4696. doi: 10.1073/pnas.072090199

Li, X. D. (2009). T1R receptors mediate mammalian sweet and umami taste. *American Journal of Clinical Nutrition, 90*(3), 733S-737S. doi: 10.3945/ajcn.2009.27462G

Lindemann, B. (1996). Taste reception. *Physiological Reviews, 76*(3), 719-766.

Lindemann, B. (2000). A taste for umami. *Nature Neuroscience, 3*(2), 99-100.

Little, T. J., & Feinle-Bisset, C. (2011). Effects of dietary fat on appetite and energy intake in health and obesity—oral and gastrointestinal sensory contributions. *Physiology & Behavior, 104*(4), 613-620.

Loliger, J. (2000). Function and importance of glutamate for savory foods. *Journal of Nutrition, 130*(4), 915S-920S.

Long, S. J., Griffiths, E. M., Rogers, P. J., & Morgan, L. M. (2000). Ad libitum food intake as a measure of satiety: comparison of a single food test meal and a mixed food buffet. *Proceedings of the Nutrition Society, 59*, A7.

Luhovyy, B. L., Akhavan, T., & Anderson, G. H. (2007). Whey Proteins in the Regulation of Food Intake and Satiety. *Journal of the American College of Nutrition, 26*(6), 704S-712S.

Luscombe-Marsh, N. D., Smeets, A. J. P. G., & Westerterp-Plantenga, M. S. (2008). Taste sensitivity for monosodium glutamate and an increased liking of dietary protein. *British Journal of Nutrition, 99*(4), 904-908. doi: 10.1017/s000711450788295x

Luscombe-Marsh, N. D., Smeets, A. J. P. G., & Westerterp-Plantenga, M. S. (2009). The addition of monosodium glutamate and inosine monophosphate-5 to high-protein meals: effects on satiety, and energy and macronutrient intakes. *British Journal of Nutrition, 102*(6), 929-937. doi: 10.1017/s0007114509297212

Marmonier, C., Chapelot, D., & Louis-Sylvestre, J. (2000). Effects of macronutrient content and energy density of snacks consumed in a satiety state on the onset of the next meal. *Appetite, 34*(2), 161-168. doi: http://dx.doi.org/10.1006/appe.1999.0302

Mathey, M.-F. A. M., Siebelink, E., de Graaf, C., & Van Staveren, W. A. (2001). Flavor Enhancement of Food Improves Dietary Intake and Nutritional Status of Elderly Nursing Home Residents. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 56*(4), M200-M205. doi: 10.1093/gerona/56.4.M200

Mattes, R. D. (2009). Is there a fatty acid taste? *Annual Review of Nutrition, 29*, 305.

McCabe, C., & Rolls, E. T. (2007). Umami: a delicious flavor formed by convergence of taste and olfactory pathways in the human brain. *European Journal of Neuroscience, 25*(6), 1855-1864. doi: 10.1111/j.1460-9568.2007.05445.x

McCrory, M. A., Fuss, P. J., Saltzman, E., & Roberts, S. B. (2000). Dietary Determinants of Energy Intake and Weight Regulation in Healthy Adults. *The Journal of Nutrition, 130*(2), 276.

McGuire, M. T., Wing, R. R., Klem, M. L., Lang, W., & Hill, J. O. (1999). What predicts weight regain in a group of successful weight losers? *Journal of Consulting and Clinical Psychology, 67*(2), 177-185. doi: 10.1037/0022-006x.67.2.177

Mennella, J. A., & Beauchamp, G. K. (1996). Developmental Changes in the Acceptance of Protein Hydrolysate Formula. *Journal of Developmental & Behavioral Pediatrics, 17*(6), 386-391.

Mennella, J. A., Ventura, A. K., & Beauchamp, G. K. (2011). Differential growth patterns among healthy infants fed protein hydrolysate or cow-milk formulas. *Pediatrics, 127*(1), 110-118.

Mobini, S., Chambers, L. C., & Yeomans, M. R. (2007). Effects of hunger state on flavour pleasantness conditioning at home: Flavour–nutrient learning vs. flavour–flavour learning. *Appetite, 48*(1), 20-28. doi: http://dx.doi.org/10.1016/j.appet.2006.05.017

Monneuse, M. O., Bellisle, F., & Louis-Sylvestre, J. (1991). Responses to an intense sweetener in humans: Immediate preference and delayed effects on intake. *Physiology & Behavior, 49*(2), 325-330. doi: http://dx.doi.org/10.1016/0031-9384(91)90051-O

Moran, T. H. (2000). Cholecystokinin and satiety: current perspectives. *Nutrition, 16*(10), 858-865. doi: http://dx.doi.org/10.1016/S0899-9007(00)00419-6

Mori, M., Kawada, T., Ono, T., & Torii, K. (1991). Taste preference and protein nutrition and L-amino acid homeostasis in male Sprague–Dawley rats. . *Physiology & Behavior, 49*, 987–995.

Mozaffarian, D., Hao, T., Rimm, E. B., Willett, W. C., & Hu, F. B. (2011). Changes in Diet and Lifestyle and Long-Term Weight Gain in Women and Men. *New England Journal of Medicine, 364*(25), 2392-2404. doi: doi:10.1056/NEJMoa1014296

Murphy, C. (Ed.). (1987). *Flavor preference for monosodium glutamate and casein hydrolysate in young and elderly persons.* . New York: Marcel Dekker.

Myers, K. P., Taddeo, M. S., & Richards, E. K. (2013). Sensory-specific appetition: Postingestive detection of glucose rapidly promotes continued consumption of a recently encountered flavor. *Physiology & Behavior*(0). doi: http://dx.doi.org/10.1016/j.physbeh.2013.03.021

Naim, M., Ohara, I., Kare, M. R., & Levinson, M. (1991). Interaction of MSG taste with nutrition: Perspectives in consummatory behavior and digestion. . *Physiology & Behavior, 49*, 1019–1024.

Nelson, G., Chandrashekar, J., Hoon, M. A., Feng, L., Zhao, G., Ryba, N. J. P., & Zuker, C. S. (2002). An amino-acid taste receptor. [10.1038/nature726]. *Nature, 416*(6877), 199-202.

Niijima, A. (2000). Reflex Effects of Oral, Gastrointestinal and Hepatoportal Glutamate Sensors on Vagal Nerve Activity. *The Journal of Nutrition, 130*(4), 971.

Ninomiya, K. (1998). Natural occurrence *Food Reviews International, 14*(2), 177-211.

Ninomiya, Y., Tanimukai, T., Yoshida, S., & Funakoshi, M. (1991). Gustatory neural responses in preweanling mice. *Physiology & Behavior, 49*(5), 913-918. doi: http://dx.doi.org/10.1016/0031-9384(91)90203-Z

O'Doherty, J., Rolls, E. T., Francis, S., Bowtell, R., & McGlone, F. (2001). Representation of Pleasant and Aversive Taste in the Human Brain. *Journal of Neurophysiology, 85*(3), 1315-1321.

Okiyama, A., & Beauchamp, G. K. (1998). Taste dimensions of monosodium glutamate (MSG) in a food system: role of glutamate in young American subjects. *Physiology & Behavior, 65*(1), 177-181. doi: http://dx.doi.org/10.1016/S0031-9384(98)00160-7

Oliveira-Maia, A. J., Roberts, C. D., Walker, Q. D., Luo, B., Kuhn, C., Simon, S. A., & Nicolelis, M. A. (2011). Intravascular food reward. *PloS one, 6*(9), e24992.

Owen, C. G., Martin, R. M., Whincup, P. H., Smith, G. D., & Cook, D. G. (2005). Effect of infant feeding on the risk of obesity across the life course: a quantitative review of published evidence. *Pediatrics, 115*(5), 1367-1377.

Parker, B., Noakes, M., Luscombe, N., & Clifton, P. (2002). Effect of a High-Protein, High–Monounsaturated Fat Weight Loss Diet on Glycemic Control and Lipid Levels in Type 2 Diabetes. *Diabetes Care, 25*(3), 425-430. doi: 10.2337/diacare.25.3.425

Piquard, F., Schaefer, A., & Haberey, P. (1978). Influence of fasting and protein deprivation on food self-selection in the rat. *Physiology & Behavior, 20*(6), 771-778. doi: http://dx.doi.org/10.1016/0031-9384(78)90304-9

Poppitt, S. D., McCormack, D., & Buffenstein, R. (1998). Short-term effects of macronutrient preloads on appetite and energy intake in lean women. *Physiology & Behavior, 64*, 279-285.

Prescott, J. (1998). Comparisons of taste perceptions and preferences of Japanese and Australian consumers: overview and implications for cross-cultural sensory research. *Food Quality and Preference, 9*(6), 393-402. doi: http://dx.doi.org/10.1016/S0950-3293(98)00021-4

Prescott, J. (2004). Effects of added glutamate on liking for novel food flavors. *Appetite, 42*(2), 143-150. doi: 10.1016/j.appet.2003.08.013

Prescott, J., & Young, A. (2002). Does information about MSG (monosodium glutamate) content influence consumer ratings of soups with and without added MSG? *Appetite, 39*(1), 25-33.

Raben, A., Agerholm-Larsen, L., Flint, A., Holst, J. J., & Astrup, A. (2003). Meals with similar energy densities but rich in protein, fat, carbohydrate, or alcohol have different effects on energy expenditure and substrate metabolism but not on appetite and energy intake. *The American Journal of Clinical Nutrition, 77*(1), 91-100.

Ramos, M., Cáceres, I, Polo, C., Alonso, L., & Juarez, M. (1987). Effect of freezing on soluble nitrogen fraction of Cabrales cheese. *Food Chemistry, 24*(4), 271-278. doi: http://dx.doi.org/10.1016/0308-8146(87)90102-6

Raynor, H. A., & Epstein, L. H. (2000). Effects of sensory stimulation and post-ingestive consequences on satiation. *Physiology & Behavior, 70*(5), 465-470. doi: http://dx.doi.org/10.1016/S0031-9384(00)00291-2

Raynor, H. A., & Epstein, L. H. (2001). Dietary variety, energy regulation, and obesity. *Psychological Bulletin, 127*(3), 325-341. doi: 10.1037/0033-2909.127.3.325

Reeds, P. J., Burrin, D. G., Stoll, B., & Jahoor, F. (2000). Intestinal glutamate metabolism. *The Journal of Nutrition, 130*(4), 978S-982S.

Ren, X., Ferreira, J. G., Yeckel, C. W., Kondoh, T., & de Araujo, I. E. (2011). Effects of ad libitum Ingestion of Monosodium Glutamate on Weight Gain in C57BL6/J Mice. *Digestion, 83*((s1)), 32-36. doi: 10.1159/000323405

Richter, C. P. (1936). Increased salt appetite in adrenalectomized rats. *American Journal of Physiology, 115*, 155-161.

Rifkin, B., & Bartoshuk, L. M. (1980). Taste synergism between monosodium glutamate and disodium 5′-guanylate. *Physiology & Behavior, 24*(6), 1169-1172. doi: http://dx.doi.org/10.1016/0031-9384(80)90066-9

Robinson, T. M., Gray, R. W., Yeomans, M. R., & French, S. J. (2005). Test-meal palatability alters the effects of intragastric fat but not carbohydrate preloads on intake and rated appetite in healthy volunteers. *Physiology & Behavior, 84*(2), 193-203. doi: http://dx.doi.org/10.1016/j.physbeh.2004.11.004

Rogers, P. J. (1990). Dietary fat, satiety and obesity. [doi: 10.1016/0950-3293(90)90048-Y]. *Food Quality and Preference, 2*(2), 103-110.

Rogers, P. J. (2011). Obesity - Is food addiction to blame? *Addiction, 106*(7), 1213-1214. doi: 10.1111/j.1360-0443.2011.03371.x

Rogers, P. J., & Blundell, J. E. (1990). Umami And Appetite - Effects of monosodium Glutamate on Hunger and Food-Intake In Human Subjects. *Physiology & Behavior, 48*(6), 801-804.

Roininen, K., Lähteenmäki, L., & Tuorila, H. (1996). Effect of umami taste on pleasantness of low-salt soups during repeated testing. *Physiology & Behavior, 60*(3), 953-958. doi: http://dx.doi.org/10.1016/0031-9384(96)00098-4

Rolls, B. J. (2009). The relationship between dietary energy density and energy intake. *Physiology & Behavior, 97*(5), 609-615. doi: http://dx.doi.org/10.1016/j.physbeh.2009.03.011

Rolls, B. J., Hetherington, M., & Burley, V. J. (1988). The specificity of satiety: The influence of foods of different macronutrient content on the development of satiety. *Physiology & Behavior, 43*(2), 145-153. doi: http://dx.doi.org/10.1016/0031-9384(88)90230-2

Rolls, B. J., Kim, S., McNelis, A. L., Fischman, M. W., Foltin, R. W., & Moran, T. H. (1991). Time course of effects of preloads high in fat or carbohydrate on food intake and hunger ratings in humans. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 260*(4), R756-R763.

Rolls, B. J., Roe, L. S., & Meengs, J. S. (2004). Salad and satiety: Energy density and portion size of a first-course salad affect energy intake at lunch. [doi: 10.1016/j.jada.2004.07.001]. *Journal of the American Dietetic Association, 104*(10), 1570-1576.

Rolls, E. T. (2000). The Representation of Umami Taste in the Taste Cortex. *The Journal of Nutrition, 130*(4), 960.

Ruiz, C. J., Wray, K., Delay, E., Margolskee, R. F., & Kinnamon, S. C. (2003). Behavioral Evidence for a Role of α-Gustducin in Glutamate Taste. *Chemical Senses, 28*(7), 573-579. doi: 10.1093/chemse/bjg049

Rundlett, K. L., & Armstrong, D. W. (1994). Evaluation of free D-glutamate in processed foods. *Chirality, 6*(4), 277-282. doi: 10.1002/chir.530060410

Salbe, A. D., DelParigi, A., Pratley, R. E., Drewnowski, A., & Tataranni, P. A. (2004). Taste preferences and body weight changes in an obesity-prone population. *The American Journal of Clinical Nutrition, 79*(3), 372-378.

San Gabriel, A. M., Maekawa, T., Uneyama, H., Yoshie, S., & Torii, K. (2007). mGluR1 in the fundic glands of rat stomach. *FEBS letters, 581*(6), 1119-1123.

Schiffman, S. S., Sattely-Miller, E. A., Zimmerman, I. A., Graham, B. G., & Erickson, R. P. (1994). Taste perception of monosodium glutamate (MSG) in foods in young and elderly subjects. *Physiology & Behavior, 56*(2), 265-275.

Ścińska, A., Koroś, E., Polanowska, E., Kukwa, A., Bogucka-Bonikowska, A., Kostowski, W., . . . Bieńkowski, P. (2000). An opioid receptor antagonist, naltrexone, does not alter taste and smell responses in humans. *Polish Journal of Pharmacology, 52*(5), 397-402.

Sclafani, A. (1987). Carbohydrate taste, appetite, and obesity: An overview. [doi: 10.1016/S0149-7634(87)80019-2]. *Neuroscience & Biobehavioral Reviews, 11*(2), 131-153.

Shi, Z. M., Luscombe-Marsh, N. D., Wittert, G. A., Yuan, B. J., Dai, Y., Pan, X. Q., & Taylor, A. W. (2010). Monosodium glutamate is not associated with obesity or a greater prevalence of weight gain over 5 years: findings from the Jiangsu

Nutrition Study of Chinese adults. *British Journal of Nutrition, 104*(3), 457-463. doi: 10.1017/s0007114510000760

Simpson, S. J., Batley, R., & Raubenheimer, D. (2003). Geometric analysis of macronutrient selection in humans. the power of protein? *Appetite, 41*, 123–140.

Simpson, S. J., & Raubenheimer, D. (2005). Obesity: the protein leverage hypothesis. *Obesity Reviews, 6*(2), 133-142.

Simpson, S. J., & Raubenheimer, D. (Eds.). (2000). *Geometric models of macronutrient selection*. Boca Raton: CRC Press.

Sinesio, F., Peparaio, M., Moneta, E., & Comendador, F. J. (2010). Perceptive maps of dishes varying in glutamate content with professional and naive subjects. [doi: 10.1016/j.foodqual.2010.06.002]. *Food Quality and Preference, 21*(8), 1034-1041.

Skov, A. R., Toubro, S., Rønn, B., Holm, L., & Astrup, A. (1999). Randomized trial on protein vs carbohydrate in ad libitum fat reduced diet for the treatment of obesity. *International Journal of Obesity, 23*(5), 528-536.

Smith, K. S., & Berridge, K. C. (2007). Opioid limbic circuit for reward: interaction between hedonic hotspots of nucleus accumbens and ventral pallidum. *The Journal of Neuroscience, 27*(7), 1594-1605.

Smriga, M., & Torii, K. (2000). Release of hypothalamic norepinephrine during MSG intake in rats fed normal and nonprotein diet. . *Physiology & Behavior, 70*, 413–415.

Sorensen, L. B., Moller, P., Flint, A., Martens, M., & Raben, A. (2003). Effect of sensory perception of foods on appetite and food intake: a review of studies on humans. *International Journal of Obesity and Related Metabolic Disorders, 27*(10), 1152-1166.

St Jeor, S. T., Howard, B. V., Prewitt, T. E., Bovee, V., Bazzarre, T., & Eckel, R. H. (2001). Dietary protein and weight reduction: a statement for healthcare professionals from the Nutrition Committee of the Council on Nutrition, Physical Activity, and Metabolism of the American Heart Association. *Circulation, 104*(15), 1869-1874.

Steiner, J. (Ed.). (1973). *The gusto-facial response, observation on normal and anencephalic newborn infants.* Washington, DC: Government Printing Office.

Stephens, D., & Krebs, J. (1986). Princeton University Press; Princeton, NJ. *Foraging theory.*

Stewart, J. E., Feinle-Bisset, C., Golding, M., Delahunty, C., Clifton, P. M., & Keast, R. S. (2010). Oral sensitivity to fatty acids, food consumption and BMI in human subjects. *British Journal of Nutrition, 104*(1), 145.

Stock, M. J. (1999). Gluttony and thermogenesis revisited. *International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity, 23*(11), 1105-1117.

Stone, L. M., Barrows, J., Finger, T. E., & Kinnamon, S. C. (2007). Expression of T1Rs and Gustducin in Palatal Taste Buds of Mice. *Chemical Senses, 32*(3), 255-262. doi: 10.1093/chemse/bjl053

Stubbs, R., Van Wyk, M., Johnstone, A., & Harbron, C. (1996). Breakfasts high in protein, fat or carbohydrate: effect on within-day appetite and energy balance. *European Journal of Clinical Nutrition, 50*(7), 409.

Stubbs, R. J., Mazlan, N., & Whybrow, S. (2001). Carbohydrates, appetite and feeding behavior in humans. *The Journal of Nutrition, 131*(10), 2775S-2781S.

Tanphaichitr, V., Leelahagul, P., & Suwan, K. (2000). Plasma Amino Acid Patterns and Visceral Protein Status in Users and Nonusers of Monosodium Glutamate. *The Journal of Nutrition, 130*(4), 1005.

Tome, D., Schwarz, J., Darcel, N., & Fromentin, G. (2009). Protein, amino acids, vagus nerve signaling, and the brain. *American Journal of Clinical Nutrition, 90*(3), 838S-843S. doi: 10.3945/ajcn.2009.27462W

Torii, K. (1998). Central mechanism of umami taste perception and effect of dietary protein on the preference for amino acids and sodium chloride in rats. *Food Reviews International, 14*(2-3), 273-308.

Torii, K., Mimura, T., & Yugari, Y. (Eds.). (1987). *Biochemical mechanism of umami taste perception and effect of dietary protein on the taste preference for amino acids and sodium chloride in rats*. New York

Marcel Dekker.

Tremblay, A., Plourde, G., Despres, J.-P., & Bouchard, C. (1989). Impact of dietary fat content and fat oxidation on energy intake in humans. *The American Journal of Clinical Nutrition, 49*(5), 799-805.

Uematsu, A., Tsurugizawa, T., Kitamura, A., Ichikawa, R., Iwatsuki, K., Uneyama, H., & Torii, K. (2011). Evaluation of the 'liking' and 'wanting' properties of umami compound in rats. *Physiology & Behavior, 102*(5), 553-558. doi: http://dx.doi.org/10.1016/j.physbeh.2011.01.005

Uematsu, A., Tsurugizawa, T., Kondoh, T., & Torii, K. (2009). Conditioned flavor preference learning by intragastric administration of L-glutamate in rats. *Neuroscience Letters, 451*(3), 190-193. doi: 10.1016/j.neulet.2008.12.054

Uneyama, H., Uematsu, A., Iwatsuki, K., & Nakamura, E. (2012). Perceived Palatability and Appetite for Protein by Umami Taste Derived from Glutamate. *Journal of Nutrition and Food Sciences*, S10:007. doi: 10.4172/2155-9600.S10-007

Van Itallie, T. (1989). The glucostatic theory 1953-1988: roots and branches. *International Journal of Obesity, 14*, 1-10.

Vazquez, M., Pearson, P. B., & Beauchamp, G. K. (1981). Flavour preferences in malnourished Mexican infants. *Physiology & Behavior, 28*, 513–519.

Veldhorst, M. A., Nieuwenhuizen, A. G., Hochstenbach-Waelen, A., van Vught, A. J., Westerterp, K. R., Engelen, M. P., . . . Westerterp-Plantenga, M. S. (2009). Dose-dependent satiating effect of whey relative to casein or soy. *Physiology & Behavior, 96*(4), 675-682.

Ventura, A. K., Beauchamp, G. K., & Mennella, J. A. (2012). Infant regulation of intake: the effect of free glutamate content in infant formulas. *The American Journal of Clinical Nutrition, 95*(4), 875-881. doi: 10.3945/ajcn.111.024919

Viarouge, C., Caulliez, R., & Nicolaidis, S. (1992). Umami taste of monosodium glutamate enhances the thermic effect of food and affects the respiratory quotient in the rat. [doi: 10.1016/0031-9384(92)90365-9]. *Physiology & Behavior, 52*(5), 879-884.

Walker, R., & Lupien, J. R. (2000). The Safety Evaluation of Monosodium Glutamate. *The Journal of Nutrition, 130*(4), 1049.

Warwick, Z. S., Hall, W. G., Pappas, T. N., & Schiffman, S. S. (1993). Taste and smell sensations enhance the satiating effect of both a high-carbohydrate and a high-fat meal in humans. *Physiology & Behavior, 53*(3), 553-563. doi: http://dx.doi.org/10.1016/0031-9384(93)90153-7

Weigle, D. S., Breen, P. A., Matthys, C. C., Callahan, H. S., Meeuws, K. E., Burden, V. R., & Purnell, J. Q. (2005). A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations. *The American Journal of Clinical Nutrition, 82*(1), 41-48.

Westerterp-Plantenga, M., Lejeune, M., Nijs, I., Van Ooijen, M., & Kovacs, E. (2004). High protein intake sustains weight maintenance after body weight loss in humans. *International Journal of Obesity, 28*(1), 57-64.

Westerterp-Plantenga, M. S. (Ed.). (1994). *Nutrient utilization and energy balance.* Boca Raton: CRC

Press.

Westerterp-Plantenga, M. S., Nieuwenhuizen, A., Tomé, D., Soenen, S., & Westerterp, K. R. (2009). Dietary Protein, Weight Loss, and Weight Maintenance. *Annual Review of Nutrition, 29*(1), 21-41. doi: doi:10.1146/annurev-nutr-080508-141056

Westerterp-Plantenga, M. S., & Verwegen, C. R. (1999). The appetizing effect of an aperitif in overweight and normal-weight humans. *The American Journal of Clinical Nutrition, 69*(2), 205-212.

Wiessing, K. R., Xin, L., McGill, A.-T., Budgett, S. C., Strik, C. M., & Poppitt, S. D. (2012). Sensitivity of ad libitum meals to detect changes in hunger. Restricted-item or multi-item testmeals in the design of preload appetite studies. *Appetite, 58*(3), 1076-1082. doi: http://dx.doi.org/10.1016/j.appet.2012.01.031

Xue, Y.-D., Wong, P.-H., & Leong, S.-K. (1997). Nitric oxide synthase-, N-methyl-D-aspartate receptor-, glutamate-and aspartate-immunoreactive neurons in the mouse arcuate nucleus: effects of neonatal treatment with monosodium glutamate. *Acta neuropathologica, 94*(6), 572-582.

Yamaguchi, S. (1967). The Synergistic Taste Effect of Monosodium Glutamate and Disodium 5′-Inosinate. *Journal of Food Science, 32*(4), 473-478. doi: 10.1111/j.1365-2621.1967.tb09715.x

Yamaguchi, S. (1998). Basic properties of umami and its effects on food flavor. *Food Reviews International, 14*(2-3), 139-176. doi: 10.1080/87559129809541156

Yamaguchi, S. (Ed.). (1987). *Fundamental properties of umami in human taste sensation*. New York, NY: Marcel Dekker.

Yamaguchi, S., & Kimizuka, A. (1979). Psychometric studies on the taste of monosodium glutamate. In L. J. Filer, Jr , S. Garattini, M. R. Kare, W. A. Reynolds & R. J. Wurtman (Eds.), *Glutamic Acid: Advances in Biochemistry and Physiology* (pp. 35–54.). New York, NY.: Raven Press.

Yamaguchi, S., & Kobori, I. (1991). *Nucleotides: as umami substances or umami enhancers.* Paper presented at the Proceedings of the 25th Japanese Symposium on Taste and Smell.

Yamaguchi, S., & Ninomiya, K. (2000). Umami and Food Palatability. *The Journal of Nutrition, 130*(4), 921.

Yamaguchi, S., & Takahashi, C. (1984). Interactions of Monosodium Glutamate and Sodium Chloride on Saltiness and Palatability of a Clear Soup. *Journal of Food Science, 49*(1), 82-85. doi: 10.1111/j.1365-2621.1984.tb13675.x

Yamamoto, K., & Ishimaru, Y. (2013). Oral and extra-oral taste perception. *Seminars in Cell & Developmental Biology, 24*(3), 240-246. doi: http://dx.doi.org/10.1016/j.semcdb.2012.08.005

Yasumatsu, K., Horio, N., Murata, Y., Shirosaki, S., Ohkuri, T., Yoshida, R., & Ninomiya, Y. (2009). Multiple receptors underlie glutamate taste responses in mice. *American Journal of Clinical Nutrition, 90*(3), 747S-752S. doi: 10.3945/ajcn.2009.27462J

Yeomans, M. R. (1996). Palatability and the Micro-structure of Feeding in Humans: the Appetizer Effect. *Appetite, 27*(2), 119-133.

Yeomans, M. R. (2000). Rating changes over the course of meals: what do they tell us about motivation to eat? *Neuroscience & Biobehavioral Reviews, 24*(2), 249-259. doi: http://dx.doi.org/10.1016/S0149-7634(99)00078-0

Yeomans, M. R., Blundell, J. E., & Leshem, M. (2004). Palatability: response to nutritional need or need-free stimulation of appetite? *British Journal of Nutrition, 92*, S3-S14. doi: 10.1079/bjn20041134

Yeomans, M. R., & Chambers, L. (2011). Satiety-relevant sensory qualities enhance the satiating effects of mixed carbohydrate-protein preloads. *The American Journal of Clinical Nutrition, 94*(6), 1410-1417. doi: 10.3945/ajcn.111.011650

Yeomans, M. R., Gould, N. J., Mobini, S., & Prescott, J. (2008). Acquired flavor acceptance and intake facilitated by monosodium glutamate in humans. *Physiology & Behavior, 93*(4-5), 958-966. doi: 10.1016/j.physbeh.2007.12.009

Yeomans, M. R., & Gray, R. W. (1997). Effects of Naltrexone on Food Intake and Changes in Subjective Appetite During Eating: Evidence for Opioid Involvement in the Appetizer Effect. [doi: 10.1016/S0031-9384(97)00101-7]. *Physiology & Behavior, 62*(1), 15-21.

Yeomans, M. R., & Gray, R. W. (2002). Opioid peptides and the control of human ingestive behaviour. *Neuroscience & Biobehavioral Reviews, 26*, 713–728.

Yeomans, M. R., Gray, R. W., & Conyers, T. H. B. (1998). Maltodextrin preloads reduce food intake without altering the appetiser effect. [doi: 10.1016/S0031-9384(98)00086-9]. *Physiology & Behavior, 64*(4), 501-506.

Yeomans, M. R., Lee, M. D., Gray, R. W., & French, S. J. (2001). Effects of test-meal palatability on compensatory eating following disguised fat and carbohydrate preloads. *International Journal of Obesity, 25*(8), 1215-1224.

Yeomans, M. R., & Mobini, S. (2006). Hunger alters the expression of acquired hedonic but not sensory qualities of food-paired odors in humans. [doi:10.1037/0097-7403.32.4.460]. *Journal of Experimental Psychology: Animal Behavior Processes, 32*(4), 460-466. doi: 10.1037/0097-7403.32.4.460

Yeomans, M. R., & Symes, T. (1999). Individual Differences in the Use of Pleasantness and Palatability Ratings. *Appetite, 32*(3), 383-394. doi: http://dx.doi.org/10.1006/appe.1999.0224

Yeomans, M. R., Weinberg, L., & James, S. (2005). Effects of palatability and learned satiety on energy density influences on breakfast intake in humans. [doi: 10.1016/j.physbeh.2005.08.019]. *Physiology & Behavior, 86*(4), 487-499.

Young, V. R., & Ajami, A. M. (2000). Glutamate: An amino acid of particular distinction. *Journal of Nutrition, 130*(4), 892S-900S.

Yu, Y., South, T., & Huang, X.-F. (2009). Inter-meal interval is increased in mice fed a high whey, as opposed to soy and gluten, protein diets. *Appetite, 52*(2), 372-379.

Zai, H., Kusano, M., Hosaka, H., Shimoyama, Y., Nagoshi, A., Maeda, M., . . . Mori, M. (2009). Monosodium L-glutamate added to a high-energy, high-protein liquid diet promotes gastric emptying. *The American Journal of Clinical Nutrition, 89*(1), 431-435.

Zhang, F., Klebansky, B., Fine, R. M., Xu, H., Pronin, A., Liu, H., . . . Li, X. (2008). Molecular mechanism for the umami taste synergism. *Proceedings of the National Academy of Sciences, 105*(52), 20930-20934. doi: 10.1073/pnas.0810174106

Zhao, Zhang, Y., Hoon, M. A., Chandrashekar, J., Erlenbach, I., Ryba, N. J., & Zuker, C. S. (2003). The receptors for mammalian sweet and umami taste. *Cell, 115*(3), 255-266.

Zhao, G. Q., Zhang, Y., Hoon, M. A., Chandrashekar, J., Erlenbach, I., & Lyba, N. J. (2003). The receptors for mammalian sweet and umami taste. *Cell, 115*, 255–266.

Zizza, C., Siega-Riz, A. M., & Popkin, B. M. (2001). Significant Increase in Young Adults' Snacking between 1977–1978 and 1994–1996 Represents a Cause for Concern! *Preventive Medicine, 32*(4), 303-310. doi: http://dx.doi.org/10.1006/pmed.2000.0817

Zukerman, S., Ackroff, K., & Sclafani, A. (2011). Rapid post-oral stimulation of intake and flavor conditioning by glucose and fat in the mouse. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 301*(6), R1635-R1647. doi: 10.1152/ajpregu.00425.2011

The development of a hedonically neutral food to assess the enhancement of umami flavours

Una Masic[a] and Martin R Yeomans[a]

[a] School of Psychology, University of Sussex, Brighton, BN1 9QH, UK

Contact details:

Una Masic

School of Psychology

University of Sussex

Brighton

BN1 9QH

UK

Tel: (01273) 872826

Email: u.masic@sussex.ac.uk

Key Words: MSG, umami, flavour

<u>Abstract</u>

Levels of glutamate in foods contribute to the pleasantness and acceptability of these foods. Monosodium glutamate (MSG) is characterised as a flavour enhancer that improves food palatability. However, attempts to isolate the flavour-enhancing effects of added MSG are confounded by the presence of natural concentrations of glutamate salts which occur in a wide range of foods. Thus to adequately assess the effects of MSG on flavour and appetite, a novel food with neutral palatability and low levels of natural glutamate is needed. Two experiments were therefore conducted to develop a low energy food (soup) to which added MSG reliably improved rated liking when added in differing concentrations (no MSG; 0.6% (w/w) or 0.8% (w/w) added MSG). Fifteen (Experiment 1) and thirteen (Experiment 2) low-restraint men evaluated two sets of six samples of either pumpkin or butternut squash soup (Experiment 1) or carrot and spice soup with or without added cream (Experiment 2). In Experiment 1, rated palatability of the soup vehicle was below neutral and was not enhanced by MSG whilst Experiment 2 revealed two hedonically neutral soups to which added MSG improved rated flavour pleasantness, with the lower glutamate carrot and spice soup deemed most appropriate. Overall, these results suggest that MSG may not affect the palatability of all savoury foods and that low glutamate content is important to understand the true effects of added MSG on appetite.

2.1 Introduction

Glutamic acid is the most abundant free amino acid found in food (Luscombe-Marsh, et al., 2008). It is present in both animal and plant sources and contributes to the flavour of 'umami' which may be described as a broth-like, 'savoury' flavour (Ikeda, 1908). The taste of glutamic acid is most commonly experienced in the form of glutamate salts which tend to increase the palatability of savoury foods. Monosodium glutamate (MSG) is a sodium salt that elicits the taste of umami and may be used as an effective flavour enhancer to improve the taste of many food items. However, too high a concentration of glutamate (both naturally occurring or by MSG enhancement) is perceived as unpalatable and aversive (Jinap & Hajeb, 2010; Yamaguchi & Ninomiya, 2000) and may in turn influence food selection and intake. Thus it is important when assessing the influence of this flavour enhancer and its complimentary ribonucleotides (such as inosine monophosphate; IMP) on appetite and intake to formulate an appropriate vehicle to test these effects.

The natural glutamate found in foods can be categorized as glutamate present in a bound or free form (Jinap & Hajeb, 2010; Ninomiya, 1998; Yamaguchi & Ninomiya, 2000). Free glutamate is experienced immediately and elicits the taste quality of umami whilst bound glutamate may give rise to umami flavours when chewed or exposed to heat (through cooking), converting bound glutamate to free glutamate (Jinap & Hajeb, 2010). Bound glutamate may also be converted to free glutamate through the ripening process as is evidenced by the difference in flavour of young and ripe cheeses (Ramos, et al., 1987) and even vegetables (Ninomiya, 1998). This suggests that the natural occurrence of glutamate in foods differs by food type and stage of maturation. The taste of free glutamate is important due to this role in changing the flavour of the food consumed, improving the complexity and overall palatability (Jinap & Hajeb, 2010; Loliger, 2000). This can be mimicked through the use of compounds extracted from high glutamate foods such as MSG (Ikeda, 1908). Indeed, MSG is commonly used to successfully improve flavour palatability (Bellisle, et al., 1996; Bellisle, et al., 1991) and as a consequence MSG has also been researched for its effects on appetite and food intake.

MSG has been shown to encourage faster hunger recovery after intake (Rogers & Blundell, 1990), increased intake of the MSG containing foods (Bellisle, et al., 1996; Mathey, et al., 2001) but also decreased intake when consumed over longer time periods (Bellisle, 1999; Essed, et al., 2007). When in combination with complimentary ribonucleotides, namely IMP, MSG has been suggested to increase hunger recovery and subsequent food intake (Luscombe-Marsh, et al., 2009), and have no effect on feelings of hunger and ensuing energy intake (Carter, et al., 2011). MSG has also been related to a higher body mass index (He, et al., 2008). These contrasting results paint a confusing picture of the efficacy of this flavour enhancer in maintaining palatability without negatively affecting the experience of satiety.

So why might this be? One reason for the disparity between results may relate to the natural free glutamate content of the foods to which MSG was added. Much of the literature makes no mention of the natural glutamate content of the test foods used, and of the test foods described, many use animal protein-based broths and other food items which are naturally high in umami (Carter, et al., 2011; Luscombe-Marsh, et al., 2009; Rogers & Blundell, 1990). Thus the baseline glutamate present in the 'control' conditions may be high. This may be a problem as higher concentrations of natural glutamate in certain foods may cause further addition of MSG to taste aversive (Jinap & Hajeb, 2010; Yamaguchi & Ninomiya, 2000). Equally, as glutamate levels in the control condition may be naturally high, any effects of added MSG on flavour, appetite and intake are hard to interpret. This may be exemplified by the analysis of the link between obesity and MSG (He, et al., 2008). When these results were assessed with the levels of natural free glutamate included as an additional factor, the initial suggestion that added MSG was a risk for obesity was reversed, suggesting that higher total glutamate intake (both naturally and by MSG) was associated with leaner participants (Shi, et al., 2010). This suggests that taking baseline free glutamate concentration into account is vital for a clearer understanding of the influence of MSG on appetite and intake. The present pilot experiments therefore set out to establish a hedonically neutral, low-glutamate soup to which added concentrations of MSG adequately enhanced pleasantness.

2.2 Method

2.2.1 Design

Pleasantness assessments and flavour characteristics of low glutamate vegetable soups were assessed in a single taste test trial with varying concentrations of added MSG (0.6% MSG or 0.8% MSG (w/w)) or no MSG (MSG-) in pumpkin or butternut squash soups (Experiment 1) and in carrot and spice soups with or without added cream (Experiment 2). Soup sample presentation order was randomized for each participant.

2.2.2 Participants

Fifteen low-restraint male participants from a student sample at the University of Sussex took part in Experiment 1 whilst thirteen low-restraint men completed Experiment 2. Participants were recruited based on their responses to a database recruitment questionnaire system and were emailed with details of the study described as 'taste test looking at the sensory qualities of different vegetable soups'. Exclusion from participation included any individuals taking prescription medication, those smoking more than 5 cigarettes a day, those with a history of diabetes, diagnosed eating disorders and/or allergies or dietary intolerances to the foods used. Additionally, those with high restraint scores (ratings above 7 on the Three Factor Eating Questionnaire (TFEQ) were also excluded.

Participants were paid £3 upon completion of the tasting session. All experimental work was conducted in accordance with the standards expressed in the Helsinki Declaration and was approved by the University of Sussex ethics committee.

2.2.3 Test Foods

The test soups assessed in Experiments 1 and 2 were developed to have a low free glutamate content to ensure no strong baseline effects of umami in control conditions. This was addressed by selecting ingredients with a lower natural level of glutamate (Jinap & Hajeb, 2010; Loliger, 2000; Ninomiya, 1998). Although the cooking procedure may have elevated glutamate content by converting bound glutamate to free glutamate, the levels estimated within the soups trialled still remained low as these were all low-protein formulations.

2.2.3.1 Experiment 1 test soup:

The two low energy vegetable soups (pumpkin or butternut squash) had a base of celery, onions and olive oil (Medium Flavour Olive Oil, Sainsbury's Plc., UK) with the pumpkin soup containing pumpkin puree (Libby's100% Pure Pumpkin, Nestle, UK) and the butternut squash recipe including chopped butternut squash (Sainsbury's Plc., UK). The base soup was prepared in batches by softening 170g diced onion and 100g diced celery in 20g oil before adding 425g pumpkin puree and 700g water for the pumpkin soup or 840g chopped butternut squash and 900g water for the butternut squash soup. The soups were brought to the boil for 15 minutes and allowed to simmer for 25 minutes before being blended and refrigerated at 4°C until needed.

Soup energy density was mainly derived from carbohydrate for both soups: nutritional values per 100g for butternut squash were 32 Kcal, carbohydrate 4.0g, fat 1.4g, protein 0.6g, estimated free glutamate 0.9g and for pumpkin were 31 Kcal, carbohydrate 4.2g, fat 1.5g, protein 0.9g; estimated free glutamate 0.1g, see Table 1.

2.2.3.2 Experiment 2 test soup:

The carrot and spice soup (with or without added cream) contained carrots (Frozen Baby Carrots, Sainsbury's Plc. UK), celery, onions, olive oil (Medium Flavour Olive Oil, Sainsbury's Plc., UK) and a commercial spice mixture (Garam Masala Schwartz, UK) with the cream soup including added cream (Double Cream, Sainsbury's Plc., UK). Soup preparation consisted of sweating 170g diced onion and 100g diced celery in 20g oil until softened. 600g frozen carrots was then added along with 350g water and the mixture was brought to the boil for 15 minutes and left to simmer for an additional 15 minutes. The soup was then blended and separated into batches with 0.1g/100g spice mixture added to each batch and 1.5g/100g cream added to the cream soup.

Energy density was derived from carbohydrate for both soups (nutritional content per 100g for the carrot were 28 Kcal, carbohydrate 3.1g, fat 1.6g, protein 0.4g, free glutamate 0.2g and for carrot with cream were: 33 Kcal, carbohydrate 3g, fat 2.2g, protein 0.4g, free glutamate 0.3g) soups, see Table 2.1.

Table 2.1. Nutritional composition of the soup preloads of butternut squash or pumpkin soups (Experiment 1) or carrot and spice soups with or without added cream (Experiment 2) per 100g.

	Experiment 1		Experiment 2	
	Butternut squash	**Pumpkin**	**Carrot**	**Carrot with cream**
Carbohydrate	4	4.2	3.1	3
Protein	0.6	0.9	0.4	0.4
Fat	1.4	1.5	1.6	2.2
Free glutamate	0.9	0.1	0.3	0.5
Carbohydrate (% energy)	50	52	44	36
Protein (% energy)	10	8	6	5
Fat (% energy)	40	40	50	59
Kcal	31.8	30.8	28	33.2

Soups in Experiments 1 and 2 were trialled with the same levels of MSG (Ajinomoto Co., Inc. Europe); no MSG, 0.6% (w/w) added MSG or 0.8% (w/w) added MSG. These levels were chosen based on concentrations used in related studies (Luscombe-Marsh, et al., 2009) and available commercially (Loliger, 2000) and were added to soups before heating the relevant sample.

2.2.4 Computerized Data Collection

Appetite, sensory and hedonic ratings were assessed using the Sussex Ingestion Pattern Monitor (SIPM version 2.0.13, University of Sussex) across both experiments. This consists of a computer monitor linked to a digital balance system (Sartorius, Model BP4100). Visual Analogue Scales (VAS) were used to assess appetite and sensory ratings on the SIPM. The ratings consisted of simple sentences ("How <word> do you feel?") with a left hand anchor reading "Not at all <word>" (coded as 0) and a right hand anchor reading "As <word> as I have ever felt/experienced" (coded as 100). To ensure compliance during ratings the software prompted participants to "Please use the mouse to drag the bar to the point on the scale that best represents how you feel right now." before each evaluation. Participants completed this and pressed "Rating

Complete". The first set of ratings completed assessed how, full, hungry and thirsty participants felt.

Participants were then provided with a tray containing the relevant soup samples and participants were asked to "Please take a mouthful of sample <letter>, count to 5 and then swallow" before completing sensory ratings after tasting the soup (assessing how filling, pleasant, salty, savoury, strong, sweet and thick). After each tasting, participants were reminded to "Please take a mouthful of water and press 'Continue'". Soup samples were randomized across session days and were given labels "A", "B", "C", "D", "E" and "F" and once the six samples were tasted the experimenter was alerted with a statement "Please call the experimenter for the next samples". The next set of six samples were then delivered which looked identical to the first and used the same lettering system. Once all soup tastings were completed, appetite ratings equal to the first set were filled in before participants were paid and permitted to leave. The same methodology was used in Experiment 1 and 2.

2.2.5 Procedure

For both Experiments 1 and 2, participants were invited to the Ingestive Behaviour Unit (IBU) at the University of Sussex for a single taste test session. Prior to the session, participants were asked to consume nothing but water for 2 hours before testing (from 1130h-1430h) to ensure mild food deprivation.

The taste test session was carried out in a windowless cubicle containing a large glass of water and the SIPM software. Participants were asked to follow the on-screen instructions, filling in the appetite ratings before alerting their experimenter when the six sample tasting tray was required. Samples were served in 10g portions in clear plastic tasting cups served at approximately 60ºC and were labelled using letters A-F. Participants were asked to taste the relevant soup sample and complete sensory ratings relating to the flavour of the soup. Once all samples were tasted and rated, a second six sample tasting tray was provided and participants completed the same tastings a second time. Participants were not made aware that the samples were the same as those previously tasted. The second tasting was conducted to allow for the generation of a more consistent average of rating scores. A final set of appetite ratings were then

completed before participants were paid and debriefed. A graphical depiction of the experimental design can be seen in Appendix 2.1.

2.2.6 Data Analysis

The two sets of ratings for each sensory evaluation of the soups tasted were averaged to give a mean score for each evaluation. This was to provide a more reliable average of liking for the relevant sample. Mean pleasantness ratings for the control condition was used to determine acceptability with ratings of 45-55 on VAS pleasantness pre-specified as appropriate for a hedonically neutral vehicle. Two way 2x3 repeated measures ANOVA assessing soup type (experiment 1: pumpkin or butternut squash; experiment 2: carrot and spice or carrot and spice with cream) and added (0.6% added or 0.8% added MSG) or no MSG were conducted on each sensory evaluation to determine the influence of MSG on the soups tested, with specific focus on pleasantness ratings. T-tests of mean hunger, fullness and thirst ratings taken at the start of the session were also assessed to ensure participants were mildly fasted before the session. In cases of violated sphericity, Greenhouse Geisser values were used.

2.3 Results

2.3.1 Experiment 1

Participants arrived hungry 59±6, not very full 30±7 and slightly thirsty 42±7 to the taste test session. A significant effect of condition ($F(1,14) = 7.57$, p=.02) indicated that the butternut squash conditions were rated as more pleasant than the pumpkin soups, however, mean pleasantness ratings were found to be aversively low across both control soup types; pumpkin: 31±4; butternut squash: 40±4. There was no significant differences found across MSG levels for pleasantness ($F(2,28) = 1.82$, p=.18) which remained consistently low across control and added MSG conditions. Similarly, no soup condition*MSG interaction was apparent ($F(2,28) = 0.62$, p=.54). As MSG concentration increased, ratings of how salty ($F(2,28) = 4.50$, p = .02) and strong ($F(2,28) = 3.79$, p = .04) the soups were evaluated also increased. Sweetness was also rated as higher in butternut squash soups as compared to pumpkin conditions (main effect of condition: $F(1,14) = 24.89$, p=<.001). All other sensory analyses were not found to differ with the addition of MSG across pumpkin or butternut squash soups (Table 2.2).Control soup pleasantness was not rated as hedonically neutral and the

addition of MSG to the test soups did not result in an enhancement of pleasantness by MSG. Thus Experiment 2 examined a different soup type with additional spice to counteract the unpalatable experiences found in Experiment 1.

Table 2.2. Mean VAS sensory ratings of two soups (pumpkin or butternut squash) with (0.6% or 0.8%) or without added MSG. Data are mean ±SE. [a] statistically significantly different from pumpkin. [b] statistically significantly different from no MSG.

Rating	No MSG		0.6% MSG		0.8% MSG	
	Pumpkin	Butternut Squash	Pumpkin	Butternut Squash	Pumpkin	Butternut Squash
Filling	53.47±5.4	49.73±3.9	52.67±4.6	46.33±3.5	50.40±4.8	46.60±3.8
Pleasant	31.76±4.7	35.90±5.2[a]	28.70±3.6	39.90±4.8[a]	32.80±4.8	45±4.4[a]
Salty	34.87±5.2	30.80±4.2	42.07±5.5[b]	35.47±6.2[b]	43.13±5.3[b]	43.37±6.1[b]
Savoury	50.80±4.9	46.03±4.0	47.40±5.0	48.37±4.1	52.70±4.4	45.23±5.1
Strong	45.67±4.8	46.80±4.0	47.83±4.0[b]	53.20±4.4[b]	56.07±5.2[b]	54.23±4.5[b]
Sweet	25.33±3.9	50.30±5.1[a]	34.83±4.4	55.37±5.8[a]	32.60±5.5	54±6.5[a]
Thick	63.27±5.2	49.73±4.8	58.40±4.4	44.30±4.3	57.30±5.0	45±4.8

2.3.2 Experiment 2

Appetite rating at the start of the session indicated that participants were hungry (64±5) and thirsty 60±6 and were not full 26±6 before the session began.

Soup pleasantness ratings were found to be similarly neutral [$F(1,12) = 0.001$, p=.97] across both control carrot and carrot with cream conditions; carrot: 52±5; carrot with cream: 52±6. Adding MSG increased pleasantness significantly ($F(2,24) = 6.11$, p = .01). This effect was most prominent between no MSG (m:46±5) and 0.8% MSG (m: 56±6) but was small between 0.6% MSG (m: 55±7) and 0.8% MSG conditions (figure 2.1). No interaction effect was apparent ($F(2,24) = 0.69$, p=.51). A difference in saltiness was also perceived according to MSG condition ($F(2,24) = 4.54$, p = .02) with higher concentrations rated as more salty. There were no significant differences in

savoury (F(2,24) = 2.17, p=.14) or strength (F(1.3,15.6) = 0.70, p=.45) ratings by MSG indicating that a less subtle manipulation of MSG concentration may have altered these assessments. All other sensory assessments revealed no significant differences with enhancement by MSG (Figure 2.1).

A) Carrot
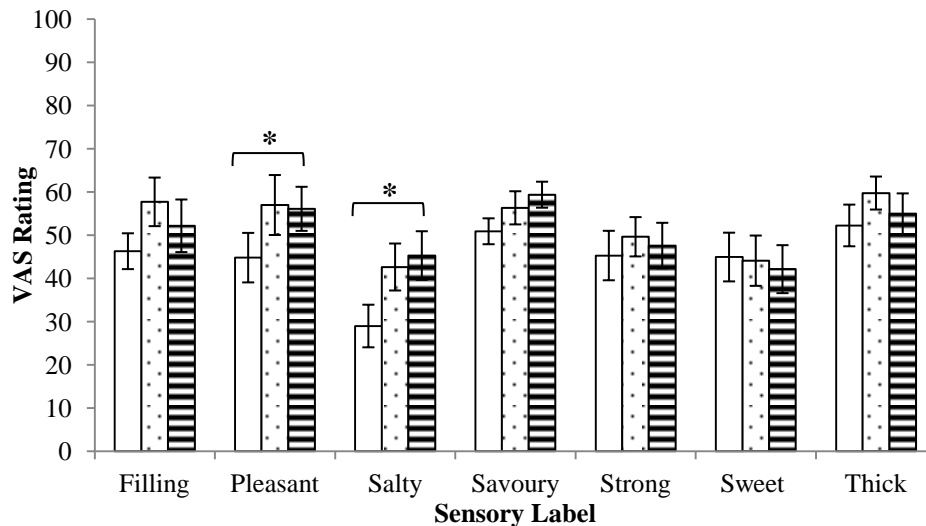
B) Carrot with Cream
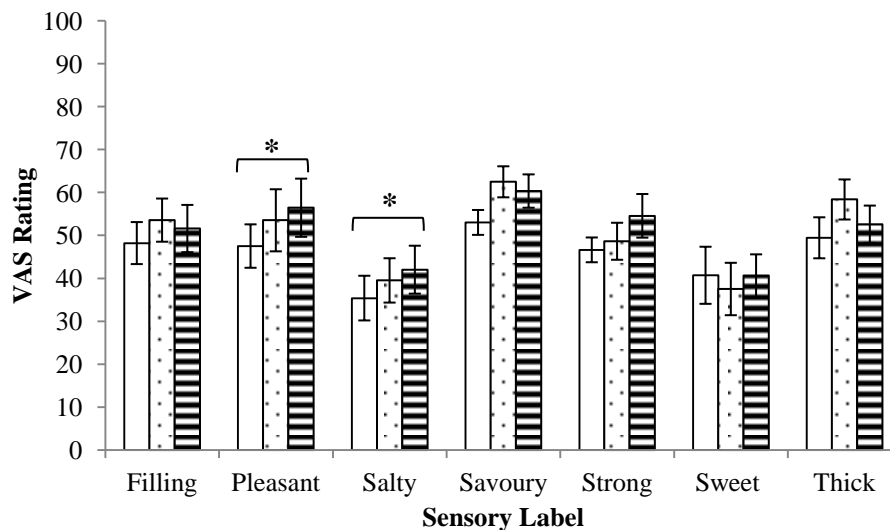
Figure 2.1. VAS sensory and hedonic ratings of two carrot soups with (Panel B) or without cream (Panel A) differing in MSG concentration (no MSG (□), 0.6% added MSG (▫) and 0.8% added MSG (▤). Data are mean ±SEM. Statistically significant difference between no and added MSG soups for pleasantness (p=.01) and saltiness (p=.02) are indicated.

2.4 Discussion

The presence of free glutamate contributes to the palatability and complexity of flavour experienced in a food (Jinap & Hajeb, 2010; Ninomiya, 1998). This may be mimicked with the use of flavour enhancers such as monosodium glutamate (MSG). However, when assessing the impact of MSG on appetite and intake it is important to note that the addition of MSG to a food naturally high in glutamate may become aversive (Jinap & Hajeb, 2010; Yamaguchi & Ninomiya, 2000). Thus it is important to formulate a vehicle that takes baseline glutamate concentrations into account to ensure an enhancement of palatability by MSG. The main findings from Experiment 1 suggested that, although the soups tested were low in free glutamate, the flavour of the control soup was deemed too low in pleasantness and no enhancement by MSG was found with both pumpkin and butternut squash conditions. Experiment 2 however identified two hedonically neutral soups to which added MSG improved rated pleasantness. As the carrot and spice soup was lower in naturally occurring glutamate and displayed a similar enhancement with added MSG despite more neutral control pleasantness ratings, this vehicle was deemed most appropriate to measure an effect by MSG.

These experiments may further support the importance of taking free glutamate content into account when assessing the influence of high glutamate flavour enhancers in foods and may explain some of the inconsistencies in previous study findings (Carter, et al., 2011; Essed, et al., 2007; Luscombe-Marsh, et al., 2009; Mathey, et al., 2001; Rogers & Blundell, 1990), further supporting the claims made by Shi and colleagues (2010). It is important to note that the flavour enhancing effects of MSG were most evident in the carrot soups in Experiment 2 even though these soups contained more free glutamate than the pumpkin soup in Experiment 1. It may be that the less pleasant flavour of the pumpkin soup was too strong to be masked by the addition of MSG. As free glutamate is vital for food pleasantness, too low a concentration may not have allowed for an improvement of flavour in the pumpkin soup with the concentrations of MSG used in the present experiments. Indeed, there were also some inconsistencies associated with the saltiness of the soups, with stronger saltiness experienced in 0.6% MSG enhanced pumpkin soups but not 0.8% enhanced soups. Whilst saltiness was found to increase more with added MSG in the carrot soup in Experiment 2, indicating a more stable effect. Savoury assessments were not affected by the addition of MSG, suggesting that

the concentrations of MSG used may have been too weak to detect a difference between conditions. Equally, the methodology used may have encouraged comparisons across the flavour samples tasted, potentially leading to more inconsistent results. This may be the case across ratings of strength of flavour found in Experiment 2 as the addition of MSG did not influence this assessment but was found to increase strength ratings in Experiment 1. As the control carrot soups were not rated as stronger tasting by participants in Experiment 2 than the control pumpkin and butternut squash soups in Experiment 1, these incompatible results may be due to cross comparisons between conditions. Similarly, the small participant numbers employed may have influenced any effect by MSG.

Overall these findings demonstrate the importance of developing an appropriate vehicle to assess effects by added MSG, ensuring a balance between maintaining low free glutamate levels and a hedonically neutral taste to allow for an enhancement by MSG. The experiments suggest that a carrot and spice soup may be used to further assess the influence of MSG on appetite and intake without potentially confounding the findings reported due to low naturally occurring free glutamate levels.

2.5 Summary of key findings and ideas for future research

- Experiment 1 indicated that the addition of MSG did not improve the palatability of pumpkin or butternut squash vegetable soups despite the low glutamate content of the developed soups
- Consequently experiment 2 was carried out and established two low glutamate soups (carrot and spice with and without added cream) to which added MSG improved palatability
- The carrot and spice soup was selected as the vehicle for testing due to its slightly lower glutamate content as compared to the carrot and cream soup
- A subsequent experiment assessing the role of a palatable MSG enhanced vehicle on appetite and intake would be important using this low glutamate soup to have a clearer understanding of the role of umami by MSG on appetite as opposed to naturally occurring umami in the diet

Appendix 2.1. Graphical depiction of the experimental design employed in experiments 1 and 2.

Taste Test

Fasted 120 min | 6 samples | 6 samples

Key: sensory rating made ▢ taste sample

2.6 References

Bellisle, F. (1999). Glutamate and the UMAMI taste: sensory, metabolic, nutritional and behavioural considerations. A review of the literature published in the last 10 years. *Neuroscience and Biobehavioral Reviews, 23*(3), 423-438.

Bellisle, F., Dalix, A. M., Chappuis, A. S., Rossi, F., Fiquet, P., Gaudin, V., . . . Slama, G. (1996). Monosodium Glutamate Affects Mealtime Food Selection in Diabetic Patients. *Appetite, 26*(3), 267-276.

Bellisle, F., Monneuse, M. O., Chabert, M., Larue-Achagiotis, C., Lanteaume, M. T., & Louis-Sylvestre, J. (1991). Monosodium glutamate as a palatability enhancer in the european diet. *Physiology & Behavior, 49*(5), 869-873.

Carter, B. E., Monsivais, P., Perrigue, M. M., & Drewnowski, A. (2011). Supplementing chicken broth with monosodium glutamate reduces hunger and desire to snack but does not affect energy intake in women. *British Journal of Nutrition, 106*(9), 1441.

Essed, N. H., van Staveren, W. A., Kok, F. J., & de Graaf, C. (2007). No effect of 16 weeks flavor enhancement on dietary intake and nutritional status of nursing home elderly. [doi: 10.1016/j.appet.2006.06.002]. *Appetite, 48*(1), 29-36.

He, K., Zhao, L., Daviglus, M. L., Dyer, A. R., Van Horn, L., Garside, D., . . . Group, I. C. R. (2008). Association of Monosodium Glutamate Intake With Overweight in Chinese Adults: The INTERMAP Study. *Obesity, 16*(8), 1875-1880. doi: 10.1038/oby.2008.274

Ikeda, K. (1908). On a New Seasoning. *Journal of Tokyo Chemistry Society, 30*, 820-836.

Jinap, S., & Hajeb, P. (2010). Glutamate. Its applications in food and contribution to health. *Appetite, 55*(1), 1-10. doi: 10.1016/j.appet.2010.05.002

Loliger, J. (2000). Function and importance of glutamate for savory foods. *Journal of Nutrition, 130*(4), 915S-920S.

Luscombe-Marsh, N. D., Smeets, A. J. P. G., & Westerterp-Plantenga, M. S. (2008). Taste sensitivity for monosodium glutamate and an increased liking of dietary protein. *British Journal of Nutrition, 99*(4), 904-908. doi: 10.1017/s000711450788295x

Luscombe-Marsh, N. D., Smeets, A. J. P. G., & Westerterp-Plantenga, M. S. (2009). The addition of monosodium glutamate and inosine monophosphate-5 to high-protein meals: effects on satiety, and energy and macronutrient intakes. *British Journal of Nutrition, 102*(6), 929-937. doi: 10.1017/s0007114509297212

Mathey, M.-F. A. M., Siebelink, E., de Graaf, C., & Van Staveren, W. A. (2001). Flavor Enhancement of Food Improves Dietary Intake and Nutritional Status of Elderly Nursing Home Residents. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 56*(4), M200-M205. doi: 10.1093/gerona/56.4.M200

Ninomiya, K. (1998). Natural occurrence *Food Reviews International, 14*(2), 177-211.

Ramos, M., Cáceres, I., Polo, C., Alonso, L., & Juarez, M. (1987). Effect of freezing on soluble nitrogen fraction of Cabrales cheese. *Food Chemistry, 24*(4), 271-278. doi: http://dx.doi.org/10.1016/0308-8146(87)90102-6

Rogers, P. J., & Blundell, J. E. (1990). Umami And Appetite - Effects of monosodium Glutamate on Hunger and Food-Intake In Human Subjects. *Physiology & Behavior, 48*(6), 801-804.

Shi, Z. M., Luscombe-Marsh, N. D., Wittert, G. A., Yuan, B. J., Dai, Y., Pan, X. Q., & Taylor, A. W. (2010). Monosodium glutamate is not associated with obesity or a greater prevalence of weight gain over 5 years: findings from the Jiangsu

Nutrition Study of Chinese adults. *British Journal of Nutrition, 104*(3), 457-463. doi: 10.1017/s0007114510000760

Yamaguchi, S., & Ninomiya, K. (2000). Umami and Food Palatability. *The Journal of Nutrition, 130*(4), 921.

Umami and the appetizer effect.

Una Masic [a] and Martin R Yeomans [a]

[a] School of Psychology, University of Sussex, Brighton, BN1 9QH, UK

Contact details:

Una Masic

School of Psychology

University of Sussex

Brighton

BN1 9QH

UK

Tel: (01273) 872826

Email: u.masic@sussex.ac.uk

Key Words: appetite, appetizer, intake, umami, MSG

Abstract

Meal intake and eating rate are said to be modulated by the hedonic properties of a meal, with rated hunger increasing early during meals evaluated as more pleasant in flavour, also termed the 'appetizer effect'. Monosodium glutamate (MSG) is a flavour enhancer which may increase flavour pleasantness and generates the taste of umami in foods but it is unclear how umami taste pleasantness influences meal intake. The effect of umami on the appetizer effect was evaluated in two studies using soups differing in MSG concentration (no MSG; 0.6% (w/w) or 1% (w/w) added MSG). Nine non-obese, low-restraint individuals (mean age: 20; BMI: 22; Experiment 1) and 18 non-obese, low-restraint individuals (mean age: 21; BMI: 22; Experiment 2) were provided with *ad-libitum* portions of soup and completed appetite and sensory evaluations before, after soup tasting, at 25g consumption for 200g intake and at meal termination. Experiment 1 revealed an increase in pleasantness after tasting added MSG conditions but this was not replicated in Experiment 2. Added MSG did not increase meal pleasantness or intake but eating rate was slower in added MSG conditions and the 1% MSG condition was rated as more satiating per gram consumed in Experiment 2. Overall, these results indicate that MSG may not influence intake according to meal pleasantness but that it may slow eating rate and increase the experience of satiation more strongly for the same quantity consumed than no added MSG conditions, and therefore may be important in maintaining satiety after a meal.

3.1 Introduction

Monosodium glutamate (MSG) is a sodium salt of glutamic acid which generates a 'savoury deliciousness' also termed 'umami' (Ikeda, 1908; Kurihara, 2009). This flavour enhancing element of MSG has been related to improving the richness and saltiness of foods (Prescott, 2004) as well as meal pleasantness (Yamaguchi & Ninomiya, 2000). Indeed, MSG containing meals have repeatedly been found to be preferred over meals containing no MSG (Bellisle, 1999; Rogers & Blundell, 1990) and have been associated with increases in short-term meal intake (Bellisle, et al., 1996; Bellisle, et al., 1991; Bellisle, et al., 1989) and a low satiety value (Rogers & Blundell, 1990). These outcomes support the literature suggesting that meal pleasantness determines meal size (Yeomans, 2000). However, the stage at which this decision occurs during intake has not been explored using MSG, suggesting that it may be valuable to understand the influence of MSG on meal pleasantness and intake at discrete time points during intake to further understand the role of MSG on meal intake.

MSG is primarily used to enhance food palatability (Loliger, 2000) and increases in meal pleasantness have been repeatedly and reliably linked with estimations of meal size (Yeomans, 2000; Yeomans, et al., 2004) and short-term overconsumption (Blundell & Cooling, 2000). Indeed, palatability assessments have been shown to relate to appetite to accurately determine portion size after simply tasting the food to be consumed. This 'appetizer effect' (Yeomans, 1996) proposes that a meal evaluated as more pleasant in flavour generates an increase in rated hunger after tasting the food which is maintained within the early stages of the meal and declines more slowly than when provided with a blander counterpart. This may be due to the orosensory reward experienced when consuming the palatable food, driving further reward-seeking translated as further intake. This opposes traditional views of homeostatic 'need' based meal-control (Yeomans, 2000; Yeomans & Gray, 1997), indicating that appetite is modulated by sensory feedback as well as satiation.

Due to the palatable nature of MSG, it may be a candidate for generating the appetizer effect. Participants provided with a novel savoury soup control which was then paired with added MSG over four training exposure sessions displayed significant increases in hunger, liking and voluntary soup intake when provided with the control soup again

without added MSG compared to those not exposed to MSG containing soups during the training phase (Yeomans, et al., 2008). This suggests that appetite and intake can indeed be stimulated by MSG as has also been found in some elderly populations (Mathey, et al., 2001; Schiffman, et al., 1994). MSG has also been anecdotally suggested to enhance eating rates due to its palatability (Bellisle & Le Magnen, 1981). However, the addition of MSG to the diet has also been repeatedly found to result in an initial increase in consumption, followed by a steady decline over time, with energy intake returning to baseline levels in long-term trials (Bellisle, et al., 1996; Bellisle, et al., 1989; Essed, et al., 2007) and has not been linked to increases in BMI in regular consumers (Shi, et al., 2010). Thus the discrepancy in the literature regarding the effects of MSG on intake indicate that the influence of short-term MSG intake results in overconsumption of the MSG-containing meal and faster hunger recovery (Mathey, et al., 2001; Rogers & Blundell, 1990) whilst other studies suggest that long-term consumption does not affect the maintenance of stable energy intake (Bellisle, et al., 1996; Bellisle, et al., 1991; Bellisle, et al., 1989; Essed, et al., 2007).

The role of meal hedonics on rated appetite and meal size of MSG containing meals may be assessed using early within-meal appetite assessments (the appetizer effect) to understand how short-term consumption is affected by MSG palatability. This was explored to understand whether enhancing the flavour of a hedonically neutral soup using increasing concentrations of MSG (0.6% and 1% added MSG) initially increases pleasantness (Experiment 1) and additionally influences rated hunger during the early stages of a meal (Experiment 2) in a concentration-specific manner relative to a bland (control) condition. As short-term intake was assessed, it was hypothesised that rated hunger and pleasantness would increase immediately after tasting (Experiments 1 and 2) and within the early stages of the meal (Experiment 2) in a linear fashion with the concentration of MSG added. Thus rated hunger, pleasantness and eating rate were predicted to be low in the bland control, higher in 0.6% MSG conditions and highest in 1% MSG conditions.

3.2 Method

3.2.1 Design

For Experiment 1, a within-participants design was used to explore the appetitive and sensory characteristics of a vegetable soup varying in the concentration of added MSG (0.6% or 1% (w/w) MSG) or no added MSG (control) during consumption. The same design was used in Experiment 2, but here an additional familiarisation session with a separate soup flavour was included prior to the target soup trials to reduce data loss. Soup condition was counterbalanced using a Latin Square design.

3.2.2 Participants

Eligible participants from a student population at the University of Sussex were emailed due to a previously expressed interest to take part in food research and based on their responses upon completion of relevant questionnaires using a database system. Both Experiments 1 and 2 were described as 'the effects of food on mood'. This was to ensure participants were not aware of the experimental manipulations and to reduce the potential for demand characteristics. Individuals with any dietary intolerances or allergies to the foods listed in the information sheet, on medications (excluding the contraceptive pill), smoking more than 5 cigarettes per week, or those with a history of diabetes or a diagnosed eating disorder were excluded from taking part. Also, those with a score higher than 7 on TFEQ restraint (Stunkard & Messick, 1985) were excluded as the literature suggests that restrained individuals tend to attend more to external cues and less to sensory and appetitive features during intake studies (Dohm, Cachelin, & Striegel-Moore, 2005; Herman & Polivy, 2005, 2008a).

Fourteen non-obese, low restraint participants took part in Experiment 1 of which 13 were eligible for analysis (6 men, 7 women, mean age: 20±0.2 , ages from: 19-21; mean BMI: 22±0.6, BMI from: 19-28 kg/m²). One participant was removed as they did not complete appetite ratings before and after the study session on the last test day.

Thirty-two low-restraint, non-obese participants were tested in Experiment 2 of which eighteen were eligible for analysis (9 men, 9 women; mean age: 21±1.5, ages from:19-24; mean BMI: 22±2.4, BMI from: 19-27 kg/m²). Three participants discontinued due to schedule conflicts, five participants consumed too little to allow for statistical analysis

and a further four did not complete an adequate number of within-meal appetite ratings and were excluded before analysis. After normality testing, two participants were excluded from further analysis due to providing ceiling responses across sessions. A further two participants had a BMI of 27, placing them in the overweight category. A correlational analysis assessing BMI and test meal intake was conducted which found no significant correlation between BMI and *ad-libitum* intake [r(18) = -0.35, p=.15] indicating that the amount consumed was not related to BMI status. Consequently these participants were not excluded from further analysis.

Upon completion of the study, participants were paid £15 (Experiment 1) or £20 (Experiment 2) or awarded credits through a participant pool scheme. All participants gave written, informed consent for their participation before the study and the research was approved by the University of Sussex ethics committee and adhered to the standards laid down in the Helsinki Declaration.

3.2.3 Test Food

3.2.3.1 Control Breakfast:

Breakfast size was determined using Food Standard Agency guidelines (FSA, 2006) for men (M) and women (W) and consisted of cereal (M:80g; W:60g; Crunchy Nut Cornflakes, Kellogg's, UK), semi-skimmed milk (M: 200g; W: 160g; Sainsbury's PLC, UK) and orange juice (M: 200g; F: 200g; Sainsbury's Pure Orange Juice, Sainsbury's PLC, UK) providing 504 Kcal for men and 404 Kcal for women.

3.2.3.2 Test Soup:

The soup consisted of a low energy density (28 Kcal/100g) carrot and spice soup containing carrots (Frozen Baby Carrots, Sainsbury's Plc., UK), onions, celery, olive oil (Medium Flavour Olive Oil, Sainsbury's Plc., UK), spice mixture (Garam Masala, Schwartz, UK) and water. The soup was prepared in large batches each week by sweating 680g diced onion and 400g diced celery in 80g oil for 8 minutes before adding 2400g frozen carrots and 1400g water. This was brought to the boil for 1 hour and then simmered for an additional hour before being blended and separated into batches. The spice mixture was then added (0.1g/100g) as well as 0.6% (w/w) MSG (Ajinomoto Co., Inc. Europe) or 1% (w/w) MSG to relevant soup conditions. The batches were refrigerated at 4ºC until needed. The soup used was specifically developed to be

hedonically neutral and low in glutamate (which produce the flavour of umami; Masic & Yeomans, 2013, Chapter 2). Soup energy density was mainly derived from fat comprising 50% fat, 44% carbohydrate and 6% protein. All nutritional information can be seen in Table 3.1.

Table 3.1. Nutritional composition of the soup preload per 100g

	Control
Carbohydrate	3.1
Protein	0.4
Fat	1.6
Carbohydrate (% energy)	44
Protein (% energy)	6
Fat (% energy)	50
Kcal	28

In Experiment 2, participants also consumed a well-accepted commercially available soup (Heinz Leek and Potato soup, Co. Ltd, UK) in a training session prior to the experimental sessions. This was to allow participants to familiarize themselves with the intake procedure, ensuring that *ad-libitum* test intake would not be influenced by external methodological factors as was found in Experiment 1. Initial tastings of the soups were served in 10g portions in plastic tasting cups before the main meal. The test soup was served in 300g portions and was consumed *ad-libitum*. Re-fill portions of the soup were provided as 300g servings.

3.2.4 Computerized Data Collection

Sensory and hedonic ratings of the soup were assessed using the Sussex Ingestion Pattern Monitor (SIPM version 2.0.13, University of Sussex) that also tracked intake. The SIPM consists of a concealed digital balance (Sartorius, Model BP4100) connected to a Dell computer which reads the balance weight (to an accuracy of 0.1g) at two-second intervals during the test meal. SIPM has previously been used in ingestive behaviour studies using soup (Yeomans & Gray, 2002; Yeomans, et al., 1998; Yeomans, et al., 2001) thus legitimizing its use in the present study.

In both Experiments 1 and 2 participants using SIPM were initially prompted to fill in mood and appetite ratings using digital Visual Analogue Scales (VAS). These ratings followed a template ("How <word> do you feel?") with the left-hand end anchor ("Not at all <word>") coded as 0 and the right-hand end anchor ("Extremely <word>") coded as 100. Participants were given the instructions "Please use the mouse to drag the bar to the point on the scale that best represents how you feel right now." before each evaluation to prevent confusion during ratings. They then completed ratings by moving the computer mouse to their desired point on the VAS and registering their selection by pressing "Rating Complete". Each participant rated how alert, anxious, clear-headed, contented, drowsy, energetic, full, happy, hungry, nauseous and thirsty they felt. All VAS ratings were presented in a randomized order across all sessions. A taster sample of the target soup was then provided with instructions to "Please take a mouthful of the soup, count to 5 and then swallow". A series of VAS questions were then presented in the form "How <taste> is the soup?" with sensory and hedonic ratings (filling, pleasant, salty, savoury, strong and sweet) followed by appetite ratings (full, hungry and thirsty). Participants were then presented with a bowl of the test soup with instructions to "Please eat as much of the soup as you like until you feel comfortably full". Participants then made further sensory and appetite ratings (Experiment 1 asked how hungry, full and thirsty and Experiment 2 additionally included how pleasant the soup tasted) after approximately every 25g consumption of the first bowl of soup (determined as the first stable balance reading where weight had decreased by at least 25g since the last rating set up to approximately 75g consumed). An on-screen message alerted the participant to request a re-fill portion of soup at the first stable balance reading after 200g had been consumed. Once replete, participants indicated that they had finished the meal by clicking "Finished eating", and were required to confirm this decision to prevent meals being completed in error. Participants completed a second set of mood and appetite ratings once they had eaten all they wanted. All VAS data and intake data were automatically stored in a data file throughout the session by the SIPM.

3.2.5 Procedure

Participants were invited to the Ingestive Behaviour Unit (IBU) at the University of Sussex on three (Experiment 1) or four (Experiment 2) non-consecutive days. Participants consumed nothing but water from 2300h the night before each session and were given the standard breakfast at pre-arranged times (from 0830h – 1030h). After

breakfast, participants were permitted to leave the IBU with instructions to consume nothing but water and returned after 3h.

Test meal sessions ran from 1130h – 1330h in windowless cubicles containing a glass of water, cutlery and the SIPM software. Participants were asked to follow the instructions on screen which explained the experimental procedure and completed the first set of appetite and mood ratings before calling the experimenter. They were then provided with a taster sample of the soup and evaluated its sensory and hedonic qualities before completing more appetite ratings. Participants were then provided with a bowl of the test soup which they were left to consume *ad-libitum* and were automatically interrupted by SIPM after 25g consumption for the first 75g of intake and at the 200g re-fill stage with appetite and sensory ratings. After the re-fill they consumed the soup without interruptions. Additional (300g) portions of soup were provided until the participants indicated that they were comfortably full by clicking through to the final set of mood and appetite ratings. Offering re-fill portions of the soup before finishing the bowl ensured that food was always present in the bowl to prevent external food cues influencing meal intake as has been previously found (Herman & Polivy, 2008a; Wansink, Painter, & North, 2005b). Once completed participants were free to leave but were weighed, measured and debriefed before payment on the final day.

As the experimental sessions involved set weight interrupts during consumption, an additional training day was included in Experiment 2 on day 1. This involved consuming a commercial brand soup and was to ensure that participants did not miss weighted interrupts during eating by allowing participants to ask any questions and familiarize themselves with the experimental procedure as participants in Experiment 1 indicated some confusion regarding what to do when meal interrupts occurred. A graphical depiction of the experimental design can be seen in Appendix 3.1.

3.2.6 Data Analysis

The main variable examined was MSG concentration, with three levels; no MSG (control), 0.6% MSG (w/w) or 1% MSG (w/w). Repeated measures ANOVAs were conducted on sensory ratings (Experiments 1 and 2), appetite ratings during the meal

(Experiment 2) and overall intake data (Experiment 2). Further within-meal analyses were not possible in Experiment 1 due to missing data leading to inadequate power required for analysis, with the number of participants reduced to 9 available cases. Instead, sensory and tasting data were analysed for 13 participants from Experiment 1. The effects of MSG concentration on assessments of appetite (hunger and fullness) were evaluated at two key points (before and after tasting the meal) in Experiment 1 and at seven key points in Experiment 2: before, after tasting, at 25g intervals during the first 75g consumption, at the 200g re-fill point and immediately after the meal. Pleasantness was rated at six key points following the same structure as the appetite evaluations after tasting in Experiment 2.

Within-meal appetite and sensory ratings assessing the microstructure of feeding require best fit quadratic function analyses to determine the more subtle effects of appetite on food intake. However, as previous microstructural analyses were mainly conducted with solid foods (Yeomans, 1996; Yeomans, et al., 1998) eating time was increased in these studies, allowing for more accurate assessments of the amount consumed to trigger the appropriate VAS appetite questions. With semi-liquids, eating time is greatly reduced due to an increased bite size (Zijlstra, Mars, De Wijk, Westerterp-plantenga, & De Graaf, 2008b) and reduced oral exposure time (de Graaf & Kok, 2010). This reduced the accuracy of triggering the appropriate VAS question, affecting the number of data points acquired. Therefore, to assess the more subtle effects of soup type on appetite stimulation and intake, the rate of satiation during consumption was assessed using a similar methodology to that which has been adopted previously when assessing the satiating capacity of a food over the time after consumption (satiety quotient; Green, Delargy, Joanes, & Blundell, 1997). These scores take into account the combined impact of hunger ratings and volume consumed (grams) over an eating episode and were calculated for each individual time point by subtracting the pre-meal hunger rating from the interrupt point hunger rating (tasting, 25g, 50g, 75g, 200g) or post-meal hunger rating and dividing this value by the grams consumed. These scores were then analysed in a 3x6 repeated measures ANOVA to assess the progression of the satiating capacity of each soup type.

As the effects of nausea may impact on subjective appetite and sensory evaluations as well as overall energy intake, repeated measures ANOVA was also conducted assessing

nausea before and after the meal in Experiment 2. Equally, thirst may also influence appetite and intake (Bellisle & Le Magnen, 1981; McKiernan, Houchins, & Mattes, 2008) and was assessed alongside appetite ratings in Experiment 2 to ensure no confounding influence of this factor. This was especially pertinent due to the sodium component of the MSG-containing soups. Eighteen participants were eligible for analysis in Experiment 2 from a sample of thirty-two. This meant that counterbalancing was incomplete across participants and was subsequently analysed to ensure no confounding effects of order. In cases where sphericity was violated, adjusted Greenhouse Geisser values are reported ($\varepsilon = <0.75$). Significant interactions were further explored through analysis of within-subjects Bonferroni protected contrasts across conditions at relevant interrupt stages and Pearson's correlation coefficient was used to determine effect sizes where interactions were evident. G*Power was used to assess post-hoc power in Experiment 2.

3.3 Results

3.3.1 Experiment 1

There was a significant overall increase in pleasantness (Figure 3.1) with the addition of MSG ($F_{(1.1,12.63)} = 20.77$, $p <.001$); with 1% MSG conditions generating the strongest pleasantness ($F_{(1,12)} = 21.87$, $p = .001$, $r = 0.80$), followed by the 0.6% MSG condition ($F_{(1,12)} = 18.06$, $p = .001$, $r = 0.78$) when compared to the control. Ratings of savouriness were also found to differ significantly across MSG concentrations ($F_{(2,24)} = 6.00$, $p = .008$; Figure 1) with 1% MSG conditions rated most savoury when compared to control ($F_{(1,12)} = 13.98$, $p = .003$, $r = 0.73$). A main effect of saltiness ($F_{(2,24)} = 3.81$, $p = .04$) also indicated an increase in saltiness perceptions in 1% ($F_{(1,12)} = 6.75$, $p = .02$, $r = 0.60$) conditions compared to control. A trend was also evident in strength of flavour ratings ($F_{(2,24)} = 3.01$, $p=.07$), suggesting that the added MSG conditions were rated as stronger than control. However, there were no significant differences between conditions in how filling the soup was expected to be ($F_{(2,24)} = 1.82$, $p=.18$), or how sweet ($F_{(2,24)} = 0.26$, $p=.77$) it tasted.

Experiment 1
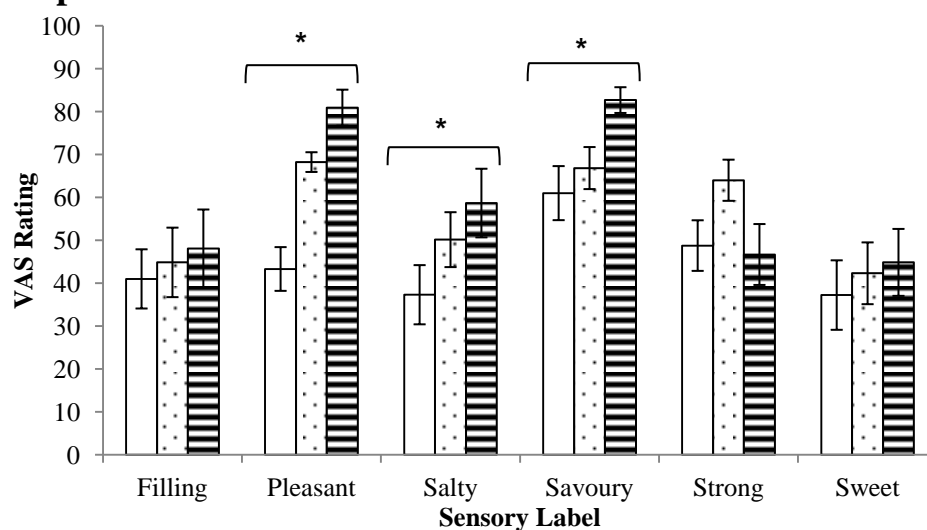
Experiment 2
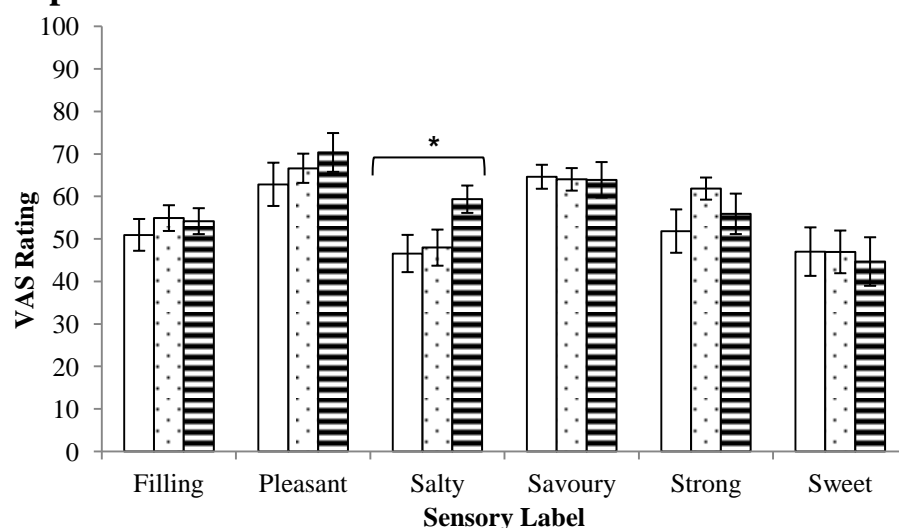
Figure 3.1. Mean Experiment 1 (Panel A) and Experiment 2 (Panel B) sensory and hedonic assessments (mm) of three soups differing in MSG concentration (no MSG (□), 0.6% added MSG (⊡) and 1% added MSG (▤). Data are mean ±SE. Significant differences between added and no MSG conditions are indicated: * P<0.05.

No baseline differences were noted in hunger ($F(1.2,14.9) = 2.32$, p=.15) or fullness ($F(1.3,16.1) = 3.50$, p=.10) immediately before intake. A significant increase in hunger was evident after tasting the soup samples (main effect of time: $F(1,12) = 24.62$, p $<.001$, r = 0.82) and an MSG*time interaction was also evident ($F(2,24) = 15.45$, p $<.001$) indicating that hunger increased significantly after tasting the soups with 1% MSG ($F(1,12) = 28.30$, p = $<.001$, r = 0.84) and 0.6% MSG ($F(1,12) = 20.16$, p = .001, r = 0.79) added when compared to control (Table 3.2).

Table 3.2. Mean Experiment 1 hunger ratings pre-meal and after tasting three soups differing in MSG concentration (no MSG control, 0.6% added MSG or 1% added MSG). Data are mean ±SE. [1]Statistically significantly different from post-taste. [2]Statistically significantly different from control using pair-wise protected contrasts, P<0.05.

	Condition		
	Control	0.6% MSG	1% MSG
Pre-meal	69.67±3.7[1]	66.56±3.6[1,2]	67.67±4.3[1,2]
Post-taste	65.44±4.5	83.22±4.5[2]	85.78±4[2]

3.3.2 Experiment 2

3.3.2.1 Sensory and hedonic evaluations of the soup

There was a significant difference in perceived saltiness across MSG conditions $(F(1.5,25.5) = 3.99, p = 0.04)$ with saltiness rated as stronger in 1% MSG conditions compared to control $(F(1,17) = 8.11, p = .01, r = 0.57)$. All other sensory assessments were found to be non-significant including ratings of soup pleasantness $(F(2,34) = 1.47, p=.24)$ and savouriness $(F(1.5, 24.9) = 0.03, p=.93)$ although pleasantness tended to increase with MSG concentration, with effect sizes indicating a moderate effect of 1% MSG when compared to control $(F(1,17) = 2.18, p=.16, r = .33)$ but a small effect of 0.6% MSG when compared to control $(F(1,17) = 0.64, p=.44, r = .19;$ Figure 3.1).

3.3.2.2 Appetite and meal pleasantness

The main variables of interest were the effects of MSG concentration on appetite and hedonic liking tracked over the course of the meal. As expected, no significant differences were noted for hunger $(F(2,34) = 1.63, p=.21)$ or fullness $(F(2,34) = 0.12, p=.87)$ before intake sessions indicating that participants arrived hungry (m:69±2.8) and not very full (m:26±3.5). Hunger ratings decreased $(F(2.5,42.2) = 57.83, p <0.001)$ and fullness ratings increased $(F(2.3,39.7) = 53.07, p <0.001)$ over the course of the meal. MSG concentration did not influence hunger $(F(2,34) = 1.84, p=.17)$ or fullness ratings $(F(2,34) = 0.65, p=.53)$ immediately after soup tasting, suggesting that the MSG added conditions did not generate an appetizer effect (Figure 2). However, appetite analysis over the duration of the meal revealed a significant MSG*time interaction for hunger

ratings (F(5.5,94.2) = 2.47, p = .03) with further protected contrasts revealing a significantly stronger hunger reducing effect at the end of the meal for the 1% MSG condition (F(1,17) = 13.02, p=.002, r = 0.66) compared to control (Figure 3.2). No significant main effect of MSG (F(2,34) = 0.11, p=.90) or MSG*time interaction (F(4.8,81.7) = 1.29, p=.22) were evident for fullness (Figure 3.2) over the course of the meal. Pleasantness declined over the course of the meal (main effect of time: F(2.1, 35.3) = 16.54, p<.001). There was no effect of MSG condition on pleasantness during intake (F(2, 34) = 1.45, p=.25; Figure 3.2) and no MSG*time interaction (F(3.8, 64.0) = 0.58, p=.83) was evident (Figure 3.2). The lack of a detectable appetizer effect between soup condition and appetite may be due to a lack of power in the sample as effect size calculations estimated a requirement for 28 participants to detect a moderate appetizer effect between conditions (at 80% power) whereas post-hoc power analyses with the current sample was too low (25% power).
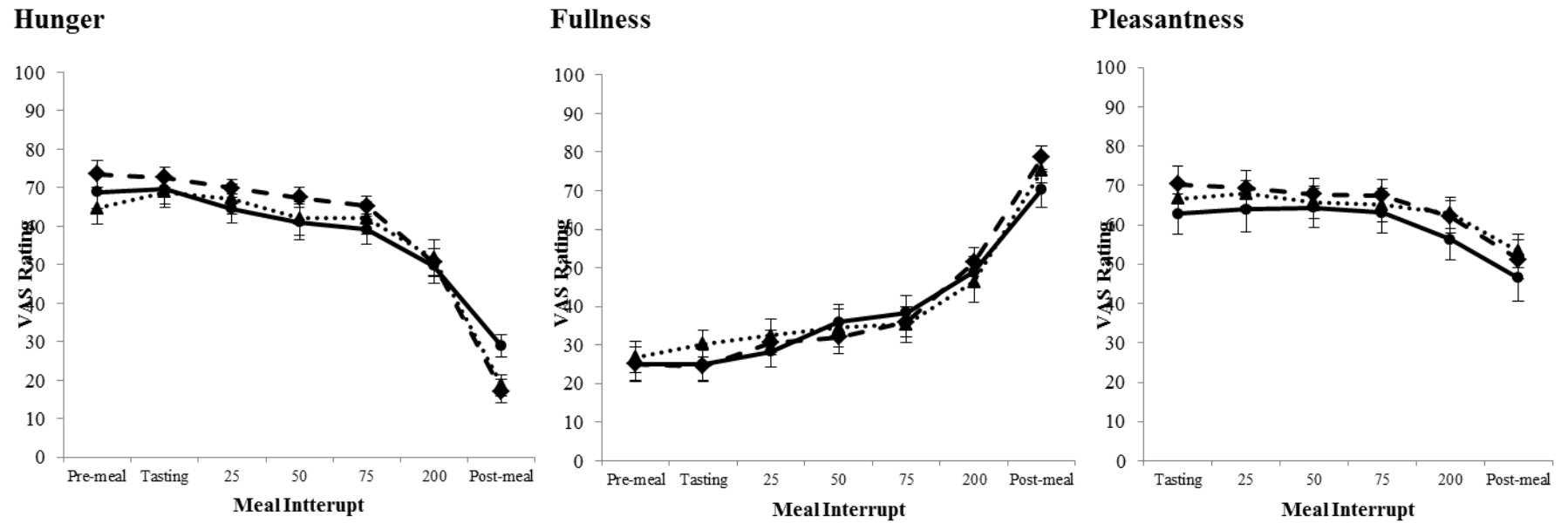
Figure 3.2. Mean (± SEM) Experiment 2 VAS ratings (mm) for hunger (Panel A), fullness (Panel B) and pleasantness (Panel C) at interrupt points (g) before, during and after ingestion of soup varying in MSG concentration (no MSG (———●———), 0.6% MSG (....▲...) or 1% MSG (– ◆ –).

3.3.2.3 Intake, eating rate and the rate of satiation during the meal

Energy intake did not differ by MSG condition (F(2,34) = 1.66, p=.21; Figure 3.3). However, eating rate was significantly slower as MSG concentration increased (main effect of condition: F(2,34) = 3.49, p = .04; Figure 3.4). Contrast analysis revealed a trend for a longer time spent eating in the 0.6% MSG condition (F(1,17) = 3.55, p = .08, r = 0.42) relative to the bland control and a significantly slower eating rate in 1% MSG conditions compared to control (F(1,17) = 5.46, p = 03, r = 0.49). Eating rate did not change over time (F(1.3, 22.5) = 1.77, p=.20), and there was no condition*time interaction (F(1.7, 28.2) = 1.25, p=.30) suggesting that the speed of eating was similar across the duration of intake and that this did not differ by condition.
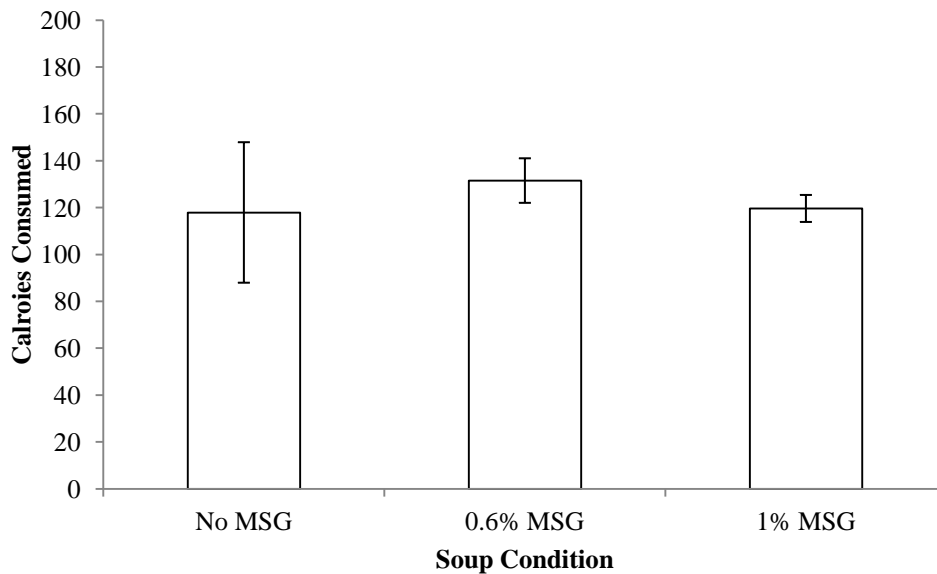
Figure 3.3. Mean (±SEM) a*d-libitum* energy intake (Kcal) of three soups differing in MSG concentration (no MSG, 0.6% MSG and 1% MSG).

The rate of satiation was assessed during the meal to understand whether there was an influence of appetite stimulation across MSG conditions during intake. This analysis was based on the satiety quotient (SQ; Green et al., 1997), which determines a food's effectiveness at maintaining satiety after intake by allocating a SQ score. This score reflects the extent to which the food consumed decreases rated appetite (after the meal) per unit of intake (*e.g.* gram consumed). Thus if rated hunger was taken into account, the greater the quotient value, the more hunger ratings were suppressed per gram consumed. In the present experiment, SQ scores could not be used as post-meal appetite was not assessed but was conducted using within-meal ratings to generate a 'satiating'

quotient. When this rate of satiation was averaged for the meal, a significant effect of MSG was evident ($F_{(2,34)} = 5.19$, $p = .01$). Consequently, ratings at discrete time points (Figure 3.4) were assessed to further understand how satiation and intake were related during the meal, a significant MSG*time interaction was evident ($F_{(4.2,71.6)} = 3.53$, $p = .01$) with further analyses revealing a stronger hunger reducing effect immediately after tasting in the 1% condition as compared to control ($F_{(1,17)} = 4.57$, $p = .05$) but not the 0.6% condition when compared to control ($F_{(1,17)} = 0.22$, $p=.65$) indicating an immediate orosensory effect of the high MSG condition. A significantly higher satiating quotient score was also evident after the meal in the 1% MSG ($F_{(1,17)} = 10.10$, $p = .006$, $r = 0.61$) and 0.6% MSG ($F_{(1,17)} = 4.52$, $p = .05$, $r = 0.46$) conditions compared to control. This suggests that, overall the 1% MSG and 0.6% conditions reduced feelings of hunger more during intake despite intake being similar across conditions (Figure 3.4). There was also a significant effect of time ($F_{(2,33.7)} = 50.91$, $p <.001$) with satiation increasing over time across all conditions.

A) Satiation Quotient
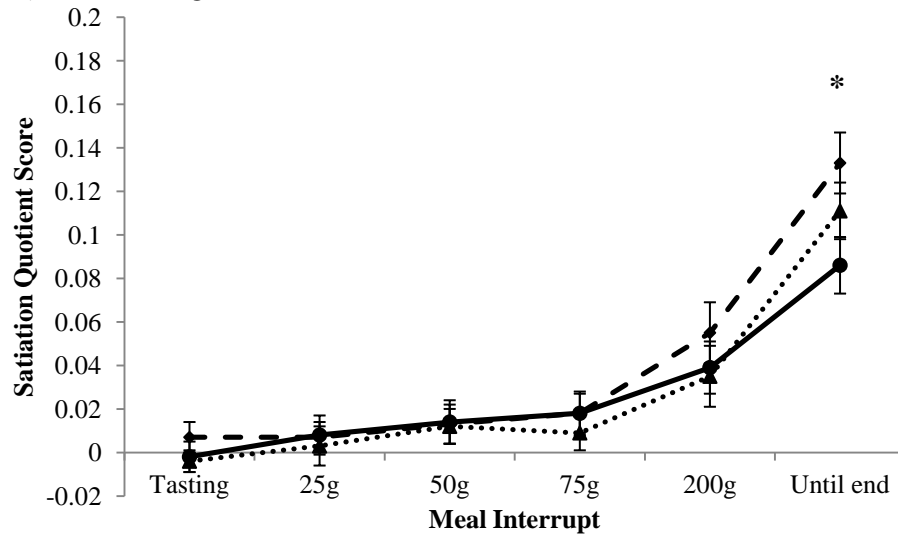
B) Eating Rate
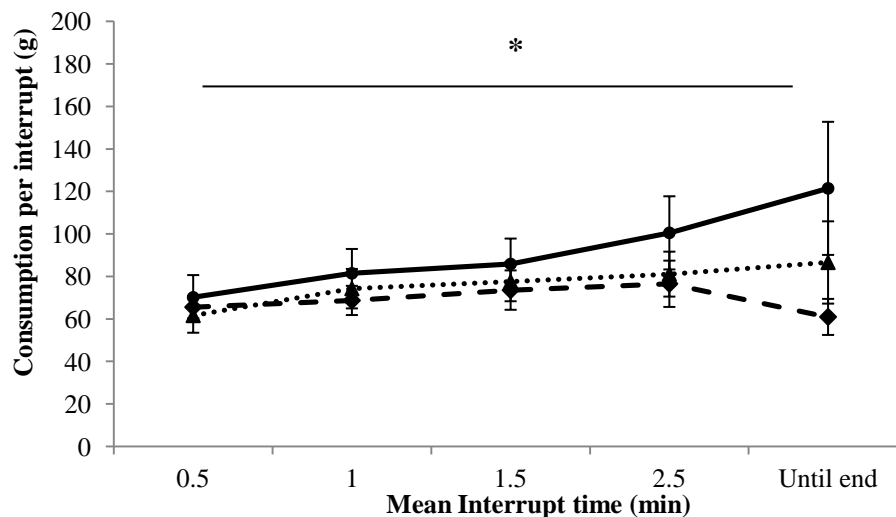
Figure 3.4. Mean (±SEM) experience of satiation (satiation quotient score) in relation to the volume consumed at specified interrupts (Panel A) and eating rate (g/min) at mean time interrupts during ingestion of soup varying in MSG concentration (no MSG (———●——), 0.6% MSG (...▲...) or 1% MSG (— ◆—)). Statistically significant protected contrasts between control and 1% MSG are indicated at the end of the meal (Panel A) and over the meal (Panel B) are indicated: * P<0.05.

3.3.2.4 Nausea, thirst and order effects

Analyses of nausea revealed a significant effect of time (F(1,17) = 5.79, p = .03) with nausea increasing after soup consumption. This may be due to the volume of soup ingested potentially making some participants feel uncomfortable. Nausea did not differ according to condition and no interaction was evident (condition: F(2,34) = 0.17, p=.85;

MSG*time interaction: F(2,34) = 0.75, p=.48). Baseline thirst analyses before lunch sessions was non-significant across conditions (F(2,34) = 0.63, 54) indicating that participants arrived in a similar state of thirst. A significant effect of time suggested that thirst decreased over the intake session (F(1,17) = 4.50, p = .05) but this did not differ according to MSG condition (F(2,34) = 2.15, p=.13) and there was no MSG*time interaction (F(5.3, 90.6) = 0.53, p=.76).

As fourteen participants were excluded from all analyses in Experiment 2 the influence of order was taken into account due to unequal counterbalancing. There was no effect of order on energy intake across test days (F(10,24) = 1.46, p=.22) however an MSG*pleasantness*order interaction trend (F(14.8,35.5) = 1.84, p = .07) was noted. This may be due to one counterbalanced sequence only being completed by one participant, potentially giving rise to a spurious interaction. As there was no significant effect of order on soup type across all other sensory and intake data, it can be assumed that previous soup flavour experiences did not affect intake and sensory assessments across the testing days.

3.4 Discussion

The main findings suggest that MSG improved the palatability of a hedonically neutral soup and increased hunger after tasting the soup compared to a no-MSG control (Experiment 1). However, MSG did not influence rated meal pleasantness or appetite during the early stages of a meal in Experiment 2 but a strong concentration of MSG did reduce rated hunger more than the no-MSG control at the end of the meal. These findings also suggested that the addition of MSG may not affect energy intake but may reduce eating speed and increase the experience of satiation immediately after intake than when no MSG is provided.

Despite no appetizer effect being apparent early during the meal in Experiment 2 and no differences in energy intake, the rate of satiation was higher immediately after tasting and at the end of the meal in the 1% MSG condition, indicating lower rated hunger with respect to the volume consumed when compared to the bland control. Similarly, eating rate tended to decline as a function of MSG concentration with the slowest speed of eating evident in the 1% MSG condition followed by 0.6% MSG and the control

condition. Slowed eating rate has been related to reduced meal intake due to improving the accuracy of recognizing satiation as it develops as was found in the 1% MSG condition. This could be attributed to the intra-meal pauses used in the present study (as has been found elsewhere (Andrade, Greene, & Melanson, 2008). However, as eating rate did not change over time, the effect of early meal pauses most likely did not influence the experience of satiation.

So why may it be that the 1% MSG condition reduced eating speed and increased satiation compared to the control condition? Indeed, these differences cannot be attributed to flavour pleasantness over the course of the meal (Yeomans, 1996) as this was found to decline equally across conditions. It may instead be that eating rate is related to improved assessments of the experience of satiation developing in the MSG conditions compared to the control condition. This interaction between slowed eating rate and a stronger experience of satiation towards the end of the meal may be related to early sensory-gut processing as umami receptors have not only been found on the tongue (potentially conveying immediate messages of satiation) but also in the stomach lining and small intestine (Beauchamp, 2009; Egan & Margolskee, 2008). It may be that sensory and gut umami receptors are conveying feedback messages of satiation during the eating episode more effectively due to a slower eating rate. Indeed this stronger experience of satiation for the volume consumed may in turn influence feelings of hunger, potentially delaying the timing of the next meal (enhancing satiety) despite the similar energy intake between conditions. This explanation is particularly pertinent when assessed alongside the existing literature on the long-term effects of MSG maintaining stable energy intake and body weight despite improving palatability assessments (Bellisle, et al., 1996; Bellisle, et al., 1991; Bellisle, et al., 1989; Essed, et al., 2007) and requires clarification.

A further question may consider why MSG may increase satiation due to early sensory-gut umami signalling? Some researchers suggest that, just as sugar sweetness indicates the presence of energy (Sclafani, 1987; Yamamoto & Ishimaru, 2013), the umami taste may detect the availability of protein within foods (Ikeda, 1908; Luscombe-Marsh, et al., 2008). As protein has been repeatedly found to be the most satiating macronutrient (Bertenshaw, et al., 2008; Bertenshaw, et al., 2013; Gosby, et al., 2011), it may be that the high concentration of MSG in the 1% soup combined with a slowed eating rate

allowed for the immediate detection of protein, thus making satiation more apparent. This may explain the inconsistencies found between short- and long-term MSG trials as both short-term (Rogers & Blundell, 1990) and long-term (Bellisle, et al., 1996; Bellisle, et al., 1991; Bellisle, et al., 1989; Essed, et al., 2007) trials did not measure eating rate. It may be that long-term trials allow for a learning effect of the satiating capacity of the MSG containing foods, especially as exposure to foods alters assessments of how satiating they are deemed to be (Gibson & Brunstrom, 2007). Eating rates in the short-term trials however may have been too high in single exposure MSG conditions (due to palatability effects) to allow for adequate sensory-gut protein detection.

The expected impact of an increase in hunger immediately after tasting MSG enhanced meals compared to the no-MSG condition was not evident in Experiment 2. This apparent lack of an appetizer effect may be due to the similar high pleasantness ratings found across conditions at the tasting stage and early within the meal as previous findings show clear differences when pleasantness ratings are not closely matched (Yeomans, 1996; Yeomans, et al., 2001) indicating that the flavour manipulation may have been too subtle. However, Experiment 1 and previous research utilizing the same and lower doses of MSG (Bellisle, et al., 1996; Luscombe-Marsh, et al., 2008, 2009; Prescott, 2004; Prescott & Young, 2002) did find significant differences in pleasantness between conditions. This may be due to participants receiving a separate training soup prior to target soup sessions in Experiment 2 which may have influenced sensory expectations. However, as there was no effect of soup order presentation on intake it may instead be that the sample sizes analysed in both experiments were too small and thus more sensitive to variation. The comparable pleasantness ratings across soup conditions in Experiment 2 may also explain the similar energy intake due to the effects of meal pleasantness on energy intake (Yeomans, 2000). However, although non-significant, pleasantness ratings remained consistently higher in 1% MSG added conditions compared to the equivalent 0.6% and no MSG conditions in Experiment 2 and yet intake in this condition was lower than both 0.6% and no MSG conditions. The effect of MSG on the appetizer effect has not previously been examined in this way before and is indicative of the potential for MSG to encourage satiation more strongly, potentially delaying the return of hunger and maintaining satiety over a longer period. Future research is warranted to further explore the time course of MSG satiation during

the meal and over the post-gastric period to examine the influence of MSG on sensory and post-oral processing, particularly in combination with macronutrients such as protein. Similarly, further research using a larger sample size and a vehicle that can more accurately track the rate of satiation as it develops would be required as inconsistencies in tracked food intake meant that this aspect of intake could not be adequately assessed.

Overall this research suggests that the addition of MSG to an *ad-libitum* meal increases the satiating capacity of a meal and delays eating speed when compared to a no-MSG control. However, added MSG did not generate an appetizer effect when provided in a more varied soup context as rated appetite and meal pleasantness remained similar across conditions during the early stages of the meal. Equally, energy intake remained stable across conditions suggesting that although added MSG may be more effective at increasing satiation and reducing eating rate towards the end of the meal, this did not translate to actual energy intake. Further research should explore the potential for an enhancement of satiety by MSG.

3.5 Summary of key findings and ideas for future research
- Experiment 1 established an appetizing effect of the MSG concentrations used, with hunger ratings increasing after tasting the MSG containing soups in a concentration specific manner
- However, further ratings of appetite could not be assessed due to limitations with the study design
- Experiment 2 included an additional training soup to reduce missed within-meal appetite ratings, however a high number of ratings were still left uncompleted
- Despite no appetizing effect of MSG in experiment 2 and no differences in early meal ratings of hunger or fullness, the rate of satiation was stronger in the added MSG conditions as compared to control suggesting that there may be a satiating component to MSG
- Future research would be important to re-assess the methodology used in the present experiment to understand whether the lack of an appetizing effect by MSG was due to this basic taste or may be as an artefact of the methods employed

- Future research should also focus on an already well-established basic taste, known for its palatable properties such as sweetness to clarify the unexpected findings of the present experiments

Appendix 3.1. Graphical depiction of the experimental procedure carried out for experiments 1 and 2. Experiment 2 also included an additional training soup day.

Key: ↓ appetite rating made ⩔ sensory ratings made ■ fixed meal ☐ ad-libitum meal
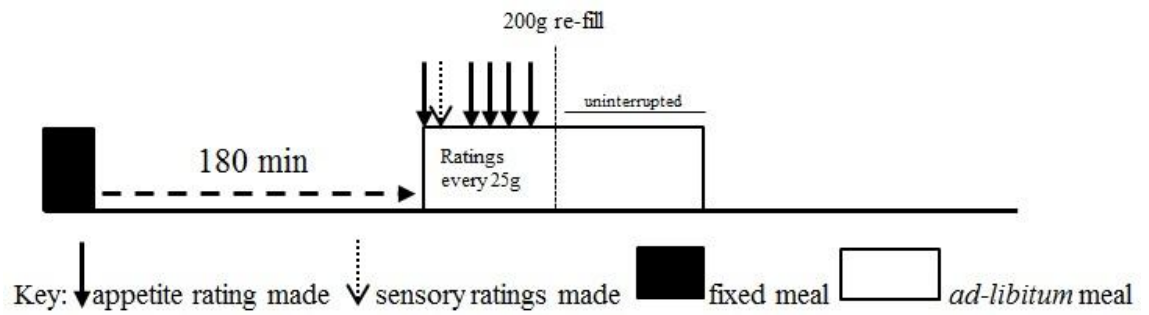
3.6 References

Andrade, A. M., Greene, G. W., & Melanson, K. J. (2008). Eating Slowly Led to Decreases in Energy Intake within Meals in Healthy Women. [doi: 10.1016/j.jada.2008.04.026]. *Journal of the American Dietetic Association, 108*(7), 1186-1191.

Beauchamp, G. K. (2009). Sensory and receptor responses to umami: an overview of pioneering work. *American Journal of Clinical Nutrition, 90*(3), 723S-727S. doi: 10.3945/ajcn.2009.27462E

Bellisle, F. (1999). Glutamate and the AMI taste: sensory, metabolic, nutritional and behavioural considerations. A review of the literature published in the last 10 years. *Neuroscience and Biobehavioral Reviews, 23*(3), 423-438.

Bellisle, F., Dalix, A. M., Chappuis, A. S., Rossi, F., Fiquet, P., Gaudin, V., . . . Slama, G. (1996). Monosodium Glutamate Affects Mealtime Food Selection in Diabetic Patients. *Appetite, 26*(3), 267-276.

Bellisle, F., & Le Magnen, J. (1981). The structure of meals in humans: Eating and drinking patterns in lean and obese subjects. [doi: 10.1016/0031-9384(81)90237-7]. *Physiology & Behavior, 27*(4), 649-658.

Bellisle, F., Monneuse, M. O., Chabert, M., Larue-Achagiotis, C., Lanteaume, M. T., & Louis-Sylvestre, J. (1991). Monosodium glutamate as a palatability enhancer in the european diet. *Physiology & Behavior, 49*(5), 869-873.

Bellisle, F., Tournier, A., & Louis-Sylvestre, J. (1989). Monosodium glutamate and the acquisition of food preferences in a European context. *Food Quality and Preference, 1*, 103-108.

Bertenshaw, E. J., Lluch, A., & Yeomans, M. R. (2008). Satiating effects of protein but not carbohydrate consumed in a between-meal beverage context. *Physiology & Behavior, 93*(3), 427-436. doi: 10.1016/j.physbeh.2007.09.014

Bertenshaw, E. J., Lluch, A., & Yeomans, M. R. (2013). Perceived thickness and creaminess modulates the short-term satiating effects of high-protein drinks. *British Journal of Nutrition, FirstView*, 1-9. doi: doi:10.1017/S0007114512005375

Blundell, J. E., & Cooling, J. (2000). Routes to obesity: phenotypes, food choices and activity. *British Journal of Nutrition, 83*(S1), S33-S38.

de Graaf, C., & Kok, F. J. (2010). Slow food, fast food and the control of food intake. [10.1038/nrendo.2010.41]. *Nat Rev Endocrinol, 6*(5), 290-293.

Dohm, F.-A., Cachelin, F. M., & Striegel-Moore, R. H. (2005). Factors That Influence Food Amount Ratings by White, Hispanic, and Asian Samples. *Obesity, 13*(6), 1061-1069.

Egan, J. M., & Margolskee, R. F. (2008). Taste cells of the gut and gastrointestinal chemosensation. *Molecular Interventions, 8*, 78–81.

Essed, N. H., van Staveren, W. A., Kok, F. J., & de Graaf, C. (2007). No effect of 16 weeks flavor enhancement on dietary intake and nutritional status of nursing home elderly. [doi: 10.1016/j.appet.2006.06.002]. *Appetite, 48*(1), 29-36.

FSA. (2006). *FSA Nutrient and Food Based Guidelines for UK Institutions*. London.

Gibson, E. L., & Brunstrom, J. M. (Eds.). (2007). *Learned influences on appetite and food intake: Evidence in human beings*. London: Elsevier

Gosby, A. K., Conigrave, A. D., Lau, N. S., Iglesias, M. A., Hall, R. M., Jebb, S. A., . . . Simpson, S. J. (2011). Testing Protein Leverage in Lean Humans: A Randomised Controlled Experimental Study. [doi:10.1371/journal.pone.0025929]. *PLoS ONE, 6*(10), e25929.

Green, S. M., Delargy, H. J., Joanes, D., & Blundell, J., E. (1997). A Satiety Quotient: A Formulation to Assess the Satiating Effect of Food. *Appetite, 29*, 291-304.

Herman, C. P., & Polivy, J. (2005). Normative influences on food intake. [doi: 10.1016/j.physbeh.2005.08.064]. *Physiology & Behavior, 86*(5), 762-772.

Herman, C. P., & Polivy, J. (2008). External cues in the control of food intake in human: the sensory-normative distinction. [10.1016/j.physbeh.2008.04.014]. *Physiol. Behav., 94*, 722-728.

Ikeda, K. (1908). On a New Seasoning. *Journal of Tokyo Chemistry Society, 30*, 820-836.

Kurihara, K. (2009). Glutamate: from discovery as a food flavor to role as a basic taste (umami). *American Journal of Clinical Nutrition, 90*(3), 719S-722S. doi: 10.3945/ajcn.2009.27462D

Loliger, J. (2000). Function and importance of glutamate for savory foods. *Journal of Nutrition, 130*(4), 915S-920S.

Luscombe-Marsh, N. D., Smeets, A. J. P. G., & Westerterp-Plantenga, M. S. (2008). Taste sensitivity for monosodium glutamate and an increased liking of dietary protein. *British Journal of Nutrition, 99*(4), 904-908. doi: 10.1017/s000711450788295x

Luscombe-Marsh, N. D., Smeets, A. J. P. G., & Westerterp-Plantenga, M. S. (2009). The addition of monosodium glutamate and inosine monophosphate-5 to high-protein meals: effects on satiety, and energy and macronutrient intakes. *British Journal of Nutrition, 102*(6), 929-937. doi: 10.1017/s0007114509297212

Mathey, M.-F. A. M., Siebelink, E., de Graaf, C., & Van Staveren, W. A. (2001). Flavor Enhancement of Food Improves Dietary Intake and Nutritional Status of Elderly Nursing Home Residents. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 56*(4), M200-M205. doi: 10.1093/gerona/56.4.M200

McKiernan, F., Houchins, J. A., & Mattes, R. D. (2008). Relationships between human thirst, hunger, drinking, and feeding. [doi: 10.1016/j.physbeh.2008.04.007]. *Physiology & Behavior, 94*(5), 700-708.

Prescott, J. (2004). Effects of added glutamate on liking for novel food flavors. *Appetite, 42*(2), 143-150. doi: 10.1016/j.appet.2003.08.013

Prescott, J., & Young, A. (2002). Does information about MSG (monosodium glutamate) content influence consumer ratings of soups with and without added MSG? *Appetite, 39*(1), 25-33.

Rogers, P. J., & Blundell, J. E. (1990). Umami And Appetite - Effects of monosodium Glutamate on Hunger and Food-Intake In Human Subjects. *Physiology & Behavior, 48*(6), 801-804.

Schiffman, S. S., Sattely-Miller, E. A., Zimmerman, I. A., Graham, B. G., & Erickson, R. P. (1994). Taste perception of monosodium glutamate (MSG) in foods in young and elderly subjects. *Physiology & Behavior, 56*(2), 265-275.

Sclafani, A. (1987). Carbohydrate taste, appetite, and obesity: An overview. [doi: 10.1016/S0149-7634(87)80019-2]. *Neuroscience & Biobehavioral Reviews, 11*(2), 131-153.

Shi, Z. M., Luscombe-Marsh, N. D., Wittert, G. A., Yuan, B. J., Dai, Y., Pan, X. Q., & Taylor, A. W. (2010). Monosodium glutamate is not associated with obesity or a greater prevalence of weight gain over 5 years: findings from the Jiangsu Nutrition Study of Chinese adults. *British Journal of Nutrition, 104*(3), 457-463. doi: 10.1017/s0007114510000760

Stunkard, A. J., & Messick, S. (1985). The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. . *Journal of Psychosomatic Research, 29*, 71-83.

Wansink, B., Painter, J. E., & North, J. (2005). Bottomless Bowls: Why Visual Cues of Portion Size May Influence Intake**. *Obesity Research, 13*(1), 93-100. doi: 10.1038/oby.2005.12

Yamaguchi, S., & Ninomiya, K. (2000). Umami and Food Palatability. *The Journal of Nutrition, 130*(4), 921.

Yamamoto, K., & Ishimaru, Y. (2013). Oral and extra-oral taste perception. *Seminars in Cell & Developmental Biology, 24*(3), 240-246. doi: http://dx.doi.org/10.1016/j.semcdb.2012.08.005

Yeomans, M. R. (1996). Palatability and the Micro-structure of Feeding in Humans: the Appetizer Effect. *Appetite, 27*(2), 119-133.

Yeomans, M. R. (2000). Rating changes over the course of meals: what do they tell us about motivation to eat? *Neuroscience & Biobehavioral Reviews, 24*(2), 249-259. doi: http://dx.doi.org/10.1016/S0149-7634(99)00078-0

Yeomans, M. R., Blundell, J. E., & Leshem, M. (2004). Palatability: response to nutritional need or need-free stimulation of appetite? *British Journal of Nutrition, 92*, S3-S14. doi: 10.1079/bjn20041134

Yeomans, M. R., Gould, N. J., Mobini, S., & Prescott, J. (2008). Acquired flavor acceptance and intake facilitated by monosodium glutamate in humans. *Physiology & Behavior, 93*(4-5), 958-966. doi: 10.1016/j.physbeh.2007.12.009

Yeomans, M. R., & Gray, R. W. (1997). Effects of Naltrexone on Food Intake and Changes in Subjective Appetite During Eating: Evidence for Opioid Involvement in the Appetizer Effect. [doi: 10.1016/S0031-9384(97)00101-7]. *Physiology & Behavior, 62*(1), 15-21.

Yeomans, M. R., & Gray, R. W. (2002). Opioid peptides and the control of human ingestive behaviour. *Neuroscience & Biobehavioral Reviews, 26*, 713–728.

Yeomans, M. R., Gray, R. W., & Conyers, T. H. B. (1998). Maltodextrin preloads reduce food intake without altering the appetiser effect. [doi: 10.1016/S0031-9384(98)00086-9]. *Physiology & Behavior, 64*(4), 501-506.

Yeomans, M. R., Lee, M. D., Gray, R. W., & French, S. J. (2001). Effects of test-meal palatability on compensatory eating following disguised fat and carbohydrate preloads. *International Journal of Obesity, 25*(8), 1215-1224.

Zijlstra, N., Mars, M., De Wijk, R. A., Westerterp-plantenga, M. S., & De Graaf, C. (2008). Liquid foods result in higher ad libitum food intake than semi-solid foods because of a higher eating rate. [doi: 10.1016/j.appet.2007.09.061]. *Appetite, 50*(2–3), 567.

Does sweetness generate an appetizer effect in a main meal?

Una Masic[a] and Martin R Yeomans[a]

[a] School of Psychology, University of Sussex, Brighton, BN1 9QH, UK

Contact details:

Una Masic

School of Psychology

University of Sussex

Brighton

BN1 9QH

UK

Tel: (01273) 872826

Email: u.masic@sussex.ac.uk

Abstract

Sweet taste is the most accepted of the five basic tastes and generates liking responses across species and from infanthood. The palatability generated by sweetness may in turn enhance early meal assessments of hunger and motivation to consume (the appetizer effect), subsequently increasing meal intake. This was assessed using ratings of appetite, motivation and pleasantness as well as intake and eating rate in an energy-matched *ad-libitum* soup lunch with or without added sucrose. 17 non-obese, low-restraint men (mean age: 21; BMI: 21) completed appetite ratings before, after soup tasting, after 25g consumption for 200g intake and at the end of the meal. No significant differences were noted for hunger, pleasantness or motivation to consume between conditions indicating that sweet taste did not generate an appetizer effect. Eating rate was slower in the sweet condition after the 200g re-fill stage but increased in the control condition, however this did not affect meal intake or the satiating quality of the soup between conditions. Overall, the findings indicate that sweetness did not affect meal intake or appetite. However, as pleasantness and sweetness ratings between conditions were similar, the true effect of sweet palatability on intake may have been masked and thus requires further research.

4.1 Introduction

Sweetness has been described as one of the five basic tastes and is elicited by carbohydrates such as simple sugars which provide dietary energy and food reward (Bellisle & Drewnowski, 2007). We display a preference for sweet taste soon after birth (Maller & Desor, 1973; Sclafani, 1987). This may be due to sweet taste being coupled with the expectation of receiving energy (Bellisle & Drewnowski, 2007; Sclafani, 1987; Yamamoto & Ishimaru, 2013) and this expectation may be maintained by exposure to sweet-nutrient associations over the lifespan (Sclafani & Ackroff, 2012). Some researchers suggest that sweet taste may stimulate hunger and therefore increase intake (Rogers & Blundell, 1989). This may be due to the known effects of palatability on increasing appetitive hunger, meal size and speed of eating (Yeomans, 1996). Thus the effects of palatability on appetite, motivation to consume and energy intake of a sweetened meal is required to gain a better understanding of the effect of this basic taste during consumption.

The taste of sucrose sweetness is one that is widely accepted as inherently appetizing (Sclafani, 1987). Indeed, sweet taste is one of the few basic tastes that infants show a preference for within a few hours of birth (Maller & Desor, 1973) and liking for sweet taste is widespread across many species (Sclafani, 1987), with an inability to detect sweetness only present in those species that do not consume food sources that are sweet (Li et al., 2006). This predominantly universal acceptance has been said to involve hormones including dopamine, serotonin and endogenous opioid peptides (Drewnowski, Krahn, Demitrack, Nairn, & Gosnell, 1995; Levine, Kotz, & Gosnell, 2003; Schwartz, 2006) which may also play a vital role in palatability evaluations (Berridge & Robinson, 1998; Blundell & Halford, 1998; Yeomans & Gray, 2002). Sweet taste palatability has also been related to the stimulation of hunger (Brala & Hagen, 1983), which in turn may increase intake (Rogers & Blundell, 1989) possibly due to the effects of sweetness signalling energy (Blundell & Rogers, 1994). This suggests that sensory feedback can alter appetite together with satiation as has been found with other basic tastes such as saltiness (Yeomans, et al., 2004) and foods such as soup (Yeomans, et al., 2008). One example of this is the appetizer effect (Yeomans, 1996) in which tasting an optimally palatable food can increase rated hunger, consumption and eating rate of the meal relative to a bland version of the same meal.

The appetizer effect has been suggested to rely on reward mechanisms with the positive experience (a pleasant taste) eliciting an approach response (eating more of the pleasant tasting food). Indeed, this positive feedback response hypothesis has been related to the opioid system, which is linked to generating feelings of satisfaction (Yeomans & Gray, 1997). A previous study has shown an appetizer effect of sucrose and aspartame containing yoghurts when provided at the most preferred concentration, with the highest energy intake evident in these conditions (Pérez, Dalix, Guy-Grand, & Bellisle, 1994). However, evidence to the contrary has found no effect of sweetness on hunger ratings and intake (Drewnowski et al., 1994a; Mattes, 1990; Rolls, Hetherington, & Laster, 1988) suggesting that sweet taste may have a range of effects on appetite (Appleton, Rogers, & Blundell, 2004; Lavin, French, & Read, 1997). As sweet taste is also an established basic taste, it is important to understand if the appetizer effects found with other basic tastes are present with sweet taste as this has not been found with umami taste (Masic & Yeomans, Chapter 3), to further understand whether umami is qualitatively different from the other basic tastes.

These inconsistencies may be related to the influence of sweet taste on perceived pleasantness of the individual. Indeed, participants categorized as sweet likers or dislikers have been shown to react differently to sweet cues and tastes, with sweet dislikers showing an earlier decline in pleasantness over the course of consumption of a sucrose solution despite not feeling satiated (Looy & Weingarten, 1991) (termed alliesthesia; Cabanac, 1971). Other research has also supported these differential responses in sweet likers and dislikers (Laeng, Berridge, & Butter, 1993), implying that taster status as well as flavour may affect the motivation to consume, even to a greater degree than the experience of satiation. Another issue in the assessment of sweet taste on appetite relates to the test foods used, with some researchers opting for sucrose solutions (Cabanac, 1971; Looy & Weingarten, 1991) whilst others used sweetened dessert-type items provided as main course meals (de Graaf, et al., 1993; Griffioen-Roose, Mars, Finlayson, Blundell, & de Graaf, 2009). Providing such varied stimuli may not be representative of the influence of sweet taste on appetite and intake as sweetened beverages such as sucrose solutions are not perceived to be as filling as whole foods (Mattes & Campbell, 2009) and thus may not indicate how satiating sweet foods are. Indeed, appetite and intake assessments have been shown to rely heavily on expectations about how filling a meal is perceived to be (Brunstrom, et al., 2008) and as

sweet taste is also inherent in many savoury dishes, the impact of this basic taste on appetite requires further research. No research to date has explicitly measured the appetizer effect with sweet taste.

Given the contradictory findings in the literature regarding the influence of sweetness on appetite, the present study addressed the effects of sucrose sweetness on the appetizer effect and intake. It was hypothesised that a optimally sucrose sweetened soup would increase rated hunger, pleasantness and motivation to consume within the early stages of a meal and result in increased energy intake and an enhanced eating rate as compared to an energy matched bland control in a group of sweet likers.

4.2 Method

4.2.1 Design
Early within-meal sensory evaluations of the flavour of a vegetable soup with or without added sweetness were assessed using a within-participants Latin Square design. A taste test was included prior to experimental sessions to ensure liking of the sweet soup was higher than the control. This was followed by a training session with a non-edible food to familiarize participants with the experimental procedure.

4.2.2 Participants
Twenty low restraint, non-obese men from a student population at the University of Sussex took part in the research of which seventeen were eligible for statistical analysis (age range: 18-25, mean age: 21±2.7; mean BMI: 21±3.2, BMI range: 18-29 kg/m²). Three participants were excluded from further analysis as outliers when the data was subjected to normality testing due to providing ceiling responses across sessions. One participant was also found to be in the overweight category (BMI of 29) however no significant correlation between BMI and *ad-libitum* intake was evident (r(17) = 0.16, p=.55) and was not found to be an outlier therefore this participant was not excluded from analysis.

Prospective participants were contacted based on their responses to an eating habits questionnaire using a database system. Subjects were emailed with details of the study

described as 'the effects of food on mood'. This was to ensure participants were not aware of the true purpose of the study to reduce possible demand characteristics. Individuals with dietary intolerances or allergies to the foods listed and those with high restraint scores (ratings above 8 on the Three Factor Eating Questionnaire (TFEQ); Stunkard & Messick, 1985) were excluded during recruitment as restraint has been found to influence perceptions about food and intake (Dohm, et al., 2005). Individuals smoking more than 5 cigarettes per week, those with a diagnosed eating disorder, a history of diabetes or those on any medications were excluded from participation. Upon completion of all sessions participants were paid £14 or granted credits through a participant pool scheme. If participants were not eligible to continue after the taste test they were paid £2 or one credit. Written informed consent was obtained before the study began and the research was approved by the University of Sussex ethics committee and was conducted in accordance with the Helsinki Declaration.

4.2.3 Test Food

4.2.3.1 Breakfast:

Breakfasts consisted of 80g cereal (Crunchy Nut Cornflakes, Kellogg's, UK), 200g semi-skimmed milk (Sainsbury's, Plc, UK) and 200g orange juice (Sainsbury's, Plc, UK) (total 503.6 Kcal). These quantities were determined based on the Food Standard Agency guidelines for male breakfast consumption (FSA, 2006).

4.2.3.2 Test soup:

The control soup comprised a low energy (32 Kcal/100g) carrot and spice soup containing carrots (Frozen Baby Carrots, Sainsbury's Plc, UK) onions, celery, olive oil (Medium Flavour Olive Oil, Sainsbury's Plc, UK), spice mixture (Garam Masala Schwartz, UK) and water with added maltodextrin (DE: 15.3, Cargill, UK; 1.05g/100g) or sugar (Granulated Sugar, Silver Spoon, UK; 1g/100g). The basic soup was specifically developed to assess the effects of sensory (taste) influences on appetite and satiety (Masic & Yeomans, 2013, Chapter 2). The concentrations of maltodextrin and sucrose were selected in line with the quantities added to commercially available soups and were deemed acceptable by all participants during the initial tasting session (control pleasantness mean: 55±1.8; sweet pleasantness mean: 72±1.4). The preparation of the soup and its serving on test days matched those used in a previous study (Masic & Yeomans, 2013, Chapter 3) with the addition of maltodextrin or sucrose added to the

appropriate condition. The energy density of the soups was mainly derived from carbohydrate comprising 51% carbohydrate, 44% fat and 5% protein. All nutritional information is available in Table 4.1.

Table 4.1. Nutritional composition of soup preloads per 100g

	Control	Sucrose
Carbohydrate	4.1	4.1
Protein	0.4	0.4
Fat	1.6	1.6
Carbohydrate (% energy)	51	51
Protein (% energy)	5	5
Fat (% energy)	44	44
Kcal	31.9	32

All soup tasting samples were served in 10g portions in plastic tasting cups during the initial tasting session and before the main meal on days 2 and 3. The soup was served in 300g portions and was consumed *ad-libitum* with additional 300g (re-fill) portions.

4.2.4 Computerized Data Collection

The Sussex Ingestion Pattern Monitor (SIPM version 2.0.13, University of Sussex) was used to track sensory and hedonic ratings at the taste test and throughout the early stages of the test meal. This consists of a balance system (Sartorius, Model BP4100) linked to a Dell computer. During the taste test trial and the experimental sessions participants were instructed to fill in mood and appetite ratings using digital Visual Analogue Scales (VAS) by the program. These ratings were presented as "How <word> do you feel?" with the left-hand end anchor ("Not AT ALL <word>") coded as 0 and the right-hand end anchor ("As <word> as I have ever felt/experienced") coded as 100. To prevent confusion during ratings the software prompted participants to "Please use the mouse to drag the bar to the point on the scale that best represents how you feel right now." before each evaluation. The ratings were completed by moving the mouse to the desired point on the scale and pressing "Rating Complete". Each participant rated how alert, anxious, clear-headed, full, happy, hungry, nauseous and thirsty they felt. In the taste test, two labelled taster samples of the target soup were then provided with instructions

to "Please take a mouthful of soup A (or B), count to 5 and then swallow." A series of VAS questions were then presented in the form "How <taste> is the soup" which examined the sensory qualities of the soup (these included how filling, pleasant, salty, savoury, strong and sweet). Once each soup was tasted and rated, participants were deemed eligible if pleasantness ratings of the control soup were between 45-65 points on the VAS and above 65 points for the sweetened soup (Table 4.2). This also ensured that participants were sweet likers, reducing the potential influence of alliesthesia on intake (Looy & Weingarten, 1991). If eligible, participants were then asked to complete a trial of the experimental procedure using a non-edible food item (dried lentils) and were asked five VAS questions relating to the auditory and visual cues related to the item. These questions included "how appealing is the colour of the lentils?"; "How strong is the colour of the lentils?"; "How appealing is the sound of the lentils?"; "How loud is the sound of the lentils?" and "How strong is your desire to buy lentils?".

Table 4.2. Mean (±SEM) pilot tasting evaluations of two soups with or without added sweetness. [1] Statistically significantly different from control P<0.05.

Rating	Control	Sweet
Filling	43.8±4.5	50.3±4.9
Pleasant	54.5±1.8	71.5±1.4[1]
Salty	29.6±3.7	37.4±5.6
Savoury	59±3.4	47.9±4.4
Strong	39.7±4.8	49.1±4.4
Sweet	50.4±4.7	64.4±3.3[1]

Within the experimental sessions, once participants had completed the taste test ratings with the target soup they were provided with a fixed portion of the test soup with instructions to "Please eat as much of the soup as you like until you feel comfortably full." Ratings of hunger, fullness, thirst, soup pleasantness and desire to consume the soup ("How much do you want to eat another mouthful of the soup right now?") were presented on-screen during consumption of the first bowl of soup and at the end of the meal. When additional (re-fill) portions of the soup were required, an on-screen message and alert sound were presented, instructing participants to call the experimenter. Once they had finished the meal, participants indicated this by clicking

"Finished eating" after which they were asked of their confidence in meal termination. Mood and appetite ratings were once again completed before the end of the session.

4.2.5 Procedure

On three non-consecutive days participants were invited to the Ingestive Behaviour Unit (IBU) at the University of Sussex. Prior to the first session participants consumed nothing but water two hours before the session to ensure mild hunger. The first session involved a taste test to determine whether participants preferred the sweetened soup. Participants then completed a trial of the experimental procedure using a non-edible food item (dried lentils) which they transferred from a bowl on the hidden balance to a separate bowl, completing questions about the appearance and sound of the lentils until a re-fill portion was required. The experimenter remained present during this training phase to ensure adequate completion of the task and to allow for any questions. At the end of the session participants were reminded that they would be provided with soup in the following sessions.

Following the training session, participants were instructed to abstain from eating from 2300h the night before testing and were provided with a control breakfast at a pre-arranged time (from 0830 h – 1030 h) for the experimental sessions. Participants were then permitted to leave the IBU with instructions to consume only water and returned 3h later for lunch which ran from 1130h – 1330h. For lunch sessions participants were taken to a windowless cubicle containing a glass of water and the SIPM software. They were told to follow the on-screen instructions which detailed the procedure and initiated the first set of mood and appetite ratings. Sensory and hedonic evaluations with a taster sample of the soup were then completed according to condition followed by ratings of appetite and motivation to eat. Participants were then provided with a bowl of the test soup which they consumed *ad-libitum* and were interrupted by the SIPM after approximately 25g consumption for the first 75g of intake and at the 200g re-fill stage with appetite and motivation ratings after which they were left to consume as much soup as desired. Additional (300g) portions of soup were provided until participants stated that they were full. Using this methodology reduced the impact of external cues such as an empty bowl from determining meal size (Herman & Polivy, 2008b; Wansink, Painter, & North, 2005a). A final set of mood and appetite ratings were then filled-in

and participants were free to leave and were weighed, measured and debriefed before payment on the final day. A graphical representation of the experimental procedure can be seen in Appendix 4.1.

4.2.6 Data Analysis

The main variable was the sweetness of the soup with two levels; a control (bland) condition and a sucrose sweetened (sweet) condition. The impact of soup condition on meal intake, eating rate, appetite, motivation to eat and pleasantness as well as sensory ratings were assessed using repeated measures ANOVAs, with condition as the main factor examined. Appetite (rated hunger and fullness) was measured at seven points throughout each session including before meal intake, after tasting the relevant soup, at 25g intervals for the first 75g consumption, at the 200g re-fill point and immediately at the end of the meal. Ratings of pleasantness were included at six points following the same structure as the appetite evaluations from the taste test. Additional ratings of desire to eat were also included to assess whether this motivational construct differed from hunger and pleasantness (Berridge, 1996; Berridge & Robinson, 1998) and highlighted any subtle effects that these two ratings may have been insensitive to pick up. This was rated in the same way as the appetite ratings from 25g consumption until the end of the meal. The data could not be subjected to best fit quadratic functions (see Masic & Yeomans, 2013; Chapter 3) thus the more subtle effects of soup condition on appetite and intake were measured to track the rate of satiation during consumption, similar to assessments used by Green et al., (1997; see Chapter 3). This analysis tracks the rate of satiety after consuming a food to establish the extent to which this food subsequently reduces rated appetite per unit of intake (grams) initially consumed (establishing a satiety quotient). As post-meal ratings were not available, the rate of satiation during the meal was taken into account using within-meal hunger ratings to establish a 'satiation quotient' (see Masic & Yeomans, 2013; Chapter 3). The amount consumed (grams) was compared with rated hunger during the meal to provide an index of how satiating the meal was at any given point during consumption. Hunger ratings over the course of the session (before, during and post-meal) and quantity consumed (grams) were assessed in a 2x6 repeated measures ANOVA. Test order was included as a factor in all analyses.

Nausea ratings were also assessed pre- and post-meal using repeated measures ANOVA. This was to ensure rated appetite, hedonic and motivational assessments as well as meal intake were not influenced by feelings of illness. The impact of thirst during the course of the meal was also assessed to ensure appetite and intake were not influenced by thirst as has been suggested previously (Bellisle & Le Magnen, 1981; McKiernan, et al., 2008). As three participants were excluded from the final analysis the effect of order was examined to ensure that this did not act as a confounding factor due to incomplete counterbalancing. Where sphericity was violated, adjusted Greenhouse Geisser values are reported (ε = <0.75). Significant interactions were assessed with pair-wise protected contrasts across soup conditions at relevant interrupt stages and effect sizes for these interactions are reported using Pearson's correlation coefficient. Power analysis was conducted using G*Power.

4.3 Results

4.3.1 Rated appetite, motivation and meal pleasantness during the meal

The main focus was to understand whether meal palatability influenced appetite, motivation to consume and intake tracked early during the meal and at the end of the meal. As expected, there were no significant differences in appetite before lunch sessions began (hunger: $F(1,16) = 0.03$, p=.86; fullness: $F(1,16) = 0.68$, p=.42). No effect of soup condition and no condition*time interaction ($F(1,16) = 0.30$, p=.59) was evident for hunger after tasting suggesting that an appetizer effect was not present despite high pleasantness ratings across conditions (Table 4.3).

Table 4.3. Mean (±SEM) sensory evaluations of two soups with or without added sweetness.

Rating	Control	Sweet
Filling	55.1±4.7	56.1±4.8
Pleasant	69.1±2.9	73±1.7
Salty	36.2±3.8	43±4.1
Savoury	59.1±3.3	57±3.3
Strong	54.1±3.1	56±3.7
Sweet	57.8±3.3	55.7±4.0

The lack of an observable appetizer effect may be due to low power as a result of the small sample size analysed with post-hoc power analysis revealing only 30% statistical power. Based on the observed findings (Figure 4.1), to ensure adequate power of 80%, approximately 52 participants would have been required to detect a significant appetizer effect between conditions. When appetite was assessed over the course of the meal (time), no significant effects of condition were evident (hunger: $F_{(1,16)} = 1.32$, p=.27; fullness: $F_{(1,16)} = 1.78$, p=.20; Figure 4.1). Perhaps unsurprisingly, ratings of desire [$F_{(1,16)} = 0.71$, p=.41] and pleasantness [$F_{(1,16)} = 0.81$, p=.38] during intake also did not differ significantly according to condition (Figure 4.1). As expected, assessments of hunger decreased ($F_{(2.5, 40.4)} = 75.04$, p <.001) and fullness increased ($F_{(3.6, 58.3)} = 115.45$, p <.001) over time. Equally, ratings of desire to eat ($F_{(1.9, 31.1)} = 231.39$, p <.001) and pleasantness ($F_{(1.7, 27.0)} = 23.22$, p <.001) declined over the course of intake.
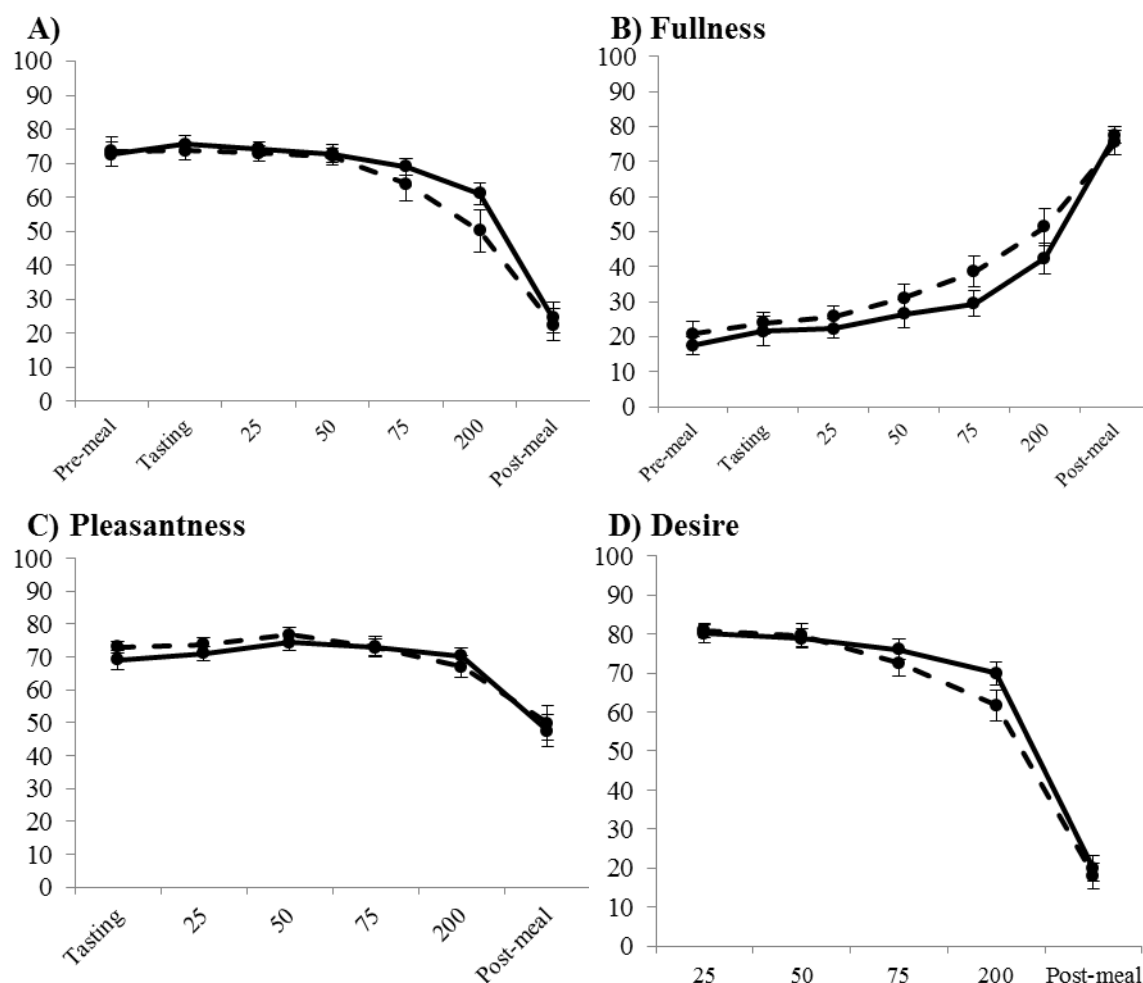
Figure 4.1. Mean (±SEM) VAS ratings (mm) for hunger (Panel A), fullness (Panel B), pleasantness (Panel C) and desire (Panel D) at interrupt points (g) before, during and after ingestion of soup with (_ •_) or without (——•) added sweetness.

4.3.2 Intake, eating rate and the experience of satiation

Energy intake did not differ between conditions (F(1,16) = 0.39, p=.54; Table 4.4). This may explain why hunger and fullness ratings were similar across soup conditions as the equivalence found in consumption matched those observed in the appetite ratings.

Table 4.4. Mean (±SEM) *ad-libitum* energy intake (grams and calories consumed; Kcal) across two soups with or without added sweetness.

	Control	Sweet
Energy intake (g)	507.01±35.16	481.37±35.03
Energy intake (Kcal)	161.74±11.77	154.04±11.15

However, a main effect of time ($F(4,64) = 19.31$, $p <.001$) suggested that eating rate initially increased during the first two interrupt stages, then slowed until the first re-fill stage. A condition*time interaction ($F(2.4, 38.1) = 10.97$, $p <.001$) also revealed that eating rate differed significantly by condition (Figure 4.2) with further analyses using pair-wise protected contrasts revealing that eating rate slowed significantly after the 200g re-fill until the end of the meal in the sweet condition but increased in the control soup ($t(16) = 9.12$, $p <.001$, $r = .86$). Analyses of order indicated that the condition initially received did not influence this interaction ($F(4,60) = 0.77$, $p=.55$). When average eating rate for the whole meal was assessed, no differences were evident between conditions ($F(1,16) = 2.28$, $p=.15$), suggesting that the effect found was sensitive to the specific interrupt point. The more subtle effects of the rate of satiation during intake were assessed using a similar analysis established by Green and colleagues (Green, et al., 1997). A significant effect of time ($F(2.0, 31.3) = 83.83$, $p <.001$) suggested that the meal was rated as more satiating as intake increased over time (Figure 4.2). However no effect of condition ($F(1,16) = 0.60$, $p=.45$) and no condition*time interaction ($F(2.8, 44.9) = 0.91$, $p=.48$) at discrete time points over the course of the intake session was evident indicating that there was no difference between soups in how satiating per gram consumed and that this was not influenced by the stage of consumption.
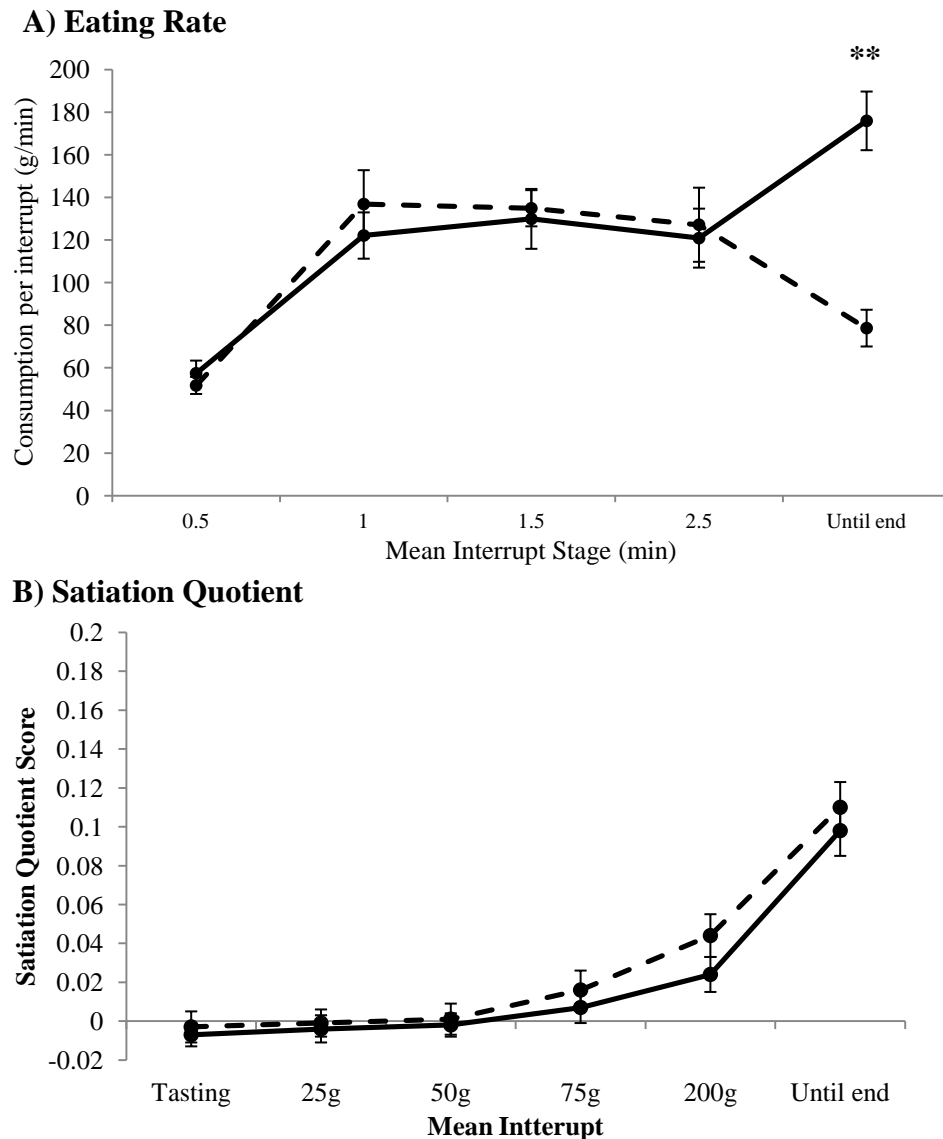
A) Eating Rate

B) Satiation Quotient

Figure 4.2. Mean (±SEM) eating rate (g/min) at mean time interrupts (Panel A) and rate of satiation in relation to volume consumed at specified interrupts (Panel B) during ingestion of a soup with (– ● –) and without (──●──) added sweetness. Statistically significant differences between no and added sweet conditions are indicated: ** $P<0.001$.

4.3.3 Sensory assessments of the meal

There were no significant differences evident across conditions for soup pleasantness ($F(1,16) = 1.74$, p=.21) or sweetness ($F(1,16) = 0.21$, p=.65; Table 4.3) despite the baseline pilot session indicating clear significant differences between conditions (Table 4.2). As expected, all other sensory analyses of how filling, savoury, salty and strong were found to be non-significant (Table 4.3).

4.3.4 Nausea, thirst and order effects

No significant effect of nausea was evident between conditions ($F(1,16) = 0.56$, p=.46) or over time ($F(1,16) = 0.72$, p=.41) indicating that intake was not confounded by nausea; m: 18.6±1.2. Equally, no interaction with order was apparent ($F(1,15) = 1.01$, p=.33) suggesting that the order of soup presentations did not influence feelings of nausea across testing days. Baseline thirst analysis before intake sessions was found to be non-significant ($F(1,16) = 0.15$, p=.71) indicating all participants arrived in a similar state of thirst. When thirst was assessed over the duration of the eating episode, no significant effect of time ($F(3.3, 52.3) = 1.88$, p=.10) or condition ($F(1,16) = 0.02$, p=.88) was observed. Despite unequal counterbalancing there was no effect of order on subjective evaluations of hedonic, motivational and appetitive responses and on intake. Thus the order of soup presentation did not affect feelings of hunger ($F(1,15) = 0.54$, p=.48), fullness ($F(1,15) = 0.55$, p=.47), pleasantness ($F(1,15) = 0.26$, p=.62), desire ($F(1,15) = 0.36$, p=.56) or intake ($F(1,15) = 1.43$, p=.58).

4.4 Discussion

The main results indicate that eating rate reduced towards the end of a sucrose sweetened meal compared to an energy-matched unsweetened meal. However, sweetness was not found to influence early meal ratings of pleasantness, appetite, motivation to consume or energy intake relative to the control condition. The sweet soup also did not influence how satiating the meal was perceived to be. These findings may be explained in terms of perceptions of sweetness as the sweet condition was not identified as significantly sweeter or more pleasant than the control condition.

The influence of sweet taste in a lunch setting was not shown to generate a detectable appetizer effect when compared to a 'non-sweet' control. The similar hunger ratings in the early stages of the meal may be due to the comparable ratings of pleasantness and sweetness found across conditions. This may explain why test meal intake and the satiating capacity of the soup were equally matched between conditions and why 'desire' ratings did not differ according to soup condition, as the hedonic effects of the soup were deemed equally rewarding (Berridge, 1996; Berridge & Robinson, 2003; Yeomans, et al., 2004). Eating rate however steadily increased during the first bowl of

soup, slowing down from the re-fill stage until the end of the meal in the sweet condition but speeding up further in the control condition. As the change in eating rate occurred after the re-fill stage, it may be that eating rate increased in the control condition due to a cessation in meal interrupts at this point. However, the speed of intake decreased in the sweet condition at this stage, indicating that this effect was not an artefact of the methods used. Equally, due to the high pleasantness assessments of the soups and the focus on sweet likers, an alliesthesia effect cannot account for the divergence found, particularly as desire and pleasantness ratings were comparable (Looy & Weingarten, 1991). Eating rate also did not differ from the participant's first session to the next, suggesting that participant naiveté cannot account for the results found. As whole meal eating rate between conditions remained similar, this divergence may have been spurious due to the limited size of the sample assessed.

The predicted effect of sweet taste increasing appetite at the early stages of a meal compared to a bland control was not evident and may be related to aspects of the design employed. Although every effort was made to ensure that participants preferred the sweet soup relative to the control before the study began, no difference in pleasantness was evident as had been found in the pilot experiment. This may be due to a contrast effect, with the difference in sweetness being more easily, and more extremely perceived when both soups were sampled together in the pilot session than when provided across test days. Indeed, it may also be that the sweetness manipulation was too subtle, or that the dextrose equivalent of the carbohydrate used was too high and thus the difference in sweetness was not detected at separate exposure sessions. The sample assessed may also have been too small to adequately detect an influence of this basic taste on early within-meal appetite ratings.

The role of sweet taste on appetite has been extensively assessed due to the innate enjoyment of sweet taste evident from birth (Maller & Desor, 1973) and across species (Sclafani, 1987). This study aimed to assess the impact of sweet taste on early appetite evaluations and how they may influence overall intake in a meal context as previous findings have been mixed (Appleton, et al., 2004; Lavin, et al., 1997; Pérez, et al., 1994), possibly due to the effects of sweetness being focussed on sweet solutions (Cabanac, 1971; Lavin, et al., 1997) and dessert-type main courses (Finlayson, King, & Blundell, 2007; Griffioen-Roose, et al., 2009). Overall, early meal assessments of

appetite, pleasantness, motivation and overall intake were not affected by a difference in sweetness in a meal context. Eating rate was found to increase after the first bowl consumed in the control condition but decrease in the sweet condition. Due to the similar hedonic value of the soups used, further investigation is warranted with less subtle taste manipulations and a larger sample size.

4.5 Summary of key findings and ideas for future research

- The main findings suggested no appetizing effect of sweetness on increasing the experience of hunger, motivation to eat, or pleasantness after tasting a sucrose sweetened soup

- Eating rate was slower in the added sucrose condition and this condition was also rated as more satiating per gram consumed

- However, no differences in energy intake were also seen between the sucrose sweetened condition and the energy-matched control despite pilot ratings of palatability indicating the sucrose condition was more pleasant in flavour

- Both sucrose sweet and control soups were deemed as equally palatable and sweet during the experimental phase, indicating that the appetizer effect may not have been easily assessed

- As a consequence it is still unclear whether basic tastes such as sweetness or umami elicit an appetizer effect that influences intake from the early stages of the meal

- Future research may wish to make the sweet distinction more clear before assessing the appetizing role of a sucrose sweetened meal and may wish to use a solid food vehicle to ensure a more sensitive methodology

Appendix 4.1. Graphical depiction of the experimental procedure carried out for the training day (Panel A) and on test days (Panel B).
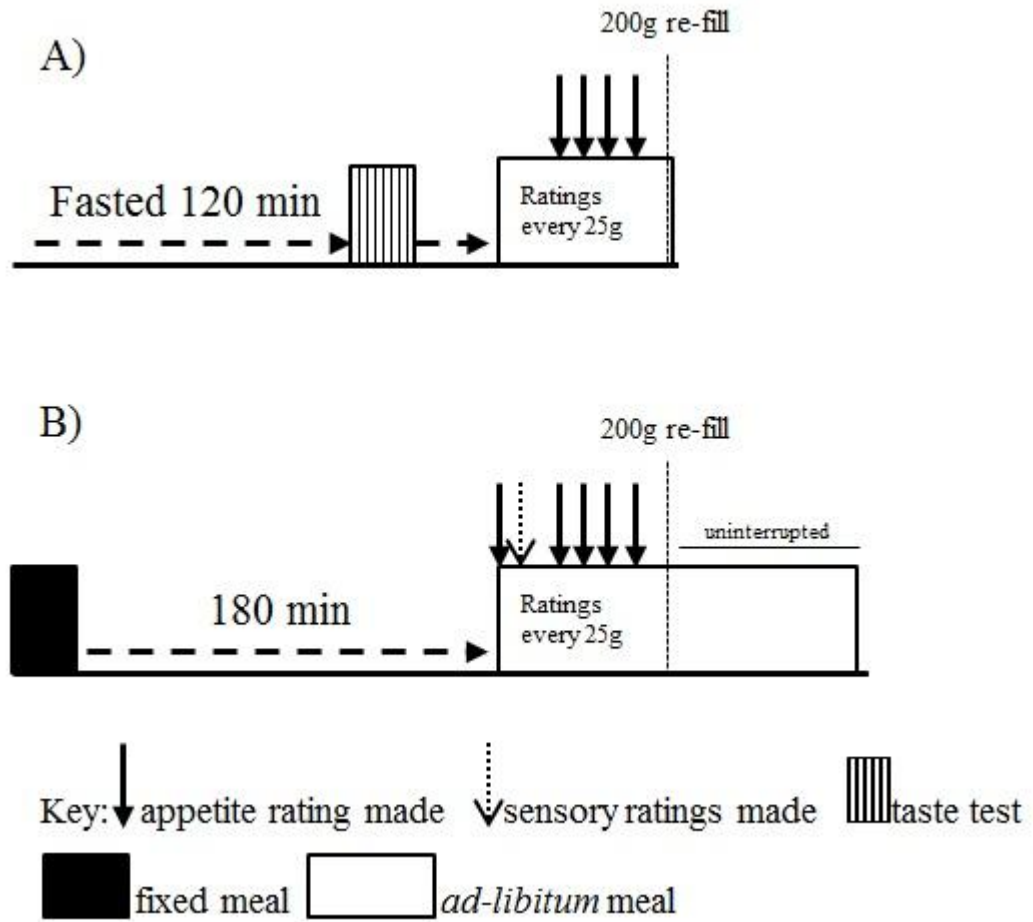
4.6 References

Appleton, K. M., Rogers, P. J., & Blundell, J. E. (2004). Effects of a sweet and a nonsweet lunch on short-term appetite: differences in female high and low consumers of sweet/low-energy beverages. *Journal of Human Nutrition and Dietetics, 17*(5), 425-434. doi: 10.1111/j.1365-277X.2004.00548.x

Bellisle, F., & Drewnowski, A. (2007). Intense sweeteners, energy intake and the control of body weight. *European Journal of Clinical Nutrition, 61*(6), 691-700.

Bellisle, F., & Le Magnen, J. (1981). The structure of meals in humans: Eating and drinking patterns in lean and obese subjects. [doi: 10.1016/0031-9384(81)90237-7]. *Physiology & Behavior, 27*(4), 649-658.

Berridge, K. C. (1996). Food reward: Brain substrates of wanting and liking. *Neuroscience & Biobehavioral Reviews, 20*(1), 1-25. doi: http://dx.doi.org/10.1016/0149-7634(95)00033-B

Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? [doi: 10.1016/S0165-0173(98)00019-8]. *Brain Research Reviews, 28*(3), 309-369.

Berridge, K. C., & Robinson, T. E. (2003). Parsing reward. *Trends in Neurosciences, 26*(9), 507-513. doi: http://dx.doi.org/10.1016/S0166-2236(03)00233-9

Blundell, J. E., & Halford, J. C. G. (1998). Serotonin and Appetite Regulation: Implications for the Pharmacological Treatment of Obesity. *CNS Drugs, 9*(6), 473-495.

Blundell, J. E., & Rogers, P. J. (Eds.). (1994). *Sweet carbohydrate substitutes (intense sweeteners) and the control of appetite: scientific issues.* (Vol. 113–124). London: CRC Press.

Brala, P. M., & Hagen, R. L. (1983). Effects of sweetness perception and caloric value of a preload on short term intake. [doi: 10.1016/0031-9384(83)90030-6]. *Physiology & Behavior, 30*(1), 1-9.

Brunstrom, J. M., Shakeshaft, N. G., & Scott-Samuel, N. E. (2008). Measuring 'expected satiety' in a range of common foods using a method of constant stimuli. *Appetite, 51*(3), 604-614. doi: http://dx.doi.org/10.1016/j.appet.2008.04.017

Cabanac, M. (1971). Physiological role of pleasure. . *Science 173*, 1103–1107.

de Graaf, C., Schreurs, A., & Blauw, Y. H. (1993). Short-term effects of different amounts of sweet and nonsweet carbohydrates on satiety and energy intake. [doi: 10.1016/0031-9384(93)90290-V]. *Physiology & Behavior, 54*(5), 833-843.

Dohm, F.-A., Cachelin, F. M., & Striegel-Moore, R. H. (2005). Factors That Influence Food Amount Ratings by White, Hispanic, and Asian Samples. *Obesity, 13*(6), 1061-1069.

Drewnowski, A., Krahn, D. D., Demitrack, M. A., Nairn, K., & Gosnell, B. A. (1995). Naloxone, an opiate blocker, reduces the consumption of sweet high-fat foods in obese and lean female binge eaters. *The American Journal of Clinical Nutrition, 61*(6), 1206-1212.

Drewnowski, A., Massien, C., Louis-Sylvestre, J., Fricker, J., Chapelot, D., & Apfelbaum, M. (1994a). Comparing the effects of aspartame and sucrose on motivational ratings, taste preferences, and energy intakes in humans. *The American Journal of Clinical Nutrition, 59*(2), 338-345.

Finlayson, G., King, N., & Blundell, J. E. (2007). Is it possible to dissociate 'liking' and 'wanting' for foods in humans? A novel experimental procedure. [doi: 10.1016/j.physbeh.2006.08.020]. *Physiology & Behavior, 90*(1), 36-42.

FSA. (2006). *FSA Nutrient and Food Based Guidelines for UK Institutions*. London.

Green, S. M., Delargy, H. J., Joanes, D., & Blundell, J., E. (1997). A Satiety Quotient: A Formulation to Assess the Satiating Effect of Food. *Appetite, 29*, 291-304.

Griffioen-Roose, S., Mars, M., Finlayson, G., Blundell, J. E., & de Graaf, C. (2009). Satiation Due to Equally Palatable Sweet and Savory Meals Does Not Differ in Normal Weight Young Adults. *The Journal of Nutrition, 139*(11), 2093-2098. doi: 10.3945/jn.109.110924

Herman, C. P., & Polivy, J. (2008). External cues in the control of food intake in humans: The sensory-normative distinction. [doi: 10.1016/j.physbeh.2008.04.014]. *Physiology & Behavior, 94*(5), 722-728.

Laeng, B., Berridge, K. C., & Butter, C. M. (1993). Pleasantness of a Sweet Taste during Hunger and Satiety: Effects of Gender and "Sweet Tooth". [doi: 10.1006/appe.1993.1043]. *Appetite, 21*(3), 247-254.

Lavin, J. H., French, S. J., & Read, N. W. (1997). The effect of sucrose- and aspartame-sweetened drinks on energy intake, hunger and food choice of female, moderately restrained eaters. *International Journal of Obesity, 21*, 37–42.

Levine, A. S., Kotz, C. M., & Gosnell, B. A. (2003). Sugars and Fats: The Neurobiology of Preference. *The Journal of Nutrition, 133*(3), 831S-834S.

Li, X., Li, W., Wang, H., Bayley, D. L., Cao, J., Reed, D. R., . . . Brand, J. G. (2006). Cats Lack a Sweet Taste Receptor. *The Journal of Nutrition, 136*(7), 1932S-1934S.

Looy, H., & Weingarten, H. P. (1991). Effects of metabolic state on sweet taste reactivity in humans depend on underlying hedonic response profile. *Chemical Senses, 16*(2), 123-130. doi: 10.1093/chemse/16.2.123

Maller, O., & Desor, J. (Eds.). (1973). *Effect of taste on ingestion by human newborns.*: NIH.

Mattes, R. D. (1990). Effects of aspartame and sucrose on hunger and energy intake in humans. [doi: 10.1016/0031-9384(90)90350-D]. *Physiology & Behavior, 47*(6), 1037-1044.

Mattes, R. D., & Campbell, W. W. (2009). Effects of Food Form and Timing of Ingestion on Appetite and Energy Intake in Lean Young Adults and in Young Adults with Obesity. *Journal of the American Dietetic Association, 109*(3), 430-437. doi: http://dx.doi.org/10.1016/j.jada.2008.11.031

McKiernan, F., Houchins, J. A., & Mattes, R. D. (2008). Relationships between human thirst, hunger, drinking, and feeding. [doi: 10.1016/j.physbeh.2008.04.007]. *Physiology & Behavior, 94*(5), 700-708.

Pérez, C., Dalix, A.-M., Guy-Grand, B., & Bellisle, F. (1994). Human Responses to Five Concentrations of Sucrose in a Dairy Product: Immediate and Delayed Palatability Effects. [doi: 10.1006/appe.1994.1044]. *Appetite, 23*(2), 165-178.

Rogers, P. J., & Blundell, J. E. (1989). Separating the actions of sweetness and calories: Effects of saccharin and carbohydrates on hunger and food intake in human subjects. [doi: 10.1016/0031-9384(89)90093-0]. *Physiology & Behavior, 45*(6), 1093-1099.

Rolls, B. J., Hetherington, M., & Laster, L. J. (1988). Comparison of the effects of aspartame and sucrose on appetite and food intake. [doi: 10.1016/S0195-6663(88)80048-5]. *Appetite, 11, Supplement 1*(0), 62-67.

Schwartz, M. W. (2006). Central Nervous System Regulation of Food Intake. *Obesity, 14*(2S), 1S-8S.

Sclafani, A. (1987). Carbohydrate taste, appetite, and obesity: An overview. [doi: 10.1016/S0149-7634(87)80019-2]. *Neuroscience & Biobehavioral Reviews, 11*(2), 131-153.

Sclafani, A., & Ackroff, K. (2012). Role of gut nutrient sensing in stimulating appetite and conditioning food preferences. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 302*(10), R1119-R1133. doi: 10.1152/ajpregu.00038.2012

Stunkard, A. J., & Messick, S. (1985). The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. . *Journal of Psychosomatic Research, 29*, 71-83.

Wansink, B., Painter, J. E., & North, J. (2005). Bottomless bowls: why visual cues of portion size may influence intake. [10.1038/oby.2005.12]. *Obes. Res., 13*, 93-100.

Yamamoto, K., & Ishimaru, Y. (2013). Oral and extra-oral taste perception. *Seminars in Cell & Developmental Biology, 24*(3), 240-246. doi: http://dx.doi.org/10.1016/j.semcdb.2012.08.005

Yeomans, M. R. (1996). Palatability and the Micro-structure of Feeding in Humans: the Appetizer Effect. *Appetite, 27*(2), 119-133.

Yeomans, M. R., Blundell, J. E., & Leshem, M. (2004). Palatability: response to nutritional need or need-free stimulation of appetite? *British Journal of Nutrition, 92*, S3-S14. doi: 10.1079/bjn20041134

Yeomans, M. R., Gould, N. J., Mobini, S., & Prescott, J. (2008). Acquired flavor acceptance and intake facilitated by monosodium glutamate in humans. *Physiology & Behavior, 93*(4-5), 958-966. doi: 10.1016/j.physbeh.2007.12.009

Yeomans, M. R., & Gray, R. W. (1997). Effects of Naltrexone on Food Intake and Changes in Subjective Appetite During Eating: Evidence for Opioid Involvement in the Appetizer Effect. [doi: 10.1016/S0031-9384(97)00101-7]. *Physiology & Behavior, 62*(1), 15-21.

Yeomans, M. R., & Gray, R. W. (2002). Opioid peptides and the control of human ingestive behaviour. *Neuroscience & Biobehavioral Reviews, 26*, 713–728.

Does acute or habitual protein deprivation influence liking for monosodium glutamate?

Una Masic[a] and Martin R Yeomans[a]

[a] School of Psychology, University of Sussex, Brighton, BN1 9QH, UK

Contact details:

Una Masic

School of Psychology

University of Sussex

Brighton

BN1 9QH

UK

Tel: (01273) 872826

Email: u.masic@sussex.ac.uk

Abstract

The umami flavour generated by monosodium glutamate (MSG) has been proposed as the marker for the presence of protein in foods. As protein is the most closely regulated macronutrient in the diet, the present study addressed whether acute protein deprivation, habitual protein intake or a combination of the two influenced liking for the taste of MSG. 24 low-restraint male participants were tested after their habitual breakfast (baseline), after a low protein breakfast and a high protein breakfast and returned at lunchtime to complete sensory assessments of flavour samples (control, MSG, sodium chloride (NaCl) or Acesulphame K (Ace K)) in low or high concentrations in a soup using a within-subjects design. Participants also completed a habitual protein intake questionnaire. Acute protein deprivation increased liking for the flavours more strongly than on the high protein day and a main effect of flavour revealed NaCl and Ace K flavours were liked less on both protein manipulation days when compared to the control sample. Habitual protein intake was not related to liking for MSG stimuli alone but habitual high protein consumers rated a high concentration of MSG as more pleasant than any other flavour when in protein deficit. Overall, these findings suggest that liking of high MSG concentrations may be moderated by nutritional need in high protein consumers.

5.1 Introduction

Protein is an essential macronutrient predominantly consumed in savoury tasting foods (Blundell & Rogers, 1994; Luscombe-Marsh, et al., 2008; Viskaal-van Dongen, Kok, & de Graaf, 2011). It is the most tightly regulated macronutrient in the diet and has been related to the overconsumption of high carbohydrate and high fat containing foods if too little has been ingested to meet protein needs (Simpson & Raubenheimer, 2005). Animals and humans are able to detect the quality and quantity of protein consumed during a meal and use this information to monitor protein intake (Kondoh, et al., 2000). This indicates that the taste system has evolved to provide information about the nutritional value of food. Umami, the fifth basic taste which can be generated by the flavour enhancer monosodium glutamate (MSG) has been linked to the detection of protein in the diet (He, et al., 2004; Ikeda, 1908; Luscombe-Marsh, et al., 2008) and may influence food selection based on nutritional need state. Therefore, it may be that liking for umami sources such as MSG in foods changes according to the amount of protein generally consumed in the diet (protein status) and may also be sensitive to acute protein deprivation.

Protein accounts for 15% dietary energy intake (Veldhorst et al., 2008; Westerterp-Plantenga, 1994) which has remained constant over time and across populations compared to other macronutrients (Simpson & Raubenheimer, 2005; Westerterp-Plantenga, 1994) and may be related to the body's efficiency in regulating protein feedback (Essed et al., 2009; He, et al., 2004) due to the limited availability of carbohydrate and fat in human evolutionary history (Simpson, et al., 2003; Simpson & Raubenheimer, 2000). Indeed, protein intake has been shown to be prioritized over carbohydrate and fat intake in *ad-libitum* meals after participants were provided with unbalanced diets (Austin, et al., 2011; Larsen, et al., 2010; Simpson, et al., 2003; Simpson & Raubenheimer, 2005). This change in food selection may be influenced by flavour, with increases in consumption of savoury foods due to their association with protein. Indeed, the savoury taste elicited by MSG has been found to increase preference for foods with stronger MSG concentrations in both adults (Murphy, 1987) and children (Vazquez, et al., 1981) in protein-deficient states with poor nutritional status compared to well-nourished controls. This indicates that long-term protein deprivation may influence the selection of and liking for MSG. Laska and Hernandez

Salazar (2004) assessed MSG taste preferences in primates naturally consuming low or high quantities of animal protein. Contrary to the previous findings, they reported that primates consuming more animal protein preferred stronger concentrations of MSG whilst low animal protein consumers enjoyed MSG more overall, particularly when delivered in weaker concentrations. These differences between malnourished humans and primates consuming low amounts of animal protein may be due to learned experiences of umami concentrations in foods. As the malnourished humans tested had previously been exposed to animal protein, which is abundant in umami (Jinap & Hajeb, 2010), their liking and preference for strong concentrations of MSG flavour may have been greater due to previously learned associations between stronger concentrations of umami linked to high protein sources. Indeed, liking for MSG in humans is correlated with liking of high protein foods (Luscombe-Marsh, et al., 2008). However, as primates who consume low amounts of protein subsist on predominantly plant-based diets, their exposure to strong concentrations of umami tastants is less likely and thus they may prefer weaker concentrations of umami, as would naturally be found in their diet. Additionally, the primates consuming low-protein diets may be more sensitive to sensory cues, including umami taste, which may predict the presence of protein, in line with optimal foraging theory (Stephens & Krebs, 1986). This influence of habitual dietary intake affecting preference for a stimulus which may be predictive of protein has not been previously assessed in humans and as protein is so tightly regulated, may provide further insight into the influence of general protein intake on hedonic preference.

The influence of protein status may even alter hedonic taste perception in short-term manipulations. Gibson and colleagues (1995) found that participants experimentally manipulated to be in acute mild protein deficit (by provision of low protein breakfasts) learned to prefer a dessert flavour that was previously paired with the delivery of more protein when compared to participants in nutritional balance. This mild protein deprivation has also been found to increase ingestion and choice of flavours that have been paired with the post-ingestive consequences of consuming protein in rats (Baker & Booth, 1989; Booth & Baker, 1990; Gibson & Booth, 1986) and protein conditioned flavour preferences have been reported to be eliminated by a protein preload, but not after an equicaloric carbohydrate preload (Baker, et al., 1987). Such flavour preferences required very few pairings of novel flavoured protein-rich food when in mild protein

need (Baker & Booth, 1989; Booth & Baker, 1990; Gibson & Booth, 1986), reflecting the tight regulation of this macronutrient within the diet.

However, although short-term protein deprivation has been shown to influence choice through learning, the effect of an acute deficit on liking for tastes that may naturally be associated with protein has not been assessed. Equally, it is not known whether habitual protein consumption in the diet influences taste preferences for these flavours in humans in the same way as has been reported in animals. Thus, the current experiment aimed to explore the effects of manipulating protein status (through the presentation of high or low protein breakfasts) and habitual protein intake in the diet (using a protein food frequency questionnaire) on subsequent sensory assessments of a hedonically neutral savoury soup with strong and weak concentrations of MSG (0.6% or 1% (w/w)), sodium chloride (NaCl; 0.3% or 0.4% (w/w)), sweetener (acesulphame K; 0.005% or 0.01% (w/w)) or an unflavoured control. The additional flavour conditions were included to assess whether the effects of protein were specific to the flavour of MSG alone as opposed to energy need (sweetness) (Bellisle & Drewnowski, 2007; Sclafani, 1987; Yamamoto & Ishimaru, 2013) or specifically the sodium found in MSG (saltiness). Baseline flavour assessments were also taken before acute protein manipulations. It was hypothesised that MSG would be optimally preferred when participants were in an acute protein deficit with stronger concentrations of MSG being most preferred. Protein status based on habitual protein intake was also hypothesised to be related to liking of MSG, with naturally high protein consumers expected to prefer stronger concentrations of MSG compared to naturally low protein consumers, particularly when in acute protein deficit.

5.2 Method

5.2.1 Design

A two way 3x7 mixed design was used to contrast protein manipulation day (baseline, low protein or high protein) with evaluations of strong or weak concentrations of samples of soup with added savouriness (0.6% or 1% w/w MSG), saltiness (0.3% or 0.4% w/w NaCl), sweetness (0.005% or 0.01% Acesulphame K (Ace K)) or nothing added (control) (within-participant). This was contrasted with participant's habitual self-reported protein consumption (between-participants). Habitual protein intake was

assessed using a protein food frequency questionnaire (FFQ). Breakfast conditions were balanced using a Williams square design (Williams, 1949).

5.2.2 Participants

24 healthy weight low restraint men from a student population at the University of Sussex took part in the research (mean age: 22; ages from 19-31; mean BMI: 23, BMI from 19-25 kg/m²). Participants were recruited based on their responses to an eating habits questionnaire using a database system. Individuals who smoked, were diabetic, had a diagnosed eating disorder, used medication, or had allergies or intolerances to the foods used were excluded. Those who scored high (ratings above 7) on TFEQ restraint (Stunkard & Messick, 1985) were also excluded due to the potential for confounding factors influencing assessments as high restraint individuals have been found to differ in their perceptions of food (Crystal, Frye, & Kanarek, 1995; Dohm, et al., 2005; Tepper & Ullrich, 2002). Eligible participants were emailed with the study details 'the effects of breakfast on mood' to ensure participants were not aware of the experimental manipulation. Study details were emailed to participants and written, informed consent was obtained before taking part. Participants were paid £10 upon completion of all sessions or were awarded credits according to a participant pool scheme.

A-priori power calculations were conducted to establish the number of participants required for a medium effect of protein test day manipulation on changes in pleasantness ratings of strong and weak concentrations of the flavours assessed using G*Power. This indicated that 24 participants would be required to provide 80% power to detect a difference in rated palatability for weak and particularly strong concentrations of MSG flavours when in mild protein deficit. All experimental work was conducted in accordance with the standards expressed in the Helsinki Declaration and was approved by the University of Sussex ethics committee.

5.2.3 Test Food

5.2.3.1 Breakfasts:

Both high and low protein breakfasts consisted of 52g cereal (Crunchy Nut Cornflakes, Kellogg's, UK). The high protein breakfast also included 170g skimmed milk (Sainsbury's PLC, UK) and a 300g high protein breakfast shake made up of 250g semi-skimmed milk (Sainsbury's PLC, UK) combined with 25g Greek yoghurt (Total 0%,

Fage, UK), 25g whey protein (MyProtein, UK), 0.1g vanilla extract (Nielsen-Massey, Netherlands) and 0.04g Acesulphame K (Beckmann-Kenko, Germany). High protein breakfasts provided 504 Kcal and consisted of 32% protein, 53% carbohydrate and 16% fat.

The low protein breakfast included 170g low protein milk consisting of 156g tara water (water mixed with tara gum (Kalys, France; 0.03g/100g) at least 24 hours before use) added to 10g double cream (Sainsbury's Plc, UK) and 4g maltodextrin (Cargill, UK). The 300g low protein breakfast shake comprised of 225g tara water added to 27g honey yoghurt (Rachel's Organics Greek Style Honey Yoghurt, UK), 24g double cream, 24g maltodextrin, 0.1g vanilla extract, 0.06g cream flavour (International Flavours and Fragrances) and 0.02g acesulphame K. The whole breakfast provided 504 Kcal and was made up of 4% protein, 59% carbohydrate and 37% fat. The low protein milk was necessary to further enhance protein deprivation and has been successfully used elsewhere (Gibson, et al., 1995). The addition of tara gum was necessary to mimic the thicker consistency of milk and the natural thickness generated by the addition of whey protein in the high protein shake. Equally, the added cream flavouring in the low protein milkshake was required to match the creaminess of the high protein milkshake. Both high and low protein breakfasts provided adequate energy requirements for men as specified in the Food Standard Agency guidelines (FSA, 2006). All nutritional information can be found in Table 5.1.

Table 5.1. Nutritional composition of high and low protein breakfasts per 100g

	High Protein Shake	Low Protein Shake	High Protein Breakfast	Low Protein Breakfast
Carbohydrate	15	28.1	66.1	74.8
Protein	30.7	1.3	39.6	4.6
Fat	6	13.6	8.7	20.9
Carbohydrate (% energy)	25	47	53	59
Protein (% energy)	52	2	32	4
Fat (% energy)	23	51	16	37
Kcal	78.4	78.3	96.5	96.5
Kcal/portion	235.1	235	503.6	503.7

5.2.3.2 Soup Samples:

A low energy density (28 Kcal/100g) carrot and spice soup was used for all taste test samples. This soup was formulated in earlier studies to be hedonically neutral and low in naturally occurring MSG (Masic & Yeomans, 2013, Chapter 2). The soup was made up of carrots (Frozen Baby Carrots, Sainsbury's Plc, UK) onions, celery, olive oil (Medium Flavour Olive Oil, Sainsbury's Plc, UK), spice mixture (Garam Masala Schwartz, UK) and water (see Masic & Yeomans, 2013, Chapter 2). The sample flavours consisted of the addition of monosodium glutamate (MSG; Ajinomoto Co., Inc. Europe) (0.6g/100g; weak MSG or 1g/100g; strong MSG) for the savoury sample, sodium chloride (NaCl; Saxa Table Salt, UK) (0.3g/100g; weak NaCl or 0.4g/100g; strong NaCl) for the salty sample and acesulphame K (0.005g/100g; weak Ace K or 0.01g/100g; strong Ace K) for the sweet sample. These concentrations were chosen in line with those commercially available in soups and used in previous research (Luscombe-Marsh, et al., 2008, 2009; Roininen, et al., 1996). All nutritional information can be seen in Table 5.2.

Table 5.2. Nutritional composition of the soup preload per 100g

	Control
Carbohydrate	3.1
Protein	0.4
Fat	1.6
Carbohydrate (% energy)	44
Protein (% energy)	6
Fat (% energy)	50
Kcal	28

Both breakfast shakes and soup flavour conditions were subject to initial pilot testing to ensure equal acceptability and adequate taste enhancement across conditions. This included 12 men (mean age: 23; range: 20-29) attending a session in which they rated the sensory and flavour characteristics of the two breakfast shakes and six samples of soup (high and low protein shakes and strong and weak MSG, NaCl, Ace K). Mean pilot study ratings are shown in Table 5.3 and indicated no significant differences in sensory ratings between protein shakes including equivalent pleasantness assessments

($F(1,11) = 0.37$, p=.56). Soup assessments across strong and weak flavour samples indicated an enhancement by MSG of saltiness ($F(1,11) = 6.81$, $p = .02$) and strength of flavour ($F(1,11) = 12.90$, $p = .004$). Equally, an increase in saltiness ($F(1,11) = 12.30$, $p = .005$) across conditions was found in NaCl conditions and enhanced sweetness across concentrations in the Ace K samples ($F(1,11) = 12.74$, $p = .004$) as well as increased familiarity in weak compared to strong ($F(1,11) = 11.57$, $p = .006$) Ace K samples. All other sensory ratings including those of pleasantness were found to be non-significant across conditions.

Table 5.3. Mean (±SEM) pilot sensory ratings of two milkshakes (low or high protein) and six soups differing in MSG (weak MSG or strong MSG), sodium chloride (weak NaCl or strong NaCl) and sweetener (weak Ace K or strong Ace K) concentration (dashes are provided where ratings were not included as part of the sensory assessment). [1] Statistically significantly different from low concentration $p<0.05$.

Rating	Breakfast Shake		Soup					
	High Protein	Low Protein	Low MSG	High MSG	Low NaCl	High NaCl	Low Ace K	High Ace K
Familiar	41.00±8.0	45.17±8.7	60.33±4.7	54.08±6.5	60.33±5.5	58.33±5.5	63.92±6.0	54.67±6.4[1]
Pleasant	56.92±4.0	59.42±4.4	58.42±5.9	53.33±7.2	67.83±3.7	65.00±5.1	67.83±5.4	60.58±6.4
Salty	-	-	52.83±3.9	66.75±3.3[1]	43.50±5.3	69.50±4.2[1]	39.91±7.2	38.83±4.7
Savoury	-	-	71.25±3.5	72.50±3.8	66.17±3.1	70.25±5.4	62.25±6.3	56.67±5.9
Strong	40.67±7.4	36.83±5.6	50.17±3.6	68.75±3.9	46.75±7.0[1]	58.25±4.5	60.42±4.1	55.92±5.1
Sweet	54.00±5.7	53.58±4.9	38.17±6.0	39.50±4.4	47.75±5.7	38.91±5.8	51.42±4.2	76.25±4.7[1]
Thick	45.33±6.1	41.58±5.9	-	-	-	-	-	-

5.2.4 Computerized Data Collection

Appetite, hedonic and sensory ratings were assessed using the Sussex Ingestion Pattern Monitor (SIPM version 2.0.13, University of Sussex). This consists of a digital balance (Sartorius, Model BP4100) linked to a Dell computer. Appetite, hedonic, sensory and mood ratings were completed using digital Visual Analogue Scales (VAS) and stored by the SIPM. Ratings were presented as sentences ("How <word> do you feel?") with the left hand anchor ("Not at all <word>") coded as 0 and the right hand anchor ("As <word> as I have ever felt") coded as 100. Participants were presented with instructions on how to use the scale before each rating was completed to prevent confusion and registered their selection (pressing "Rating Completed") before moving on to the next question. The first rating set completed assessed how energetic, full, happy, hungry, nauseous, and thirsty participants felt as well as how much they desired to eat something savoury or sweet ("how much would you like to eat something savoury/sweet right now?"). All VAS ratings were randomized across trials and sessions.

A tray containing seven soup samples (strong and weak concentrations of MSG, NaCl, and Ace K soups and the control soup) served hot in 10g plastic tasting cups and labelled using randomly generated 3-digit number sequences was then provided and participants were asked to "Please take a mouthful of sample <number>, count to 5 and then swallow" before completing sensory ratings upon tasting each soup (how familiar, pleasant, salty, savoury, strong and sweet). After each tasting, participants were reminded to "Please take a mouthful of water and press Continue". Soup samples were randomized across session days. Once all soup tastings were completed, participants repeated the set of mood and appetite ratings from the start of the session before they were free to leave.

5.2.5 Protein Food Frequency Questionnaire

The protein food frequency questionnaire (FFQ) was based on the National Health and Nutrition Examination Survey (NHANES) FFQ developed and validated by the National Cancer Institute (NCI, 2007). The NHANES questionnaire comprises a set of multiple choice questions assessing intake of a variety of foods which are grouped into categories (such as fruit and vegetables, dairy, cereals and beverages amongst others). The questionnaire originally focuses on how often a particular food is consumed over a

12 month period. The NHANES FFQ contains over 130 questions, which were tailored in the present study to allow for a more specific focus on the frequency of consumption of high protein foods. Based on this, a 39 item protein FFQ was developed assessing intake of protein-rich items (dairy, nut, meat, seafood, tofu, egg) and specific sources of protein (beverages, bars and supplements) over the last six months. Also noted was the exclusion of any of these items over that time.

The questions asked took the form "Over the past 6 months, how often did you (drink/eat) (relevant food and distinction; *e.g.* milk **as a beverage (not in coffee, cereal or tea)**?". Possible answers were "Never (go to question __)" (awarded 0 points), "1 time per month or less" (1 point), "2-3 times per month" (2 points), "1-2 times per week" (3 points), "3-4 times per week" (4 points), "5-6 times per week" (5 points), "1 time per day" (6 points), "2-3 times per day" (7 points) or "2 or more times per day" (8 points) for meals and additional ratings "4-5 times per day" (9 points) or "6 or more times per day" (10 points) for beverages. Equally, with dairy products, questions regarding the type of product ("whole milk" (3 points), "semi-skimmed (2% fat) milk" (4 points), "skimmed milk"(4 points), "soy milk"(2 points), "rice milk" (1 point), "raw unpasteurized milk"(3 points) or "other" (1 point)) was important to include as points were awarded based on the available protein in these products. With dairy products it was also important to include the proportion of times lower fat options were chosen ("How often was the yoghurt you ate low fat or fat free?" – "Almost never or never" (0 points), "about ¼ of the time"(1 point), "about ½ of the time"(2 points), "about ¾ of the time" (3 points) or "almost always or always" (4 points)).This was important as protein content tends to be higher in low fat dairy products. Additionally, consumption of meat, animal products, seafood and added protein (in the form of milkshakes/supplements or bars) was awarded double points across ratings of how often and how much of the food was consumed in line with the higher protein content of these foods.

In addition to assessing how often high protein foods were consumed, the protein FFQ also included questions relating to approximate portion sizes each time the food was consumed. For beverages this ranged from "Less than 1 mug/glass (less than 250ml)" (1 point), "1-2 mugs/glasses (250-500 ml)" (2 points) or "more than 2 mugs/glasses (more than 500ml)" (3 points) whilst for solid foods this differed based on average portion size information (NCI, 2007). Again, for very high protein foods (protein supplemented

beverages and foods, meat, fish and eggs) double points were awarded for each possible portion size response. To allow for a more concrete estimation, participants were provided with a portion size estimation sheet (Appendix 5.1), which provided pictures of approximate portion sizes based on hand symbols (also provided on the sheet was ounce measurements). For instance the question: "Each time you ate **beef in mixtures** (such as meatballs, chilli, meatloaf, beef stew, beef pies, beef and noodles, beef and vegetables), how much did you usually eat?" provided the answers "Less than 125g or less than 3 ounces (palm of hand)", "125-250g or 3 – 6 ounces (1 – 2 palms of hands)" or "More than 250g or more than 6 ounces (more than 2 palms of hand)". This ensured participants were able to make as accurate a selection as possible. An additional question regarding the exclusion of high protein foods from the diet: "For **ALL** of the past 6 months, have you followed any type of **vegetarian diet**?"; "Which of the following foods did you **TOTALLY EXCLUDE** from your diet **(mark all that apply)**?" with answers "Meat (beef, pork, lamb, etc)", "Poultry (chicken, turkey, duck, etc)", "Fish and/or seafood", "Eggs" and "Dairy products (milk, cheese, yoghurt etc)" resulted in negative marking (deducting 4 points for all animal or seafood products and 2 points for dairy products). If participants had not consumed a particular food they were directed to the next question "(go to question __')". An example of the questionnaire used can be seen in Appendix 5.2)

Six participants completed the Protein FFQ 2-3 weeks before starting the study whilst the remaining 18 participants filled-in the questionnaire at the end of session 3. There were no differences in awareness between those participants provided with the FFQ before the study compared to those who completed the FFQ after the study had ended when contingency awareness questions were assessed.

5.2.6 Procedure
Participants were invited to the Ingestive Behaviour Unit (IBU) at the University of Sussex on three non-consecutive sessions. On the first (baseline) session day participants were allowed to consume their normal breakfast and were then asked to consume nothing but water for the two hours before testing (from 1200h-1400h) to ensure mild food deprivation. For sessions two and three, participants were required to eat nothing and consume only water from 2300h on the previous evening, with breakfast served in the IBU between 9000h-1100h after which they were free to leave

but were asked to return after three hours for the taste test session (from 1200h-1400h) having consumed nothing but water.

The taste test sessions were completed in a windowless cubicle containing a large glass of water and the SIPM software. Participants were asked to follow the on-screen instructions, filling in all mood, appetite and desire for savoury and sweet ratings before alerting their experimenter when the relevant samples were required. Across all sessions participants were asked to taste the relevant soup sample and complete sensory ratings relating to the flavour of that soup. The order in which the samples were evaluated was randomised at each session. Once all samples were tasted and rated a final set of mood and appetite ratings were filled in. At the end of sessions one and two participants were free to leave but their weight, height and age were recorded on the last day and they were provided with a copy of the FFQ which they completed before being debriefed and paid. A graphical depiction of the experimental design can be seen in Appendix 5.3.

5.2.7 Data Analysis

In the short-term protein manipulation, sensory ratings of the flavour (MSG, NaCl and Ace K) samples were transformed to examine the change in the sensory rating (*e.g.* pleasantness) of the relevant flavour (control, MSG, NaCl or Ace K) and concentration (control, weak or strong) on the protein manipulation days (low or high protein) relative to the baseline test day. These change scores were calculated taking the sample flavour first on the low protein day in control, weak or strong concentrations and expressing the difference in this value from the relevant flavour and concentration on the baseline day. This was then done for the high protein test day (control was contrasted with no inclusion of concentration over the low or high protein manipulation). Two way repeated measures 2x7 ANOVAs assessing test condition (low protein breakfast or high protein breakfast) and sample taste for weak and strong concentrations (control, weak MSG, strong MSG, weak NaCl, strong NaCl, weak Ace K or strong Ace K) were conducted to determine any differences in sensory assessments. Similarly, change from baseline measures were used in assessments of desire for sweet or savoury to assess whether protein manipulation day influenced these assessments in 2x2 ANOVA comparing test day manipulation (low or high protein) and time (pre and post flavour sample tasting). Ratings of baseline appetite, thirst and nausea were not transformed and were examined over session days (baseline test day, low protein manipulation and high

protein manipulation) and over time (ratings made before and after the session) using 3x2 repeated measures ANOVA.

The influence of habitual protein intake in the diet (long-term protein status) on sensory assessments of the relevant sample tastes (control, MSG, NaCl or Ace K) and concentrations (weak or strong for MSG, NaCl and Ace K) were assessed using simple linear regression analyses for each individual taste and each individual concentration to assess whether protein status was related to liking for specific tastes and concentrations on each day (baseline, low protein day and high protein day). Additional multiple regression analyses were also conducted for each individual taste and concentration including familiarity alongside protein status as separate blocks in the analysis to determine whether familiarity influenced pleasantness ratings more than protein status alone. This was due to the known effects of familiarity on influencing pleasantness (Appleton, 2013; Blissett & Fogel, 2013; Pliner, 1982).

The influence of long-term protein status was further explored over low and high protein manipulation test days to determine whether protein status influenced palatability ratings of the taste samples and concentrations differently when in acute protein deprivation (after a low protein breakfast) as opposed to when a high protein load was received (after the high protein breakfast). The baseline test day was not included in this analysis as breakfast consumption (and thus protein intake) on this day was not controlled. This was assessed taking each simple linear regression analysis of pleasantness for each individual relevant taste (control, MSG, NaCl or Ace K) and concentration (weak or strong) and comparing low protein to high protein manipulation days using a one tailed t-test. Where there were cases of violated sphericity, Greenhouse Geisser values ($\varepsilon = <0.75$) were used. If Greenhouse Geisser ($\varepsilon = >0.75$), Huynh-Feldt values were reported. Bonferroni adjusted comparisons were used to assess significant interactions between patterns of data using within-subjects contrasts and effect sizes are reported using Pearson's correlation coefficient for specific effects. G*Power was used to determine the number of participants a-priori for 80% power and was also assessed post-hoc. Data are shown for all 24 participants.

5.3 Results

5.3.1 Hedonic change from baseline ratings after protein manipulations

When the change from baseline pleasantness for all flavours and concentrations were assessed across test days, a significant effect of test day was evident ($F(1,23) = 4.66$, p =.04), indicating that the change in palatability was higher for all flavours on the low protein day than the high protein day in relation to baseline test day (Figure 5.1). Flavour samples also differed significantly in rated palatability (main effect of flavour: $F(6,138) = 3.37$, p=.004) with strong NaCl samples ($F(1,23) = 7.63$, p =.01, r=0.50) and weak Ace K samples ($F(1,23) = 5.32$, p=.03, r=0.43) rated as more aversive on both protein manipulation days when compared to the control sample. Strong MSG samples were also rated as most pleasant in flavour over all other flavour samples but this did not differ significantly from control ($F(1,23) = 0.70$, p=.41, r=0.17) and may be due to inadequate power as post-hoc power calculations indicated only 12% power in this sample. No test day*flavour interaction was evident ($F(6,138) = 1.01$, p=.43).
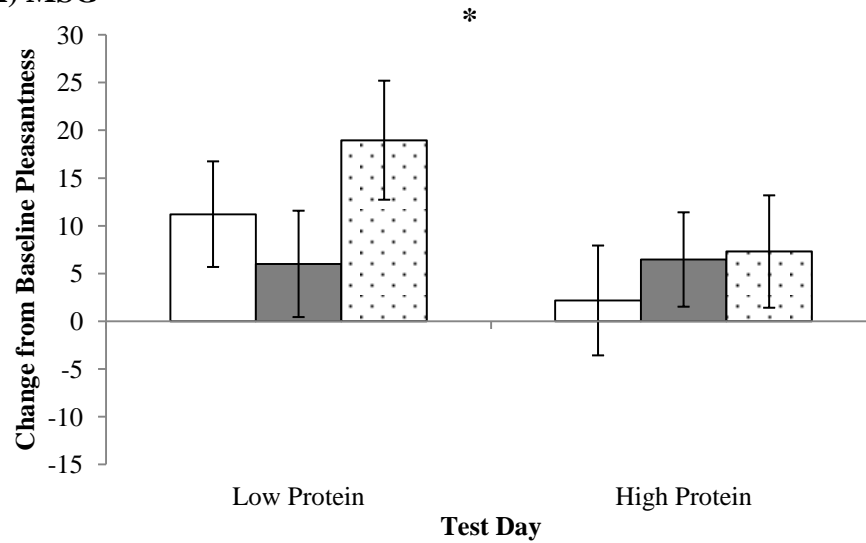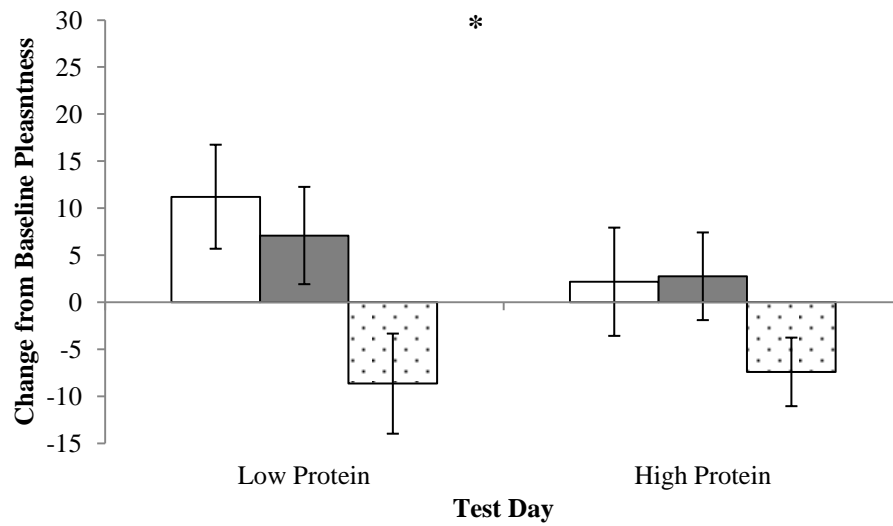
A) MSG
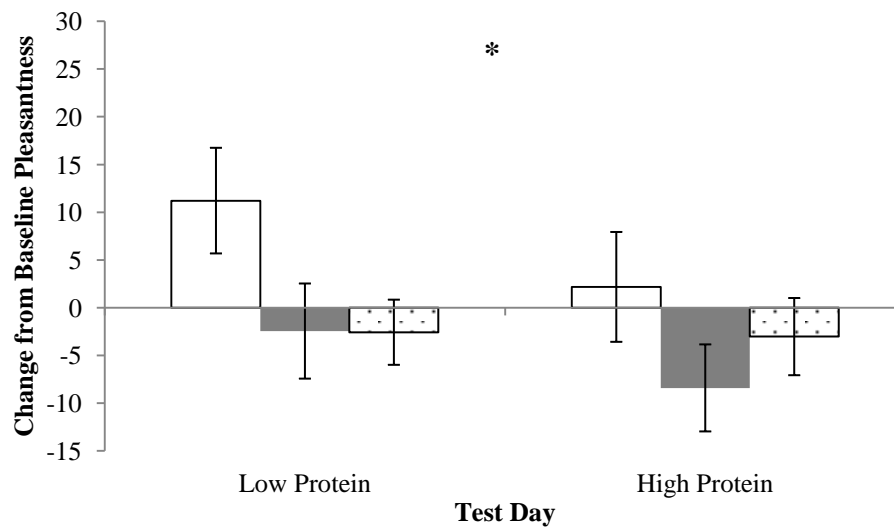

B) NaCl

C) Ace K

Figure 5.1. Change from baseline pleasantness assessments (mm) of three flavours

(Monosodium glutamate; MSG, Panel A, sodium chloride; NaCl, Panel B and

Acesulphame K; Ace K, Panel C) of a soup delivered in weak (■) or strong (⊡) flavour concentrations and compared to a bland control (□) across high and low protein breakfast exposure days. Data are mean±SEM. Statistically significant difference between low and high protein test day (main effect of day) is indicated: * P<0.05.

5.3.2 Sensory change from baseline assessments after protein manipulations

Sensory ratings were also assessed as change from baseline test day to determine whether the protein manipulation influenced these ratings differently from the baseline day. A significant effect of flavour was evident in familiarity ratings ($F_{(6,130)} = 3.88$, $p=.001$) with some flavours rated as more familiar on protein manipulation days as compared to the baseline day. Indeed, within-subjects contrasts indicated that strong NaCl samples were rated as more familiar than the control condition ($F_{(1,23)} = 6.79$, $p=.02$, $r=0.50$). Similarly, weak MSG conditions were rated as more familiar than control but this was not statistically significant ($F_{(1,23)} = 2.92$, $p=.10$, $r=0.34$) although effect size calculations indicated a medium effect ($r = 0.33$; Table 5.4). A significant effect of flavour was also apparent in strength ratings ($F_{(6,138)} = 2.52$, $p =.02$) with all but weak Ace K samples rated as stronger than control in flavour. In particular, weak MSG ($F_{(1,23)} = 5.62$, $p=.02$, $r=0.44$) and weak NaCl ($F_{(1,23)} = 6.93$, $p =.02$, $r=0.48$) concentrations were rated as stronger tasting than control on protein manipulation days as compared to the baseline test day. All other sensory ratings were not found to be influenced by flavour type or test day and no interactions were apparent.

Table 5.4. Mean (±SEM) change from baseline sensory ratings across weak and strong concentrations of flavour samples (monosodium glutamate; MSG, sodium chloride; NaCl and Acesulphame K; Ace K) and the control sample after protein manipulations (low protein breakfast or high protein breakfast) during lunchtime taste test sessions.[1] Significantly different from all flavour manipulations P<0.05.

| | | **Control** | **MSG** | | **NaCl** | | **Ace K** | |
			Low	High	Low	High	Low	High
Low Protein	Familiar	10.17±5.4[1]	8.25±5.2	21.75±6.5	3.71±5.2	-2.63±5.5	11.08±6.3	1.29±5.5
	Pleasant	11.21±5.5[1]	6±5.6	18.96±6.2	7.08±5.2	-8.67±5.3	-2.46±5	-2.58±3.4
	Salty	2.21±4[1]	3.83±4.4	5.38±4.3	10.87±3.3	0.38±4.3	0.79±3.8	-0.25±3.3
	Savoury	-0.42±4.6[1]	2.88±4	5.63±4.5	5.12±3.8	-6.20±4.2	6.25±5.4	-2.45±3.5
	Strong	-2.54±4[1]	8.83±4.6	8.08±4	8.83±4.68	-1.50±5.6	0.04±3.9	1.29±4.2
	Sweet	-2.08±3.7[1]	-4.13±3.7	-0.50±5.9	0.63±5	-2.75±3.9	-5.58±4.6	-3.42±4.1
High Protein	Familiar	8.63±6.4[1]	12.46±4.4	16.58±5.8	5.08±4.5	-5.67±3.9	5.38±6	3.71±5.5
	Pleasant	2.17±5.8[1]	6.46±4.9	7.29±5.89	2.75±4.7	-7.42±3.6	-8.2±4.6	-3.04±4.1
	Salty	1.42±4.3[1]	7.42±4.3	1.21±4.3	7.58±4.6	7.58±3.6	0.67±3	-3.54±5.3
	Savoury	-3.58±5.2[1]	6.13±4.6	-0.21±4.3	-0.04±4.1	-1.21±3.8	-1.54±4.1	0.50±5.1
	Strong	-5.79±4.3[1]	11.67±5	0.33±4.8	13.92±4.8	1.96±3.8	-0.29±3.8	2.17±3.7
	Sweet	-5.67±5.03[1]	-5.67±6	6.58±4.9	-2.04±5.22	-1.88±4.5	-0.25±3.5	-0.38±3.3

5.3.3 Appetite and desire ratings over test day sessions

Hunger (F(2,46) = 0.82, p=.85) and fullness (F(2,46) = 1.10, p=.34) evaluations before soup tasting sessions were found to be non-significant, indicating that participants arrived hungry; m: 61.8±2.1 and not very full; m: 33.6±1.3. Change from baseline desire for sweet foods was not significantly different irrespective of protein manipulation (F(1,23) = 1.20, p=.28), did not differ from pre- to post- session (F(1,23) = 1.49, p=.23) and no interaction was apparent (F(1,23) = 0.28, p=.60) when compared to the baseline test day. However, change from baseline desire for savoury was significantly higher on low as compared to high protein days (Main effect of test day: F(1,23) = 5.48, p=.03; Table 5.5). This did not change over time (F(1,23) = 0.002, p=.97) and no interaction was apparent (F(1,23) = 0.02, p=.89).

Table 5.5. Mean (±SEM) change from baseline appetite and desire ratings across protein manipulations (low protein breakfast or high protein breakfast) before (pre) and after (post) lunchtime taste test sessions. [1] Significantly different from high protein P<0.05.

	Low Protein		High Protein	
	Pre	Post	Pre	Post
Desire for Savoury	6.5±3.3[1]	6.3±3.3[1]	-0.8±3.9	-0.3±4.2
Desire for Sweet	7.1±4.4	-3.2±6	1.8±4	-5.1±5.8

5.3.4 Long-term protein status and pleasantness assessments

It was predicted that habitual protein intake in the diet (protein status) would influence liking for MSG sources, with those self-reporting higher dietary protein intake expected to show increased palatability assessments for stronger concentrations of MSG. There was no significant effect of a higher protein status alone increasing pleasantness for MSG on baseline sessions for weak (*b* = 0.03, t(23) = 0.39, p=.70) or strong (*b* = 0.02, t(23) = 0.19, p=.85) concentrations or on low protein (weak concentration: *b* = 0.01, t(23) = 0.19, p=.85; strong concentration: *b* = 0.05, t(23) = 0.70, p=.49) or high protein (weak concentration: *b* = 0.06, t(23) = 1.04, p=.31; strong concentration: *b* = -0.06, t(23) = -0.98, p=.34) test days (Table 5.6). The influence of protein status on other sensory ratings of MSG was also not found to be significant and can be seen in Table 5.6. Protein status was also not predictive of desire for savoury after baseline (*b* = -0.004,

t(23) = -0.10, p=.92) , low protein (b = -0.004, t(23) = -0.08, p=.94) or high protein (b = 0.004, t(23) = -0.07, p=.87) manipulations.

Table 5.6. Regression results across sensory ratings for weak and strong MSG soup sample concentrations after protein manipulations (baseline no manipulation, low protein breakfast or high protein breakfast).

	Sensory Rating	B		β		t	
		Low	High	Low	High	Low	High
Baseline	Familiar	0.06±0.06	-0.02±0.06	0.20	-0.57	0.97	-0.26
	Pleasant	0.03±0.1	0.02±0.1	0.08	0.04	0.39	0.19
	Salty	0.05±0.07	-0.04±0.06	0.16	-0.16	0.75	-0.76
	Savoury	-0.03±0.05	-0.01±0.04	-0.14	-0.82	-0.64	-0.23
	Strong	0.07±0.07	-0.05±0.06	0.20	-0.19	0.94	-0.92
	Sweet	-0.01±0.05	-0.04±0.06	-0.05	-0.13	-0.24	-0.59
Low Protein	Familiar	-0.01±0.06	-0.02±0.06	-0.04	-0.06	-0.17	0.78
	Pleasant	0.01±0.07	0.05±0.07	0.04	0.15	0.19	0.70
	Salty	0.07±0.05	0.09±0.05	0.29	0.37	1.42	1.88
	Savoury	0.03±0.05	-0.03±0.04	0.15	-0.15	0.49	-0.71
	Strong	-0.05±0.05	-0.05±0.03	-0.20	-0.36	-0.94	-1.80
	Sweet	-0.06±0.06	0.03±0.07	-0.21	0.09	-0.98	0.40
High Protein	Familiar	0.04±0.05	-0.040.07	0.15	-0.13	0.72	-0.59
	Pleasant	0.06±0.06	-0.06±0.07	0.21	-0.21	1.04	-0.98
	Salty	0.02±0.05	0.001±0.07	0.07	0.003	0.32	0.01
	Savoury	0.01±0.06	-0.13±0.06	0.05	-0.42	0.23	-1.22
	Strong	0.005±0.05	-0.01±0.05	0.02	-0.04	0.11	-0.20
	Sweet	-0.09±0.06	0.004±0.07	-0.28	0.01	-1.38	0.06

When familiarity was included as a separate block alongside protein status in multiple regression analyses, familiarity was found to significantly predict pleasantness for weak and strong concentrations of MSG, NaCl and Ace K and for control conditions after the baseline and high protein test days but was not found to be significant for MSG on the low protein test day at weak ($b = 0.33$, t(23) = 1.47, p = 0.16) or strong ($b = 0.35$, t(23) = 1.44, p = 0.16) concentrations or for Ace K at strong concentrations ($b = 0.27$, t(23) = 1.56, p = 0.14) or for the control condition ($b = 0.24$, t(23) = 1.47, p = 0.16) on the low protein day. Correlations between the sensory and appetite ratings revealed no significant correlations between these conditions on the test days thus no additional sensory assessments were added to the regression model.

It was also predicted that acute protein deprivation would increase liking of stronger concentrations of MSG on low protein days as a function of protein status with the expectation that the higher the protein status, the more palatable the strong concentration of MSG would be rated on the low protein test day as compared to the high protein test day. Simple regression analyses for rated pleasantness of strong concentrations of MSG on low protein test days were contrasted with high protein test days using one tailed t-tests. This was also conducted across weak concentrations of MSG as well as weak and strong concentrations of all of the other taste samples tested (control, NaCl and Ace K) but was not found to significantly differ between days for the other flavour samples. As protein status increased, palatability ratings were found to increase after strong concentrations of MSG on low protein test days as compared to high protein test days (t(23) = -0.012, p = .05; Figure 5.2). Figure 5.2 also suggests that those with higher protein status disliked strong MSG concentrations on the high protein test day. Protein status did not influence palatability across weak concentrations of MSG on low as compared to high protein test days (t(23) = -0.84, p=.79) and there were no other significant differences found across all other tastes or concentrations.
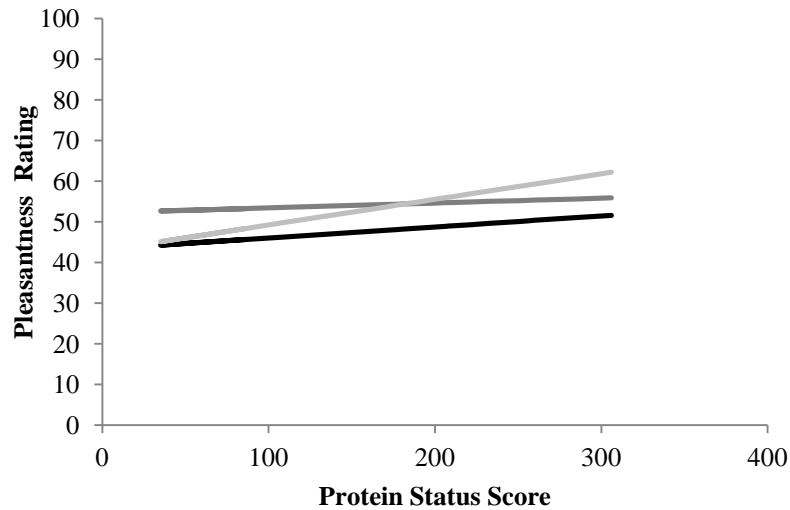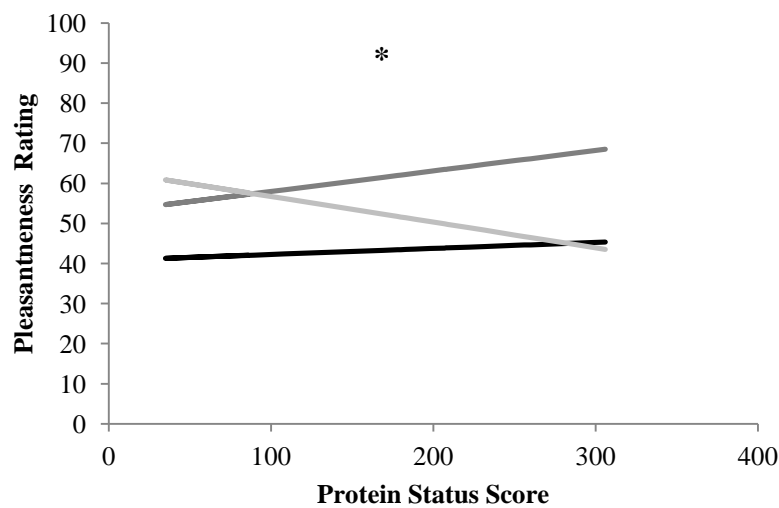
A) Low Concentration MSG

B) High Concentration MSG

Figure 5.2. Pleasantness assessments (mm) of weak (Panel A) and strong (Panel B) concentrations of MSG soup samples after protein manipulations (baseline no manipulation (——), low protein breakfast (——) or high protein breakfast (——)) as a function of habitual protein consumption (Protein Status Score) using linear regression coefficients. Significant t-test difference on high concentration day between manipulation conditions P=0.05.

5.3.5 Rated thirst and nausea across test days

There were no significant differences in thirst ($F_{(2,46)} = 1.33$, p=.28) or nausea ($F_{(1.5,33.7)} = 0.51$, p=.60) assessments before each tasting session. Participants arrived relatively thirsty; m: 56±0.1 and were not nauseous; m: 10±1.1 before the sessions. Thirst significantly decreased over time across all test sessions ($F_{(1,23)} = 18.40$, p =

<.001) and this did not interact with test day (F(2,46) = 3.00, p=.38). Nausea ratings did not change across the test session (F(1,23) = 0.00, p=.98) and there was no condition*time interaction (F(2,46) = 0.77, p=.47) indicating that neither test day nor taste sample caused any detrimental effects.

5.4 Discussion

The main results suggest that acute protein deprivation increased liking for all flavours and increased desire for savoury foods indicating that short-term manipulations of protein may act to increase liking and desire for a range of foods but not specifically for umami flavours. These differences in preferences were seen despite there being no overall difference in rated hunger at the two test sessions, suggesting the effects were driven by the macronutrient manipulation and not appetite per se. Although liking for strong concentrations of MSG tended to be greater on low protein days, this was not significant. Strong NaCl and weak Ace K flavours were liked less when protein status was manipulated but liking for MSG tended to remain high. Habitual protein intake was not a predictor of liking for MSG when assessed alone however, higher habitual protein intake was associated with higher pleasantness assessments of strong concentrations of MSG when these individuals were in acute protein deficit and lower pleasantness ratings when these individuals were provided with high protein loads. These findings may be contradictory based on the evidence put forward by Laska and Hernandez Salazar (2004) but do suggest that protein status may influence liking of foods that may predict protein.

The hypothesis that acute protein deprivation would increase liking of a strong concentration of MSG relative to other flavour samples was not supported. This may be due to the research being underpowered as the results did suggest stronger liking for the stronger concentration of MSG when in protein deficit as compared to baseline. Similarly, the desire for savoury was higher after the low protein manipulation irrespective of hunger. This may be understood in terms of an innate desire for foods which predict protein (Austin, et al., 2011; Larsen, et al., 2010; Simpson, et al., 2003; Simpson & Raubenheimer, 2005) as savoury foods contain more reliable and higher quantities of protein than sweet foods (Blundell & Rogers, 1994; Luscombe-Marsh, et al., 2008; Viskaal-van Dongen, et al., 2011). However this did not translate to liking,

with a general increase in liking of all flavours found on the low protein day, instead suggesting that pleasantness assessments were extended to any flavour which offered a nutrient source. Liking of the flavours was also lower on the high protein day, with strong NaCl and weak Ace K flavours in particular liked less on this day than after baseline sessions. Although it is not clear why these flavours in particular were liked less, the general decline in liking after the high protein manipulation may be related to macronutrient sensing, with a less urgent need for nutrients in high as opposed to low protein manipulation reflected in the pleasantness ratings. It is however surprising to note that hunger assessments did not differ across low and high protein manipulations as liking has been related to hunger, with stronger hunger increasing liking (Gibson & Wardle, 2001) and as protein has been shown to be particularly satiating (Bertenshaw, et al., 2008). Only weak NaCl samples were rated as more familiar after baseline sessions, although weak MSG soups were also perceived as more familiar (but this was not statistically significant). This may be due to this quantity of NaCl used falling within the range usually consumed by this sample thus it was assessed as more familiar on the remaining sessions. It may also be that participants were focussed more on flavours that predicted a savoury source and this salience caused NaCl and MSG flavours to be perceived as more familiar. Indeed, ratings of strength of flavour were also higher across all flavour conditions apart from weak Ace K samples in comparison to control soups on protein manipulation days. This suggests that for the most part 'strength' may have been assumed to denote 'additional flavour' across protein manipulation days indicating that more clarification may have been required with the ratings used. It also may suggest that the samples low in sweetness began to taste more similar to the control after the baseline test day, possibly due to the less important signal of sweetness encountered. However, this remains as speculation.

Although habitual protein intake was also not found to be associated with liking of MSG concentrations alone, when individuals who regularly consumed higher proportions of protein were exposed to an acute protein deprivation, pleasantness ratings of strong MSG concentrations were greater than when these individuals consumed large protein loads. This is similar to the findings observed in primates (Laska & Hernandez Salazar, 2004), with primates who ingested higher quantities of animal protein shown to prefer stronger concentrations of MSG. These individuals were also found to rate strong concentrations of MSG as less palatable after a high protein

load. This may be due to these participants regulating their protein intake more efficiently due to their regular exposure to protein (Simpson, et al., 2003). Unlike the findings reported by Laska and Hernandez Salazar (2004), lower protein consumers were not found to prefer weaker concentrations of MSG. This may be due to the nature of the habitual protein intake questionnaire used which may not have been sensitive enough to pick up subtle differences in protein intake due to the questions asked. Similarly, there may have been a potential for errors of omission and under-reporting as has frequently been found in questionnaire designs (Mendez et al., 2011). Indeed, the effects reported must also be treated with caution as they were found to be subtle and were underpowered.

When the influences of familiarity and protein status on the rated pleasantness of soups were assessed across all session days (baseline, low protein and high protein) for all soup flavours, habitual protein intake was not found to be associated with liking of MSG concentrations, suggesting that regular consumption of high protein sources did not influence liking of MSG on baseline, low or high protein test days. However, a significant effect was apparent for familiarity. Indeed, the salt enhanced samples in particular were rated as more pleasant due to familiarity across all session days irrespective of protein status. This may be due to the known effects of familiarity on liking (Appleton, 2013; Blissett & Fogel, 2013; Pliner, 1982). Indeed, participants may have been more familiar with the NaCl conditions overall, again due to the greater likelihood of repeated exposure to salt-containing stimuli. This was also evident with the MSG samples on baseline and high protein days, with familiarity (but not protein status) predicting liking but was not evident on the low protein day. It may be that familiarity was not a dominant factor in liking for MSG on low protein test days due to other cues potentially acting as more dominant sources. However as no clear change in pleasantness for MSG samples when given a high or low protein load was apparent in the earlier analyses mentioned, speculations about the mechanisms that may be more salient cannot be made. Familiarity did not influence liking of control and strong concentrations of Ace K on the low protein day either.

Despite the modest increase in liking for strong concentrations of MSG during protein deprivation relative to baseline and high protein sessions in habitually high protein consumers, it is still not clear whether this liking would elicit approach responses in

- There was no effect of low protein manipulation on specific liking for umami and habitual protein status score did not predict liking for MSG sources however high protein consumers subjected to a protein deprivation showed a stronger liking for high concentrations of MSG than low protein consumers

- Future research is required to further understand whether the effects for umami palatability seen in high protein consumers undergoing a mild protein deprivation are specific to the effects of protein

- Similarly, if high protein consumers are more responsive to umami flavours, future research may wish to further explore this distinction in relation to appetite and intake using MSG and protein cues

- As high protein consumers were receptive to strong concentrations of MSG, it would additionally be useful to assess the potential link between umami flavours and protein and whether umami may act as a cue for protein in the diet, particularly in relation to the aspects of protein related to improving satiation and satiety

Appendix 5.1. Portion size estimation sheet

Hand Symbol	Equivalent	Foods
	Palm 3 ounces	Meat Fish Poultry
	Handful 1 ounce	Nuts Raisins Yoghurt Cottage cheese
	Thumb 1 ounce	Peanut butter Hard cheese
	Thumb tip 1 teaspoon	Cooking oil Mayonnaise, butter Sugar

Appendix 5.2. Protein Food Frequency Questionnaire

This questionnaire will ask you about your food intake over the last **6 months**. **To mark your selections check the boxes next to the options listed by pressing the square box to the left of the option e.g.**

☒ This box is checked
☐ This box is not checked

With a **new** question, either the **question number** and/or the **question text** will be written in blue; this is to allow you to locate the next question if the current question is not relevant.

Included is a **portion sizing guide** to estimate how much of certain foods you have eaten within this period. Please use it when making your selections as the questionnaire explicitly refers to it.

Please ensure that you read all of the questions thoroughly and answer them to the best of your knowledge. All questionnaire data will be anonymised and assigned coded values to maintain your anonymity.

1. Please check the box next to each beverage that you drank at least once in the **past 6 months**.

☐ *1. Milk as a beverage (NOT in coffee, cereal or tea)*

☐*2. Chocolate milk or milkshakes (including hot chocolate) (NOT including high protein milkshakes)*

☐*3. High-protein beverages or milkshakes*

☐*None of the above* [GO TO QUESTION 4]

1a. Over the past 6 months, how often did you drink **milk as a beverage (NOT in coffee, cereal or tea)**?

☐Never [GO TO QUESTION 2]

☐1 time per month or less

☐2-3 times per month

☐1-2 times per week

☐3-4 times per week

☐5-6 times per week

☐1 time per day

☐2-3 times per day

☐4-5 times per day

☐6 or more times per day

1b. Each time you drank **milk as a beverage (NOT in coffee, cereal or tea),** how much did you usually drink?

☐Less than 1 mug/glass (less than 250ml)

☐1 – 2 mugs/glasses (250ml – 500ml)

☐More than 2 mugs/glasses (more than 500ml)

1c. Each time you drank **milk as a beverage (NOT in coffee, cereal or tea),** what **kind** of milk did you usually drink?

☐Whole milk

☐Semi-skimmed (2% fat) milk

☐Skimmed milk

☐Soy milk

☐Rice milk

☐Raw unpasteurized milk

☐Other

2a. Over the past 6 months, how often did you drink **chocolate milk or milkshakes (including hot chocolate) (not including high protein milkshakes)**?

☐Never [GO TO QUESTION 3]
☐1 time per month or less
☐2-3 times per month
☐1-2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2-3 times per day
☐4-5 times per day
☐6 or more times per day

2b. Each time you drank **chocolate milk or milkshakes (including hot chocolate) (not including high protein milkshakes),** how much did you usually drink?
☐Less than 1 mug/glass (less than 250ml)
☐1 – 2 mugs/glasses (250ml – 500ml)
☐More than 2 mugs/glasses (more than 500ml)

2c. Each time you drank **chocolate milk or milkshakes (including hot chocolate) (not including high protein milkshakes),** what **kind** of milk did you usually drink?
☐Whole milk
☐Semi-skimmed (2% fat) milk
☐Skimmed milk
☐Soy milk
☐Rice milk
☐Raw unpasteurized milk
☐Other

3a. Over the past 6 months, how often did you drink **high protein beverages or milkshakes**?
☐Never [GO TO QUESTION 4]
☐1 time per month or less
☐2-3 times per month
☐1-2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2-3 times per day

☐4-5 times per day
☐6 or more times per day

3b. Each time you drank **high protein beverages or milkshakes,** how much did you usually drink?
☐Less than 1 mug/glass (less than 250ml)
☐1 – 2 mugs/glasses (250ml – 500ml)
☐More than 2 mugs/glasses (more than 500ml)

4. Please check the box next to each food that you ate at least once in the **past 6 months**.
☐*4. Porridge or other cooked cereal*
☐*5. Cold cereal*
☐*None of the above* [GO TO QUESTION 6]

4a. How often did you eat **porridge or other cooked cereal** over the past 6 months?
☐Never [GO TO QUESTION 5]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

4b. Each time you ate **porridge or other cooked cereal,** how much did you usually eat?
☐Less than 1 instant sachet or less than one serving size (26g)
☐1 instant sachet or one serving size (27-50g)
☐More than 1 instant sachet or more than one serving size (more than 50g)

4c. Was milk added to make up your **porridge or other cooked cereal?**
☐Yes
☐No

4d. If yes, what kind of milk was usually added?
☐Whole milk
☐Semi-skimmed (2% fat) milk

☐Skimmed milk
☐Soy milk
☐Rice milk
☐Raw unpasteurized milk
☐Other

4e. Each time milk was added to your **porridge or other cooked cereal,** how much milk was usually added?
☐Less than 1 mug/glass (less than 250ml)
☐1 – 2 mugs/glasses (250ml – 500ml)
☐More than 2 mugs/glasses (more than 500ml)

5a. How often did you eat **cold cereal** over the past 6 months?
☐Never [GO TO QUESTION 6]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

5b. Each time you ate **cold cereal,** how much did you usually eat?
☐Half of a breakfast bowl or less than 1 serving size (29g)
☐1- 1½ breakfast bowl or 1 to 2 serving sizes (30g-60g)
☐More than 1½ breakfast bowls or more than 2 serving sizes (more than 60g)

5c. Was milk added to make up your **cold cereal?**
☐Yes
☐No [GO TO QUESTION 6]

5d. If yes, what kind of milk was usually added?
☐Whole milk
☐Semi-skimmed (2% fat) milk
☐Skimmed milk
☐Soy milk
☐Rice milk

☐Raw unpasteurized milk
☐Other

5e. Each time milk was added to your cold cereal, how much milk was usually added?
☐Less than 1 mug/glass (less than 250ml)
☐1 – 2 mugs/glasses (250ml – 500ml)
☐More than 2 mugs/glasses (more than 500ml)

6. Please check the box next to each food that you ate at least once in the past 6 months
☐Never [GO TO QUESTION 8]
☐*6. Peanut butter or other nut or seed butters*
☐*7.Peanuts, walnuts, almonds, seeds or other nuts*

6a. How often did you eat peanut butter or other nut or seed butters over the past 6 months?
☐Never [GO TO QUESTION 7]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

6b. Each time you ate peanut butter or other nut or seed butters, how much did you usually eat?
☐Less than 15g (less than 1 tablespoon or less than 1 thumb size (1 ounce))
☐15-30g (1-2 tablespoons or 1-2 thumb sizes (1-2 ounces))
☐More than 30g (more than 2 tablespoons or more than 2 thumb sizes (more than 2 ounces))

7a. How often did you eat peanuts, walnuts, almonds, seeds or other nuts in the last 6 months?
☐Never [GO TO QUESTION 8]
☐1 time per month or less
☐2-3 times per month

☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

7b. Each time you ate **peanuts, walnuts, almonds, seeds or other nuts,** how much did you usually eat?
☐Less than 25g (less than 1 ounce or less than 1 handful)
☐25g to 50g (1-2 ounces or 1-2 handfuls)
☐More than 50g (more than 2 ounces or more than 2 handfuls)

8. Please check the box next to each food that you ate at least once in the **past 6 months (including in sandwiches and/or in other meals)**.
☐*8. Roast beef or steak in sandwiches*
☐*9. Turkey or chicken COLD CUTS (such as luncheon meat, turkey or chicken slices or pastrami)*
☐*10. Luncheon or deli-style ham*
☐*11. Other cold cuts or luncheon meats (such as salami, corned beef, pastrami etc) (Please do not include ham, turkey or chicken cold cuts)*
☐*12. Hot dogs or frankfurters (Please do not include sausages or vegetarian hot dogs)*
☐*None of the above* [GO TO QUESTION 12]

8a. How often did you eat **roast beef or steak in sandwiches**?
☐Never [GO TO QUESTION 9]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

8b. Each time you ate **roast beef or steak in sandwiches,** how much did you usually eat?
☐Less than 1 slice (or less than 26g or less than 1 ounce/ 1 handful)
☐1 to 2 slices (or 27- 54g or 1 – 2 ounces/ 1-2 handfuls)

☐3 or more slices (more than 54g or more than 2 ounces/ 2 handfuls)

9a. How often did you eat **Turkey or chicken COLD CUTS** (such as luncheon meat, turkey ham or turkey pastrami)?
☐Never [GO TO QUESTION 10]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

9b. Each time you ate **Turkey or chicken COLD CUTS** (such as luncheon meat, turkey ham or turkey pastrami)**,** how much did you usually eat?
☐Less than 1 slice (or less than 26g or less than 1 ounce/ 1 handful)
☐1 to 2 slices (or 27- 54g or 1 – 2 ounces/ 1-2 handfuls)
☐3 or more slices (more than 54g or more than 2 ounces/ 2 handfuls)

10a. How often did you eat **luncheon or deli-style ham**?
☐Never [GO TO QUESTION 11]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

10b. Each time you ate **luncheon or deli-style ham,** how much did you usually eat?
☐Less than 1 slice (or less than 26g or less than 1 ounce/ 1 handful)
☐1 to 2 slices (or 27- 54g or 1 – 2 ounces/ 1-2 handfuls)
☐3 or more slices (more than 54g or more than 2 ounces/ 2 handfuls)

11a. How often did you eat **other cold cuts or luncheon meats** (such as salami, corned beef, pastrami etc) (Please do not include ham, turkey or chicken cold cuts)?

☐Never [GO TO QUESTION 12]

☐1 time per month or less

☐2-3 times per month

☐1 time per week

☐2 times per week

☐3-4 times per week

☐5-6 times per week

☐1 time per day

☐2 or more times per day

11b. Each time you ate **other cold cuts or luncheon meats,** how much did you usually eat?

☐Less than 1 slice (or less than 26g or less than 1 ounce/ 1 handful)

☐1 to 2 slices (or 27- 54g or 1 – 2 ounces/ 1-2 handfuls)

☐3 or more slices (more than 54g or more than 2 ounces/ 2 handfuls)

12a. How often did you eat **hot dogs or frankfurters** (Please do not include sausages or vegetarian hot dogs)?

☐Never [GO TO QUESTION 13]

☐1 time per month or less

☐2-3 times per month

☐1 time per week

☐2 times per week

☐3-4 times per week

☐5-6 times per week

☐1 time per day

☐2 or more times per day

12b. Each time you ate **hot dogs or frankfurters,** how much did you usually eat?

☐Less than 1 hot dog/frankfurter

☐1-2 hot dogs/frankfurters

☐More than 2 hot dogs/frankfurters

13. Please check the box next to each food that you ate at least once in the past 6 months.

☐*13. Minced chicken or turkey*

☐*14. Beef hamburgers or cheeseburgers*

☐*15. Beef mixtures (such as meatballs, chilli, meatloaf, beef stew, beef pies, beef and noodles, beef and vegetables)*

☐*16. Roast beef, pot roasts or beef steak (NOT in sandwiches)*
☐*17. Pork or beef spareribs*
☐*18. Baked ham or ham steaks (e.g. gammon)*
☐*19. Bacon*
☐20. *Pork Sausages*
☐*21. Pork (including chops, roasts and mixed in dishes) (Please do not include ham, bacon or sausages)*
☐*22. Lamb (including chops, roasts and mixed in dishes)*
☐*23. Roast turkey, turkey nuggets, or turkey cutlets (including sandwiches)*
☐*24. Chicken mixtures (such as salads, sandwiches, casseroles, stews and other mixtures)*
☐*25. Roasted, baked, boiled or fried chicken (including nuggets)*
☐*26. Offal such as heart, kidneys, liver (all kinds)*
☐*27. Canned tuna (including in salads, sandwiches or casseroles)*
☐*28. Shellfish (including fried)*
☐*29. Salmon, fresh tuna or trout*
☐*30. Other fish, fish fingers (not including shellfish)*
☐*None of the above* [GO TO QUESTION 30]

13a. How often did you eat **minced chicken or turkey**?
☐Never [GO TO QUESTION 14]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

13b. Each time you ate **minced chicken or turkey,** how much did you usually eat?
☐Less than 125g or less than 3 ounces (palm of hand)
☐125-250g or 3 – 6 ounces (1 – 2 palms of hands)
☐More than 250g or more than 6 ounces (more than 2 palms of hand)

14a. How often did you eat **beef hamburgers** or **cheeseburgers**?
☐Never [GO TO QUESTION 15]

☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

14b. Each time you ate **beef hamburgers** or **cheeseburgers,** how much did you usually eat?
☐Less than 1 patty (less than 70g)
☐1 patty (approximately 70g)
☐More than 1 patty (more than 70g)

15a. How often did you eat **beef in mixtures** (such as meatballs, chilli, meatloaf, beef stew, beef pies, beef and noodles, beef and vegetables)?
☐Never [GO TO QUESTION 16]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

15b. Each time you ate **beef in mixtures** (such as meatballs, chilli, meatloaf, beef stew, beef pies, beef and noodles, beef and vegetables), how much did you usually eat?
☐Less than 125g or less than 3 ounces (palm of hand)
☐125-250g or 3 – 6 ounces (1 – 2 palms of hands)
☐More than 250g or more than 6 ounces (more than 2 palms of hand)

16a. How often did you eat **roast beef, pot roasts or beef steaks** (NOT in sandwiches)?
☐Never [GO TO QUESTION 17]
☐1 time per month or less
☐2-3 times per month
☐1 time per week

☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

16b. Each time you ate **roast beef, pot roasts or beef steaks** (NOT in sandwiches), how much did you usually eat?
☐Less than 125g or less than 3 ounces (palm of hand)
☐125-250g or 3 – 6 ounces (1 – 2 palms of hands)
☐More than 250g or more than 6 ounces (more than 2 palms of hand)

17a. How often did you eat **pork or beef spareribs**?
☐Never [GO TO QUESTION 18]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

17b. Each time you ate **pork or beef spareribs**, how much did you usually eat?
☐Less than 4 spareribs
☐4 to 12 spareribs
☐More than 12 spareribs

18a. How often did you eat **baked ham or ham steaks** (e.g. gammon)?
☐Never [GO TO QUESTION 19]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

18b. Each time you ate **baked ham or ham steaks** (e.g. gammon), how much did you usually eat?
☐Less than 125g or less than 3 ounces (palm of hand)
☐125-250g or 3 – 6 ounces (1 – 2 palms of hands)
☐More than 250g or more than 6 ounces (more than 2 palms of hand)

19a. How often did you eat **bacon**?
☐Never [GO TO QUESTION 20]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

19b. Each time you ate **bacon**, how much did you usually eat?
☐Less than 2 rashers
☐2 to 3 rashers
☐More than 3 rashers

20a. How often did you eat **pork sausages**?
☐Never [GO TO QUESTION 21]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

20b. Each time you ate **pork sausages**, how much did you usually eat?
☐Less than 2 links
☐2 to 3 links
☐More than 3 links

21a. How often did you eat **pork** (including chops, roasts and mixed in dishes) (Please do not include ham, bacon or sausages)?
☐Never [GO TO QUESTION 22]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

21b. Each time you ate **pork**, how much did you usually eat?
☐Less than 125g or less than 3 ounces (palm of hand)
☐125-250g or 3 – 6 ounces (1 – 2 palms of hands)
☐More than 250g or more than 6 ounces (more than 2 palms of hand)

22a. How often did you eat **lamb** (including chops, roasts and mixed in dishes)?
☐Never [GO TO QUESTION 23]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

22b. Each time you ate **lamb**, how much did you usually eat?
☐Less than 125g or less than 3 ounces (palm of hand)
☐125-250g or 3 – 6 ounces (1 – 2 palms of hands)
☐More than 250g or more than 6 ounces (more than 2 palms of hand)

23a. How often did you eat **roast turkey, turkey nuggets, or turkey cutlets** (including sandwiches)?
☐Never [GO TO QUESTION 24]
☐1 time per month or less
☐2-3 times per month

☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

23b. Each time you ate **roast turkey, turkey nuggets, or turkey cutlets**, how much did you usually eat? (Please note: 4 to 8 turkey nuggets = 85g or 3 ounces)
☐Less than 125g or less than 3 ounces (palm of hand)
☐125-250g or 3 – 6 ounces (1 – 2 palms of hands)
☐More than 250g or more than 6 ounces (more than 2 palms of hand)

24a. How often did you eat **chicken mixtures** (such as salads, sandwiches, casseroles, stews and other mixtures)?
☐Never [GO TO QUESTION 25]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

24b. Each time you ate **chicken mixtures**, how much did you usually eat?
☐Less than 125g or less than 3 ounces (palm of hand)
☐125-250g or 3 – 6 ounces (1 – 2 palms of hands)
☐More than 250g or more than 6 ounces (more than 2 palms of hand)

25a. How often did you eat **roasted, baked, boiled or fried chicken** (including nuggets)?
☐Never [GO TO QUESTION 26]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week

☐5-6 times per week
☐1 time per day
☐2 or more times per day

25b. Each time you ate roasted, baked, boiled or fried chicken, how much did you usually eat?
☐Less than 2 drumsticks or wings, or less than 1 breast or thigh, or less than 4 nuggets
☐2 drumsticks or wings, or 1 breast or thigh, or 4 nuggets-8 nuggets
☐More than 2 drumsticks or wings, or 1 breast or thigh, or 4 nuggets-8 nuggets

26a. How often did you eat offal such as heart, kidneys, liver (all kinds)?
☐Never [GO TO QUESTION 27]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

26b. Each time you ate offal such as heart, kidneys, liver (all kinds), how much did you usually eat?
☐Less than 125g or less than 3 ounces (palm of hand)
☐125-250g or 3 – 6 ounces (1 – 2 palms of hands)
☐More than 250g or more than 6 ounces (more than 2 palms of hand)

27a. How often did you eat canned tuna (including in salads, sandwiches or casseroles)?
☐Never [GO TO QUESTION 28]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

27b. Each time you ate **canned tuna**, how much did you usually eat?

☐Less than 125g or less than 1 can, less than 3 ounces (palm of hand)

☐125-250g or 1- 1½ cans or 3 – 6 ounces (1 – 2 palms of hands)

☐More than 250g or more than 1½ cans or more than 6 ounces (more than 2 palms of hand)

28a. How often did you eat **shellfish** (including fried)?

☐Never [GO TO QUESTION 29]

☐1 time per month or less

☐2-3 times per month

☐1 time per week

☐2 times per week

☐3-4 times per week

☐5-6 times per week

☐1 time per day

☐2 or more times per day

28b. Each time you ate **shellfish**, how much did you usually eat?

☐Less than 125g or less than 3 ounces (palm of hand)

☐125-250g or 3 – 6 ounces (1 – 2 palms of hands)

☐More than 250g or more than 6 ounces (more than 2 palms of hand)

29a. How often did you eat **salmon, fresh tuna or trout**?

☐Never [GO TO QUESTION 30]

☐1 time per month or less

☐2-3 times per month

☐1 time per week

☐2 times per week

☐3-4 times per week

☐5-6 times per week

☐1 time per day

☐2 or more times per day

29b. Each time you ate **salmon, fresh tuna or trout**, how much did you usually eat?

☐Less than 1 or 1 fillet, less than 125g or less than 3 ounces (palm of hand)

☐1 to 2 fillets or 125-250g or 3 – 6 ounces (1 – 2 palms of hands)

☐More than 2 fillets or more than 250g or more than 6 ounces (more than 2 palms of hand)

30a. How often did you eat **other fish or fish fingers** (not including shellfish)?
☐Never [GO TO QUESTION 31]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

30b. Each time you ate **other fish or fish fingers** (not including shellfish), how much did you usually eat?
☐Less than 3 fish fingers or less than 1 fillet (less than 125g or less than 3 ounces (palm of hand)
☐3 to 4 fish fingers or 1 - 2 fillets (125-250g or 3 – 6 ounces (1 – 2 palms of hands)
☐More than 4 fish fingers or more than 2 fillets (more than 250g or more than 6 ounces (more than 2 palms of hands)

31. Please check the box next to each food that you ate at least once in the **past 6 months**.
☐*31. Tofu, soy burgers, vegetarian sausages or other soy-meat substitutes*
☐*32. Eggs or egg whites (Please include eggs in egg salads, quiche or soufflés) (not counting eggs in baked goods and desserts)*
☐*None of the above* [GO TO QUESTION 33]

31a. How often did you eat **tofu, soy burgers, vegetarian sausages** or **other soy-meat substitute**s?
☐Never [GO TO QUESTION 32]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week

☐1 time per day
☐2 or more times per day

31b. Each time you ate **tofu, soy burgers, vegetarian sausages** or **other soy-meat substitute**s, how much did you usually eat?
☐Less than 2 burgers or sausages or less than 125g (palm of hand) tofu or soy substitute
☐2-3 burgers or sausages or 125-250g (1 – 2 palms of hands) tofu or soy substitute
☐More than 3 burgers or sausages or more than 250g (2 palms of hands) tofu or soy substitute

32a. How often did you eat **eggs or egg whites** (Please include eggs in egg salads, quiche or soufflés) (not counting eggs in baked goods and desserts)?
☐Never [GO TO QUESTION 33]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

32b. Each time you ate **eggs or egg whites**, how many did you usually eat?
☐1 egg or 1 egg white
☐2 eggs or 2 egg whites
☐More than 2 eggs or more than 2 egg whites

33a. How often did you eat **high protein bars** (e.g. Maximuscle, Promax etc) in the last 6 months?
☐Never [GO TO QUESTION 34]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

33b. Each time you ate **high protein bars,** how much did you usually eat?
☐Less than 1 bar (less than 60g)
☐1 bar (60g)
☐More than 1 bar (more than 60g)

34. Please check the box next to each food that you ate at least once in the **past 6 months**.
☐*34. Yoghurt (including low-fat and fat-free and fruit yoghurts) (NOT including frozen yoghurt)*
☐*35. Cottage cheese or cream cheese (including low-fat and fat-free)*
☐*36. Cheese (including low-fat and fat-free; including on cheeseburgers or in sandwiches or subs)*
☐*None of the above* [GO TO QUESTION 37]

34a. How often did you eat **yoghurt** (including low-fat and fat-free and fruit yoghurts) (NOT including frozen yoghurt)?
☐Never [GO TO QUESTION 35]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

34b. Each time you ate **yoghurt**, how much did you usually eat?
☐Less than 125g (less than ¼ large pot or less than 3 ounces (palm of hand)
☐125g – 250g (¼ to ½ large pot or 3 – 6 ounces (1-2 palms of hand)
☐More than 250g (more than ¼ to ½ large pot or more than 6 ounces (2 palms of hands)

34c. How often was the **yoghurt** you ate **low-fat** or **fat-free**?
☐Almost never or never
☐About ¼ of the time
☐About ½ of the time
☐About ¾ of the time
☐Almost always or always

35a. How often did you eat **cottage cheese**?
☐Never [GO TO QUESTION 35d]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

35b. Each time you ate **cottage cheese**, how much did you usually eat?
☐Less than 28g (less than ⅙ large pot or less than 1 handful)
☐28g – 56g (⅙ to ⅓ large pot or 1-2 handfuls)
☐More than 56g (more than ⅙ to ⅓ large pot or more than 2 handfuls)

35c. How often was the **cottage cheese** you ate **low-fat** or **fat-free**?
☐Almost never or never
☐About ¼ of the time
☐About ½ of the time
☐About ¾ of the time
☐Almost always or always

35d. How often did you eat **cream cheese**?
☐Never [GO TO QUESTION 36]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

35e. Each time you ate **cream cheese**, how much did you usually eat?
☐Less than 15g (less than 1 tablespoon or less than 1 thumb size (1 ounce))
☐15-30g (1-2 tablespoons or 1-2 thumb sizes (1-2 ounces))

☐More than 30g (more than 2 tablespoons or more than 2 thumb sizes (more than 2 ounces))

35f. How often was the cheese you ate low-fat or fat-free?
☐Almost never or never
☐About ¼ of the time
☐About ½ of the time
☐About ¾ of the time
☐Almost always or always

36a. How often did you eat cheese (including low-fat and fat-free; including on cheeseburgers or in sandwiches or subs)?
☐Never [GO TO QUESTION 37]
☐1 time per month or less
☐2-3 times per month
☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

36b. Each time you ate cheese, how much did you usually eat?
☐Less than 25g (less than 1 slice/ less than 1 ounce or 1 thumb's worth)
☐25-50g (1 to 2 slices/ 1-2 ounces or 1-2 thumb's worth)
☐More than 50g (more than 2 slices/ more than 2 ounces or more than 2 thumb's worth)

36c. How often was the cheese you ate low-fat or fat-free?
☐Almost never or never
☐About ¼ of the time
☐About ½ of the time
☐About ¾ of the time
☐Almost always or always

37a. How often did you eat frozen yoghurt, ice cream, ice cream bars or ice cream cones in the last 6 months?
☐Never [GO TO QUESTION 38]
☐1 time per month or less
☐2-3 times per month

☐1 time per week
☐2 times per week
☐3-4 times per week
☐5-6 times per week
☐1 time per day
☐2 or more times per day

37b. Each time you ate frozen yoghurt, ice cream, ice cream bars or ice cream cones, how much did you usually eat?
☐Less than ½ cup (or less than 1 scoop), less than 1 bar or cone
☐½ cup (1 scoop), 1 bar or cone
☐More than ½ cup (more than 1 scoop), more than 1 bar or cone

38a. For ALL of the past 6 months, have you followed any type of vegetarian diet?
☐Yes
☐No [GO TO QUESTION 39]

38b. Which of the following foods did you TOTALLY EXCLUDE from your diet (mark all that apply)
☐Meat (beef, pork, lamb, etc)
☐Poultry (chicken, turkey, duck, etc)
☐Fish and/or seafood
☐Eggs
☐Dairy products (milk, cheese, yoghurt etc)

39a. Have you taken any protein supplements (not in protein shakes or bars) over the last 6 months? (N.B. this does not include multivitamin or mineral supplements)
☐Yes
☐No

39b. How often did you take protein supplements?
☐Less than 1 day per month
☐1-3 days per month
☐1-3 days per week
☐4-6 days per week
☐Every day

39c For how many years have you taken protein supplements?

☐Less than 1 year
☐1-4 years
☐5-9 years
☐10 or more years

That completes the questionnaire, thank you for your participation. Please email your completed questionnaire as an attachment to Una Masic (u.masic@sussex.ac.uk) with the words 'FFQ' in the subject title.

Appendix 5.3 Graphical depiction of the study design employed

Day 3: Protein
Food Frequency
Questionnaire

120 min

7 sample tasting

Key: appetite rating made   unassessed, high or low protein breakfast

taste test
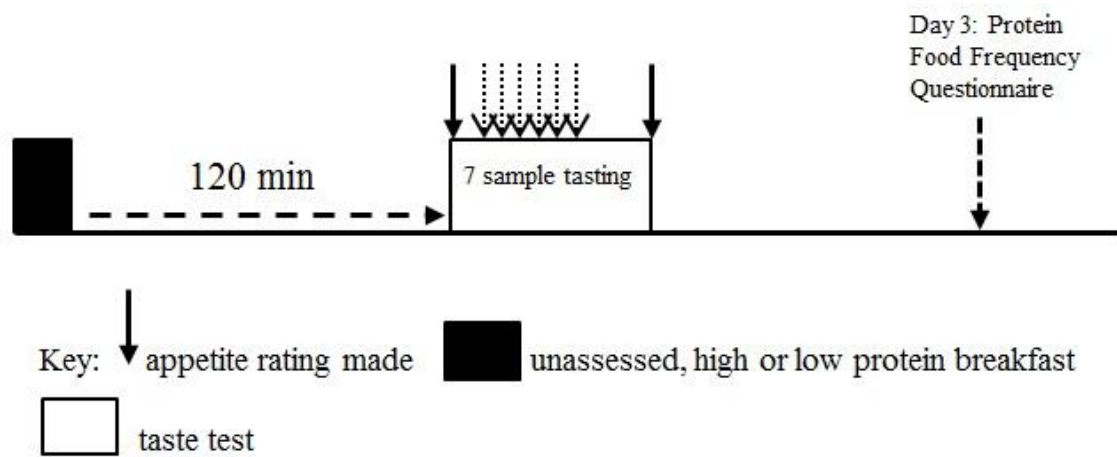
5.6 References

Appleton, K. M. (2013). Increases in fruit intakes in older low consumers of fruit following two community-based repeated exposure interventions. *British Journal of Nutrition, 109*(05), 795-801. doi: doi:10.1017/S0007114512002188

Austin, G. L., Ogden, L. G., & Hill, J. O. (2011). Trends in carbohydrate, fat, and protein intakes and association with energy intake in normal-weight, overweight, and obese individuals: 1971–2006. *The American Journal of Clinical Nutrition, 93*(4), 836-843. doi: 10.3945/ajcn.110.000141

Baker, B. J., & Booth, D. A. (1989). Preference conditioning by concurrent diets with delayed proportional reinforcement. *Physiology & Behavior, 46*(4), 585-590. doi: http://dx.doi.org/10.1016/0031-9384(89)90336-3

Baker, B. J., Booth, D. A., Duggan, J. P., & Gibson, E. L. (1987). Protein appetite demonstrated: Learned specificity of protein-cue preference to protein need in adult rats. *Nutrition Research, 7*(5), 481-487. doi: http://dx.doi.org/10.1016/S0271-5317(87)80004-0

Bellisle, F., & Drewnowski, A. (2007). Intense sweeteners, energy intake and the control of body weight. *European Journal of Clinical Nutrition, 61*(6), 691-700.

Berridge, K. C. (1996). Food reward: Brain substrates of wanting and liking. *Neuroscience & Biobehavioral Reviews, 20*(1), 1-25. doi: http://dx.doi.org/10.1016/0149-7634(95)00033-B

Berridge, K. C. (2009). 'Liking' and 'wanting' food rewards: Brain substrates and roles in eating disorders. *Physiology & Behavior, 97*(5), 537-550. doi: http://dx.doi.org/10.1016/j.physbeh.2009.02.044

Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? [doi: 10.1016/S0165-0173(98)00019-8]. *Brain Research Reviews, 28*(3), 309-369.

Bertenshaw, E. J., Lluch, A., & Yeomans, M. R. (2008). Satiating effects of protein but not carbohydrate consumed in a between-meal beverage context. *Physiology & Behavior, 93*(3), 427-436. doi: 10.1016/j.physbeh.2007.09.014

Blissett, J., & Fogel, A. (2013). Intrinsic and extrinsic influences on children's acceptance of new foods. *Physiology & Behavior*(0). doi: http://dx.doi.org/10.1016/j.physbeh.2013.02.013

Blundell, J. E., & Rogers, P. J. (Eds.). (1994). *Sweet carbohydrate substitutes (intense sweeteners) and the control of appetite: scientific issues.* (Vol. 113–124). London: CRC Press.

Booth, D. A., & Baker, B. J. (1990). dl-Fenfluramine challenge to nutrient-specific textural preference conditioned by concurrent presentation of two diets. *Behavioral Neuroscience, 104*(1), 226.

Born, J. M., Martens, M. J. I., Lemmens, S. G. T., Goebel, R., & Westerterp-Plantenga, M. S. (2013). Protein v. carbohydrate intake differentially affects liking- and wanting-related brain signalling. *British Journal of Nutrition, 109*(02), 376-381. doi: doi:10.1017/S0007114512001092

Crystal, S., Frye, C. A., & Kanarek, R. B. (1995). Taste preferences and sensory perceptions in female varsity swimmers. *Appetite, 24*(1), 25-36. doi: http://dx.doi.org/10.1016/S0195-6663(95)80003-4

Dohm, F.-A., Cachelin, F. M., & Striegel-Moore, R. H. (2005). Factors That Influence Food Amount Ratings by White, Hispanic, and Asian Samples. *Obesity, 13*(6), 1061-1069.

Essed, N., Oerlemans, P., Hoek, M., Van Staveren, W., Kok, F., & De Graaf, C. (2009). Optimal preferred MSG concentration in potatoes, spinach and beef and their effect on intake in institutionalized elderly people. *The Journal of Nutrition, Health & Aging, 13*(9), 769-775. doi: 10.1007/s12603-009-0212-9

Finlayson, G., King, N., & Blundell, J. (2008). The role of implicit wanting in relation to explicit liking and wanting for food: Implications for appetite control. *Appetite, 50*(1), 120-127. doi: http://dx.doi.org/10.1016/j.appet.2007.06.007

Finlayson, G., King, N., & Blundell, J. E. (2007). Is it possible to dissociate 'liking' and 'wanting' for foods in humans? A novel experimental procedure. [doi: 10.1016/j.physbeh.2006.08.020]. *Physiology & Behavior, 90*(1), 36-42.

FSA. (2006). *FSA Nutrient and Food Based Guidelines for UK Institutions*. London.

Gibson, E. L., & Booth, D. A. (1986). Acquired protein appetite in rats: Dependence on a protein-specific need state. *Experientia, 42*(9), 1003-1004. doi: 10.1007/bf01940706

Gibson, E. L., Wainwright, C. J., & Booth, D. A. (1995). Disguised protein in lunch after low-protein breakfast conditions food-flavor preferences dependent on recent lack of protein intake. [doi: 10.1016/0031-9384(95)00068-T]. *Physiology & Behavior, 58*(2), 363-371.

Gibson, E. L., & Wardle, J. (2001). Effect of contingent hunger state on development of appetite for a novel fruit snack. *Appetite, 37*(2), 91-101. doi: http://dx.doi.org/10.1006/appe.2001.0420

He, W., Yasumatsu, K., Varadarajan, V., Yamada, A., Lem, J., Ninomiya, Y., . . . Damak, S. (2004). Umami Taste Responses Are Mediated by α-Transducin and α-Gustducin. *The Journal of Neuroscience, 24*(35), 7674-7680. doi: 10.1523/jneurosci.2441-04.2004

Ikeda, K. (1908). On a New Seasoning. *Journal of Tokyo Chemistry Society, 30*, 820-836.

Jinap, S., & Hajeb, P. (2010). Glutamate. Its applications in food and contribution to health. *Appetite, 55*(1), 1-10. doi: 10.1016/j.appet.2010.05.002

Kondoh, T., Mori, M., Ono, T., & Torii, K. (2000). Mechanisms of Umami Taste Preference and Aversion in Rats. *Journal of Nutrition, 130*(4), 966-.

Larsen, T. M., Dalskov, S.-M., van Baak, M., Jebb, S. A., Papadaki, A., Pfeiffer, A. F. H., . . . Astrup, A. (2010). Diets with High or Low Protein Content and Glycemic Index for Weight-Loss Maintenance. *New England Journal of Medicine, 363*(22), 2102-2113. doi: doi:10.1056/NEJMoa1007137

Laska, M., & Hernandez Salazar, L. T. (2004). Gustatory responsiveness to monosodium glutamate and sodium chloride in four species of nonhuman primates. *Journal of Experimental Zoology Part A: Comparative Experimental Biology, 301A*(11), 898-905. doi: 10.1002/jez.a.118

Luscombe-Marsh, N. D., Smeets, A. J. P. G., & Westerterp-Plantenga, M. S. (2008). Taste sensitivity for monosodium glutamate and an increased liking of dietary protein. *British Journal of Nutrition, 99*(4), 904-908. doi: 10.1017/s000711450788295x

Luscombe-Marsh, N. D., Smeets, A. J. P. G., & Westerterp-Plantenga, M. S. (2009). The addition of monosodium glutamate and inosine monophosphate-5 to high-protein meals: effects on satiety, and energy and macronutrient intakes. *British Journal of Nutrition, 102*(6), 929-937. doi: 10.1017/s0007114509297212

Mendez, M. A., Popkin, B. M., Buckland, G., Schroder, H., Amiano, P., Barricarte, A., . . . González, C. A. (2011). Alternative Methods of Accounting for Underreporting and Overreporting When Measuring Dietary Intake-Obesity

Relations. *American Journal of Epidemiology, 173*(4), 448-458. doi: 10.1093/aje/kwq380

Murphy, C. (Ed.). (1987). *Flavor preference for monosodium glutamate and casein hydrolysate in young and elderly persons.* . New York: Marcel Dekker.

NCI. (2007). Diet History Questionnaire, Version 1.0. National Institutes of Health, Applied Research Program, National Cancer Institute.

Peciña, S., Cagniard, B., Berridge, K. C., Aldridge, J. W., & Zhuang, X. (2003). Hyperdopaminergic Mutant Mice Have Higher "Wanting" But Not "Liking" for Sweet Rewards. *The Journal of Neuroscience, 23*(28), 9395-9402.

Pliner, P. (1982). The Effects of Mere Exposure on Liking for Edible Substances. *Appetite, 3*(3), 283-290. doi: http://dx.doi.org/10.1016/S0195-6663(82)80026-3

Roininen, K., Lähteenmäki, L., & Tuorila, H. (1996). Effect of umami taste on pleasantness of low-salt soups during repeated testing. *Physiology & Behavior, 60*(3), 953-958. doi: http://dx.doi.org/10.1016/0031-9384(96)00098-4

Sclafani, A. (1987). Carbohydrate taste, appetite, and obesity: An overview. [doi: 10.1016/S0149-7634(87)80019-2]. *Neuroscience & Biobehavioral Reviews, 11*(2), 131-153.

Simpson, S. J., Batley, R., & Raubenheimer, D. (2003). Geometric analysis of macronutrient selection in humans. the power of protein? *Appetite, 41*, 123–140.

Simpson, S. J., & Raubenheimer, D. (2005). Obesity: the protein leverage hypothesis. *Obesity Reviews, 6*(2), 133-142.

Simpson, S. J., & Raubenheimer, D. (Eds.). (2000). *Geometric models of macronutrient selection*. Boca Raton: CRC Press.

Stephens, D., & Krebs, J. (1986). Princeton University Press; Princeton, NJ. *Foraging theory*.

Stunkard, A. J., & Messick, S. (1985). The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. . *Journal of Psychosomatic Research, 29*, 71-83.

Tepper, B. J., & Ullrich, N. V. (2002). Influence of genetic taste sensitivity to 6-n-propylthiouracil (PROP), dietary restraint and disinhibition on body mass index in middle-aged women. *Physiology & Behavior, 75*(3), 305-312. doi: http://dx.doi.org/10.1016/S0031-9384(01)00664-3

Vazquez, M., Pearson, P. B., & Beauchamp, G. K. (1981). Flavour preferences in malnourished Mexican infants. *Physiology & Behavior, 28*, 513–519.

Veldhorst, M., Smeets, A., Soenen, S., Hochstenbach-Waelen, A., Hursel, R., Diepvens, K., . . . Westerterp-Plantenga, M. (2008). Protein-induced satiety: Effects and mechanisms of different proteins. [doi: 10.1016/j.physbeh.2008.01.003]. *Physiology & Behavior, 94*(2), 300-307.

Viskaal-van Dongen, M., Kok, F. J., & de Graaf, C. (2011). Eating rate of commonly consumed foods promotes food and energy intake. *Appetite, 56*(1), 25-31. doi: http://dx.doi.org/10.1016/j.appet.2010.11.141

Westerterp-Plantenga, M. S. (Ed.). (1994). *Nutrient utilization and energy balance.* Boca Raton: CRC Press.

Williams, E. J. (1949). Experimental designs balanced for the estimation of residual effects of treatment. *Australian Journal of Scientific Research, Series A*(2), 149-168.

Yamamoto, K., & Ishimaru, Y. (2013). Oral and extra-oral taste perception. *Seminars in Cell & Developmental Biology, 24*(3), 240-246. doi: http://dx.doi.org/10.1016/j.semcdb.2012.08.005

Zandstra, E. H., de Graaf, C., van Trijp, H. C. M., & van Staveren, W. A. (1999). Laboratory hedonic ratings as predictors of consumption. *Food Quality and Preference, 10*(4–5), 411-418. doi: http://dx.doi.org/10.1016/S0950-3293(98)00050-0

This paper was published in Physiology & Behavior in May, 2013.

Does monosodium glutamate interact with macronutrient composition to influence subsequent appetite?

Una Masic [a] and Martin R Yeomans [a]

[a] School of Psychology, University of Sussex, Brighton, BN1 9QH, UK

Running head:

MSG and satiety

Contact details:

Una Masic

School of Psychology

University of Sussex

Brighton

BN1 9QH

UK

Tel: (01273) 872826

Email: u.masic@sussex.ac.uk

Abstract

The influence of flavour enhancers such as monosodium glutamate (MSG) on satiation and satiety is unclear, and the present study aimed to explore this by examining the effects consumption of soups varying in MSG (1% added MSG or no MSG) and macronutrient content (added carbohydrate, protein or control) had on appetite. 24 non-obese, low-restraint male participants (mean age: 21; BMI: 24) consumed a fixed portion of soup and rated their appetite before, immediately after intake and at 15 minute intervals for 120 minutes post-ingestion across six sessions. Added MSG significantly increased flavour pleasantness and tended to result in a smaller decrease in hunger immediately after soup ingestion. MSG also reduced rather than enhanced feelings of fullness immediately after ingestion of the high protein soup. As expected, hunger increased, and fullness decreased, over the subsequent 120 min, but the increase in hunger was significantly lower in the MSG than no-MSG conditions with the protein soup between 30 and 60 minutes post-ingestion. Overall these data suggest that MSG may have a bi-phasic effect on appetite, with reduced satiation mediated by effects on palatability, but potential for enhanced post-ingestive satiety particularly in the context of protein ingestion.

6.1 Introduction

Monosodium glutamate (MSG) has been shown to elicit the sensory experience of 'umami', evoking the taste of glutamate and nucleotides found in animal protein, vegetables and matured foods such as cheeses. MSG's savoury flavour profile (McCabe & Rolls, 2007) improves the palatability and acceptability of many foods (Yamaguchi & Ninomiya, 2000) and (Prescott, 2004), highlighting its potential for sustaining higher energy intake due to its flavour enhancing properties (Mathey, et al., 2001) and apparent low satiety value (Rogers & Blundell, 1990). However, MSG supplemented diets have been repeatedly linked to the maintenance of stable energy intake over time (Essed, et al., 2007) and (Schiffman, et al., 1994). Likewise, and in contrast to claims of a low satiety-value for MSG, some studies suggest the potential for MSG enhancing satiety over time due to interactions with specific macronutrients, with supporting evidence for the role of protein reported in animal-studies (Viarouge, et al., 1992). This has led to the idea that MSG may have a dual role in appetite control since it has been found to increase hedonic satisfaction (which may stimulate appetite and intake (Yeomans, 1996; Yeomans & Gray, 1997; Yeomans, et al., 1998 and Yeomans, et al., 2004) and yet maintain stable energy intake over time. Inconsistencies in the literature may be related to the role of MSG on the experience of appetite after meal cessation. Thus there is a need for more clarification of the impact of MSG at discrete points during satiation (before and immediately after meal consumption) and satiety (over the post-ingestive period).

MSG has the potential for multiple effects on appetite. The role of MSG as a flavour enhancer would suggest that the presence of MSG in foods will lead to increased consumption. Indeed, the effects of palatability on intake are well established and robust (see Yeomans, et al., 2004). In brief, as liking for a food increases so too does voluntary intake. However, there have been few studies specifically testing whether MSG-enhancement of liking actually results in increased intake. Bellisle et al., (1989) assessed the acceptability of novel foods supplemented with 0.6% (w/w) or 1.2% (w/w) MSG, finding that the 0.6% condition increased intake and eating rate whilst the 1.2% condition increased meal size. However these initial increases did not remain over time with energy intake (EI) returning to baseline values. Bellisle also found no differences

in energy intake in elderly (Bellisle, et al., 1991) or diabetic (Bellisle, et al., 1996) participants with the addition of MSG to the diet.

One possible explanation for the apparent disparity between the acute effects of MSG on liking but failure to find evidence of increased intake over time might be that MSG initially stimulates appetite through flavour enhancement but then increases satiety by acting on post-ingestive physiological responses. If so, analyses need to look separately at the effects of MSG before and immediately after a meal (to measure satiation effects), and during the post-ingestive time span (to examine effects on satiety). Indeed, some recent research does suggest enhanced satiety after consumption of MSG. Carter et al., (Carter, Monsivais, & Drewnowski, 2011) compared MSG-containing and no-MSG soup preloads over two morning exposures separated by two hours with appetite ratings completed every 15 minutes after consumption of the first preload and for a further 10 minutes after ingestion of the second preload. Participants were then provided with a buffet-style lunch 2 or 30 minutes after preload ingestion. The addition of MSG significantly reduced the experience of hunger and desire to snack after the second preload relative to control and may have reduced the experience of hunger over time but the authors reported no subsequent energy intake differences between no-MSG and MSG conditions in the *ad-libitum* meal. Earlier studies also report no differences in intake 2 or 30 minutes after consumption of low energy soup consommé preloads supplemented with MSG compared to no-MSG controls (Rogers & Blundell, 1990). However, the authors also reported a more rapid recovery of hunger after ingestion of the MSG preloads whilst the rate of hunger recovery was found to be equal across low energy control and MSG conditions in the previous study (Carter, et al., 2011). These inconsistencies between studies may be due to the procedures employed. For example, Carter et al., (Carter, et al., 2011) provided chicken broth as a breakfast item which may have been unusual for participants and consequently affected appetite assessments (de Castro, 1987; Lipps Birch, Billman, & Salisbury Richards, 1984; Means, Ginn, Arolfo, & Pence, 2000; Rodin, 1980). Also, although MSG improved soup acceptability compared to control, the palatability enhancing effects of MSG may not have been accounted for due to low soup pleasantness ratings across conditions in both studies.

The lack of consensus regarding the effects of MSG on immediate appetite over time may be related to the macronutrient compositions of the foods tested. Indeed, the key

macronutrients (carbohydrate, fat and protein) have been repeatedly shown to differentially alter the experience of satiety with protein reported to be more effective at encouraging both satiation and satiety than carbohydrate or fat (Dove et al., 2009; Poppitt, et al., 1998; Porrini, Crovetti, Testolin, & Silva, 1995; Ratliff et al., 2010). Equally, the experience of umami has been linked to a protein detection function in foods (Ikeda, 1908), and may guide food selection practices (Bellisle, et al., 1996; Luscombe-Marsh, et al., 2008) and regulate protein intake (Laska & Hernandez Salazar, 2004; Smriga & Torii, 2000) because of the glutamate sensing component of MSG which has been suggested to be a taste constituent of dietary protein (Finlayson, Bordes, Griffioen-Roose, de Graaf, & Blundell, 2012; Naim, et al., 1991; Narukawa et al., 2011).Thus, umami itself may provide a 'protein taste' (de Araujo, et al., 2003; Rolls, 2000) or act as a learned cue for protein with experience (Gibson, 2001) only signalling the presence of protein when accompanied by other nutrients or flavours that reinforce this message (Gibson, 2001; Beauchamp, 2009).This potential for a learned regulatory effect of umami by protein signalling may be traced to infanthood, affecting future assessments of appetite by initially associating umami flavours as being more satiating (Mennella & Beauchamp, 1996; Ventura, et al., 2012). These findings may provide an explanation for the lack of direct evidence for increased intake over time with the addition of MSG. It may be that the sensory experience of MSG acts to prepare for protein ingestion and so contributes to the enhanced satiating effects of protein. Thus paradoxically MSG could theoretically enhance appetite, through its effects on flavour, and at the same time enhance satiety by allowing the body to prepare for ingestion of protein. If so, then MSG may lead to reduced satiation (as a consequence of its effects on palatability) but enhanced satiety, by acting as a cue for protein ingestion. Although one study (Luscombe-Marsh, et al., 2009) found no differences in satiety between protein preloads varying in MSG, there is evidence that gut glutamate sensing mechanisms enhance satiety in animals (Kondoh & Torii, 2008) and MSG has been related to gastric increases in GLP-1 secretion 30 minutes after a test meal when compared with sodium chloride (NaCl; Hosaka et al., 2012).

Given the uncertainly in the literature, the present research consequently assessed the time course of the effects of added MSG (MSG+) relative to a control (MSG-) across different macronutrient compositions and energy levels (high energy carbohydrate-rich (CHO) or protein-rich (PRO) or low energy controls) in a hedonically neutral low-

glutamate soup. It was hypothesised that the flavour enhancing properties of MSG would increase appetitive hunger sensations compared to the no-MSG conditions immediately and so result in lower satiation from consumption of a fixed portion, whilst in contrast MSG would retard the recovery of hunger post-ingestion. It was also hypothesised that the protein containing preloads would enhance satiety more strongly than the control and carbohydrate conditions (Bertenshaw, Lluch, & Yeomans, 2008), but this effect of protein on satiety would be further enhanced by MSG.

6.2 Method

6.2.1 Design

A within-subjects design was used to assess the time-course of changes in hunger and fullness both during ingestion (to assess satiation) and over the subsequent two hours (to assess satiety) for a fixed portion of a vegetable soup differing in macronutrient content, energy (high energy carbohydrate-rich (CHO) or protein-rich (PRO) or low energy control) and MSG content (1% MSG w/w (MSG+) or no MSG (MSG-)). Condition order was counterbalanced using a Williams square design (Williams, 1949).

6.2.2 Participants

Twenty-four low restraint male participants from a student population (mean age: 21±1.4, ages from 18-53; mean BMI: 24±0.5, BMI from 19-26 kg/m²) at the University of Sussex took part in the research. Prospective participants who had previously expressed an interest in participating in appetite research were emailed with details of the study described as 'the effects of food on motor skills' to reduce demand characteristics due to awareness of the experimental manipulations. Individuals on medications, those smoking more than 5 cigarettes a day, those with a history of diabetes, diagnosed eating disorders and allergies or dietary intolerances to the foods used were excluded as were those with high restraint scores (ratings above 7 on the Three Factor Eating Questionnaire (TFEQ; Stunkard & Messick, 1985) as restrained individuals may not be representative of general eating behaviour (Dohm, et al., 2005).

Participants gave written informed consent before participation and were paid £60 on completion of the final session. The study was approved by the University of Sussex

ethics committee and was conducted in accordance with the standards laid down in the Helsinki Declaration.

6.2.3 Test Food

6.2.3.1 Control Breakfasts:

Breakfasts comprised of 80g cereal (Crunchy Nut Cornflakes, Kellogg's, UK), 200g semi-skimmed milk (Sainsbury's Plc., UK) and 200g orange juice (Sainsbury's Plc., UK) (total 503.6 Kcal). These quantities were established based on the UK Food Standard Agency guidelines for male breakfast consumption (FSA, 2006).

6.2.3.2 Soups:

A low energy density (ED) control soup was used as a base for all flavour and energy manipulations. This consisted of a carrot and spice soup containing carrots (Frozen Baby Carrots, Sainsbury's Plc. UK) onions, olive oil (Medium Flavour Olive Oil, Sainsbury's Plc., UK), spice mixture (Garam Masala Schwartz, UK) and water. The base soup was prepared in large batches at the start of each week by sweating 680g diced onion and 400g chopped celery in 80g oil before adding 2400g frozen carrots combined with 1400g water. The mixture was brought to the boil for 1 hour and then simmered for an additional hour. The soup was then blended and separated into batches and the spice mixture and MSG (according to soup type) was added before being stored at 4^oC until needed. Soup formulation and serving followed a standardized procedure and preload portions were fixed at 450g as this has previously been found to be an adequate portion size for male participants (Yeomans & Gray, 2002).The addition of 52g maltodextrin (DE: 15.3, Cargill, UK) was used in the CHO soup and 17.86g maltodextrin (Cargill, UK) combined with 36g whey protein isolate (MyProtein UK) was added to the PRO soup to make up 450g portions. The addition of maltodextrin to the PRO preload was necessary to ensure both high energy conditions were similar in ED while minimizing differences in flavour pleasantness. The addition of 1% (w/w) MSG (Ajinomoto Co., Inc. Europe) was also added to all MSG+ soup conditions. The final formulations followed extensive pilot testing (data not shown) to identify a base soup that was low in glutamate, and which was rated as moderately pleasant to allow for enhancement by MSG.

The high ED conditions contained around180 Kcal more per portion than the low ED conditions (CHO: 178 Kcal, PRO: 184 Kcal). The small (6 Kcal) difference between high ED CHO and PRO conditions arose due to efforts to minimise the flavour effects of maltodextrin added in the CHO condition. All soups were tested in a final pilot experiment to ensure similar pleasantness ratings across MSG+ compared to MSG- conditions. This involved 10 unrestrained male participants (mean age: 20±1.3, ages from: 18-23; mean BMI: 22.8±2.7, BMI from: 19-29 kg/m²) attending a single session in which they evaluated the sensory characteristics of two samples of each of the six soups in random order. Mean ratings from the pilot study are shown in Table 6.1 and indicate a significant sensory enhancement in pleasantness ($F_{(1,9)} = 10.76$, $p = 0.01$), saltiness ($F_{(1,9)} = 19.82$, $p = 0.002$), and strength ($F_{(1,9)} = 9.27$, $p = 0.01$) with the addition of MSG. Sweetness was also found to differ significantly by condition ($F_{(2,18)} = 3.78$, $p = .04$) with carbohydrate and protein conditions deemed sweeter than control. The pilot also indicated no significant differences in pleasantness between the three soups without added MSG ($F_{(2,18)} = 1.68$, $p = NS$) and no effect of MSG on savoury assessments ($F_{(1,9)} = 2.62$, $p = NS$). All nutritional information can be found in Table 6.2.

Table 6.1. Mean (±SEM) pilot sensory ratings of six soups differing in macronutrient and MSG concentration.

Rating	Control		Carbohydrate		Protein	
	No MSG	MSG	No MSG	MSG	No MSG	MSG
Pleasant	48.6±7.4	59.8±3.9	46.1±7.4	62.4±3.6	39.6±6.0	61.5±5.2
Salty	36.7±6.2	57.1±5.5	36.0±5.8	52±5.6	31.1±5.2	41.9±5.3
Strong	33.8±5.1	53.3±3.5	45.1±4.6	57.7±4.8	37.8±5.8	52.2±5.7
Savoury	50.1±5.2	57.4±3.7	49.8±4.9	53.8±4.1	44.3±4.1	53.9±4.1
Sweet	42.2±4.7	42.1±4.1	58.1±3.4	49.4±6.2	54.0±5.3	53.2±11.0

Table 6.2. Nutritional composition of soup preloads per 100g

	Control	Carbohydrate	Protein
Carbohydrate (g)	3	14	7
Protein (g)	0.4	0.3	8
Fat (g)	2	1	1
Carbohydrate (% energy)	44	81	38
Protein (% energy)	6	2	45
Fat (% energy)	50	17	19
Kcal	28	67	69
Kcal/450g portion	126	303	310

6.2.4 Computerized Data Collection

Sensory and hedonic ratings were tracked using the Sussex Ingestion Pattern Monitor (SIPM version 2.0.13, University of Sussex) which consists of a digital balance system linked to a computer monitor similar to the Universal Eating Monitor (UEM). In all trials participants were asked to fill in appetite and mood ratings using digital Visual Analogue Scales (VAS) by the SIPM. The ratings were presented as simple sentences ("How <word> do you feel?") with a left hand anchor reading "Not at all <word>" (coded as 0) and a right hand anchor reading "As <word> as I have ever felt/experienced" (coded as 100). To ensure compliance during ratings the software prompted participants to "Please use the mouse to drag the bar to the point on the scale that best represents how you feel right now." before each evaluation. Participants completed this action and pressed "Rating Complete". Initial appetite and mood ratings included how alert, clear-headed, energetic, full, hungry, nauseous and thirsty they felt.

Following the appetite and mood ratings, participants were provided with a fixed portion of the target soup according to condition and first completed a taste test in which they were asked to "Please take a mouthful of the soup, count to 5 and then swallow". VAS ratings regarding the sensory features of the soup (how filling, pleasant, salty, savoury, strong and sweet) were then completed before being notified to "Please

consume all of the soup". Participants were also reminded to "Make sure you consume all of the soup before moving on to the next stage". Once finished, meal termination was indicated by clicking "Finished eating". A second set of mood and appetite ratings mirroring the first set were then filled-in before participants were asked to sit in the waiting room and complete further ratings of hunger, fullness and thirst every 15 minutes for 2 hours from meal cessation. All VAS ratings were randomized across all trials.

6.2.5 Procedure

Participants attended sessions at the Ingestive Behaviour Unit (IBU) at the University of Sussex on six non-consecutive days under the impression that they were taking part in an experiment assessing "the effects of food on motor skills". Prior to each testing session participants were asked to consume nothing but water from 2300h the night before testing and were given a control breakfast at a pre-arranged time (from 0900h - 1100h) for all sessions. Following breakfast, participants were permitted to leave the IBU with instructions to consume only water and returned 3 h later for the soup session.

Upon their return, participants were taken to a cubicle containing a glass of water and the SIPM software. They were asked to follow the instructions on-screen which explained the experimental procedure and instigated the first set of mood and appetite ratings. Participants then informed the experimenter who provided the soup according to condition. Ratings of the sensory and hedonic features of the soup were carried out after consuming one mouthful of soup after which participants were prompted to consume the soup in its entirety. This was followed by further ratings of appetite before participants were provided with a pencil and paper and were asked to draw within an outline of a star using their non-dominant hand. This star drawing task was used as means of concealing the true purpose of the experiment.

Participants were then taken to the waiting room and returned to the cubicle every 15 minutes for 120 minutes post-ingestion to fill-in appetite ratings. Star drawings were also completed at baseline and after each hour in keeping with the cover story. At the end of sessions 1-5 participants were free to leave but their height and weight was recorded and they were debriefed before payment on the final test day. A graphical depiction of the experimental design can be seen in Appendix 6.1.

6.2.6 Data Analysis

Repeated measures two way 3x2 ANOVAs were conducted on initial sensory evaluations after tasting and on appetite (hunger and fullness) ratings after test meal consumption assessing the two main variables; soup type (PRO, CHO, or control) and MSG condition (MSG+ or MSG-). Additional repeated measures 3x2x8 ANOVAs were also carried out assessing the effect of soup type and MSG condition over distinct time points after soup ingestion (15, 30, 45, 60, 75, 90, 105 and 120 minutes post-ingestion). Satiation (defined as the experience of hunger and fullness before and immediately after ingestion) data and satiety (hunger and fullness after ingestion) data were analysed separately to understand the effects of MSG on immediate and prolonged appetite sensations independently. All of the variables examined were transformed to track the change in subjective experience either from the start of the meal until immediately after ingestion (satiation) or from the end of the meal over 2 hours post-consumption (satiety). Pair-wise protected contrasts of MSG and no-MSG conditions at each time point were carried out where significant interactions were apparent. Effect sizes are reported for specific effects using Pearson's correlation coefficient.

Greenhouse Geisser values were used in cases of violated sphericity ($\varepsilon = <0.75$) and time based within-subject contrasts were analysed to understand specific effects of different patterns of data. Data are shown for all 24 participants.

6.3 Results

6.3.1 Sensory and Hedonic Assessments

MSG+ conditions were rated as more pleasant tasting than the equivalent MSG- conditions (main effect of MSG: $F_{(1,23)} = 10.25$, $p<0.01$, $r=.55$) and MSG enhanced soups were also rated as stronger tasting, ($F_{(1,23)} = 12.85$, $p <0.01$, $r=.60$) and more salty, ($F_{(1,23)} = 14.94$, $p<0.01$, .63) than MSG- soups (Figure 6.1). This may be due to the sodium ion found in MSG increasing the perception of saltiness. There was also a trend for a significant effect of soup condition on pleasantness ratings ($F_{(2,46)} = 2.99$, $p=.06$) and a similar trend for sweetness to differ between soups ($F_{(2,46)} = 2.76$, $p=.07$) with CHO soups tending to be rated as more pleasant ($F_{(1,23)} = 6.55$, $p=.02$, $r=.47$) than control soups and sweeter ($F_{(1,23)} = 6.24$, $p=.02$, $r=.46$) than protein soups at

baseline. Interestingly there was no effect of savouriness between no MSG and added MSG soups ($F_{(2,46)} = 0.16$, $p=.45$). This may be because participants were not trained to associate the MSG flavour with savouriness, which may be a more ambiguous food label in the current context.
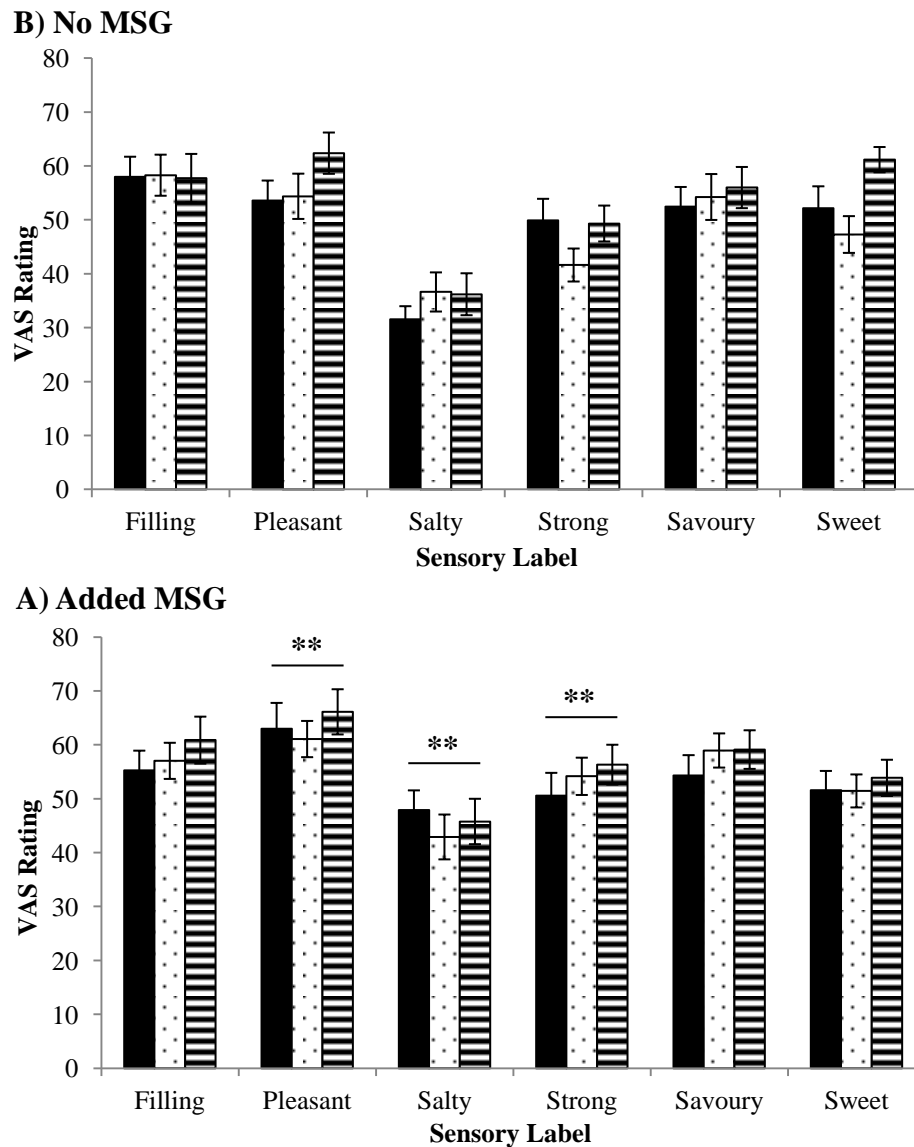
B) No MSG

A) Added MSG

Figure 6.1. Sensory and hedonic assessments (mm) of three levels of soup (low energy control (■), high energy protein (□) and high energy carbohydrate (▤) with (Panel A) and without (Panel B) added MSG. Data are mean ±SE. Significant differences between no and added MSG are indicated: ** $P<0.01$.

6.3.2 Satiation Ratings

The data of interest were how hunger and fullness changed over ingestion. As expected, there were no significant differences in hunger, $(F_{(2,46)} = 1.19, p=.31)$ or fullness, $(F_{(2,46)} = 0.54, p=.54)$ before the food was tasted. Analyses looked at overall changes from pre- to post-meal intake. No significant changes in appetite were noted between soup types for either hunger, $(F_{(2,46)} = 1.63, p=.21)$ or fullness, $(F_{(2,46)} = 1.25, p=.30)$ suggesting that the macronutrient content of the soup did not affect the immediate post-meal experience of satiation. The predicted smaller decrease in hunger after MSG approached significance $(F_{(1,23)} = 3.63, p = 0.07)$ with a moderate effect size $(r=.37)$ suggesting a smaller overall decrease in hunger in the MSG+; m: -41.6±3.9 compared to MSG-; m: -46.9±4.2 condition. The equivalent data for fullness was not significant $(F_{(1,23)} = 0.35, p=.56)$. However, for changes in fullness the soup*MSG interaction approached significance $(F_{(2,46)} = 2.85, p=.07)$ but surprisingly the increase in fullness after consumption of the protein soup was lower with added MSG; m: 46.4±4.5 than without MSG; m: 53 ($r=.43$: Figure 6.2). However, this effect was reversed for both the carbohydrate and control soups with fullness increasing more in the MSG+ than MSG- conditions. There was no significant effect of order of testing on hunger $(F_{(2,36)} = 1.92, p=.82)$ or fullness $(F_{(2,36)} = 1.33, p=.34)$.
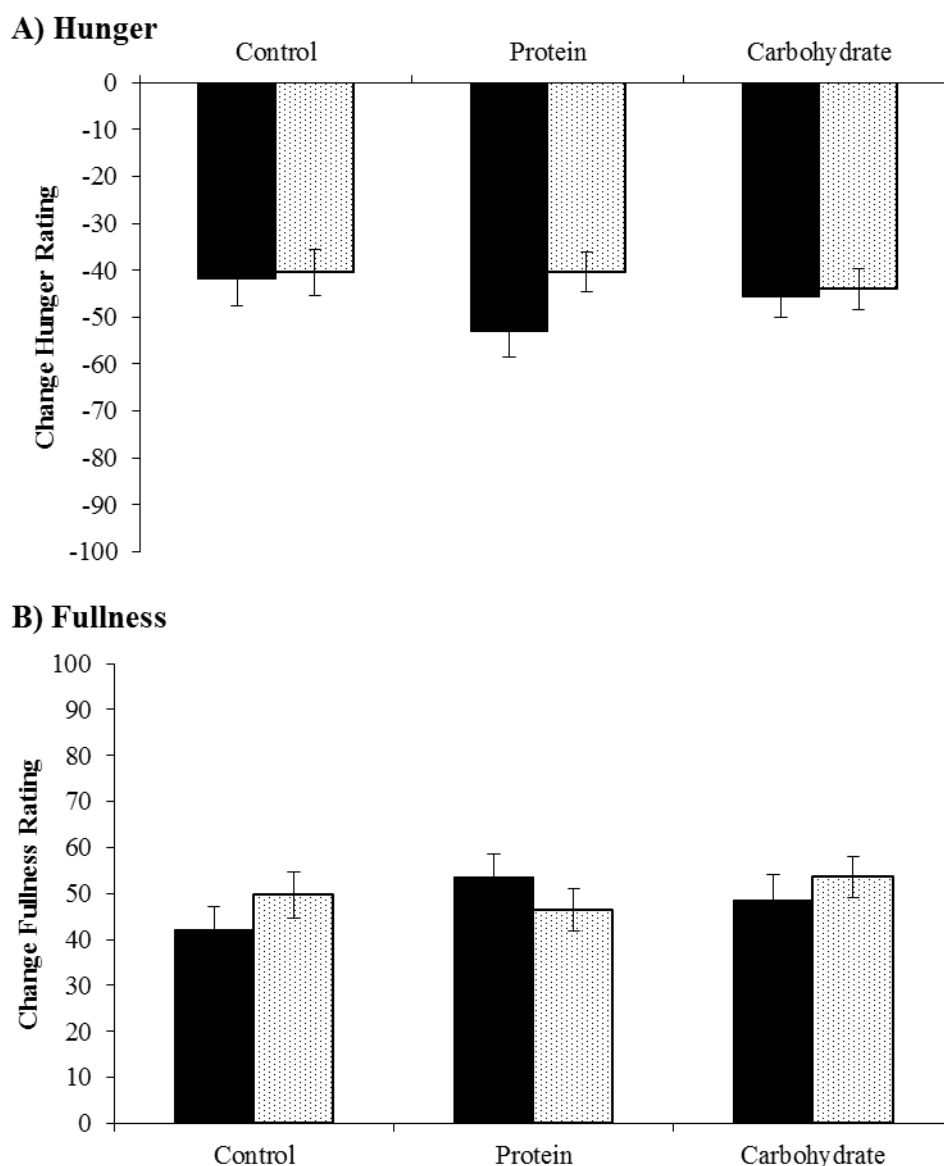
A) Hunger

B) Fullness

Figure 6.2. Change from baseline VAS hunger (Panel A) (mm) and fullness (Panel B) ratings across three levels of soup (low energy control, high energy carbohydrate and high energy protein) containing no (■) and added (□) MSG. Data are mean ± SE.

6.3.3 Satiety Evaluations

Effects of the preload nutrient and MSG manipulations on satiety were assessed by examining changes in rated hunger and fullness over the subsequent 120 minutes (Figures 6.3 and 6.4). Since the prediction was that both the recovery of hunger and decline in fullness would depend on a combination of MSG and macronutrient content, we examined how hunger and fullness changed using linear contrasts within multivariate ANOVA to test for these effects. As expected, hunger increased ($F(1,23 =$

45.88, p<.001) and fullness decreased (F(1,23) = 48.32, p<.001) over the 120 minutes. However, with hunger these changes over time depended both on the MSG and soup manipulations (time*soup*MSG interaction: F(1,23) = 4.76, p=.04, r=.41). The main effect of soup overall was not significant (F(1,23) = 0.19, p=.67), which was surprising given that two of the soups had higher energy content than the control. However, effect size calculations indicated a large effect of protein compared to control (r=.41), indicating that the protein condition suppressed hunger more strongly than the equivalent control condition, while the main effect of MSG approached significance (F(1,23) = 3.18, p=.09). No two-way interactions were significant in these analyses. Pair-wise protected contrasts of equivalent MSG and no-MSG conditions at each time point revealed hunger to be significantly lower in the MSG than no-MSG condition at 3 time points: 30, 45 and 60 minutes after the soup was consumed. There were no equivalent effects of MSG on fullness ratings: the main effects of MSG (F(1,23) 0.83, p=.48) and soup (F(1,23) = 0.81, p=.46) were not significant, and there were no significant interactions between MSG, soup and time. The lower panels of Figure 6.3 show no clear pattern of difference in fullness between soups or MSG conditions over the 120 minutes post-ingestion.

Figure 6.3. Change in hunger ratings (mm) post-ingestion across three levels of soup (low energy control (Panel A) and high energy protein (Panel B) and high energy carbohydrate (Panel C)) with (_ _•_ _) and without (___•___) added MSG over time (minutes). Data are mean ± SEM. Significant protected contrasts (p = <.02) are denoted with *.
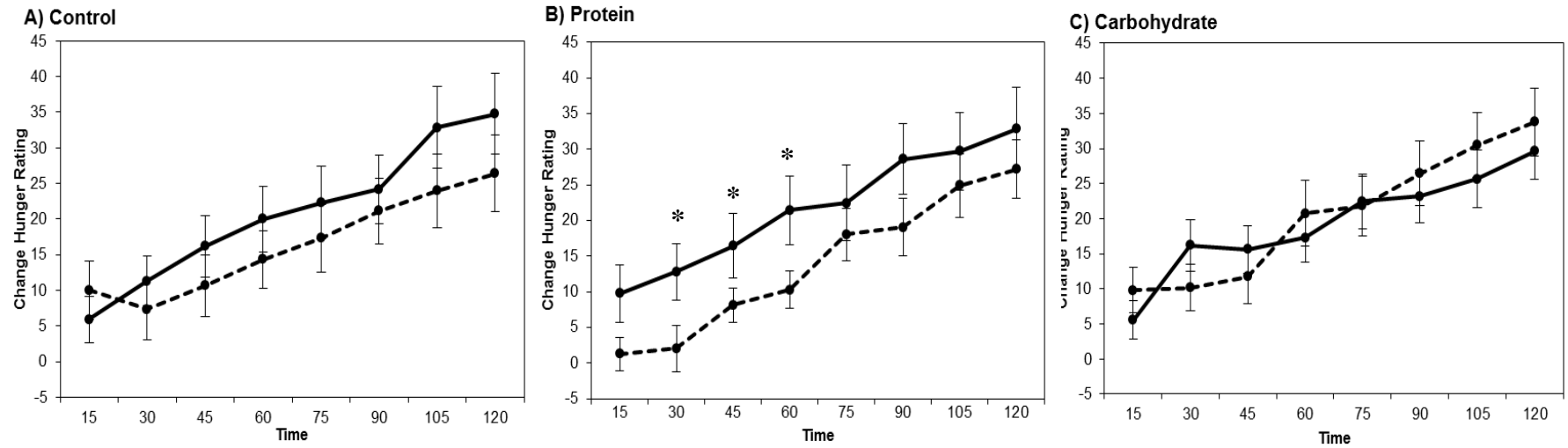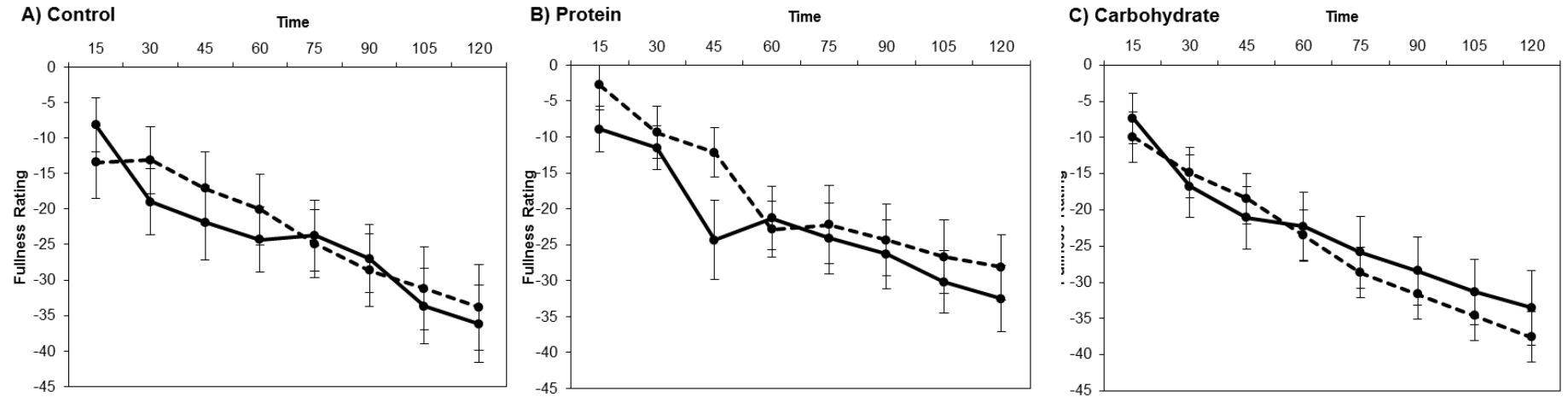
Figure 6.4. Change in fullness ratings (mm) post-ingestion across three levels of soup (low energy control (Panel A) and high energy protein (Panel B) and high energy carbohydrate (Panel C)) with (– ● – .) and without (——●——) added MSG over time (minutes). Data are mean ± SEM.

6.4 Discussion

This research aimed to elucidate the impact of MSG on experiences of short-term hunger and fullness (satiation) and appetite over time (satiety) when combined with different macronutrients in a vegetable soup context. The main findings suggest that MSG may reduce satiation due to the effects of MSG on pleasantness enhancing appetite but that MSG may subsequently enhance satiety, especially when provided in a protein context.

The predicted impact of MSG enhancing immediate post-meal hunger across soup conditions may be explained in terms of hedonic satisfaction (Bellisle, et al., 1989) and the appetizer effect (Yeomans, 1996). Hunger remained elevated after the more appetizing MSG+ soup across all conditions and sensory analysis confirmed that MSG+ soups tasted more pleasant than were MSG-. When MSG was assessed with soup type, the MSG+ protein soup resulted in a lower increase in fullness compared to MSG+ carbohydrate and MSG- conditions instead of further maximising satiation as predicted. Why might the addition of MSG reduce this fullness response in combination with protein? It may be that the oral protein sensing cue (Bellisle, 1999) which may be implicitly learned to be more filling (Tome, et al., 2009), was masked by the oral glutamate protein cue as this additional cue in the vehicle used (a low protein soup) was not expected. In contrast, the glutamate cue in the MSG carbohydrate and control conditions may have increased the experience of immediate fullness due to the expectation of receiving protein(Kondoh, et al., 2009).

When looking at the influence of MSG after the meal (post-meal satiety), the addition of MSG seemed to decrease hunger sensations across soup type compared to the equivalent no MSG conditions in the control and protein contexts, and these differences were significant in the protein condition between 30 and 60 minutes post-ingestion. This is consistent with the idea that the glutamate protein signal may be a more potent cue post-ingestion due to the impact on gut glutamate receptors (Niijima, 2000; San Gabriel, et al., 2007) with the MSG cue acting to maximise satiety more effectively when provided with protein. Alternatively, it may be that the mismatch between the expectation of protein in the MSG+ carbohydrate soup and subsequent experience of carbohydrate but no protein reversed any satiating effects of the added MSG in that

context. This mismatch may have led to rebound hunger within the MSG+ carbohydrate condition (Appleton & Blundell, 2007; Tordoff & Alleva, 1990). However, there was no protein in the low-energy control condition and here MSG+ did slow the recovery of hunger post-ingestion. Further research is therefore needed to elucidate the apparent short-term satiating effects of MSG in this context.

One surprising outcome was the lack of differences in rated appetite between the soup conditions despite differences in energy and macronutrient content. This may be due to the effect of sweetness as the carbohydrate and protein conditions were evaluated as sweeter during pilot testing which may have affected hunger recovery due to the difference in taste. This outcome may also be due to the context of the soup as a single lunchtime item. Indeed, preload energy has been found to affect subsequent energy intake and appetite assessments (Perrigue, Monsivais, & Drewnowski, 2009). Thus high energy conditions were required to ensure any appetite suppressing effects could be related to the impact of MSG rather than due to energy effects (Rogers & Blundell, 1990). Nonetheless, participants' expectations of how filling a single serving of soup is as a meal immediately after ingestion may be cognitively mediated by other factors such as portion size and usual intake at lunch. For instance, Blatt et al., (2011) indicated that palatability and appearance are more salient features during satiation than the immediate post-ingestive effects of the food.

No significant differences were noted in the experience of fullness during the post-ingestive period with the addition of MSG despite indications in the literature suggesting that fullness assessments are more accurately judged over time (Stuart & Davis, 1972). This may be due to the fullness responses relying on the volume consumed, making them more prone to variability over time (Allirot et al., 2013) than hunger ratings, which tend to be more sensitive to assessments of hedonic eating motivation (Yeomans, 2010).

The time dependent effects of MSG have not been previously examined in this way to consider the generation of satiety across different macronutrient conditions. Thus, future research exploring the impact of MSG on satiety by assessing subsequent test meal intake is warranted to further understand the role of MSG-macronutrient relationships on consumption as well as subjective appetite.

Overall, MSG may increase hunger sensations immediately post-ingestion and may also reduce feelings of fullness in high energy protein soups relative to high energy carbohydrate and low energy control conditions. MSG may also delay the experience of hunger across macronutrient conditions without affecting long-term evaluations of fullness. However, these effects remain as trends within the data and require further investigation.

6.5 Acknowledgements

6.6 Summary of main findings and directions for future research

- The addition of MSG to a fixed portion of a low energy soup or one with added energy in the form of carbohydrate or protein acted to increase feelings of hunger immediately after consuming the soup, with no differences in appetite between the high and low energy conditions

- However, trends in the data suggested that MSG was also found to slow the recovery of hunger over time across conditions

- This was found to be significant in added MSG protein conditions as compared to no MSG protein conditions within 30-60 minutes post-ingestion

- This suggests that MSG and protein may act to further enhance feelings of satiety post-ingestion, with the critical period of satiety strongest soon after intake

- However, ratings were based on subjective appetite measures and future research would be required to assess whether self-rated appetite resulted in true reductions in intake at a subsequent course as a concrete measure of satiety

Appendix 6.1. Graphical depiction of the study design employed.
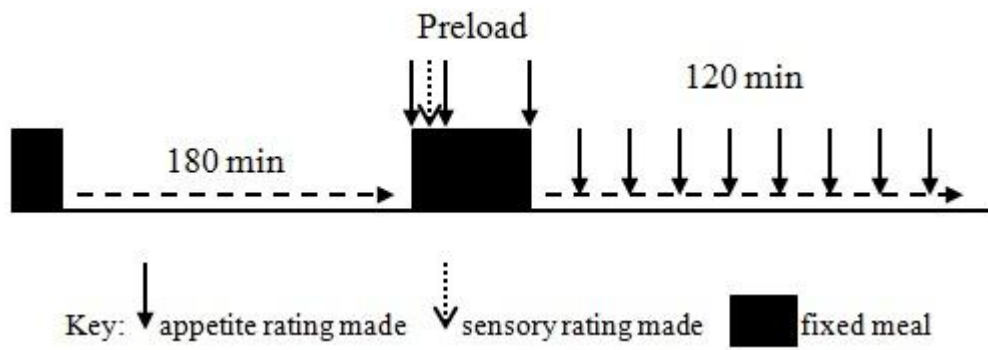
6.7 References

Allirot, X., Saulaisa, L., Disseb, E., Nazareb, J., Cazalb, C., & Lavilleb, M. (2013). Integrating behavioral measurements in physiological approaches of satiety. *Food Quality and Preference*.

Appleton, K. M., & Blundell, J. E. (2007). Habitual high and low consumers of artificially-sweetened beverages: Effects of sweet taste and energy on short-term appetite. [doi: 10.1016/j.physbeh.2007.04.027]. *Physiology & Behavior, 92*(3), 479-486.

Beauchamp, G. K. (2009). Sensory and receptor responses to umami: an overview of pioneering work. *American Journal of Clinical Nutrition, 90*(3), 723S-727S. doi: 10.3945/ajcn.2009.27462E

Bellisle, F. (1999). Glutamate and the UMAMI taste: sensory, metabolic, nutritional and behavioural considerations. A review of the literature published in the last 10 years. *Neuroscience and Biobehavioral Reviews, 23*(3), 423-438.

Bellisle, F., Dalix, A. M., Chappuis, A. S., Rossi, F., Fiquet, P., Gaudin, V., . . . Slama, G. (1996). Monosodium Glutamate Affects Mealtime Food Selection in Diabetic Patients. *Appetite, 26*(3), 267-276.

Bellisle, F., Monneuse, M. O., Chabert, M., Larue-Achagiotis, C., Lanteaume, M. T., & Louis-Sylvestre, J. (1991). Monosodium glutamate as a palatability enhancer in the european diet. *Physiology & Behavior, 49*(5), 869-873.

Bellisle, F., Tournier, A., & Louis-Sylvestre, J. (1989). Monosodium glutamate and the acquisition of food preferences in a European context. *Food Quality and Preference, 1*, 103-108.

Bertenshaw, E. J., Lluch, A., & Yeomans, M. R. (2008). Satiating effects of protein but not carbohydrate consumed in a between meal beverage context. *Physiology and Behavior, 93*(3), 427-436.

Blatt, A. D., Roe, L. S., & Rolls, B. J. (2011). Increasing the Protein Content of Meals and Its Effect on Daily Energy Intake. *Journal of the American Dietetic Association, 111*(2), 290-294.

Carter, B. E., Monsivais, P., & Drewnowski, A. (2011). The sensory optimum of chicken broths supplemented with calcium di-glutamate: A possibility for reducing sodium while maintaining taste. *Food Quality and Preference, 22*(7), 699-703.

de Araujo, I. E. T., Kringelbach, M. L., Rolls, E. T., & Hobden, P. (2003). Representation of umami taste in the human brain. [Article]. *Journal of Neurophysiology, 90*(1), 313-319. doi: 10.1152/jn.00669.2002

de Castro, J. M. (1987). Circadianrhythms of the spontaneous meal pattern, macronutrient intake, and mood of humans. *Physiology & Behavior, 40*(4), 437-446.

Dohm, F.-A., Cachelin, F. M., & Striegel-Moore, R. H. (2005). Factors That Influence Food Amount Ratings by White, Hispanic, and Asian Samples. *Obesity, 13*(6), 1061-1069.

Dove, E. R., Hodgson, J. M., Puddey, I. B., Beilin, L. J., Lee, Y. P., & Mori, T. A. (2009). Skim milk compared with a fruit drink acutely reduces appetite and energy intake in overweight men and women. *The American Journal of Clinical Nutrition, 90*(1), 70-75. doi: 10.3945/ajcn.2008.27411

Essed, N. H., van Staveren, W. A., Kok, F. J., & de Graaf, C. (2007). No effect of 16 weeks flavor enhancement on dietary intake and nutritional status of nursing home elderly. [doi: 10.1016/j.appet.2006.06.002]. *Appetite, 48*(1), 29-36.

Finlayson, G., Bordes, I., Griffioen-Roose, S., de Graaf, C., & Blundell, J. E. (2012). Susceptibility to Overeating Affects the Impact of Savory or Sweet Drinks on Satiation, Reward, and Food Intake in Nonobese Women. *The Journal of Nutrition, 142*(1), 125-130. doi: 10.3945/jn.111.148106

FSA. (2006). *FSA Nutrient and Food Based Guidelines for UK Institutions*. London.

Gibson, E. L. (Ed.). (2001). *Learning in the development of food cravings.* Leatherhead: Leatherhead Publishing.

Hosaka, H., Kusano, M., Zai, H., Kawada, A., Kuribayashi, S., Shimoyama, Y., . . . Mori, M. (2012). Monosodium glutamate stimulates secretion of glucagon-like peptide-1 and reduces postprandial glucose after a lipid-containing meal. *Alimentary Pharmacology & Therapeutics, 36*(9), 895-903. doi: 10.1111/apt.12050

Ikeda, K. (1908). On a New Seasoning. *Journal of Tokyo Chemistry Society, 30*, 820-836.

Kondoh, T., Mallick, H. N., & Torii, K. (2009). Activation of the gut-brain axis by dietary glutamate and physiologic significance in energy homeostasis. *American Journal of Clinical Nutrition, 90*(3), 832S-837S. doi: 10.3945/ajcn.2009.27462V

Kondoh, T., & Torii, K. (2008). MSG intake suppresses weight gain, fat deposition, and plasma leptin levels in male Sprague-Dawley rats. . *Physiology & Behavior, 95*, 135–144.

Laska, M., & Hernandez Salazar, L. T. (2004). Gustatory responsiveness to monosodium glutamate and sodium chloride in four species of nonhuman primates. *Journal of Experimental Zoology Part A: Comparative Experimental Biology, 301A*(11), 898-905. doi: 10.1002/jez.a.118

Lipps Birch, L., Billman, J., & Salisbury Richards, S. (1984). Time of day influences food acceptability. *Appetite, 5*(2), 109-116.

Luscombe-Marsh, N. D., Smeets, A. J. P. G., & Westerterp-Plantenga, M. S. (2008). Taste sensitivity for monosodium glutamate and an increased liking of dietary protein. *British Journal of Nutrition, 99*(4), 904-908. doi: 10.1017/s000711450788295x

Luscombe-Marsh, N. D., Smeets, A. J. P. G., & Westerterp-Plantenga, M. S. (2009). The addition of monosodium glutamate and inosine monophosphate-5 to high-protein meals: effects on satiety, and energy and macronutrient intakes. *British Journal of Nutrition, 102*(6), 929-937. doi: 10.1017/s0007114509297212

Mathey, M.-F. A. M., Siebelink, E., de Graaf, C., & Van Staveren, W. A. (2001). Flavor Enhancement of Food Improves Dietary Intake and Nutritional Status of Elderly Nursing Home Residents. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 56*(4), M200-M205. doi: 10.1093/gerona/56.4.M200

McCabe, C., & Rolls, E. T. (2007). Umami: a delicious flavor formed by convergence of taste and olfactory pathways in the human brain. *European Journal of Neuroscience, 25*(6), 1855-1864. doi: 10.1111/j.1460-9568.2007.05445.x

Means, L. W., Ginn, S. R., Arolfo, M. P., & Pence, J. D. (2000). Breakfast in the nook and dinner in the dining room: time-of-day discrimination in rats. *Behavioural Processes, 49*, 21-33.

Mennella, J. A., & Beauchamp, G. K. (1996). Developmental Changes in the Acceptance of Protein Hydrolysate Formula. *Journal of Developmental & Behavioral Pediatrics, 17*(6), 386-391.

Naim, M., Ohara, I., Kare, M. R., & Levinson, M. (1991). Interaction of MSG taste with nutrition: Perspectives in consummatory behavior and digestion. . *Physiology & Behavior, 49*, 1019–1024.

Narukawa, M., Morita, K., Uemura, M., Kitada, R., Oh, S.-H., & Hayashi, Y. (2011). Nerve and Behavioral Responses of Mice to Various Umami Substances. *Bioscience, Biotechnology, and Biochemistry, advpub*, 1109292677-1109292677.

Niijima, A. (2000). Reflex Effects of Oral, Gastrointestinal and Hepatoportal Glutamate Sensors on Vagal Nerve Activity. *The Journal of Nutrition, 130*(4), 971.

Perrigue, M. M., Monsivais, P., & Drewnowski, A. (2009). Added Soluble Fiber Enhances the Satiating Power of Low-Energy-Density Liquid Yogurts. *Journal of the American Dietetic Association, 109*(11), 1862-1868.

Poppitt, S. D., McCormack, D., & Buffenstein, R. (1998). Short-term effects of macronutrient preloads on appetite and energy intake in lean women. *Physiology & Behavior, 64*, 279-285.

Porrini, M., Crovetti, R., Testolin, G., & Silva, S. (1995). Evaluation of satiety sensations and food intake after different preloads. *Appetite, 25*, 17-30.

Prescott, J. (2004). Effects of added glutamate on liking for novel food flavors. *Appetite, 42*(2), 143-150. doi: 10.1016/j.appet.2003.08.013

Ratliff, J., Leite, J. O., de Ogburn, R., Puglisi, M. J., VanHeest, J., & Fernandez, M. L. (2010). Consuming eggs for breakfast influences plasma glucose and ghrelin, while reducing energy intake during the next 24 hours in adult men. *Nutrition Research 30*(2), 96-103.

Rodin, J. (1980). Social and immediate environmental influences on food selection. *International Journal of Obesity, 4*, 364-370.

Rogers, P. J., & Blundell, J. E. (1990). Umami And Appetite - Effects of monosodium Glutamate on Hunger and Food-Intake In Human Subjects. *Physiology & Behavior, 48*(6), 801-804.

Rolls, E. T. (2000). The Representation of Umami Taste in the Taste Cortex. *The Journal of Nutrition, 130*(4), 960.

San Gabriel, A. M., Maekawa, T., Uneyama, H., Yoshie, S., & Torii, K. (2007). mGluR1 in the fundic glands of rat stomach. *FEBS letters, 581*(6), 1119-1123.

Schiffman, S. S., Sattely-Miller, E. A., Zimmerman, I. A., Graham, B. G., & Erickson, R. P. (1994). Taste perception of monosodium glutamate (MSG) in foods in young and elderly subjects. *Physiology & Behavior, 56*(2), 265-275.

Smriga, M., & Torii, K. (2000). Release of hypothalamic norepinephrine during MSG intake in rats fed normal and nonprotein diet. . *Physiology & Behavior, 70*, 413–415.

Stuart, R. B., & Davis, B. (1972). *Slim chance in a fat world: Behavioral control of obesity*. Champaign, Illinois: Research Press Co.

Stunkard, A. J., & Messick, S. (1985). The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. . *Journal of Psychosomatic Research, 29*, 71-83.

Tome, D., Schwarz, J., Darcel, N., & Fromentin, G. (2009). Protein, amino acids, vagus nerve signaling, and the brain. *American Journal of Clinical Nutrition, 90*(3), 838S-843S. doi: 10.3945/ajcn.2009.27462W

Tordoff, M. G., & Alleva, A. M. (1990). Oral stimulation with aspartame increases hunger. [doi: 10.1016/0031-9384(90)90126-O]. *Physiology & Behavior, 47*(3), 555-559.

Ventura, A. K., Beauchamp, G. K., & Mennella, J. A. (2012). Infant regulation of intake: the effect of free glutamate content in infant formulas. *The American Journal of Clinical Nutrition, 95*(4), 875-881. doi: 10.3945/ajcn.111.024919

Viarouge, C., Caulliez, R., & Nicolaidis, S. (1992). Umami taste of monosodium glutamate enhances the thermic effect of food and affects the respiratory quotient in the rat. [doi: 10.1016/0031-9384(92)90365-9]. *Physiology & Behavior, 52*(5), 879-884.

Williams, E. J. (1949). Experimental designs balanced for the estimation of residual effects of treatment. *Australian Journal of Scientific Research, Series A*(2), 149-168.

Yamaguchi, S., & Ninomiya, K. (2000). Umami and Food Palatability. *The Journal of Nutrition, 130*(4), 921.

Yeomans, M. R. (1996). Palatability and the Micro-structure of Feeding in Humans: the Appetizer Effect. *Appetite, 27*(2), 119-133.

Yeomans, M. R. (2010). Understanding Individual Differences in Acquired Flavour Liking in Humans. *Chemosensory Perception, 3*(1), 34-41. doi: 10.1007/s12078-009-9052-6

Yeomans, M. R., Blundell, J. E., & Leshem, M. (2004). Palatability: response to nutritional need or need-free stimulation of appetite? *British Journal of Nutrition, 92*, S3-S14. doi: 10.1079/bjn20041134

Yeomans, M. R., & Gray, R. W. (1997). Effects of Naltrexone on Food Intake and Changes in Subjective Appetite During Eating: Evidence for Opioid Involvement in the Appetizer Effect. [doi: 10.1016/S0031-9384(97)00101-7]. *Physiology & Behavior, 62*(1), 15-21.

Yeomans, M. R., & Gray, R. W. (2002). Opioid peptides and the control of human ingestive behaviour. *Neuroscience & Biobehavioral Reviews, 26*, 713–728.

Yeomans, M. R., Gray, R. W., & Conyers, T. H. B. (1998). Maltodextrin preloads reduce food intake without altering the appetiser effect. [doi: 10.1016/S0031-9384(98)00086-9]. *Physiology & Behavior, 64*(4), 501-506.

This paper was accepted by Journal of Nutritional Sciences in April, 2014.

Monosodium glutamate delivered in a protein-rich soup improves subsequent energy compensation

Una Masic [a] and Martin R Yeomans [a]

[a] School of Psychology, University of Sussex, Brighton, BN1 9QH, UK

Running head:

MSG and satiety

Contact details:

Una Masic

School of Psychology

University of Sussex

Brighton

BN1 9QH

UK

Tel: (01273) 872826

Email: u.masic@sussex.ac.uk

Key Words: intake; appetite; MSG; hunger

Abstract

Previous research suggests that monosodium glutamate (MSG) may have a bi-phasic effect on appetite, increasing appetite within a meal with its flavour-enhancing effect, but enhancing subsequent satiety due to its proposed role as a predictor of protein content. The present study explored this by assessing the impact of a 450g soup preload differing in MSG concentration (1% MSG added, MSG+ or no MSG, MSG-) and nutrient content (low energy control or high energy carbohydrate or high energy protein) on rated appetite and *ad-libitum* intake of a test meal in 35 low-restraint male volunteers using a within-participant design. Protein preloads significantly reduced intake at the test meal and resulted in more accurate energy compensation than did carbohydrate preloads. This effect was stronger in the MSG+ protein conditions when compared to MSG+ carbohydrate conditions. No clear differences in rated appetite were seen in MSG or macronutrient conditions alone during preload ingestion or 45 minutes after intake. Overall, these findings indicate that MSG may act to further improve energy regulation when provided in a protein context.

7.1 Introduction

Monosodium glutamate (MSG) is a flavour enhancer which improves the savoury experience of foods and is the prototypical chemical associated with the 'umami' taste (Ikeda, 1908; Jinap & Hajeb, 2010; Ninomiya, 1998). Because of its role as a flavour enhancer, it was initially believed that MSG increased appetite and intake (Bellisle, et al., 1991; Bellisle, et al., 1989; Essed, et al., 2009; Mathey, et al., 2001; Schiffman, et al., 1994; Yeomans, 1996; Yeomans & Gray, 2002; Yeomans, et al., 1998). However, initial enhanced intake tends to decrease over time (Bellisle, 1999; Bellisle, et al., 1996; Essed, et al., 2007; Schiffman, et al., 1994) and recent research suggests that MSG may delay hunger recovery over time; particularly when in combination with protein (Masic & Yeomans, 2013).

So why might any appetite-enhancing effects of MSG be short-lived? One explanation is that appetite is stimulated by MSG during the course of the meal (reducing satiation), but then suppressed during the post-ingestive stage to delay later intake (enhancing satiety). This suggestion is based on the idea that our ability to detect MSG evolved as a means of detecting the presence of protein in foods (Ikeda, 1908) and regulates protein consumption (Laska & Hernandez Salazar, 2004; Smriga & Torii, 2000). There is clear evidence that protein enhances satiety more effectively than does either carbohydrate (Fischer, et al., 2004; Marmonier, et al., 2000; Poppitt, et al., 1998) or fat (Simpson & Raubenheimer, 2000; Weigle, et al., 2005) when delivered acutely as a preload (Bertenshaw, et al., 2008; Latner & Schwartz, 1999) and longer term in the diet (Leidy, et al., 2011; Lejeune, et al., 2005; Simpson & Raubenheimer, 2005). Recent data suggest that the satiating effects of protein are in part due to the sensory characteristics of protein foods acting to enhance post-ingestive satiety (Bertenshaw, et al., 2013), and umami could be one of the critical cues generating these sensory-nutrient interactions. Indeed, umami taste has been linked to the satiating effects of protein in infants (Mennella & Beauchamp, 1996; Ventura, et al., 2012) and may explain why intake of MSG meals over time remains stable despite increases in palatability. A previous study assessing the time course of changes in rated appetite over 120 minutes after consumption of a fixed volume of soup varying in macronutrient content (high energy carbohydrate or protein or low energy control) with and without added MSG also supports these findings. Hunger decreased less after MSG soup intake (consistent with

the stimulation of appetite through flavour enhancement), but slower hunger recovery in the MSG protein condition compared to protein alone which was not seen for MSG in a low-energy or carbohydrate context (Masic & Yeomans, 2013). However, another study has found no effect of MSG and protein on satiety when measured at a test-meal (Luscombe-Marsh, et al., 2009). This discrepancy might be due to the previous study (Masic & Yeomans, 2013a) relying on measures of rated appetite at pre-defined time points whilst other research has assessed intake at *ad-libitum* test meal sessions (Luscombe-Marsh, et al., 2009).

Given this ambiguity in the literature about the role of MSG in appetite control, the present research examined the effects of a soup preload differing in macronutrient content (high energy soups enhanced with protein or carbohydrate contrasted with a low energy control) either with MSG added (MSG+) or no MSG (MSG-) on appetite and intake at a subsequent *ad-libitum* meal. A preload meal interval of 45 minutes was used as this was found to be the optimum time for differences in hunger and fullness ratings between MSG+ and MSG- conditions in our recent study (Masic & Yeomans, 2013). It was hypothesised that the flavour enhancing effects of MSG would mean less decrease in hunger when consuming a fixed portion of the MSG+ versions regardless of nutrient content, but that hunger would recover more slowly, and consequently that test-meal intake would be less in the protein than carbohydrate preload conditions relative to the control, with MSG+ enhancing the satiating effects of protein.

7.2 Method

7.2.1 Design
The study used a within-participant design to examine the effects of consumption of a fixed soup preload differing in macronutrient content/energy (high energy carbohydrate or protein or low energy control) and MSG content (1% MSG w/w; MSG+ or no MSG; MSG-). Preload condition order was balanced using a Williams square design (Williams, 1949).

7.2.2 Participants
Thirty-six low restraint males initially participated in the research but one participant failed to complete all sessions. The 35 remaining participants (mean age: 21±0.4, ages

from 18-28; mean BMI: 22±0.5, BMI from 18-33 kg/m²) were mainly students at the University of Sussex. This number was used based on a previously under powered study (Masic & Yeomans, 2013) with effect size calculations indicating that 36 participants would be required to reach 80% statistical power. Potential participants were initially contacted based on their responses to a general eating habits recruitment questionnaire using a database system. Individuals who smoked, those on medications, those with a history of diabetes, diagnosed eating disorders, allergies or dietary intolerances to the foods used were not contacted. Also, those with high restraint scores (ratings above 7 on the Three Factor Eating Questionnaire (TFEQ; Stunkard & Messick, 1985) were excluded as restrained individuals may not be representative of general eating behaviour (Fedoroff, Polivy, & Herman, 1997, 2003). One participant had a BMI of 33, placing him in the obese category, thus a correlational analysis between test meal intake and BMI was performed. No significant correlation was found (r(35) = 0.06, p=.72) therefore this participant was not excluded from further analysis.

Prospective subjects were emailed with details of the study disguised as 'assessing the effects of food on motor skills'. This was to ensure eating behaviour would not be affected by awareness of the experimental manipulation. Details about the study procedure and exclusion criteria were specified and written informed consent was given before participation. Upon completion of all testing sessions participants were paid £60. The study was conducted in accordance with the standards expressed in the Helsinki Declaration and was approved by the University of Sussex ethics committee.

7.2.3 Test Food
7.2.3.1 Control Breakfast:
Breakfast on all test days consisted of 80g cereal (Crunchy Nut Cornflakes, Kellogg's, UK), 200g semi-skimmed milk (Sainsbury's Plc, UK) and 200g orange juice (Sainsbury's Plc, UK) (total 503.6 Kcal). These quantities were established based on UK Food Standard Agency guidelines for male breakfast consumption (FSA, 2006).

7.2.3.2 Soup preloads:
All flavour and energy manipulations used the same low energy density (ED) control soup. The soup consisted of a carrot and spice soup containing carrots (Frozen Baby Carrots, Sainsbury's Plc, UK) onions, celery, olive oil (Medium Flavour Olive Oil,

Sainsbury's Plc, UK), spice mixture (Garam Masala Schwartz, UK) and water (see (Masic & Yeomans, 2013). Soup formulation and serving on test days followed standard operating procedures and portion size was fixed at 450g as this quantity has been established as an adequate portion size for males (Yeomans & Gray, 2002) and was deemed adequate in a previous study (Masic & Yeomans, 2013). The addition of 52g/450g portion maltodextrin (DE: 15.3, Cargill, UK) was used in the carbohydrate soup and 17.86g/450g maltodextrin (Cargill, UK) combined with 36g/450g whey protein isolate (MyProtein UK) was added to the protein soup. Maltodextrin was added to the protein preload to reduce differences in flavour pleasantness whilst maintaining similar ED across the two conditions. 1% w/w MSG (Ajinomoto Co., Inc. Europe) was added to all MSG+ soup conditions. Base soup formulation followed extensive pilot testing to formulate a novel soup low in MSG, and which was rated as moderately pleasant to allow for enhancement by MSG. Pilot testing was also carried out on the energy and macronutrient soup combinations used (see Masic & Yeomans, 2013).

The high ED conditions contained approximately 180 Kcal more per portion than the low ED condition (carbohydrate: 177.5 Kcal, protein: 184.3 Kcal). The 6 Kcal difference between high ED carbohydrate and protein conditions was due to efforts to regulate the quantity of maltodextrin used in the carbohydrate condition without affecting the flavour of the preload due to the sweetness that can be experienced with the use of high quantities of maltodextrin. All nutritional information can be found in Table 7.1.

Table 7.1. Nutritional composition of soup preloads (all data are per 100g).

	Control	Carbohydrate	Protein
Carbohydrate (g)	3	14	7
Protein (g)	0.4	0.3	8
Fat (g)	2	1	1
Carbohydrate (% energy)	44	81	38
Protein (% energy)	6	2	45
Fat (% energy)	50	17	19
Kcal	28	67	69
Kcal/450g portion	126	303	310

7.2.3.3 Ad-libitum meal:

The two course *ad-libitum* lunch was comprised of pasta in tomato sauce followed by ice cream purchased from a local supermarket. Due to an unexpected change in the formulation of the pasta sauce part-way through the study, two versions of the meal had to be used but each participant was only tested with one version. Both versions used 250g cooked pasta (Conchiglie Pasta, Sainsbury's PLC, UK) which was combined with 250g of pasta sauce (Tomato and Basil Sauce, Sainsbury's PLC, UK) for version 1, giving a 500g served portion (total 543.4 Kcal). Re-fill portions were provided as 500g servings. As the reformulated version of the sauce had a higher energy density, the portion size was re-adjusted to 200g for version 2 to ensure a similar energy density across test meals (total 500.4 Kcal). Fifteen participants were tested with 500g (version 1) whilst the remaining 20 received the reformulated meal (version 2). Repeated measures analyses of sensory ratings and intake of the test meal depending on the served version found a significant difference in rated saltiness between the two versions $(F(5,165) = 2.78, p = .02)$ but all other ratings were found to be non-significant including pleasantness ratings $(F(5,165) = 0.73, p=.74)$. Intake did not differ significantly between those consuming version 1 and version 2 $(F(5,165) = 0.85, p=.71)$ and when test meal version was included as a covariate, no differences in pasta meal intake was apparent [MSG*test meal version: $F(1,33) = 2.57, p=.12$;

MSG*condition*test meal version: F(2,66) = 1.34, p=.21]. The dessert consisted of vanilla ice cream (Carte D'or, Unilever, UK; total 315 Kcal/150g) with re-fill portions provided (total 210 Kcal/100g). The preparation and serving of all meals on test days adhered to a standardized protocol.

7.2.4 Computerized Data Collection

Sensory, hedonic and appetite ratings were tracked using the Sussex Ingestion Pattern Monitor (SIPM version 2.0.13, University of Sussex), an extension of the Universal Eating Monitor (Kissileff, Klingsberg, & Van Itallie, 1980) which is comprised of a digital balance (Sartorius, Model BP4100) linked to a computer. Participants were asked to complete appetite, sensory, hedonic and mood ratings using digital Visual Analogue Scales (VAS) by the SIPM. All ratings were presented as sentences ("How <word> do you feel?") with a left hand anchor reading "Not at all <word>" (coded as 0) and a right hand anchor reading "As <word> as I have ever felt/experienced" (coded as 100). Instructions on how to use the scale were presented to participants before each evaluation to ensure compliance. Participants registered their selection by pressing "Rating Complete". The first set of ratings presented before each trial assessed how alert, clear-headed, energetic, full, hungry, nauseous and thirsty subjects felt. All VAS ratings were randomized across all trials and sessions.

Participants were then presented with their fixed preload portion and completed hedonic assessments of the target soup (how familiar, pleasant, salty, savoury, strong and sweet) and appetite ratings of hunger, fullness and thirst after tasting the soup ("Please take a mouthful of the soup, count to 5 and then swallow".) before being asked to "Please consume all of the soup". Participants were also reminded to "Make sure you consume all of the soup before moving on to the next stage". Meal termination was indicated by pressing "Finished eating". Mood and appetite ratings mirroring the first set completed were then presented and participants were free to leave but were told to return at a specific time (45 min after ingestion) for the second part of the meal.

The *ad-libitum* lunch followed a similar structure with an initial set of mood and appetite ratings completed. Before each course, participants were provided with samples of the food to be consumed and completed taste tests (assessing how familiar, pleasant and strong; for both of the courses, salty and savoury; for the pasta course, and sweet;

for the dessert course) and then filled in appetite ratings (hunger, fullness and thirst) before being given a portion of the food with instructions to "Please eat as much (pasta/ice cream) as you like until you feel comfortably full". Additional (re-fill) portions of the course being consumed were prompted by the SIPM with an on-screen message and alert sound which instructed participants to call the experimenter. Pasta course termination was indicated by pressing "Ready for dessert" which triggered further appetite assessments before the dessert course began while whole meal termination was registered by pressing "Finished the meal" which prompted a confidence in meal termination statement. A final set of appetite and mood ratings were then completed mirroring the initial set.

7.2.5 Procedure

The research took place over six non-consecutive sessions at the Ingestive Behaviour Unit (IBU) at the University of Sussex. Participants were asked to consume nothing but water from 2300h the night before each testing session and were provided with the control breakfast at pre-arranged times (from 0900h – 1030h) across testing days. Participants were free to leave the IBU after breakfast with instructions to consume nothing but water and returned after 3 h for the soup preload.

For all consumption trials (preload and test meal) participants were tested in a windowless cubicle and were provided with water throughout testing. In compliance with the cover story to conceal the true purpose of the experiment, participants were first asked to complete a bogus motor skills task (the star motor task, which consisted of tracing the outline of a star with their non-dominant hand).

Within the preload session which ran from 1200h – 1330h, mood and appetite ratings were completed and followed by the provision of the soup which was served according to condition. Taste test and appetite ratings were then completed with further appetite ratings made after soup intake. Participants were then permitted to leave but were asked to return after 40 min for the main course which was provided 45 min post-ingestion as this has been found to be the most sensitive time window for detecting preloading effects with this test soup as revealed by a previous experiment (Masic & Yeomans, 2013).

Upon their return participants once again completed the star motor task, mood and appetite ratings and completed a taste test with the pasta main course before being provided with an *ad-libitum* 500g (or 450g) portion of the pasta in sauce served on a white ceramic plate. Re-fills were registered by the SIPM and were provided in 500g (or 450g) portions after approximately 450g (or 400g) consumption. This ensured that food was always present on the plate to prevent normative external cues such as an empty plate from influencing meal intake (Herman & Polivy, 2008b; Wansink, et al., 2005b). After main meal consumption, further ratings of appetite were filled in before a taste test was completed for the dessert course. After the ice cream sample was evaluated, a 150g portion of ice cream was provided in a white ceramic bowl with additional 100g portions provided after 100g ice cream consumption. The session was completed after the final set of mood and appetite ratings were filled in. At the end of sessions 1-5 participants were free to leave but their height and weight was recorded and they were debriefed before payment on the final test day. A graphical depiction of the experimental design can be seen in Appendix 7.1.

7.2.6 Data Analysis

Test meal intake (Kcal consumed) was contrasted using two-way 3x2 repeated measures ANOVA with soup type (control, protein or carbohydrate) and MSG condition (MSG+ or MSG-) as variables. Intake was also analysed across the whole test meal and by individual courses (savoury course and dessert) to determine whether preload conditions impacted intake overall or differed by course. Additional analyses were conducted to determine energy compensation (COMPX) at the test meal after high energy (protein or carbohydrate) preloads across MSG conditions (MSG+ or MSG-). COMPX values were calculated by subtracting test meal intake in the relevant control (low energy) MSG+ or MSG- condition from the corresponding protein or carbohydrate (high energy) MSG+ or MSG- condition and expressing this value as a percentage of the actual difference in soup preload energy between low and high energy preloads (protein: 184 Kcal; carbohydrate: 177 Kcal). The resulting COMPX scores were contrasted using two-way 2x2 repeated measures ANOVA. To control for repeat testing, test order was included as a factor in all analyses.

Satiation analyses (pre-meal, post-taste and post-course) were conducted for each eating episode using 3x2x3 repeated measures ANOVA assessing appetite (hunger and

fullness) ratings over time for the preload and equivalent 3x2x5 repeated measures ANOVA assessing appetite over time for the test meal and were analysed separately due to the expected hunger enhancing effects of MSG during the preload but maintaining satiety during the main course. A separate 3x2 repeated measures ANOVA analysing the preload effects over 45 min post ingestion was also carried out to assess the effects of MSG or condition on satiety. Hunger and fullness variables during preload satiation were transformed to track the changes from baseline over the eating episode. Sensory evaluations of the soup and test meal were analysed using repeated measures two-way 3x2 ANOVA.

In cases of violated sphericity, Greenhouse Geisser values ($\varepsilon = <0.75$) were adopted. In cases of violated Greenhouse Geisser assumptions ($\varepsilon = >0.75$), Huynh-Feldt values were reported. Within-subjects contrasts were assessed using Bonferroni adjusted comparisons to understand significant interactions between different patterns of data. Effect sizes are reported for specific effects using Pearson's correlation coefficient. Data are shown for all 35 participants.

7.3 Results

7.3.1 Test meal intake

There was a significant effect of soup preload on energy consumed at the test meal ($F(1.75, 59.55) = 4.58$, $p = .02$), with significantly less energy consumed in protein conditions compared to control ($F(1,34) = 7.47$, $p = .01$, $r = .42$; Figure 7.1). Overall, less energy tended to be consumed in MSG+; 442 Kcal compared to MSG-; 450 Kcal conditions but this small difference was not significant ($F(1,34) = 0.31$, NS, $r = .09$). The predicted enhancement of protein satiety by MSG was not supported: the interaction between MSG and soup conditions was not significant ($F(2,68) = 0.37$, NS, $r = .13$) although in line with predictions the lowest intake was in the protein MSG+ condition (Figure 7.1). An order*condition interaction effect was noted for intake of the savoury course ($F(10,58) = 2.41$, $p = .02$) and sweet course ($F(10,58) = 2.05$, $p = .04$) with inspection of the data across test sessions suggesting that intake was highest in the first session after which consumption was adjusted to the nutrients ingested in the preload. No significant effects of condition or MSG were found for the dessert course.
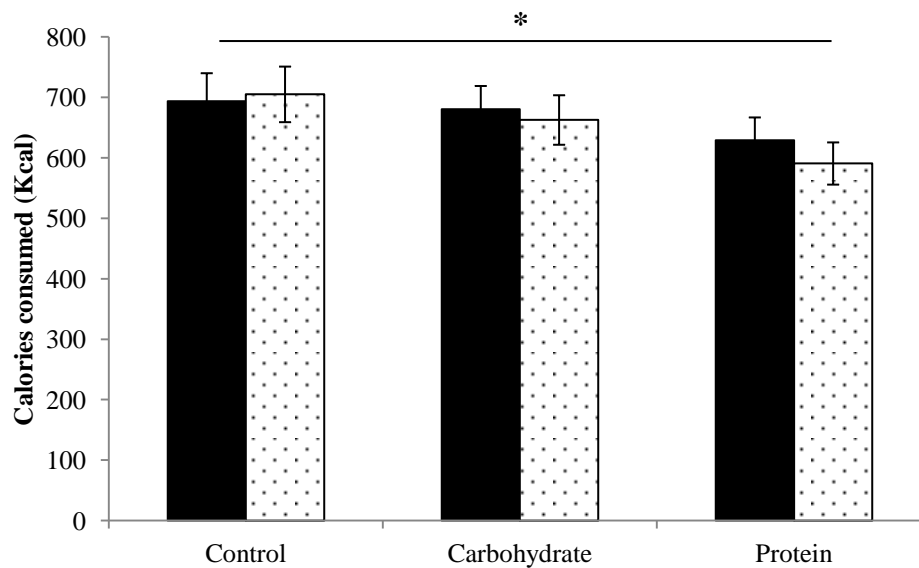
Figure 7.1. *Ad-libitum* lunch intake after fixed preload consumption of three versions of soup (low energy control, high energy carbohydrate and high energy protein) with (⊡) and without (■) added MSG. Data are mean ±SEM. Significant main effect of condition is indicated: * P<0.05.

7.3.2 Compensation for preload energy

Overall energy compensation at test meal intake for the difference in soup preload energy was significantly better in the protein than carbohydrate condition ($F_{(1,34)} = 4.19$, $p = .05$, $r = .33$), with 48% compensation in the protein and only 16% compensation in the carbohydrate conditions (Figure 7.2). Although there was no significant effect of MSG overall ($F_{(1,34)} = 0.86$, NS, $r = .16$), there was a significant difference in compensation between protein and carbohydrate conditions with added MSG ($F_{(1,34)} = 5.45$, $p = .03$, $r = .37$) with 24% compensation in the carbohydrate MSG+ condition but 62% compensation in the protein MSG+ condition. This effect was largely driven by differences in savoury course intake ($F_{(1,34)} = 5.63$, $p = .02$, $r = .38$) with 51% compensation in the MSG+ protein condition but only 16% in the carbohydrate MSG+ condition. No significant differences in compensation were found when comparing carbohydrate and protein MSG- conditions overall ($F_{(1,34)} = 1.21$, NS, $r = .04$).
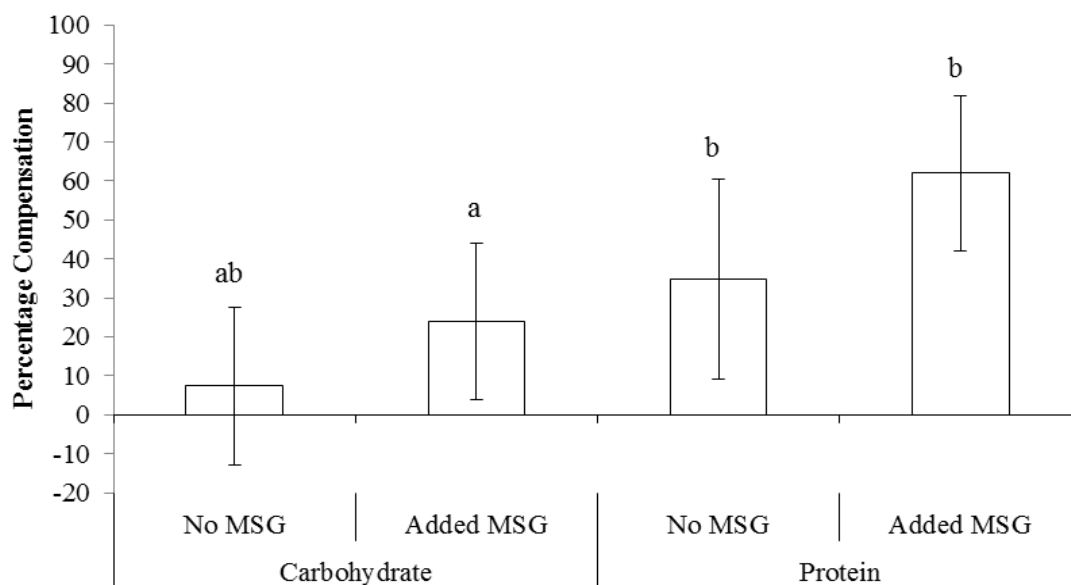

Figure 7.2. Energy compensation overall at an *ad-libitum* test meal (Panel A) after fixed consumption of high energy carbohydrate and high energy protein soup preloads with and without added MSG. Different letters indicate statistically significant differences (p = <.05) between conditions (using within-subjects Bonferroni corrected contrasts); statistically significant contrast between no and added MSG carbohydrate conditions (a); no and added MSG protein conditions (b) and no MSG carbohydrate and all protein conditions (ab). Data are mean±SEM.

7.3.3 Rated hunger and fullness during the preload and test meal

7.3.3.1 Preload Satiation and Satiety

There were no significant spurious differences in rated hunger ($F_{(2,68)} = .06$, p = NS) or fullness ($F_{(2,68)} = .68$, p = NS) before the soup was tasted. Thus, change from baseline hunger and fullness were analysed to assess the influence of MSG manipulations when the soup was first tasted (assessing the appetizer effect (Yeomans, 1996)) and immediately after consuming the soup preload (assessing effects on satiation).

Hunger and fullness ratings at the start of the test meal (45 min after soup preload intake) were also assessed, with hunger ratings immediately after tasting the meal to be consumed of particular interest as these have been shown to correlate with selected meal-size during *ad-libitum* intake sessions (Yeomans, et al., 2001). A significant condition*MSG interaction ($F_{(2,68)} = 4.10$, p = .02) was noted in the hunger ratings

immediately after tasting the soup. Hunger increased after tasting the MSG+ control and carbohydrate soups but, unexpectedly, decreased after tasting in the MSG+ protein condition whilst in the no added MSG conditions, hunger was lower after control and carbohydrate conditions but increased after tasting the protein soup ($F(1,34) = 5.70$, $p = .02$, $r = .38$) (Table 7.2). No significant differences were noted between conditions for changes in fullness after tasting the soup ($F (2,68) = 0.27$, NS; Table 7.2). When changes in appetite ratings immediately after soup consumption were assessed, no significant effects were found for either hunger ($F (2,68) = 0.31$, NS) or fullness ($F (2,68) = <.001$, NS) across conditions indicating that the nutrients or energy in the soup did not affect appetite immediately after intake.

Satiety analyses post-preload to pre-lunch revealed a significant effect of time with hunger increasing ($F(1,34) = 38.30$, $p = <.001$) and fullness decreasing ($F(1,34) = 59.55$, $p = <.001$) over the 45 min period. There was no effect of soup condition on hunger ($F(2,68) = 0.53$, NS) or fullness ($F(2,68) = 0.81$, NS) despite the energy and nutrient differences between the conditions (Table 7.2). However, effect size calculations suggested stronger hunger in carbohydrate conditions compared to control upon return for the test meal ($r = .32$) than when protein was compared to control ($r = .11$). No differences in hunger ($F(1,34) = 0.87$, NS) or fullness ($F(1,34) = <0.001$, NS) were observed between MSG+ and MSG- soups and no MSG*condition interaction effects were noted for hunger ($F(1.7, 57.2) = 0.21$, NS) or fullness ($F(1.8, 57.3) = 0.33$, NS).

Table 7.2. Change from baseline Visual Analogue Scale (VAS) appetite ratings for three versions of soups (low energy control, high energy carbohydrate, and high energy protein) with (MSG+) and without (MSG-) added MSG. [1] Significantly different from post-preload. [2] Significantly different pair-wise contrast from control at P<0.05.

	Time	Control				Carbohydrate				Protein			
		MSG-	SEM	MSG+	SEM	MSG-	SEM	MSG+	SEM	MSG-	SEM	MSG+	SEM
Hunger	Taste	-4.2	3.3	1.1[1]	1.3	-3.0	2.2	0.1[1]	1.5	2.0	1.1	-3.4[1,2]	2.1
	Post-Preload	-29.2	4.0	-27.2	3.4	-27.4	3.6	-25.0	3.7	-24.8	4.4	-28.3[2]	4.0
Fullness	Taste	1.9	2.0	1.5	1.4	2.8	2.3	2.8	1.8	2.1	1.5	3.5	2.4
	Post-Preload	32.7	4.6	32.3	4.1	32.9	4.0	32.1	3.6	32.2	3.7	32.8	3.9

7.3.3.2 Test Meal Satiation

The final analyses of appetite ratings focussed on how satisfying the test meal was to see if this was influenced by the earlier soup preload manipulation. As expected, hunger decreased (F $(1,34)$ = 213.73 , p<.001) and fullness increased (F$(1,34)$ = 211.39, p<.001) over the course of the *ad-libitum* test meal.

Over the course of the test meal a significant effect of soup condition on changes in rated hunger (F $(2,68)$ = 15.18, p = <.001) indicated that protein was found to be more satiating across the meal when compared to control conditions (F $(1,34)$ = 23.80, p = <.001, r = .64). A significant condition*time interaction (F$(2,68)$ = 10.31, p = <.001) suggested that appetite was most suppressed post-meal after protein preloads followed by carbohydrate and control conditions. The overall decrease in hunger across the test meal was significantly greater after MSG- soup preloads compared to MSG+ (main effect of MSG: F$(1,34)$ = 4.52, p = .04, r = .34) but this may be driven by MSG effects on the control and carbohydrate conditions as a significant condition*MSG*time interaction F$(2,68)$ = 5.39, p = .007) revealed that the addition of MSG to carbohydrate soups suppressed hunger less over the course of the *ad-libitum* meal when compared to control (F $(1,34)$ = 4.15, p = .05, r = .33) but acted to reduce hunger more in the protein condition when compared to control (F $(1,34)$ = 9.77, p = .004, r = .47). Changes in fullness over the meal were significantly influenced by the preload condition initially received (F$(2,68)$ = 4.01, p = .02), with lowest fullness ratings after the control condition followed by carbohydrate and protein conditions. This suggests that satiation was lowest in the control condition despite test meal intake being highest in this condition (Figure 7.1). No significant effects of added MSG were found (F $(1,34)$ = 0.43, NS) and no MSG*condition interaction was evident (F$(2,68)$ = 1.08, NS).

Inspection of each individual course was carried out to assess which courses were most affected by the preload manipulations. Overall, hunger ratings declined after savoury course intake (F$(1,34)$ = 151.81, p = < .001) and fullness ratings increased (F$(1,34)$ = 174.61, p = < .001) as expected. There was a significant appetizer effect on hunger ratings immediately after tasting the savoury course (main effect of time F $(1,34)$ = 15.09, p <.001), and this varied between soup conditions (F $(2,68)$ = 3.24, p = .05), with higher hunger ratings after tasting in control and protein conditions compared to the carbohydrate conditions. A significant condition*MSG*time interaction (F$(2,68)$ =

3.60, p = .03) revealed significant appetizer effects across all macronutrient conditions in MSG- soups which was also found in MSG+ control and protein conditions but there was no increase in hunger immediately after tasting the MSG+ carbohydrate condition. However, these increases in hunger after tasting did not translate to actual savoury course consumption, which was lower in the MSG added conditions (Figure 7.1).

When assessing changes in appetite over the duration of the savoury course there were no main effects of macronutrient condition on hunger (F(2,68) = 0.82, NS) or fullness (F(2,68) = 2.56, NS), no main effect of MSG on hunger (F(1,34) = 0.01, NS) or fullness (F(1,34) = 0.07, NS) and no interaction effects found for hunger (F(2,68) = 1.98, NS) or fullness (F(1.7, 58.5) = 0.88, NS). Hunger increased (F (1,34) = 12.59, p = .001) and fullness decreased (F(1,34) = 9.35, p = .004) immediately after the dessert was first tasted. A significant effect of soup condition (F(2,68) = 10.80, p = <.001) was evident in changes in hunger from the end of the savoury course to the end of the meal with hunger remaining elevated in the control compared to carbohydrate and protein preload conditions. There was also a significant Condition*MSG*time interaction (F(2,68) = 8.54, p = <.001) with protected contrasts indicating that the hunger reducing effects of protein were stronger with added MSG at the end of the meal compared to control (F(1,34) = 15.20, p = <.001, r = .56) and compared to carbohydrate (F(1,34) = 5.48, p = .03, r = .37) conditions. Equally, fullness evaluations before the dessert course to after the meal differed significantly between soup conditions (F(2,68) = 3.34, p = .04) with a smaller increase in fullness experienced after control preload soup than protein or carbohydrate conditions.

7.3.4 Sensory ratings of the preload and test meal

7.3.4.1 Soups

There were no significant differences noted for familiarity (F (2,68) = 0.08, p=.93), pleasantness (F (2,68) = 2.93, p=.07), or sweetness (F (2,68) = 2.31, p=.09) across soup conditions; all soups were deemed familiar; m: 70, pleasant; m: 63 and marginally sweet; m: 54 (Table 7.3). MSG+ soups were rated as more salty (F (1,34) = 26.26, p = <.001) and stronger tasting (F (1,34) = 12.57, p = .001) than MSG- soups and there was a significant soup*MSG interaction found for savoury assessments (F (2,68) = 4.37, p = .02) with MSG+ control and protein soups deemed more savoury than no added MSG but MSG+ carbohydrate soups rated as less savoury than the MSG- condition (Table 7.3). This may be due to the ambiguous nature of the 'savoury' label affecting sensory judgements as trained sensory panels were not tested.

7.3.4.2 Test Meal

The main meal items were deemed equally familiar across conditions. There was a significant difference in savoury course pleasantness (F (2,68) = 5.93, p =.004), and strength of flavour (F (2,68) = 3.16, p = .05) ratings across conditions with higher ratings made after protein preload consumption, followed by control and carbohydrate conditions. There were no significant differences in sensory ratings across conditions for the dessert course.

Table 7.3. VAS ratings of the sensory characteristics of three versions of soup (low energy control, high energy carbohydrate and high energy protein with (MSG+) and without (MSG-) added MSG. [1] Significantly different from MSG-. [2] Significantly different pair wise comparison from control P<0.05.

Rating	Control				Carbohydrate				Protein			
	MSG-	SEM	MSG+	SEM	MSG-	SEM	MSG+	SEM	MSG-	SEM	MSG+	SEM
Familiar	70.3	3.6	68.6	3.3	72.3	2.5	69.4	3.5	69.9	3.5	70.1	3.2
Pleasant	60.5	3.4	65.9	1.8	66.9	2.2	65.8	2.7	60.6	3.2	61.1	3.5
Salty	45.4	3.1	50.7[1]	3.2	40.1	2.7	49.9[1]	3.1	41.5	3.6	53.9[1]	3.7
Savoury	61.2	2.4	63.8	2.3	61.3	2.0	57.2[2]	3.2	57.7	3.2	63.7	2.9
Strong	51.5	3.5	56.5[1]	2.6	49.6	2.7	56.4[1]	3.0	49.9	3.6	54.3[1]	3.4
Sweet	55.1	2.6	50.0	3.0	56.7	2.3	57.6	2.6	54.3	2.8	51.8	2.8

7.4 Discussion

The main findings of the study indicate that nutrients received largely as protein in a soup preload allow for an adequate adjustment of energy consumed at a later *ad-libitum* meal. Increasing the overall energy content of the test soup with added protein resulted in a greater reduction in lunch intake, and consequently more accurate compensation for this added energy, than was seen when energy was increased by addition of carbohydrate, in line with several recent studies (Bertenshaw, et al., 2008; Bertenshaw, et al., 2009; Yeomans & Chambers, 2011). Although the predicted enhancement of protein-induced satiety by addition of MSG was not significant based on intake data, the addition of MSG did result in greater compensation for added protein energy. However there were no differences between MSG or specific macronutrient conditions in rated satiety over testing after preload intake.

As predicted, test meal intake after consumption of the protein enriched preload was lowest followed by the carbohydrate and control conditions. Thus, there was better compensation for the energy received in the protein condition when later provided with an *ad-libitum* meal, consistent with the broader literature suggesting that protein is more satiating than is carbohydrate (Fischer, et al., 2004; Marmonier, et al., 2000; Poppitt, et al., 1998). Importantly, compensation became even more accurate when MSG was added to the protein- compared to the carbohydrate-enriched preload, with this effect strongest in the savoury course of the test meal. This compensation effect was evident despite the relatively small energy difference between low and high energy conditions. In previous studies compensation effects have only been apparent with larger energy differences between preloads (Bertenshaw, et al., 2008; Yeomans & Chambers, 2011; Yeomans, et al., 2001). This suggests that moderate increases in the energy content of a food through the addition of protein and MSG, for example as a savoury snack, may reduce the likelihood of subsequent overconsumption.

We suggest two possible explanations for how MSG may enhance compensation for energy added as protein. Firstly, the sensory quality generated by the addition of MSG may have cued the experience of protein, and so enhanced the actual satiating effects of protein at the test meal. This idea is supported by recent data showing no significant differences in compensation at a test meal following high protein and high carbohydrate

preloads when these were matched for thickness and creaminess (Bertenshaw, et al., 2013). Indeed, it may be that the savoury characteristics of MSG are more strongly associated with protein than are the sensory characteristics of thickness and creaminess identified in the earlier study and thus act as a more reliable cue for protein-based satiety. Alternatively, the enhanced satiating effects of protein in the MSG+ condition could be related to post-ingestive stimulation of gut glutamate sensors (Kitamura, Tsurugizawa, & Torii, 2011; Masic & Yeomans, 2013) which have been related to enhanced satiety in animals (Kondoh & Torii, 2008; Niijima, 2000; San Gabriel, et al., 2007).

An appetizing effect (Yeomans, 1996; Yeomans & Gray, 1997) of MSG was seen in both the control and carbohydrate-rich soups with added MSG but surprisingly was not found in the equivalent protein-rich soup. This may be due to the immediate sensory experience of protein and MSG eliciting lower feelings of hunger in the protein-rich MSG+ condition, however as *ad-libitum* intake of the soup course was not assessed, this remains as speculation. Despite the immediate stimulation of appetite by added MSG in some conditions, no significant differences in hunger were seen immediately after ingesting the soup, in contrast to our recent study (Masic & Yeomans, 2013). This may be related to volume and hedonic assessments, with participants expecting each soup to be equally satiating due to the equivalent volumes received (Allirot, et al., 2013). These predetermined portion sizes may then have influenced hedonic hunger (Yeomans, 2010). No difference in appetite was also evident immediately before the *ad-libitum* meal 45 minutes after preload ingestion and may be due to participants responding in anticipation of the meal to be received (Weingarten, 1984). Hunger also remained stronger at the *ad-libitum* meal in added MSG control and carbohydrate conditions but was suppressed in added MSG protein conditions. Such an effect may be indicative of delayed rebound hunger (Yeomans & Chambers, 2011), as participants consuming added MSG preloads without the accompanying protein may have been anticipating stronger satiation due to the MSG protein cue. However, as protein was not delivered, hunger was not as satisfied at the end of the test meal as when no protein cue was present (in no MSG conditions). But when added MSG was presented in combination with protein, the additional protein cue from MSG acted to reduce feelings of hunger more strongly; suggesting that the added MSG may have increased the salience of this cue as has been found previously (Masic & Yeomans, 2013) and is evident in infants

experiencing umami taste in mother's milk (Mennella & Beauchamp, 1996; Ventura, et al., 2012).

The present study also noted that most of the effects of the preload manipulations on lunch intake were evident for the first (savoury) course whereas intake at the dessert course was not affected by preload type. This may be due to the high palatability of this course overriding sensory and energy effects as has been found previously (Yeomans, et al., 2001) as sweet appetite relies less on the experience of hunger (de Graaf, et al., 1993) and more on the hedonic effects of palatability (Blundell & Rogers, 1994; Rogers & Blundell, 1989). Thus consumption of a sweet course may be less suppressed by a previously consumed savoury course (Appleton, et al., 2004), with the critical impact on behaviour being an earlier switch from savoury to sweet courses. There were also a number of limitations in the present design that constrained the conclusions drawn. Due to reformulations of the *ad-libitum* main course item, the different versions of the main course may have influenced test meal intake. However, further analyses of intake taking this into account indicated that this was most likely not the case. Similarly, effects of order on *ad-libitum* intake indicated that consumption was greater after the first test day but thereafter consumption was in line with the nutrients ingested, indicating that these order effects should not have influenced the sensory-nutrient interactions reported. Initial power analyses indicated that the sample tested would yield adequate power, however, although rated appetite and intake was in the direction predicted it was not found to be significant. This may suggest that a larger sample would be required to assess the more subtle effects of MSG on appetite.

Overall, the addition of protein to a soup preload reduced subsequent intake and allowed for more accurate energy compensation at a test meal and this was enhanced by the addition of MSG. When protein was consumed with added MSG, meal satisfaction remained the same despite reduced intake at a later test meal. However, subjective satiety ratings were not influenced by MSG, macronutrients or energy 45 minutes after preload intake. Further research is required to understand the influence of MSG and protein on sensory and gut responding and how this interacts with the subjective experience of appetite and subsequent intake.

7.5 Acknowledgements

7.6 Financial Support

7.7 Conflict of Interest

None

7.8 Authorship

Una Masic and Martin Yeomans formulated the research question and designed the study. Una Masic carried out the testing, analysed the data and wrote the article with revisions read by Martin Yeomans and corrections made accordingly.

7.9 Summary of main findings and directions for future research

- The main findings suggested that the addition of energy in a fixed preload (added carbohydrate or added protein) acted to improve the compensation for this extra energy received in a subsequent two-course meal
- The addition of MSG further improved this compensation, particularly when in combination with added energy in the form of protein but no changes in meal intake between conditions were apparent after MSG conditions
- Added MSG also increased palatability and rated hunger immediately after tasting in added control and carbohydrate conditions but not protein conditions
- This suggests that MSG increases palatability and hunger during intake of the MSG containing meal but that it may contribute to satiety when combined with protein by improving energy regulation over time
- Future research may wish to further assess the effects of MSG in relation to protein; as the effects found were subtle, the use of a more reliable protein cue such as one derived from umami in the form of inosine monophosphate (IMP) in combination with MSG may reveal whether the energy compensation effects

found may be more specific rather than a general effect of umami on appetite and intake

Appendix 7.1. Graphical depiction of the study design employed.
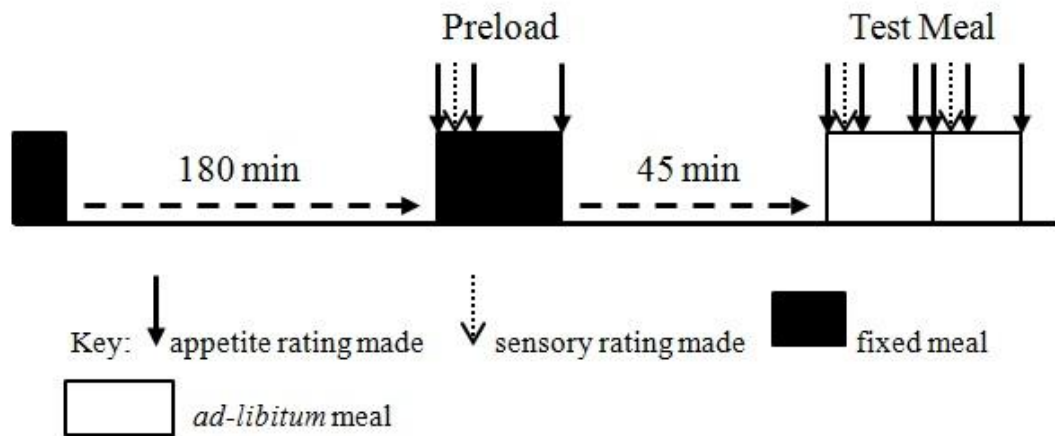
7.10 References

Allirot, X., Saulais, L., Disse, E., Nazare, J. A., Cazal, C., & Laville, M. (2013). Integrating behavioral measurements in physiological approaches of satiety. *Food Quality and Preference, 31*(0), 181-189. doi: http://dx.doi.org/10.1016/j.foodqual.2011.05.005

Appleton, K. M., Rogers, P. J., & Blundell, J. E. (2004). Effects of a sweet and a nonsweet lunch on short-term appetite: differences in female high and low consumers of sweet/low-energy beverages. *Journal of Human Nutrition and Dietetics, 17*(5), 425-434. doi: 10.1111/j.1365-277X.2004.00548.x

Bellisle, F. (1999). Glutamate and the UMAMI taste: sensory, metabolic, nutritional and behavioural considerations. A review of the literature published in the last 10 years. *Neuroscience and Biobehavioral Reviews, 23*(3), 423-438.

Bellisle, F., Dalix, A. M., Chappuis, A. S., Rossi, F., Fiquet, P., Gaudin, V., . . . Slama, G. (1996). Monosodium Glutamate Affects Mealtime Food Selection in Diabetic Patients. *Appetite, 26*(3), 267-276.

Bellisle, F., Monneuse, M. O., Chabert, M., Larue-Achagiotis, C., Lanteaume, M. T., & Louis-Sylvestre, J. (1991). Monosodium glutamate as a palatability enhancer in the european diet. *Physiology & Behavior, 49*(5), 869-873.

Bellisle, F., Tournier, A., & Louis-Sylvestre, J. (1989). Monosodium glutamate and the acquisition of food preferences in a European context. *Food Quality and Preference, 1*, 103-108.

Bertenshaw, E. J., Lluch, A., & Yeomans, M. R. (2008). Satiating effects of protein but not carbohydrate consumed in a between-meal beverage context. *Physiology & Behavior, 93*(3), 427-436. doi: 10.1016/j.physbeh.2007.09.014

Bertenshaw, E. J., Lluch, A., & Yeomans, M. R. (2009). Dose-dependent effects of beverage protein content upon short-term intake. *Appetite, 52*(3), 580-587. doi: http://dx.doi.org/10.1016/j.appet.2009.01.010

Bertenshaw, E. J., Lluch, A., & Yeomans, M. R. (2013). Perceived thickness and creaminess modulates the short-term satiating effects of high-protein drinks. *British Journal of Nutrition, FirstView*, 1-9. doi: doi:10.1017/S0007114512005375

Blundell, J. E., & Rogers, P. J. (Eds.). (1994). *Sweet carbohydrate substitutes (intense sweeteners) and the control of appetite: scientific issues.* (Vol. 113–124). London: CRC Press.

de Graaf, C., Schreurs, A., & Blauw, Y. H. (1993). Short-term effects of different amounts of sweet and nonsweet carbohydrates on satiety and energy intake. [doi: 10.1016/0031-9384(93)90290-V]. *Physiology & Behavior, 54*(5), 833-843.

Essed, N., Oerlemans, P., Hoek, M., Van Staveren, W., Kok, F., & De Graaf, C. (2009). Optimal preferred MSG concentration in potatoes, spinach and beef and their effect on intake in institutionalized elderly people. *The Journal of Nutrition, Health & Aging, 13*(9), 769-775. doi: 10.1007/s12603-009-0212-9

Essed, N. H., van Staveren, W. A., Kok, F. J., & de Graaf, C. (2007). No effect of 16 weeks flavor enhancement on dietary intake and nutritional status of nursing home elderly. [doi: 10.1016/j.appet.2006.06.002]. *Appetite, 48*(1), 29-36.

Fedoroff, I. D. C., Polivy, J., & Herman, C. P. (1997). The Effect of Pre-exposure to Food Cues on the Eating Behavior of Restrained and Unrestrained Eaters. [doi: 10.1006/appe.1996.0057]. *Appetite, 28*(1), 33-47.

Fedoroff, I. D. C., Polivy, J., & Herman, C. P. (2003). The specificity of restrained versus unrestrained eaters' responses to food cues: general desire to eat, or craving for the cued food? *Appetite, 41*(1), 7-13. doi: http://dx.doi.org/10.1016/S0195-6663(03)00026-6

Fischer, K., Colombani, P. C., & Wenk, C. (2004). Metabolic and cognitive coefficients in the development of hunger sensations after pure macronutrient ingestion in the morning. [doi: 10.1016/S0195-6663(03)00116-8]. *Appetite, 42*(1), 49-61.

FSA. (2006). *FSA Nutrient and Food Based Guidelines for UK Institutions*. London.

Herman, C. P., & Polivy, J. (2008). External cues in the control of food intake in humans: The sensory-normative distinction. [doi: 10.1016/j.physbeh.2008.04.014]. *Physiology & Behavior, 94*(5), 722-728.

Ikeda, K. (1908). On a New Seasoning. *Journal of Tokyo Chemistry Society, 30*, 820-836.

Jinap, S., & Hajeb, P. (2010). Glutamate. Its applications in food and contribution to health. *Appetite, 55*(1), 1-10. doi: 10.1016/j.appet.2010.05.002

Kissileff, H. R., Klingsberg, G., & Van Itallie, T. B. (1980). Universal eating monitor for continuous recording of solid or liquid consumption in man. *The American Journal of Physiology, 238*(1), R14-22.

Kitamura, A., Tsurugizawa, T., & Torii, K. (2011). Biological Significance of Glutamate Signaling during Digestion of Food through the Gut-Brain Axis. *Digestion, 83(suppl 1)*(Suppl. 1), 37-43.

Kondoh, T., & Torii, K. (2008). MSG intake suppresses weight gain, fat deposition, and plasma leptin levels in male Sprague-Dawley rats. . *Physiology & Behavior, 95*, 135–144.

Laska, M., & Hernandez Salazar, L. T. (2004). Gustatory responsiveness to monosodium glutamate and sodium chloride in four species of nonhuman primates. *Journal of Experimental Zoology Part A: Comparative Experimental Biology, 301A*(11), 898-905. doi: 10.1002/jez.a.118

Latner, J. D., & Schwartz, M. (1999). The Effects of a High-carbohydrate, High-protein or Balanced Lunch upon Later Food Intake and Hunger Ratings. *Appetite, 33*(1), 119-128. doi: http://dx.doi.org/10.1006/appe.1999.0237

Leidy, H. J., Tang, M., Armstrong, C. L. H., Martin, C. B., & Campbell, W. W. (2011). The Effects of Consuming Frequent, Higher Protein Meals on Appetite and Satiety During Weight Loss in Overweight/Obese Men. *Obesity, 19*(4), 818-824.

Lejeune, M. P. G. M., Kovacs, E. M. R., & Westerterp-Plantenga, M. S. (2005). Additional protein intake limits weight regain after weight loss in humans. *British Journal of Nutrition, 93*(02), 281-289. doi: doi:10.1079/BJN20041305

Luscombe-Marsh, N. D., Smeets, A. J. P. G., & Westerterp-Plantenga, M. S. (2009). The addition of monosodium glutamate and inosine monophosphate-5 to high-protein meals: effects on satiety, and energy and macronutrient intakes. *British Journal of Nutrition, 102*(6), 929-937. doi: 10.1017/s0007114509297212

Marmonier, C., Chapelot, D., & Louis-Sylvestre, J. (2000). Effects of macronutrient content and energy density of snacks consumed in a satiety state on the onset of the next meal. *Appetite, 34*(2), 161-168. doi: http://dx.doi.org/10.1006/appe.1999.0302

Masic, U., & Yeomans, M. R. (2013). Does monosodium glutamate interact with macronutrient composition to influence subsequent appetite? *Physiology & Behavior, 116–117*(0), 23-29. doi: http://dx.doi.org/10.1016/j.physbeh.2013.03.017

Mathey, M.-F. A. M., Siebelink, E., de Graaf, C., & Van Staveren, W. A. (2001). Flavor Enhancement of Food Improves Dietary Intake and Nutritional Status of Elderly Nursing Home Residents. *The Journals of Gerontology Series A: Biological*

Sciences and Medical Sciences, 56(4), M200-M205. doi:
10.1093/gerona/56.4.M200

Mennella, J. A., & Beauchamp, G. K. (1996). Developmental Changes in the Acceptance of Protein Hydrolysate Formula. *Journal of Developmental & Behavioral Pediatrics, 17*(6), 386-391.

Niijima, A. (2000). Reflex Effects of Oral, Gastrointestinal and Hepatoportal Glutamate Sensors on Vagal Nerve Activity. *The Journal of Nutrition, 130*(4), 971.

Ninomiya, K. (1998). Natural occurrence *Food Reviews International, 14*(2), 177-211.

Poppitt, S. D., McCormack, D., & Buffenstein, R. (1998). Short-term effects of macronutrient preloads on appetite and energy intake in lean women. *Physiology & Behavior, 64*, 279-285.

Rogers, P. J., & Blundell, J. E. (1989). Separating the actions of sweetness and calories: Effects of saccharin and carbohydrates on hunger and food intake in human subjects. [doi: 10.1016/0031-9384(89)90093-0]. *Physiology & Behavior, 45*(6), 1093-1099.

San Gabriel, A. M., Maekawa, T., Uneyama, H., Yoshie, S., & Torii, K. (2007). mGluR1 in the fundic glands of rat stomach. *FEBS letters, 581*(6), 1119-1123.

Schiffman, S. S., Sattely-Miller, E. A., Zimmerman, I. A., Graham, B. G., & Erickson, R. P. (1994). Taste perception of monosodium glutamate (MSG) in foods in young and elderly subjects. *Physiology & Behavior, 56*(2), 265-275.

Simpson, S. J., & Raubenheimer, D. (2005). Obesity: the protein leverage hypothesis. *Obesity Reviews, 6*(2), 133-142.

Simpson, S. J., & Raubenheimer, D. (Eds.). (2000). *Geometric models of macronutrient selection*. Boca Raton: CRC Press.

Smriga, M., & Torii, K. (2000). Release of hypothalamic norepinephrine during MSG intake in rats fed normal and nonprotein diet. . *Physiology & Behavior, 70*, 413–415.

Stunkard, A. J., & Messick, S. (1985). The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. . *Journal of Psychosomatic Research, 29*, 71-83.

Ventura, A. K., Beauchamp, G. K., & Mennella, J. A. (2012). Infant regulation of intake: the effect of free glutamate content in infant formulas. *The American Journal of Clinical Nutrition, 95*(4), 875-881. doi: 10.3945/ajcn.111.024919

Wansink, B., Painter, J. E., & North, J. (2005). Bottomless Bowls: Why Visual Cues of Portion Size May Influence Intake**. *Obesity Research, 13*(1), 93-100. doi: 10.1038/oby.2005.12

Weigle, D. S., Breen, P. A., Matthys, C. C., Callahan, H. S., Meeuws, K. E., Burden, V. R., & Purnell, J. Q. (2005). A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations. *The American Journal of Clinical Nutrition, 82*(1), 41-48.

Weingarten, H. P. (1984). Meal initiation controlled by learned cues: Basic behavioral properties. *Appetite, 5*(2), 147-158. doi: http://dx.doi.org/10.1016/S0195-6663(84)80035-5

Williams, E. J. (1949). Experimental designs balanced for the estimation of residual effects of treatment. *Australian Journal of Scientific Research, Series A*(2), 149-168.

Yeomans, M. R. (1996). Palatability and the Micro-structure of Feeding in Humans: the Appetizer Effect. *Appetite, 27*(2), 119-133.

Yeomans, M. R. (2010). Understanding Individual Differences in Acquired Flavour Liking in Humans. *Chemosensory Perception, 3*(1), 34-41. doi: 10.1007/s12078-009-9052-6

Yeomans, M. R., & Chambers, L. (2011). Satiety-relevant sensory qualities enhance the satiating effects of mixed carbohydrate-protein preloads. *The American Journal of Clinical Nutrition, 94*(6), 1410-1417. doi: 10.3945/ajcn.111.011650

Yeomans, M. R., & Gray, R. W. (1997). Effects of Naltrexone on Food Intake and Changes in Subjective Appetite During Eating: Evidence for Opioid Involvement in the Appetizer Effect. [doi: 10.1016/S0031-9384(97)00101-7]. *Physiology & Behavior, 62*(1), 15-21.

Yeomans, M. R., & Gray, R. W. (2002). Opioid peptides and the control of human ingestive behaviour. *Neuroscience & Biobehavioral Reviews, 26*, 713–728.

Yeomans, M. R., Gray, R. W., & Conyers, T. H. B. (1998). Maltodextrin preloads reduce food intake without altering the appetiser effect. [doi: 10.1016/S0031-9384(98)00086-9]. *Physiology & Behavior, 64*(4), 501-506.

Yeomans, M. R., Lee, M. D., Gray, R. W., & French, S. J. (2001). Effects of test-meal palatability on compensatory eating following disguised fat and carbohydrate preloads. *International Journal of Obesity, 25*(8), 1215-1224.

This paper was submitted to the American Journal of Clinical Nutrition in November, 2013.

Original article reference: Masic, U., & Yeomans, M.R. (2013). Umami flavour enhances appetite but also enhances satiety. *American Journal of Clinical Nutrition*, *under review*.

Umami flavour enhances appetite but also increases satiety[1]

Una Masic and Martin R Yeomans

[1] School of Psychology, University of Sussex, Brighton, BN1 9QH, UK

Running head: MSG IMP and satiety

Address for reprint requests and correspondence:

Una Masic

School of Psychology

University of Sussex

Brighton

BN1 9QH

UK

Tel: +44-1273-872826

Email: u.masic@sussex.ac.uk

Abbreviations: Monosodium glutamate, MSG; inosine 5'-monophosphate, IMP; guanosine 5'-monophosphate, GMP; visual analogue scale, VAS; body mass index, BMI; Three Factor Eating Questionnaire, TFQ; Sussex Ingestion Pattern Monitor, SIPM; Ingestive Behaviour Unit, IBU; men, M; women, W; energy compensation, COMPX.

Abstract

Monosodium glutamate (MSG) has been shown to increase satiety when in combination with protein. Inosine 5'-monophosphate (IMP) acts synergistically with MSG and is present in high protein sources and may potentially enhance satiety further. This experiment assessed the effects of a combination of MSG and IMP either provided alone or in combination with protein on appetite during ingestion and post-ingestive satiety. Fixed portions (450g) of low energy control and high energy protein soup preloads with (MSG/IMP+) or without (MSG/IMP-) added MSG/IMP were consumed on four non-consecutive days and changes in appetite during soup intake and at a subsequent *ad-libitum* lunch were assessed in 26 low-restraint volunteers using in a within-participant design. MSG/IMP+ preloads significantly reduced subsequent intake more than MSG/IMP- conditions irrespective of protein. The addition of protein also reduced intake independently of MSG/IMP. Energy compensation was greater in MSG/IMP+ protein conditions than protein alone and the addition of MSG/IMP+ also increased soup pleasantness and caused an immediate increase in appetite when soup was first tasted. This suggests that the addition of MSG/IMP to a low energy preload had a biphasic effect on appetite, stimulating appetite during ingestion but then enhancing post-ingestive satiety.

8.1 Introduction

Monosodium glutamate (MSG) has been used for over a century to increase the savoury umami taste of food (Ikeda, 1908; Jinap & Hajeb, 2010; Ninomiya, 1998). When MSG is combined with the purine nucleotide inosine 5'-monophosphate (IMP), these chemicals act synergistically to further increase the flavour of umami (Chaudhari, et al., 2009; Garcia-Bailo, et al., 2009; Yamamoto & Ishimaru, 2013). Since MSG enhances flavour and thereby palatability, and palatability is known to increase short-term intake (Yeomans, et al., 2004), it was initially thought that MSG would increase short-term intake (Bellisle, et al., 1991; Mathey, et al., 2001). However, the short-term effects of MSG on appetite have been found to reduce over time (Bellisle, 1999; Essed, et al., 2007). This raises the possibility that MSG may also modulate post-ingestive satiety, giving umami a dual role in appetite control.

It has been suggested that the ability to taste umami arose as a way of detecting the presence of protein (Kurihara, 2009; Luscombe-Marsh, et al., 2008; Naim, et al., 1991) and it may be this potential to signal the presence of protein in foods that contributes to satiety (Ikeda, 1908; Ninomiya, 1998). There is considerable evidence that protein is more satiating than either carbohydrate (Bertenshaw, et al., 2008; Fischer, et al., 2004) or fat (Simpson & Raubenheimer, 2000; Weigle, et al., 2005). In support of this idea, two recent studies in our laboratory found that MSG reduced post-ingestive recovery of hunger (Masic & Yeomans, 2013a) and improved energy compensation (Masic & Yeomans, 2013b) when consumed in combination with protein. Similarly, it has been found that increasing MSG content in infant formula causes the same increase in satiation and satiety as when protein hydrolysate formula milk is used, which is known for its effects on improving appetite control due to added protein and free glutamate (Ventura, et al., 2012). However, not all studies have found evidence that MSG enhances satiety (Mathey, et al., 2001). IMP is predominantly found with MSG in animal protein (Ninomiya, 1998), thus it may be a strong candidate for enhancing sensory associations with protein, further increasing the salience of the protein signal provided by MSG and in turn reducing appetite and intake in a protein context. However, few studies have looked at effects of MSG+IMP on measures of appetite and satiety. Indeed, the only studies to date examining effects of MSG+IMP on acute satiety in adults found no evidence of enhanced satiety (Carter, et al., 2011; Luscombe-Marsh,

excluded. Individuals with a high restraint score (above 7 on the Three Factor Eating Questionnaire (TFEQ; Stunkard & Messick, 1985) were also excluded as restrained eaters display different eating behaviours to those seen more generally in the population (Dohm, et al., 2005). Eligible participants were contacted with details of the study described as 'determining the effects of food on motor skills'. This was to ensure awareness of the experimental manipulation would not influence eating behaviour during the testing sessions. Written, informed consent was given before participation and participants were paid £40 on completion of the study. The study was conducted in accordance with the standards expressed in the Helsinki Declaration and was approved by the University of Sussex ethics committee.

8.2.3 Test Food

8.2.3.1 Breakfast and lunch:

The size of breakfasts on test days were based on Food Standard Agency guidelines for men (M) and women (W) (FSA, 2006) and comprised of cereal (M:80g; W:60g; Crunchy Nut Cornflakes, Kellogg's, UK), semi-skimmed milk (M: 200g; W: 160g; Sainsbury's Plc, UK) and orange juice (M: 200g; W: 200g; Sainsbury's Pure Orange Juice, Sainsbury's Plc, UK) providing 504 Kcal for men and 404 Kcal for women. The *ad-libitum* lunch consisted of pasta (Conchiglie, Sainsbury's Plc, UK) served in a tomato sauce (Tomato and Basil Sauce, Sainsbury's Plc, UK). Each serving consisted of 250g cooked pasta combined with 200g sauce (total: 500 Kcal, 12% fat, 13% protein, 75% carbohydrate). Re-fill portions were provided as 450g servings. Tasting samples were served in 10g portions in small white ceramic bowls while pasta tasting portions were served on white ceramic plates.

8.2.3.2 Soup Preloads:

As in our earlier studies (Masic & Yeomans, 2013a, 2013b), a low energy density control spiced carrot soup (per 100g: 28 Kcal, 50% fat, 6% protein, 44% carbohydrate) was used as the base for all preloads which was served in a 450g portion. This contained carrots (Frozen Baby Carrots, Sainsbury's Plc, UK) onions, celery, olive oil (Medium Flavour Olive Oil, Sainsbury's Plc, UK), spice mixture (Garam Masala Schwartz, UK) and water (see Masic & Yeomans, 2013a) (368). Protein-rich soups (per 100g: 69 Kcal, 19% fat, 45% protein, 36% carbohydrate) additionally contained 36g/450g whey protein isolate (MyProtein UK) and 18g/450g maltodextrin (DE: 15.3, Cargill, UK).

Maltodextrin was added to the protein-rich soup in line with our previous studies (Masic & Yeomans, 2013a, 2013b) to ensure an adequate energy difference between low and high energy conditions. 0.6% w/w MSG and 0.25% w/w IMP (Ajinomoto Co., Inc. Europe) were added to MSG/IMP+ soup conditions. The formulation of the control soup followed extensive pilot testing to prepare a moderately pleasant soup low in MSG to which added MSG/IMP would allow for an enhancement of pleasantness. Pilot testing was also carried out on the high energy protein-rich soup used to ensure its acceptability (see Masic & Yeomans, 2013a). The levels of MSG/IMP used were determined based on previous successful flavour manipulations (Luscombe-Marsh, et al., 2008, 2009) and are in-line with commercial usage (Bellisle, 1999; Loliger, 2000; Yamaguchi & Ninomiya, 2000).

8.2.4 Procedure

The study was conducted over four non-consecutive test days at the Ingestive Behaviour Unit (IBU) at the University of Sussex. Participants arrived in a fasted state having consumed nothing but water from 2300h the night before and were given a standard breakfast in the IBU at a pre-arranged time (from 0900h – 1030h) . After breakfast, participants were free to leave with instructions to abstain from eating and drink only water and to return to the IBU 3 h later.

Preload and test meal sessions ran from 1200 h-1330 h and were conducted in windowless cubicles. To conceal the purpose of the experiment and in compliance with the cover story used, participants completed a motor skills task (the star motor task, which entailed tracing the outline of a star using their non-dominant hand) to minimise demand effects from the preload/intake tests. Before preload and test lunch sessions participants completed digital Visual Analogue Scale (VAS) ratings assessing how alert, clear-headed, energetic, full, hungry, nauseous and thirsty they felt using the Sussex Ingestion Pattern Monitor (SIPM) which consists of a digital balance (Sartorius, Model BP4100) linked to a Dell personal computer. All scales were presented as sentences ("How <word> do you feel?") with a left hand anchor reading "Not at all <word>" (coded as 0) and a right hand anchor reading "As <word> as I have ever felt/experienced" (coded as 100). Participants were given instructions on screen of how to use the scale before each evaluation. Participants registered their response by selecting "Rating Complete" and data were logged by SIPM. A sample of the relevant

preload soup was then provided in a clear plastic beaker and participants rated how filling, pleasant, salty, savoury, strong and sweet the sample was before completing appetite ratings (how full, hungry and thirsty). A bowl of the tasted soup was then provided and ratings of how full, hungry and thirsty they felt, and how pleasant the soup tasted, were made after every 50g soup consumed until all of the soup was ingested (the change in the weight of the bowl was tracked and registered by the SIPM), after which additional appetite and mood ratings were completed. Participants were then free to leave but told to consume only water until they returned 40 minutes later for the test lunch. This was provided 45 min after the soup had been consumed as this interval was previously found to be the most sensitive for detecting appetite suppression by MSG (Masic & Yeomans, 2013a). As with the soup preload, participants first completed the star motor task, mood and appetite ratings and taste test, before being provided with a 450g portion of the pasta in sauce with instructions to "Please eat as much pasta as you like until you feel comfortably full". Re-fill portions of the course were prompted by the SIPM after 400g consumed with an alert sound and on-screen message instructing participants to call the experimenter. Re-fills were provided in this way to reduce external cues such as an empty plate from influencing intake (Herman & Polivy, 2008b; Wansink, et al., 2005a). When participants had indicated that they had finished their meal, a final set of appetite and mood ratings were completed matching those at the start of the test meal. The order of VAS ratings was randomized for all rating sets. Drinking water was available during both the preload and test lunch. Participants were free to leave once the test meal was complete on days 1-3. The final session ended with a structured debriefing during which height and weight was recorded before payment. A graphical depiction of the experimental design can be seen in Appendix 8.1.

8.2.5 Data Analysis

Initial analysis of intake data identified one outlier who had a higher BMI and was consequently removed from all analyses. To assess post-ingestive satiety, two-way repeated measures ANOVA was used to assess test meal intake (Kcal) between soup (control or protein-rich) and flavour (MSG/IMP+ or MSG/IMP-) conditions. To further determine effects of flavour and energy manipulations on satiety, energy compensation (COMPX) values (Rolls, Dimeo, & Shide, 1995) were calculated by subtracting *ad-libitum* meal intake in the control MSG/IMP- condition from that in the protein-rich MSG/IMP+ or MSG/IMP- condition and expressing this as a percentage of the

difference in preload energy between low and high energy protein preloads (187 Kcal). COMPX values were contrasted using repeated measures ANOVA with the prediction that MSG/IMP+ would reduce intake resulting in a unidirectional hypothesis. Appetite (hunger and fullness) and food pleasantness across the test meal were contrasted using ANOVA. As there were no baseline differences in rated hunger or fullness at the start of each session, immediate changes in rated hunger and fullness after first tasting the soup was calculated as this has been shown to measure sensory-impact on appetite (Yeomans & Bertenshaw, 2008), and these change data were contrasted using two-way ANOVA, with a prediction of enhanced appetite in MSG/IMP conditions. The overall change in these measures after tasting until the end of soup ingestion was also contrasted between conditions. Eating rate (grams per minute) was assessed for the whole preload duration using 2x2 repeated measures ANOVA. Soup and test meal sensory evaluations were contrasted using two-way repeated measures ANOVA. Gender was included as a between-subjects factor in all analyses as was test order. Within-subjects contrasts were assessed using Bonferroni adjusted comparisons to further understand the nature of specific interactions. Effect sizes are reported using Pearson's correlation coefficient. Data are shown for 26 out of 27 participants. All data were analysed using IBM SPSS 20.0 for Windows.

8.3 Results

8.3.1 Intake at the test meal

Test meal intake was lower in the MSG/IMP+ than MSG/IMP- conditions (P=.02) and after addition of protein (P=.001) (Figure 8.1 panel A). The interaction between MSG/IMP and protein was non-significant. Energy compensation (COMPX) was significantly better (P=.04) after the high energy MSG/IMP+ protein-rich preload (70%) than the high energy MSG/IMP- protein condition (44%) (Figure 8.1 panel B). No significant effect of eating rate was observed. Similarly, no significant effects of order or gender were apparent on intake or COMPX measures.

A) Main Meal Intake
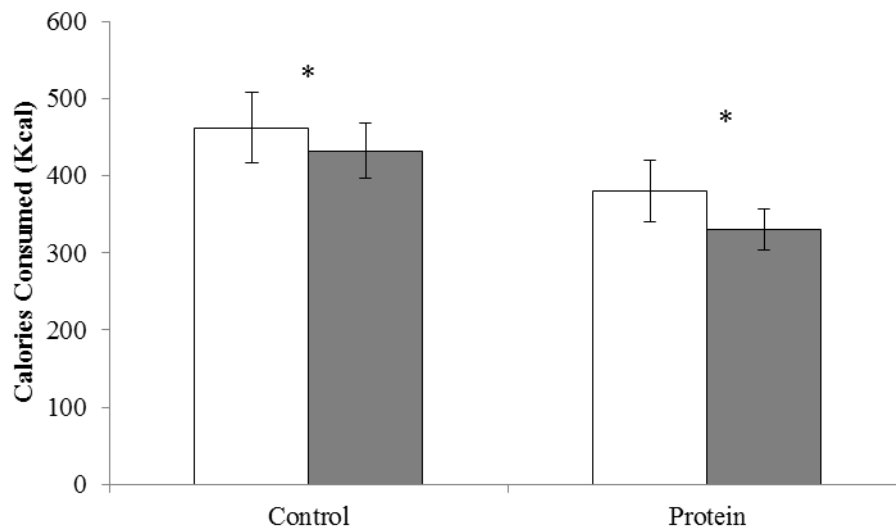
B) Energy Compensation
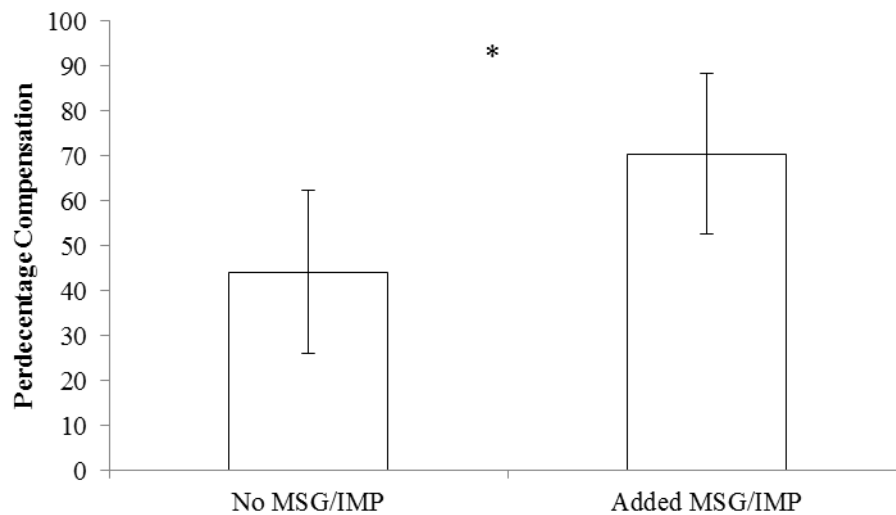
Figure 8.1. *Ad-libitum* lunch intake (A) after fixed preload consumption of a high energy protein or low energy control with (■) and without (□) added MSG/IMP and (B) compensation for the extra energy ingested in the high energy protein-rich condition across added and no MSG/IMP conditions. Data are mean ±SE. Significant differences between added and no MSG/IMP conditions are indicated: * P<.05.

8.3.2 Rated hunger and fullness during the preload and test meal

Appetite was predicted to change over the course of both preload and test meal intake depending on the presence of MSG/IMP and protein. Ingestion of MSG/IMP+ preloads resulted in an immediate significant increase in hunger after tasting compared to MSG/IMP- preloads (P=.03) but the addition of high energy protein did not alter hunger after tasting these soups (Figure 8.2), and fullness did not change significantly in any condition. Hunger and fullness also did not differ significantly between preload conditions once all of the soup had been ingested, suggesting that the initial increase in hunger in MSG/IMP+ conditions was not sustained overall, although women were hungrier after consuming the soup in the MSG/IMP+ conditions than were men (P=.05), possibly suggesting a stronger appetizing effect of MSG/IMP in women.
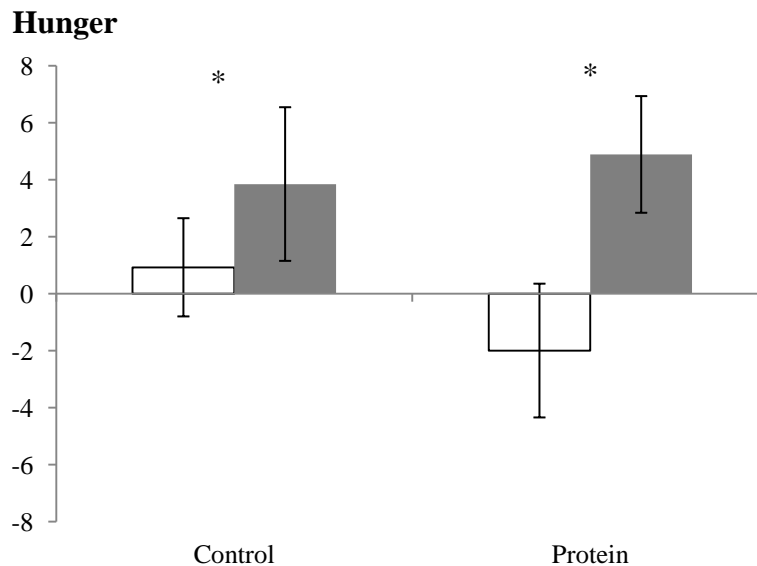
Hunger

Figure 8.2. Change from baseline VAS hunger ratings after tasting low energy control or high energy protein soups with (■) and without (□) added MSG/IMP. Data are mean±SE. Significant differences between added and no MSG/IMP conditions are indicated: * P<.05.

Hunger increased and fullness decreased significantly during the 45 minute inter-meal interval between preload and lunch but no significant differences were found between conditions. Men were significantly more hungry and less full after the 45 minute interval after MSG/IMP+ conditions than were women (P=.04).

The test meal significantly increased feelings of hunger after tasting (P=.01), possibly due to its palatability. However, an MSG/IMP*protein interaction indicated that hunger increased more than the MSG/IMP- control after tasting the pasta in the control MSG/IMP+ preload but was lower after tasting after protein MSG/IMP+ preload than the protein MSG/IMP- condition (P=.05) whilst fullness decreased after tasting the pasta after MSG/IMP- preloads (P=.05; Table 8.1). Hunger remained significantly elevated during the *ad-libitum* test meal after the high protein MSG/IMP- than MSG/IMP+ preloads (P=.05) and fullness remained higher during the test meal after the added protein MSG/IMP- condition than MSG/IMP+ (P=.03). However, rated fullness was lower during the test meal after the MSG/IMP+ than MSG/IMP- control condition. This indicates that the additional protein consumed may have improved the regulation of fullness but this does not relate to the lower test meal intake evident after MSG/IMP+ as compared to MSG/IMP- conditions (Figure 8.1).

When gender was included in the analysis over the course of the meal a significant MSG/IMP*gender (p = .04) and time*gender interaction (p = .02) was evident in fullness evaluations with men rating fullness as lower and women rating fullness as higher during the test meal after MSG/IMP+ preloads. Indeed, men were less full before the meal, reduced feelings of fullness after tasting the meal but showed similar fullness to women at the end of the test meal whilst fullness increased in a linear fashion in women. No effects of order were found across any preload or test meal analyses of hunger or fullness.

Table 8.1. Changes in VAS hunger and fullness ratings before, after tasting and after intake of an *ad-libitum* main course after consumption of a low energy control or high energy protein-rich preload with and without added MSG/IMP[1].

Rating		Control		Protein	
		MSG/IMP-	MSG/IMP+	MSG/IMP-	MSG/IMP+
Hunger	Pre-meal	51.85 ± 2.2	55.37 ± 1.9	54.37 ± 1.7	51.37 ± 1.9
	Taste	57.74 ± 2.1[a,b]	59.59 ± 1.7[a,b]	58.22 ± 2.3[a]	53.52 ± 1.5[a]
	Post-meal	12.37 ± 1.5	12.44 ± 0.8	10.11 ± 0.9	12.19 ± 1.1
Fullness	Pre-meal	47.59 ± 2.4	40.96 ± 1.9	44.04 ± 1.6	47.67 ± 2.6
	Taste	44.30 ± 2.4[c]	40.63 ± 2.1	40.30 ± 2.3[c]	47.81 ± 2.3
	Post-meal	82.11 ± 1.3	80.63 ± 2.5	82.59 ± 1.3	80.81 ± 1.3

[1] All data are mean ±SEM. All data were analysed using two-way repeated measures ANOVA with pair-wise analyses assessed using Bonferroni adjusted comparisons. [a] Statistically significantly different from pre-meal, main effect of time ($P = 0.01$). [b] Statistically significantly different from protein, interaction between MSG/IMP and protein ($P = 0.05$). [c] Statistically significantly different from MSG/IMP+, main effect of MSG/IMP ($P = 0.05$)

8.3.3 Sensory evaluations of the preload and test meal

The MSG/IMP+ preloads were formulated to increase pleasantness and savouriness compared to control and protein-rich conditions. Sensory assessments confirmed that MSG/IMP+ soups were significantly more pleasant (P=.001), salty (P=.001) and stronger tasting (P=.002) than MSG/IMP- conditions. The protein-rich and control soups were perceived to be similarly pleasant and not aversive (Table 8.2). There were no significant differences across conditions for ratings of how filling, salty, savoury or strong the main course was but a significant effect of protein condition was found for ratings of pleasantness of the test meal (P=.01) with lower pleasantness ratings made after initially consuming the added protein preloads as compared to the control conditions.

Table 8.2. VAS sensory ratings of low energy control and high energy protein-rich soups with (MSG/IMP+) or without (MSG/IMP-) added MSG/IMP.[a]

Rating	Control		Protein	
	MSG/IMP-	MSG/IMP+	MSG/IMP-	MSG/IMP+
Filling	52.55 ± 1.7	52.59 ± 1.7	51.51 ± 1.9	49.78 ± 1.5
Pleasant	60.41 ± 1.7	66.88 ± 1.5[b]	62.56 ± 1.5	70.48 ± 1.6[b]
Salty	43.26 ± 2.1	56.48 ± 1.9[b]	40.59 ± 2.5	50.89 ± 2.4[b]
Savoury	59.85 ± 1.8	62.37 ± 1.4	58.85 ± 1.5	63.37 ± 1.8
Strong	51.96 ± 2.4	60.67 ± 1.8[b]	50.33 ± 2.3	58.00 ± 1.8[b]
Sweet	53.33 ± 2.2	54.81 ± 2.4	54.92 ± 2.4	51.44 ± 1.9

[a] All data are mean ±SEM. All data was analysed using two-way repeated measures ANOVA. [b] Statistically significantly different from MSG/IMP- (Pleasant: P=0.001, Salty: P=0.001 and Strong: P=0.002) (N = 27)

8.4 Discussion

The main results suggest that the addition of MSG/IMP to a test soup reduced subsequent intake at a test lunch, both when the soup was low-energy or had added protein, while addition of energy in the form of protein also reduced test lunch intake. The addition of MSG/IMP also increased the degree to which extra energy (largely from added protein) was compensated for by reduced lunch intake. When the soup was consumed, Addition of MSG/IMP+ also stimulated hunger on first tasting the soup but when combined with protein resulted in a faster decrease in appetite during the subsequent test meal. These results imply a bi-phasic effect of MSG/IMP on appetite, with umami flavour stimulating hunger due to increased palatability but subsequently acting to enhance satiety. Although we hypothesised that effects of umami on satiety may be due to MSG/IMP acting as a signal for the satiating characteristics of protein, here effects of IMP/MSG were independent of effects of added protein.

The provision of a MSG/IMP+ preload acted to reduce subsequent test meal intake irrespective of the additional energy received in a protein-rich form. This effect of MSG+IMP combinations was not found previously (Carter, et al., 2011; Luscombe-Marsh, et al., 2009), possibly due to those studies using control preloads naturally high in MSG+IMP. Likewise, our earlier studies on MSG alone (Masic & Yeomans, 2013a, 2013b) only found enhanced satiety when MSG was consumed with added energy in the form of protein, but not carbohydrate only suggesting that the addition of IMP, which is naturally consumed in protein sources (Ninomiya, 1998), may have acted synergistically with MSG to amplify the umami signal and thus act as a more robust cue. Indeed, if MSG/IMP signalled the likely presence of protein, even in the low energy condition, this signal alone may have been sufficient to induce satiety, whereas a weaker MSG only cue needed actual protein to be present to be effective. It is important to note that the addition of protein decreased intake more strongly than did addition of MSG/IMP, (further confirming the potency of protein in inducing satiety; Poppitt, McCormack, & Buffenstein, 1998; Weigle et al., 2005). However, compensation for the energy consumed in the high energy protein-rich condition was better with the addition of MSG/IMP, suggesting that energy regulation was further improved with the addition of these flavour enhancers than providing protein alone. The lack of interaction between protein and MSG/IMP on intake here contrasted with effects

of MSG alone (Masic & Yeomans, 2013b). However, the combination of MSG/IMP and energy largely from protein generated evidence of stronger satiety during the *ad-libitum* meal in terms of changes in ratings of appetite (more rapid decrease in hunger and increase in fullness) than did addition of MSG/IMP+ in the low-energy soup, suggesting that MSG/IMP may have enhanced satiety to some extent, but not sufficient to result in suppression of lunch intake beyond the additive effects of protein and MSG/IMP.

As well as enhancing satiety, addition of MSG/IMP increased flavour pleasantness and increased rated hunger on tasting the soup, (the appetizer effect; Yeomans, 1996), irrespective of the addition of energy largely as protein. This is the first study to report a clear appetizer effect of MSG/IMP, although appetizing effects of flavours associated with the presence of MSG have been reported (Yeomans, et al., 2008). Despite an enhancement of pleasantness in the MSG/IMP+ conditions giving rise to an appetizer effect (Yeomans, 1996), no significant differences were noted in eating rates between the conditions. This effect may be more subtle and was not detected as the baseline eating rate was high due to the nature of the preload (as semi-liquids tend to be consumed at a faster rate than solids (Zijlstra, Mars, de Wijk, Westerterp-Plantenga, & de Graaf, 2008a)). A pronounced increase in saltiness and strength of flavour was observed in MSG/IMP+ soups but no influence of savoury was evident in line with our previous experiments with MSG (Masic & Yeomans, 2013a, 2013b). This may be due to the ambiguity surrounding the 'savoury' label for untrained assessors. It is also noteworthy that whereas addition of MSG/IMP increased soup pleasantness, addition of energy in the form of protein tended to reduce soup pleasantness, yet both manipulations subsequently increased satiety.

There was some suggestion that women may have been more responsive to the appetizing effects of MSG/IMP+ preloads as hunger was maintained more strongly during preload intake by women than men. However, men felt hungrier 45 minutes after preload intake and early on during the *ad-libitum* meal than women after MSG/IMP+ preloads. This suggests that the women may have been more sensitive to the bi-phasic effect of MSG/IMP, maintaining higher hunger during preload intake due to palatability but then reducing hunger and maintaining fullness more over the post-ingestive period,

although the imbalance in numbers between men and women mean this has to be interpreted with caution.

A potential way of further dissociating the potential for IMP as a protein cue would be to repeat this design using guanosine 5'-monophosphate (GMP) which also enhances MSG-induced umami flavour (Jinap & Hajeb, 2010) but is typically found in low protein foods (Yamaguchi & Ninomiya, 2000). Additional research should also explore the gastric effects of ingestion of MSG, IMP and GMP on the generation of satiety as peak effects are seen after 45 minutes (Masic & Yeomans, 2013a), potentially implicating the gut-based glutamate receptors (Kondoh & Torii, 2008; Niijima, 2000; Tome, et al., 2009). This may also determine whether added MSG/IMP encourages slower gastric emptying in line with effects of protein (Tome, et al., 2009).

Overall, combining MSG and IMP in a soup preload acted to both increase flavour and immediate appetite and reduce subsequent *ad-libitum* test meal intake irrespective of the macronutrient content of the soup. This is the first study to report a biphasic action of MSG/IMP, and implies that increased liking at one eating occasion can lead to better regulation at the next.

8.5 Author's contributions to the manuscript

MY and UM designed the research, UM conducted all experimental work and data collection. UM conducted the data analysis and manuscript writing with contributions from MY. UM had primary responsibility for the final content. Neither UM nor MY had any conflict of interest in the data collection, analysis or writing up of the study.

8.6 Summary of key findings and directions for future research

- The main findings suggest that the addition of MSG and IMP to a fixed portion of soup can act to reduce subsequent intake of a separate course
- This was also found with the addition of energy in the form of protein but no interaction between MSG/IMP and protein was evident
- Appetite ratings were not found to be influenced by added MSG/IMP; participants did not rate hunger or fullness as lower after added MSG/IMP

conditions despite consuming less at the subsequent course however, when MSG/IMP was combined with added protein rated hunger recovery was slower

- This indicates that MSG/IMP may be a useful means of controlling appetite although future research is required to assess whether such an effect would occur in a high energy carbohydrate source or with added umami in the form of GMP which is found predominantly in plant sources

- Future research should also aim to assess the effects of MSG on post-gastric satiety mechanisms and hormones to further understand the processes influencing appetite control

Appendix 8.1. Graphical depiction of the study design employed.
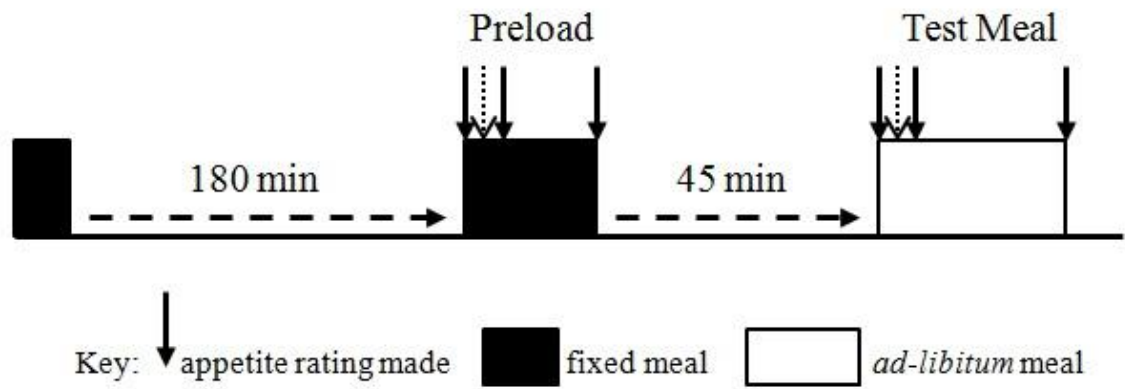
8.7 References

Bellisle, F. (1999). Glutamate and the UMAMI taste: sensory, metabolic, nutritional and behavioural considerations. A review of the literature published in the last 10 years. *Neuroscience and Biobehavioral Reviews, 23*(3), 423-438.

Bellisle, F., Monneuse, M. O., Chabert, M., Larue-Achagiotis, C., Lanteaume, M. T., & Louis-Sylvestre, J. (1991). Monosodium glutamate as a palatability enhancer in the european diet. *Physiology & Behavior, 49*(5), 869-873.

Bertenshaw, E. J., Lluch, A., & Yeomans, M. R. (2008). Satiating effects of protein but not carbohydrate consumed in a between-meal beverage context. *Physiology & Behavior, 93*(3), 427-436. doi: 10.1016/j.physbeh.2007.09.014

Carter, B. E., Monsivais, P., Perrigue, M. M., & Drewnowski, A. (2011). Supplementing chicken broth with monosodium glutamate reduces hunger and desire to snack but does not affect energy intake in women. *British Journal of Nutrition, 106*(9), 1441.

Chaudhari, N., Pereira, E., & Roper, S. D. (2009). Taste receptors for umami: the case for multiple receptors. *American Journal of Clinical Nutrition, 90*(3), 738S-742S. doi: 10.3945/ajcn.2009.27462H

Dohm, F.-A., Cachelin, F. M., & Striegel-Moore, R. H. (2005). Factors That Influence Food Amount Ratings by White, Hispanic, and Asian Samples. *Obesity, 13*(6), 1061-1069.

Essed, N. H., van Staveren, W. A., Kok, F. J., & de Graaf, C. (2007). No effect of 16 weeks flavor enhancement on dietary intake and nutritional status of nursing home elderly. [doi: 10.1016/j.appet.2006.06.002]. *Appetite, 48*(1), 29-36.

Fischer, K., Colombani, P. C., & Wenk, C. (2004). Metabolic and cognitive coefficients in the development of hunger sensations after pure macronutrient ingestion in the morning. [doi: 10.1016/S0195-6663(03)00116-8]. *Appetite, 42*(1), 49-61.

FSA. (2006). *FSA Nutrient and Food Based Guidelines for UK Institutions*. London.

Garcia-Bailo, B., Toguri, C., Eny, K. M., & El-Sohemy, A. (2009). Genetic Variation in Taste and Its Influence on Food Selection *OMICS: A Journal of Integrative Biology, 13*(1), 69-80. doi: 10.1089/omi.2008.0031

Herman, C. P., & Polivy, J. (2008). External cues in the control of food intake in humans: The sensory-normative distinction. [doi: 10.1016/j.physbeh.2008.04.014]. *Physiology & Behavior, 94*(5), 722-728.

Ikeda, K. (1908). On a New Seasoning. *Journal of Tokyo Chemistry Society, 30*, 820-836.

Jinap, S., & Hajeb, P. (2010). Glutamate. Its applications in food and contribution to health. *Appetite, 55*(1), 1-10. doi: 10.1016/j.appet.2010.05.002

Kondoh, T., & Torii, K. (2008). MSG intake suppresses weight gain, fat deposition, and plasma leptin levels in male Sprague-Dawley rats. . *Physiology & Behavior, 95*, 135–144.

Kurihara, K. (2009). Glutamate: from discovery as a food flavor to role as a basic taste (umami). *American Journal of Clinical Nutrition, 90*(3), 719S-722S. doi: 10.3945/ajcn.2009.27462D

Loliger, J. (2000). Function and importance of glutamate for savory foods. *Journal of Nutrition, 130*(4), 915S-920S.

Luscombe-Marsh, N. D., Smeets, A. J. P. G., & Westerterp-Plantenga, M. S. (2008). Taste sensitivity for monosodium glutamate and an increased liking of dietary protein. *British Journal of Nutrition, 99*(4), 904-908. doi: 10.1017/s000711450788295x

Luscombe-Marsh, N. D., Smeets, A. J. P. G., & Westerterp-Plantenga, M. S. (2009). The addition of monosodium glutamate and inosine monophosphate-5 to high-protein meals: effects on satiety, and energy and macronutrient intakes. *British Journal of Nutrition, 102*(6), 929-937. doi: 10.1017/s0007114509297212

Masic, U., & Yeomans, M. R. (2013a). Does monosodium glutamate interact with macronutrient composition to influence subsequent appetite? *Physiology & Behavior, 116–117*(0), 23-29. doi: http://dx.doi.org/10.1016/j.physbeh.2013.03.017

Masic, U., & Yeomans, M. R. (2013b). How do macronutrients interact with monosodium glutamate to affect subsequent appetite and intake? . *British Journal of Nutrition, under review*.

Mathey, M.-F. A. M., Siebelink, E., de Graaf, C., & Van Staveren, W. A. (2001). Flavor Enhancement of Food Improves Dietary Intake and Nutritional Status of Elderly Nursing Home Residents. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 56*(4), M200-M205. doi: 10.1093/gerona/56.4.M200

Naim, M., Ohara, I., Kare, M. R., & Levinson, M. (1991). Interaction of MSG taste with nutrition: Perspectives in consummatory behavior and digestion. . *Physiology & Behavior, 49*, 1019–1024.

Niijima, A. (2000). Reflex Effects of Oral, Gastrointestinal and Hepatoportal Glutamate Sensors on Vagal Nerve Activity. *The Journal of Nutrition, 130*(4), 971.

Ninomiya, K. (1998). Natural occurrence *Food Reviews International, 14*(2), 177-211.

Rolls, B. J., Dimeo, K. A., & Shide, D. J. (1995). Age-related impairments in the regulation of food intake. *The American Journal of Clinical Nutrition, 62*(5), 923-931.

Simpson, S. J., & Raubenheimer, D. (Eds.). (2000). *Geometric models of macronutrient selection*. Boca Raton: CRC Press.

Stunkard, A. J., & Messick, S. (1985). The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. . *Journal of Psychosomatic Research, 29*, 71-83.

Tome, D., Schwarz, J., Darcel, N., & Fromentin, G. (2009). Protein, amino acids, vagus nerve signaling, and the brain. *American Journal of Clinical Nutrition, 90*(3), 838S-843S. doi: 10.3945/ajcn.2009.27462W

Ventura, A. K., Beauchamp, G. K., & Mennella, J. A. (2012). Infant regulation of intake: the effect of free glutamate content in infant formulas. *The American Journal of Clinical Nutrition, 95*(4), 875-881. doi: 10.3945/ajcn.111.024919

Wansink, B., Painter, J. E., & North, J. (2005). Bottomless bowls: why visual cues of portion size may influence intake. [10.1038/oby.2005.12]. *Obes. Res., 13*, 93-100.

Weigle, D. S., Breen, P. A., Matthys, C. C., Callahan, H. S., Meeuws, K. E., Burden, V. R., & Purnell, J. Q. (2005). A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations. *The American Journal of Clinical Nutrition, 82*(1), 41-48.

Williams, E. J. (1949). Experimental designs balanced for the estimation of residual effects of treatment. *Australian Journal of Scientific Research, Series A*(2), 149-168.

Yamaguchi, S., & Ninomiya, K. (2000). Umami and Food Palatability. *The Journal of Nutrition, 130*(4), 921.

Yamamoto, K., & Ishimaru, Y. (2013). Oral and extra-oral taste perception. *Seminars in Cell & Developmental Biology, 24*(3), 240-246. doi: http://dx.doi.org/10.1016/j.semcdb.2012.08.005

Yeomans, M. R. (1996). Palatability and the Micro-structure of Feeding in Humans: the Appetizer Effect. *Appetite, 27*(2), 119-133.

Yeomans, M. R. (2000). Rating changes over the course of meals: what do they tell us about motivation to eat? *Neuroscience & Biobehavioral Reviews, 24*(2), 249-259. doi: http://dx.doi.org/10.1016/S0149-7634(99)00078-0

Yeomans, M. R., & Bertenshaw, E. J. (2008). Appetite and food intake: a human experimental perspective. *Appetite and food intake: behavioral and physiological considerations. Boca Raton, FL: Taylor and Francis*, 61-80.

Yeomans, M. R., Blundell, J. E., & Leshem, M. (2004). Palatability: response to nutritional need or need-free stimulation of appetite? *British Journal of Nutrition, 92*, S3-S14. doi: 10.1079/bjn20041134

Yeomans, M. R., Gould, N. J., Mobini, S., & Prescott, J. (2008). Acquired flavor acceptance and intake facilitated by monosodium glutamate in humans. *Physiology & Behavior, 93*(4-5), 958-966. doi: 10.1016/j.physbeh.2007.12.009

Zijlstra, N., Mars, M., de Wijk, R. A., Westerterp-Plantenga, M. S., & de Graaf, C. (2008). The effect of viscosity on ad libitum food intake. [10.1038/sj.ijo.0803776]. *Int. J. Obes., 32*, 676-683.