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

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MSG and protein liking

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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 (mean age: 22; BMI: 23) consumed either their habitual breakfast (baseline), a low protein breakfast (breakfast meal with low protein milk and milkshake) or a high protein breakfast (breakfast meal with high protein milk and milkshake) on three different days, and then evaluated the acceptability of umami (MSG), salty (NaCl) or sweet (Acesulphame K) tastes at low or high concentrations in a soup context at lunchtime. Participants also completed a habitual protein intake questionnaire (39-item protein Food Frequency Questionnaire). Liking for all tastes was higher on the low than on the high protein day, and NaCl and Acesulphame K were liked less on both protein manipulation days when compared to the no added flavour control. 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 taste when in protein deficit. Overall, these findings suggest that liking for high MSG concentrations may be moderated by nutritional need in high protein consumers.

Key words: MSG, protein, liking, taste, flavour, deprivation

1. Introduction

Protein is an essential macronutrient predominantly consumed in savoury tasting foods [1-3]. 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 protein needs have not been met [4]. 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 [5]. This suggests that the taste system may have 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 particularly linked to the detection of protein in the diet [2, 6-9] and may influence selection of protein-rich foods 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 [10, 11] which has remained constant over time and across populations compared to other macronutrients [4, 11, 12]. This may be related to the body's efficiency in regulating protein feedback [6, 13] due to the limited availability of carbohydrate and fat in human evolutionary history [14, 15]. 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 [4, 14, 16-18]. 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 [19] and children [20] 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 [21] 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 and familiarity with umami concentrations in foods. As the malnourished humans tested had previously been exposed to animal protein, which is abundant in umami [22], their familiarity, 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 [2]. However, as primates who consume low amounts of protein subsist on predominantly plant-based diets, their familiarity with and 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. 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 [23] 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 [24-26] and protein conditioned flavour preferences have been reported to be eliminated by a protein preload, but not after an equicaloric carbohydrate preload [27]. Such flavour preferences required very few pairings of novel flavoured protein-rich food when in mild protein need [24-26], 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. It is also not clear whether familiarity with the concentrations generally consumed can account for the differences found in liking. 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 (FFQ)) 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 [28-30]) or specifically the sodium found in MSG (saltiness). Familiarity ratings as well as baseline flavour assessments were also taken before acute

protein manipulations. It was hypothesised that (1) MSG would be optimally preferred when participants were in an acute protein deficit with stronger concentrations of MSG being most preferred (this was manipulated in the test sessions); (2) rated pleasantness of strong concentrations of MSG would be higher in naturally high protein consumers based on protein status derived from habitual protein intake (not manipulated in test sessions) as compared to naturally low protein consumers; (3) this would particularly be evident when these naturally high protein consumers were in acute protein deficit.

2 Method

2.1 Design

Acute effects of protein intake on liking for tastes were tested within-participant using a two way design combining acute protein intake (a baseline, low protein or high protein breakfast) with evaluations of liking for 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) or nothing added (control). Additional influences of habitual protein intake were assessed by testing the effects of participant's habitual self-reported protein consumption using a protein FFQ (between-participants) on taste responses. Breakfast conditions were balanced using a Williams square design [31]. Please see Figure 1 for a schematic of the study design employed.

2.2 Participants

Twenty four healthy weight 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 an online 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 the Three Factor Eating Questionnaire restraint scale [32] 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 [33-35]. Participants who wished to take part were provided an Information Sheet which stated that the study was assessing the effects of 'food on mood'. A cover story was necessary

to ensure participants did not respond in line with the experimental manipulations. Written, informed consent was obtained before taking part and participants were paid £10 or were awarded credits in a participant pool scheme upon completion of all sessions.

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 pleasantness 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.

2.3 Test Food

2.3.1 Breakfasts:

All nutritional information for the breakfasts can be found in Table 1. 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 [23]. 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 [36].

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 were formulated to be hedonically neutral and low in naturally occurring MSG (for soup formulation see [37, 8, 9]). The sample flavours consisted of no added flavour for the control sample, 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 Acesulphame K or 0.01g/100g strong Acesulphame K) for the sweet sample. These concentrations were chosen in line with those

commercially available in soups and used in previous research [2, 38, 39]. All nutritional information can be seen in Table 2.

Both breakfast shakes and soup flavour conditions were subject to initial pilot testing in a separate group of participants to ensure equal acceptability and adequate taste enhancement across conditions. This involved 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, Acesulphame K). Mean pilot study ratings indicated no significant differences in sensory ratings between protein shakes including equivalent pleasantness assessments ($F(1,11) = 0.37, p = 0.56$). Soup assessments across strong and weak flavour samples indicated an enhancement by MSG of saltiness ($F(1,11) = 6.81, p = 0.02$) and strength of flavour ($F(1,11) = 12.90, p = 0.004$). Equally, an increase in saltiness ($F(1,11) = 12.30, p = 0.005$) across conditions was found in NaCl conditions and enhanced sweetness across concentrations in the Acesulphame K samples ($F(1,11) = 12.74, p = 0.004$) as well as increased familiarity in weak compared to strong ($F(1,11) = 11.57, p = 0.006$) Acesulphame K samples. All other sensory ratings including those of pleasantness were found to be non-significant across conditions (see supplementary Table 1).

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. Ratings were completed using digital Visual Analogue Scales (VAS) and stored by the SIPM. They 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. All VAS ratings were randomized across trials and sessions.

A tray containing seven soup samples (strong and weak concentrations of MSG, NaCl, and Acesulphame 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.

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 [40]. 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 focus specifically on 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 purposeful 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 because 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. This was based on average portion size information [40]. To allow for a more concrete estimation, participants were provided with a portion size estimation sheet which provided pictures of approximate portion sizes based on hand symbols (also provided on the sheet was ounce measurements).

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.

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 asked to consume their normal breakfast and 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 0900h-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. Sessions were conducted with a minimum four and maximum seven-day wash out period between test day sessions.

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 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. Please see Figure 1 for a study day schematic.

2.7 Data Analysis

In the short-term protein manipulation, sensory ratings of the flavour (MSG, NaCl and Acesulphame K) samples were transformed to examine the change in the sensory rating (*e.g.* pleasantness) of the relevant flavour (control, MSG, NaCl or Acesulphame 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 (control was contrasted with no inclusion of concentration over the low or high protein manipulation). The change from baseline scores were calculated and used to account for any

differences between the protein manipulations to understand how the manipulation itself influenced ratings (therefore taking individual differences at baseline in rating into account). As these ratings most likely influence palatability and pleasantness ratings, change from baseline scores were required to account for this individual variation. 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 Acesulphame K or strong Acesulphame K) were conducted to determine any differences in these assessments. This was to assess hypothesis 1 relating to acute protein deficit on the pleasantness of MSG relative to other flavours and concentrations.

The influence of habitual protein intake in the diet (long-term protein status) on the rated pleasantness of the relevant sample tastes (control, MSG, NaCl or Acesulphame K) and concentrations (weak or strong for MSG, NaCl and Acesulphame K) were assessed using simple linear regression analyses for each individual taste 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) to assess hypothesis 2. Additional multiple regression analyses were also conducted for each individual taste and concentration including familiarity alongside protein status as a separate block in the multiple regression analysis to determine whether familiarity influenced pleasantness ratings more than protein status alone. Familiarity was assessed as a separate block due to the robust effects of familiarity on influencing pleasantness ratings [41-43] and as an additional means of addressing prior experience with the taste.

The influence of long-term protein status was further explored over low and high protein manipulation test days to determine whether protein status influenced pleasantness 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; hypothesis 3). 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 Acesulphame K) and concentration (weak or strong) and comparing low protein to high protein manipulation days using a one tailed t-test. 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 whilst change from baseline desire for sweet or savoury were examined to determine whether protein manipulation day (low or high) influenced these assessments in 2x2 ANOVA. Where there were cases of violated sphericity, Greenhouse Geisser values ($\epsilon = <0.75$) were used. If Greenhouse Geisser ($\epsilon = >0.75$), 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. All data are mean \pm SEM.

3. Results

Hunger ($F(2,46) = 0.82, p = 0.85$) and fullness ($F(2,46) = 1.10, p = 0.34$) evaluations before soup tasting sessions were found to be non-significant, indicating that participants arrived hungry; $m \pm SEM: 61.8 \pm 2.1$ and not very full; $m \pm SEM: 33.6 \pm 1.3$. There were also no significant differences in thirst ($F(2,46) = 1.33, p = 0.28$) or nausea ($F(1,5,33.7) = 0.51, p = 0.60$) assessments before each tasting session; participants arrived relatively thirsty; $m \pm SEM: 56 \pm 0.1$ and were not nauseous; $m \pm SEM: 10 \pm 1.1$ before the sessions. Change from baseline desire for sweet foods was not significantly different irrespective of protein manipulation ($F(1,23) = 1.20, p = 0.28$) whilst change from baseline desire for savoury was significantly higher on low as compared to high protein days (Main effect of test day: $F(1,23) = 5.48, p = 0.03$).

3.1 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 ($F(1,23) = 4.66, p = 0.04$), indicating that the change in pleasantness was higher for all flavours on the low protein day than on the high protein day relative to the baseline test day (Figure 2). Flavour samples also differed significantly in rated pleasantness (main effect of flavour: $F(6,138) = 3.37, p = 0.004$) with strong NaCl samples ($F(1,23) = 7.63, p = 0.01, r=0.50$) and weak Acesulphame K samples ($F(1,23) = 5.32, p = 0.03, r=0.43$) rated as more aversive on both protein manipulation days when compared to the control sample. Strong MSG samples were rated as most pleasant in flavour over all other flavour samples. No test day*flavour interaction was

evident ($F(6,138) = 1.01, p = 0.43$). Raw sensory ratings by day are shown in Supplementary Tables 2-4.

3.2 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 find the stronger concentrations of MSG more pleasant. There was no significant effect of a higher protein status alone increasing pleasantness for MSG on baseline sessions for weak ($b = 0.03, t(23) = 0.39, p = 0.70$) or strong ($b = 0.02, t(23) = 0.19, p = 0.85$) concentrations or on low protein (weak concentration: $b = 0.01, t(23) = 0.19, p = 0.85$; strong concentration: $b = 0.05, t(23) = 0.70, p = 0.49$) or high protein (weak concentration: $b = 0.06, t(23) = 1.04, p = 0.31$; strong concentration: $b = -0.06, t(23) = -0.98, p = 0.34$) test days (See supplementary Table 5 for all regression results). The influence of protein status on other sensory ratings of MSG was also not found to be significant. However, 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 Acesulphame K and for control conditions after the baseline and high protein test days but not 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. 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 Acesulphame K) but was not found to significantly differ between days for the other flavour samples. As protein status increased, pleasantness ratings were found to increase after strong concentrations of MSG on low protein test days as compared to high protein test days ($t(23) = -0.012$, $p = 0.05$; Figure 3). Also, those with higher protein status disliked strong MSG concentrations on the high protein test day. Protein status did not influence liking across weak concentrations of MSG on low as compared to high protein test days ($t(23) = -0.84$, $p = 0.79$) and there were no other significant differences found across all other tastes or concentrations.

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 Acesulphame 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 [21] 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 despite desire for savoury ratings being higher after the low protein manipulation irrespective of hunger. An initial increase in desire for savoury on the low protein manipulation day may be understood in terms of an innate desire for foods which predict protein [4, 14, 16-18] as savoury foods contain more reliable and higher quantities of protein than sweet foods [1-3]. 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 Acesulphame 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 [44] and as protein has been shown to be particularly satiating [45-47].

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 were given a large protein load. This is similar to the findings observed in primates [21], with primates who ingested higher quantities of animal protein shown to prefer stronger concentrations of MSG. However, contrary to these findings, 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 [14].

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. This may be due to the known effects of familiarity on liking [41-43]. This was 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.

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 individuals. Indeed, the distinction between behaviours influenced by 'liking' and 'wanting' is well documented in the literature [48-51] with researchers showing approach behaviours to be influenced by 'wanting' with an absence of 'liking' [52] and behaviours related to 'liking' without 'wanting' [53]. Thus, it may be that the liking response observed may not translate to an increase in free choice consumption of high MSG containing foods, particularly as liking ratings in taste test paradigms may give biased estimations of food palatability [54]. Some research also suggests that receiving a high protein or carbohydrate load differentially influences the brain areas associated with 'wanting' and 'liking' [55], with participants satiated on high protein loads found to decrease 'liking' task related brain signalling to food items in the putamen whilst high carbohydrate loads decreased 'wanting' responses to food

items in the hypothalamus. Thus, further research is required to ascertain whether the increase in liking found in high protein consumers translates to increased intake in a free choice paradigm as participants were not fed to satiation. It must also be noted that there may have been a potential for errors of omission and under-reporting in the protein FFQ as has frequently been found in questionnaire designs [56] thus the effects reported must also be treated with caution as they were found to be subtle. Additionally, as the high protein breakfast was matched for energy density and volume, the preload also contained a higher fat content than the low protein breakfast. Although the protein difference was 50%, the fat difference was 28% between the high and low protein preloads, thus it may be argued that the effects found here may additionally relate to a difference in fat. Future research is required to further isolate the effect of protein alone, while keeping all other macronutrients constant across conditions.

Overall, acutely manipulating protein need may increase desire for savoury foods and more generally increases liking of umami flavours. This liking is not predicted by habitual protein intake alone but when habitual intake is compared across acute protein manipulations, individuals who generally consume more protein in their diet show increased liking for a strong concentration of MSG when in acute protein deficit but lower liking after a high protein load. Future research is necessary to explore the role of acute protein deprivation and general protein intake on food choice and consumption to assess whether food selection practices are influenced by protein-need with MSG added and no MSG foods; further broadening our understanding about the predictive nature of flavour pleasantness and nutritional need.

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Author's Contributions

All experimental work including the study design, data collection, analysis of the data and writing of the report was carried out by the authors. UM designed the study and drafted the manuscript. UM and MRY analysed the data, MRY edited and revised the manuscript and approved the final version.

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Table 1. Nutritional composition of high and low protein breakfasts per 100g

	High Protein Shake	Low Protein Shake	High Protein Breakfast	Low Protein Breakfast
Carbohydrate (g)	15	28.1	66.1	74.8
Protein (g)	30.7	1.3	39.6	4.6
Fat (g)	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
Energy (kcal)	78.4	78.3	96.5	96.5
Energy (kcal)/portion	235.1	235	503.6	503.7
Total weight (g)/portion	300	300	222	222

Table 2. Nutritional composition of the soup preload per 100g

	Control
Carbohydrate (g)	3.1
Protein (g)	0.4
Fat (g)	1.6
Carbohydrate (% energy)	44
Protein (% energy)	6
Fat (% energy)	50
Energy (kcal)	28
Total weight (g)/portion	10

Table 3. Mean (\pm 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 $P < 0.05$.

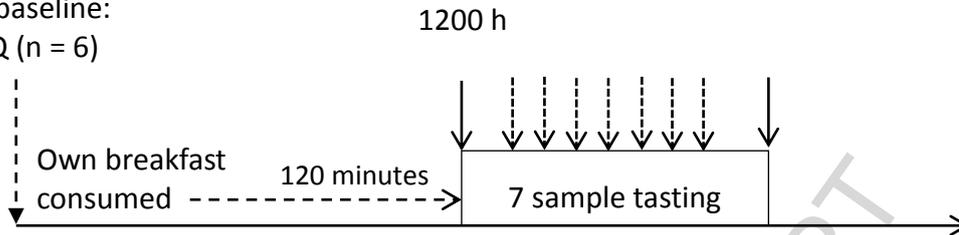
		Control	MSG		NaCl		Ace K	
			Low	High	Low	High	Low	High
Low Protein	Familiarity	10.17 \pm 5.4 ¹	8.25 \pm 5.2	21.75 \pm 6.5	3.71 \pm 5.2	-	11.08 \pm 6.3	1.29 \pm 5.5
	Pleasant	11.21 \pm 5.5 ¹	6 \pm 5.6	18.96 \pm 6.2	7.08 \pm 5.2	-	-2.46 \pm 5.3	-
	Salty	2.21 \pm 4 ¹	3.83 \pm 4.4	5.38 \pm 4.3	10.87 \pm 3.3	0.38 \pm 4.3	0.79 \pm 3.8	-
	Savoury	-0.42 \pm 4.6 ¹	2.88 \pm 4	5.63 \pm 4.5	5.12 \pm 3.8	-	6.25 \pm 5.4	-
	Strong	-2.54 \pm 4 ¹	8.83 \pm 4.6	8.08 \pm 4	8.83 \pm 4.6	-	0.04 \pm 3.9	1.29 \pm 4.2
	Sweet	-2.08 \pm 3.7 ¹	-4.13 \pm 3.7	-0.50 \pm 5.9	0.63 \pm 5	-	-5.58 \pm 4.6	-
High Protein	Familiarity	8.63 \pm 6.4 ¹	12.46 \pm 4.4	16.58 \pm 5.8	5.08 \pm 4.5	-	5.38 \pm 6	3.71 \pm 5.5
	Pleasant	2.17 \pm 5.8 ¹	6.46 \pm 4.9	7.29 \pm 5.8	2.75 \pm 4.7	-	-8.2 \pm 4.6	-

t			9			7.42±3.		3.04±4.
						6		1
Salty	1.42±4.3 ¹	7.42±4.3	1.21±4.3	7.58±4.6	7.58±3.	0.67±3	-	-
						6		3.54±5.
								3
Savoury	-3.58±5.2 ¹	6.13±4.6	-0.21±4.3	-0.04±4.1	-	-1.54±4.1	0.50±5.	
						1.21±3.		1
						8		
Strong	-5.79±4.3 ¹	11.67±5	0.33±4.8	13.92±4.	1.96±3.	-0.29±3.8	2.17±3.	
				8	8			7
Sweet	-	-5.67±6	6.58±4.9	-	-	-0.25±3.5	-	-
	5.67±5.03 ¹			2.04±5.2	1.88±4.		0.38±3.	
				2	5			3

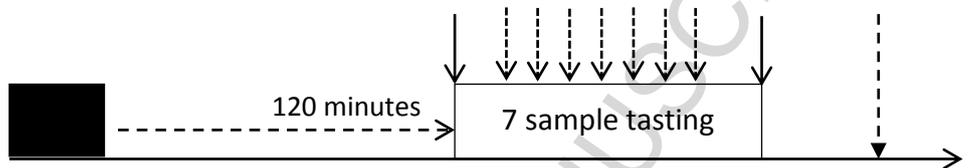
Figure 1. Graphical depiction of the study design employed. 18 participants completed the pFFQ (protein Food Frequency Questionnaire) after the trial and six completed the questionnaire 2-3 weeks before Baseline Day 1.

Baseline (Day 1)

Pre-baseline:
pFFQ (n = 6)



Study Day (Days 2 & 3)



Key: ↓ appetite rating made ■ High or low protein breakfast □ Taste test

Figure 2. Change from baseline pleasantness assessments (mm) of three flavours (Monosodium glutamate; MSG, Panel A, sodium chloride; NaCl, Panel B and Acesulphame 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.

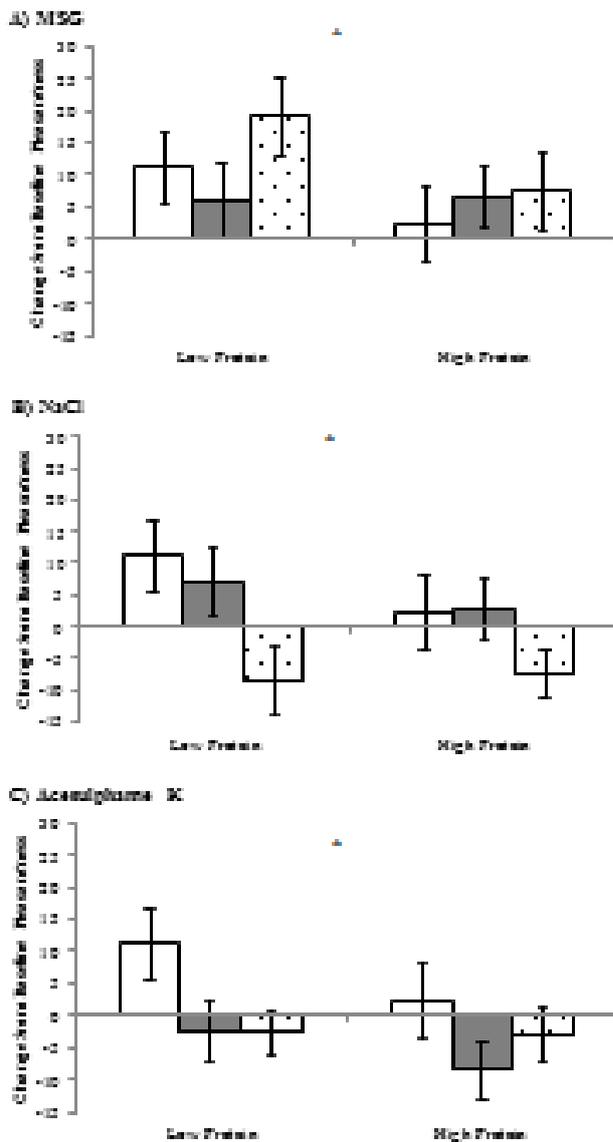
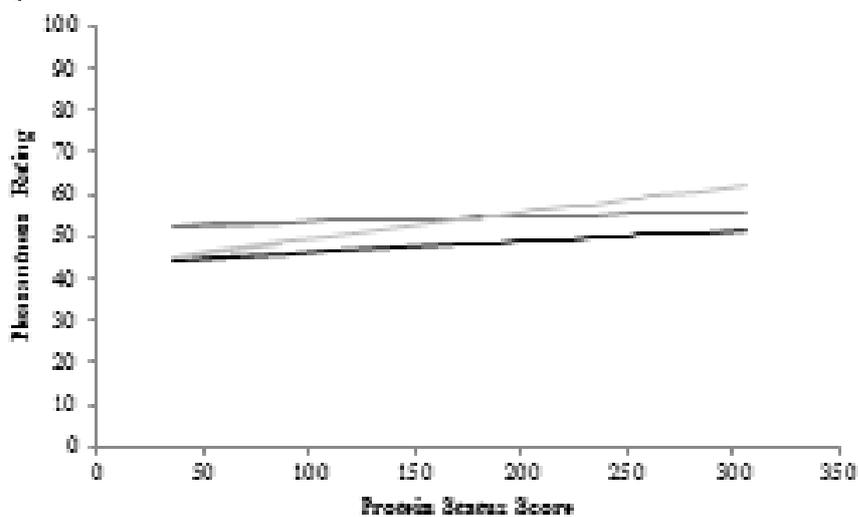
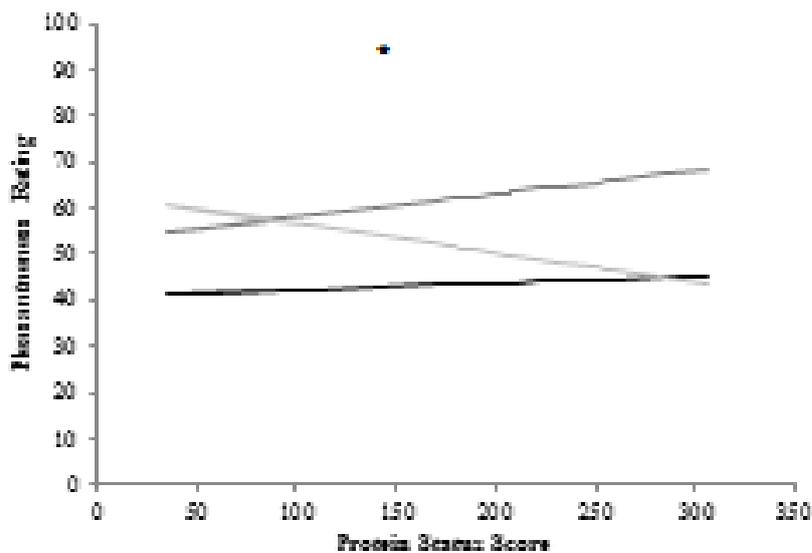


Figure 3. Pleasantness assessments (mm) of weak (Panel A) and strong (Panel B) concentrations of MSG soup samples after protein manipulations (baseline no manipulation (—), low protein breakfast (—) or high protein breakfast (—)) as a function of habitual protein consumption (Protein Status Score) using linear regression coefficients. Significant t-test difference on high concentration day between manipulation conditions $P=0.05$.

A) Low Concentration MSG



B) High Concentration MSG



Highlights

- Protein is well regulated in the diet and can be detected by the taste system
- Habitual protein use or acute protein deprivation may alter liking for protein-predictive tastes such as those experienced with strong cheese or animal protein
- Acute protein deprivation increases liking for umami, salty and sweet tastes
- High protein users like high MSG doses most when in acute protein deficit

ACCEPTED MANUSCRIPT