EFFECT OF PROTEIN SOURCE ON THE SUPPRESSION OF FOOD INTAKE IN YOUNG MEN

By

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A thesis submitted in conformity with the requirements for the degree of Master of Science
Graduate Department of Nutritional Sciences
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ABSTRACT

The research hypothesis was that the effect of protein on short-term satiety and food intake in humans depends on the source. Two experiments were conducted, in which young men consumed 180 kcal treatments. Food intake was measured 60 minutes later.

In experiment 1, subjects chose to arrive at either 9AM or 11AM. Control, intact soy, whey and egg proteins, as well as sucrose were given, and only whey and soy suppressed food intake. An interaction (p=0.04) was found between arrival time and treatment, where egg increased intake compared to control at 11AM, but had no effect at 9AM. In experiment 2, control, egg and whey were administered; only whey suppressed intake. No interaction was found (p=0.11), but intake following egg tended to be less for later arrival group (p=0.07).

In conclusion, the effect of protein on the feeding response is determined by its source, as well as time of testing.
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TABLE OF CONTENTS

List of Tables .................................................................................................................... vii
List of Figures ........................................................................................................................ ix
List of Appendices .................................................................................................................. x

Chapter 1. INTRODUCTION ................................................................................................. 1

Chapter 2. LITERATURE REVIEW

2.1 Introduction ...................................................................................................................... 5
2.2 Food Intake Regulation .................................................................................................... 5
  2.2.1 Energy Intake and Energy Balance ............................................................................ 6
  2.2.2 Macronutrient Intake and Energy Balance ............................................................... 6
  2.2.3 Proteins, Amino Acids and Peptides and Food Intake ............................................... 7
    2.2.3.1 Protein and Food Intake ...................................................................................... 7
    2.2.3.2 Protein Source and Food Intake .......................................................................... 12
    2.2.3.3 Peptides and Food Intake .................................................................................. 16
    2.2.3.4 Amino Acids and Food Intake ............................................................................. 17
  2.3 Mechanisms of Protein-Induced Satiety ....................................................................... 18
    2.3.1 Post-absorptive Signals ........................................................................................... 18
      2.3.1.1 Plasma Amino Acids: The Aminostatic Hypothesis .............................................. 19
      2.3.1.2 Brain Amino Acids: The Brain Neurotransmitter Hypothesis ............................ 20
      2.3.1.3 Post-Absorptive Hormonal Response ................................................................. 23
    2.3.2 Pre-absorptive Signals – The Role of the Gut .......................................................... 27
      2.3.2.1 Mechanoreceptors, Osmoreceptors and Chemoreceptors ................................ 27
      2.3.2.2 Gastrointestinal Hormones .............................................................................. 30
  2.4 Summary ......................................................................................................................... 34

Chapter 3. EXPERIMENTAL OUTLINE

3.1 Hypothesis ......................................................................................................................... 36
3.2 Objectives .......................................................................................................................... 36

Chapter 4. MATERIALS AND METHODS

4.1 Subjects ............................................................................................................................. 38
4.2 Experimental Design ......................................................................................................... 42
  4.2.1 Experiment 1 ............................................................................................................... 42
  4.2.2 Experiment 2 ............................................................................................................... 46
4.3 Experimental Procedure .................................................................................................... 49
  4.3.1 Experiment 1 ............................................................................................................... 50
  4.3.2 Experiment 2 ............................................................................................................... 51
Chapter 5. RESULTS AND DISCUSSION

Experiment 1: THE EFFECT OF PROTEIN SOURCE AND SUCROSE ON SUBJECTIVE APPETITE AND FOOD INTAKE

5.1. Results ........................................................................................................ 62
  5.1.1. Food and Water Intake ........................................................................ 62
  5.1.2. Average Appetite ................................................................................ 68
  5.1.3. Motivation to Eat ............................................................................... 70
  5.1.4. Physical Comfort ............................................................................... 75
  5.1.5. Palatability ......................................................................................... 76
  5.1.6. Sweetness ......................................................................................... 77
  5.1.7. Characteristics of Subjects in the Two Arrival Time Groups ............. 78
  5.1.8. Correlations ....................................................................................... 79

5.2. Discussion .................................................................................................... 81

Experiment 2: THE EFFECT OF PROTEIN SOURCE AND TIME OF ARRIVAL ON FOOD INTAKE

5.3. Results ........................................................................................................ 89
  5.3.1. Food and Water Intake ........................................................................ 89
  5.3.2. Average Appetite ............................................................................... 95
  5.3.3. Motivation to Eat ............................................................................... 97
  5.3.4. Physical Comfort .............................................................................. 101
  5.3.5. Palatability ....................................................................................... 102
  5.3.6. Sweetness ....................................................................................... 103
  5.3.7. Characteristics of Subjects in the Two Arrival Time Groups ............. 104
  5.3.8. Correlations ....................................................................................... 105

5.4. Discussion .................................................................................................... 107
LIST OF TABLES

Table 1: Experiment 1. Subject Characteristics ............................................................... 40
Table 2: Experiment 2. Subject Characteristics ............................................................... 41
Table 3: Experiment 1. Pre-Experiment Trials of Drink Sweetness and Palatability .... 44
Table 4: Experiment 1. Composition of Treatment Drinks For a Subject with 53 kg Fat Free Mass ........................................................................... 45
Table 5: Experiment 2. Pre-Experiment Trials of Drink Sweetness and Palatability .... 47
Table 6: Experiment 2. Composition of Treatment Drinks For a Subject with 53 kg Fat Free Mass ........................................................................... 48
Table 7: Experiment 1. Effect of Treatments on Food and Water Intake ....................... 65
Table 8: Experiment 1. Effect of Arrival Time on Food Intake for Individual Treatments ......................................................................................... 67
Table 9: Experiment 1. Effect of Treatment on Absolute Average Appetite Scores .... 69
Table 10: Experiment 1. Effect of Treatments on Change from Baseline Average Appetite Scores ............................................................................... 69
Table 11: Experiment 1. Effect of Treatments on Overall Desire Change from Baseline Scores (Marginal Means) ................................................................. 72
Table 12: Experiment 1. Effect of Treatments on Questions 1 to 4 Absolute Motivation to Eat Scores ...................................................................................... 73
Table 13: Experiment 1. Effect of Treatment on Questions 1 to 4 Change From Baseline Motivation to Eat Scores ................................................................. 74
Table 14: Experiment 1. Effect of Treatment on Change from Baseline Physical Comfort Scores ................................................................................................. 75
Table 15: Experiment 1. Palatability Ratings of Beverage and Pizza after Treatment.. 76
Table 16: Experiment 1. Characteristics of Subjects in the Two Arrival Time Groups 78
Table 17: Experiment 1. Correlations Between Dependent Measures ......................... 80
Table 18: Experiment 1. Effect of Treatment Order on Food Intake ............................. 88
Table 19: Experiment 2. Effect of Treatments on Food and Water Intake .......................... 92

Table 20: Experiment 2. Effect of Arrival Time on Differences in Food Intake for Individual Treatments ........................................................... 94

Table 21: Experiment 2. Energy Intake Prior to Test Days ...................................................... 94

Table 22: Experiment 2. Effect of Treatments on Absolute Average Appetite Scores . 96

Table 23: Experiment 2. Effect of Treatments on Change from Baseline Average Appetite Scores ........................................................................ 96

Table 24: Experiment 2. Effect of Treatment on Questions 1 to 4 Absolute Motivation to Eat Scores .......................................................................... 99

Table 25: Experiment 2. Effect of Treatment on Questions 1 to 4 Change from Baseline Motivation to Eat Scores ....................................................... 100

Table 26: Experiment 2. Effect of Treatment on Physical Comfort Scores ................. 101

Table 27: Experiment 2. Palatability Ratings of Beverage and Pizza After Treatment 102

Table 28: Experiment 2. Characteristics of Subjects in the Two Arrival Time Groups 104

Table 29: Experiment 2. Correlations Between Dependent Measures ...................... 106
LIST OF FIGURES

Figure 1. Experiment 1. Test Protocol ................................................................. 53

Figure 2. Experiment 2. Test Protocol ................................................................. 54

Figure 3. Test meal food intake: calories consumed from a pizza meal one hour after treatments ................................................................. 64

Figure 4. Effect of treatments on energy intake from the test pizza meal at two arrival times ........................................................................ 66

Figure 5. Perceived sweetness of treatments ....................................................... 77

Figure 6. Test meal food intake: calories consumed from a pizza test meal one hour after treatments ................................................................. 91

Figure 7. Effect of treatments on energy intake from the test pizza meal at two arrival times ........................................................................ 93

Figure 8. Perceived sweetness of treatments ....................................................... 103
LIST OF APPENDICES

APPENDIX

1. Sample Size Calculation ................................................................. 138

2. Screening Questionnaires ............................................................... 140
   Baseline Information Questionnaire ............................................... 141
   Food Acceptability List .................................................................. 142
   Eating Habits Questionnaire ......................................................... 143
   Outline of Participant's Role ............................................................ 144
   Consent Form .................................................................................. 146

3. Study Day Questionnaires ............................................................... 147
   Sleep Habits and Stress Factors Questionnaire ............................... 147
   VAS-Motivation to Eat .................................................................... 149
   VAS-Physical Comfort ................................................................... 150
   VAS-Palatability ............................................................................. 151
   VAS-Sweetness .............................................................................. 152

4. Food Diary ....................................................................................... 153
   Instructions ...................................................................................... 154
   Pre-session meal ............................................................................ 155
   Recording Sheet ............................................................................. 156

5. Composition of Protein Powders .................................................... 157

6. Pizza Composition .......................................................................... 159

7. Mood ............................................................................................... 161

8. Memory ............................................................................................ 176
CHAPTER 1.

INTRODUCTION
1. INTRODUCTION

The prevalence of obesity in North America has risen at an alarming rate over the past few decades. Health hazards include risk of hypertension, atherosclerotic heart disease and diabetes.

Despite a high incidence of obesity, North Americans are far from oblivious to the mass media that markets the newest diet and the best way to lose weight. Not only are they aware of the campaigns for thinness, they are buying into it and thus helping fuel a multi-billion-dollar industry. Diet fads have upheld a constant presence in advertising mediums since the 1960s, changing from high protein, to low fat, to high carbohydrates and back to high protein diets again. The popularity of the latest trend of high protein diets was illustrated with a cover story in TIME magazine’s November 1st 1999 issue that read “Low-Carb Diets: Meat loving bread-banning regimes are the rage. Do they work? Are they healthy? Here’s the skinny.” The article went on to describe some of the most popular high protein diets of the day, the number of books sold on the topic and the philosophies behind them.

These high protein diets receive so much attention because their use is supported by testimonials of obese people claiming weight losses that they could only dream of prior to their adherence to one of these programs. Because no scientifically sound investigation of the efficiency of these diets has been reported, more research is needed to understand the effects of protein on satiety in humans and to examine the role of high protein diets in weight loss and maintenance programs.

The scientific literature provides clear evidence that protein is more satiating in experimental animals and humans than either carbohydrate or fat. However, the majority
of the reports to date are based on studies conducted in experimental animals, in which the mechanisms of protein-induced food intake suppression, and the behavioural consequences, have been of the primary interest. Although protein source and composition have been shown to be factors affecting the feeding response of experimental animals, this aspect of protein induced-satiety has not been investigated in humans. In an effort to address the lack of human studies in this subject area, the objective of this research was to determine the effects of common dietary proteins on short-term (next meal) food intake of lean young men.
CHAPTER 2.

LITERATURE REVIEW
2. LITERATURE REVIEW

2.1. INTRODUCTION

Protein, and its relationship with satiety, is gaining more interest with the re-emergence of the popular "high protein" diets. The role of protein in food intake regulation has been studied extensively for many years in animal models, but less so in humans. The following literature review begins with a general discussion of food intake regulation, followed by a focus on the role of protein and its constituents in suppressing appetite. The next section describes pre- and post-absorptive mechanisms involved in protein satiety.

2.2. FOOD INTAKE REGULATION

The regulation of food intake is a complex process that involves physiological and psychological mechanisms. Metabolic and physiologic cues that arise from perception, ingestion, digestion, absorption and metabolism of nutrients may be responsible for the signaling that initiates satiation and terminates feeding (Anderson 1994a). Regulatory mechanisms have been investigated in small laboratory animals because of their small energy reserves compared to energy turnover and because their food consumption regulation is not obscured by sensory and cognitive cues to the extent that they are in humans (Flatt 1995). However, it is also essential to study these mechanisms in the human population because cognitive and sensory cues, such as thought, sight, smell and
taste of food, influence the beginning and end of a meal (Fedoroff 1997) as well as
physiologic and metabolic factors.

2.2.1. ENERGY INTAKE AND ENERGY BALANCE

Much of the past research on food intake has focused on the regulation of intake and
energy balance. This focus on energy balance is due mostly to evidence that shows that
experimental animals are capable of controlling and regulating energy intake to maintain
energy balance, even when requirements are altered from their normal state. For ex-
ample, exposure to cold (Kraly and Brass 1976), or to exercise (Collier et al. 1969), or
alterations in the energy density of diet (Brossardt et al. 1946), or in food availability and
choice (Ashley and Anderson 1975) result in rats making quantitative adjustments of
food intake, thus maintaining energy balance. Humans are also able to adjust their
energy intake to compensate for changes in their energy expenditure. This observation,
in addition to the recent increase in the prevalence of obesity, has led to considerable
research aimed at understanding factors that lead to energy imbalance.

2.2.2. MACRONUTRIENT INTAKE AND BALANCE

Animals regulate not only energy intake, but also macronutrient intake (Anderson et
al. 1994a). Specific regulatory mechanisms have been proposed for the regulation of
intake of the three macronutrients: protein, carbohydrates and fat (Anderson 1996).
These nutrients each have properties that may provide signals to the central nervous
system independent of their contribution to energy needs (Anderson 1984).
Of primary interest to the present research is the role of proteins and their digestion products - peptides and amino acids - on the feeding behaviour in humans. At present, little is known about the distinct roles of intact protein, hydrolysed protein and free amino acids in food intake regulation. Evidence for macronutrient specific food intake regulatory mechanisms is less clear in humans than in animal models. However, it has been proposed that these mechanisms do exist (Anderson 1979; Anderson et al. 1986).

2.2.3. PROTEINS, AMINO ACIDS, PEPTIDES AND FOOD INTAKE

Proteins are made up of amino acids, which are the building blocks of endogenous proteins and nitrogen bearing molecules. Digestive enzymes can hydrolyse peptide bonds that form the polypeptide chains of protein, producing shorter peptide fragments, ranging from 2 to 4 free amino acids. While peptidases break most peptides into free amino acids for absorption, small amounts of peptides are absorbed in the small intestine by transport systems independent of and faster than the amino acid uptake system (Fairclough et al. 1980; Grimble et al. 1986, 1987).

2.2.3.1. Protein and Food Intake

Protein is the source of indispensable amino acids for all animals; thus, it is not surprising to find strong evidence that animals are able to regulate their overall protein consumption. Animals are able to adjust consumption from dietary choices to maintain protein intake within a range consistent with optimal growth (Anderson and Li 1987; Leathwood and Ashley 1983). For example, when given the choice between 0% and 18% casein diets, rats ate almost all their meals from the diet containing protein. When
given the choice between two diets (i.e. 0% and 50%, 15% and 55% or 25% and 65% protein), they regulated their protein intake at a constant proportion of their food consumed, averaging 33% to 35% of their dietary energy (Musten et al. 1974). Although this amount of protein consumed was far greater than the 20% of dietary energy required for growing rats, it has been hypothesized that the protein intake regulatory mechanism in rats evolved to ensure that their protein needs were met. The ability of rats to regulate protein intake is also evidenced by their reduced consumption in diets that are excessively high or low in protein or of diets unbalanced or deficient in essential amino acids (Anderson et al. 1988).

Rats regulate protein intake not only on a day-to-day basis, but also on a meal-to-meal basis (Johnson et al. 1979). When rats were given a protein preload and then presented with a choice between a low-protein/high-carbohydrate diet and a high-protein/low-carbohydrate diet, rats selected more of the high carbohydrate diet and less of the high protein diet (Anderson and Li 1983).

In addition to being a regulated component of the diet, protein is the most satiating of the three macronutrients in humans and in animals (Anderson et al. 1986; Trigazis et al. 1997). Protein suppresses food intake beyond its energy content alone (Li and Anderson 1982; Trigazis et al. 1997). This effect of increased satiety also appears to depend on the source of protein. In rats, food intake suppression is readily observed when rats are fed high protein diets or imbalanced amino acid diets or are gavaged with proteins before given access to food (Anderson 1979). As well, it has been shown that protein source modifies the feeding response. For example, in rats, food intake
suppression is greatest following consumption of whey, followed by egg-albumin, soy and casein (Morgan 1998; Cochi 2001).

The effect of protein on food intake and satiety has received little investigation in humans. Short-term studies to date, however, have generally established an increased satiating capacity of protein-rich foods over foods rich in either fat or carbohydrate (Hill and Blundell 1987; Porrini et al. 1995, 1997; Rolls et al. 1988). For example, when adolescent girls were given breakfasts that contained different amounts of protein (9 g, 15 g or 24 g), the breakfast with the highest protein content suppressed energy intake to a greater extent than the lower protein meals over the remainder of the day (Ohlson et al. 1965).

A number of studies have shown that a high-protein lunch suppresses subsequent food intake more than a protein-poor lunch of similar energy. Healthy men and women, fed a protein-rich lunch, defined as containing approximately three times the recommended minimum requirement, suppressed caloric intake by 76 kcal compared to a protein-poor lunch defined as containing almost no protein, when a supplement was consumed 2 to 3 hours later (Booth et al. 1970). The same observations have been made when the test lunch was either primarily liquid or solid food. When young women consumed 450 kcal liquid lunches that were high in protein (80 g of a commercial whey protein, 71.5% of energy) food intake at dinner was suppressed by approximately 30% compared to a liquid lunch high in carbohydrates (113 g of a polyose supplement, 99% of energy) and approximately 30% compared to a mixed lunch (40 g protein and 62 g carbohydrate, 36% and 55% of energy, respectively) (Latner and Schwartz 1999).
Similarly, when young women were fed equicaloric meals (~600 kcal) at lunch, a meat casserole high in protein (43% of energy) suppressed food intake compared to a vegetarian casserole high in carbohydrate (69% of energy) at the evening meal 4 hours later by 12%. This study controlled for the fat, fiber and energy content of both meals (Barkeling et al. 1990).

Young men also respond to high protein meals. In one study, healthy young men were given either a high protein (274 kcal, 37 g, 54% of energy) omelette preload that included egg and ham or a high fat (284 kcal, 25 g, 79% of energy) omelette preload that included egg and cream at 11:00 AM. After 2 hours, those who consumed the high protein preload ate 211 kcal less than those who consumed the high fat preload. While the suppression of 211 kcal 2 hours later was not statistically significant, it is possible that the sample size of 13 was too small to detect significance in this study (Porrini et al. 1997). Another group of researchers fed 11 young men in a state of satiety, 4 hours after lunch, a mandatory snack in randomized order, either high in protein (996 kcal, 77% of energy), high in fat (984 kcal, 58% of energy), or high in carbohydrate (1009 kcal, 84% of energy). The subjects were isolated in a windowless room, devoid of any temporal cues, and were brought to a buffet dinner at their own request. On the control day, when no snack was served, subjects requested dinner approximately 6 hours after the beginning of lunch. Dinner requests were delayed by 60 minutes on the day that a high protein snack was consumed, by 34 minutes on the day that a high carbohydrate snack was consumed and by 25 minutes on the day that a high fat snack was consumed by subjects. These men rated their subjective hunger using visual analogue scales and there was no
difference in their hunger between treatments before eating the snack. However, all treatments suppressed hunger significantly compared to the no snack condition 60 and 30 minutes after the lunch. The food intakes at dinner following the snacks were not significantly different, prompting the authors to suggest that subjects were able to request dinner when they were hungry. Furthermore, no caloric compensation for the snack was found with any of the treatments at dinner. It was also suggested that snacking might be detrimental behaviour in subjects with a genetic predisposition to adiposity (Marmonier et al. 2000).

The role of protein in regulating food intake over the longer term in humans has received little study. However an isocaloric weight-reducing diet (1800 kcal/day) high in protein (26%) was reported to be more satiating than an isocaloric diet, which was lower in protein (8%). Twelve obese men were observed over 9 weeks and the proportion of macronutrients was adjusted for each of the 3-week phases. The macronutrient ratios (percent calories from protein: fat: carbohydrate) for the first phase was rounded to 26: 51: 23, the second phase 26: 25: 50, and the third phase was 8: 51: 41. The diet lowest in protein was rated as having the lowest satiety value by 6 of the 8 subjects who made adequate assessments. Furthermore, comparing the 2 phases higher in protein (26% of energy for both groups), the diet higher in protein was rated to have the highest satiety value by 5 of the 6 subjects, whereas only 2 subjects rated the diet higher in carbohydrate with the greatest satiety rank (Fryer et al. 1955).

The habitual protein intake of subjects might affect subsequent feeding responses to protein preloads, adding variability to the food intake measurement. For example, a
recent study found that protein induced satiety in young people was inversely related to the subjects' habitual protein intake. In this study, 14 men and women were divided into two groups based on their habitual protein intakes (estimated from 4-day food diaries) that were either on average high- (14 g/kg/day) or low-protein (10 g/kg/day). Three identical high-protein test meals (35% of energy) were given over the course of the day. Subjective appetite, rated using visual analogue scales, was not different for either hunger or satiety between the habitual high protein and low protein groups. However, a significant diet group by time interaction was interpreted to suggest that the habitual low-protein group developed hunger over a slightly slower time course than the habitual high-protein group over the high protein loading test day (Long et al. 2000).

2.2.3.2. Protein Source and Food Intake

The effect of protein on satiety in animals is affected by protein source, as mentioned earlier. There is also some evidence that the source determines the satiating efficacy of protein in humans. Young men fed a 50 g meal of lean fish found it to be more effective in suppressing appetite, measured by visual analogue scales, over a three-hour period than an equivalent amount of either beef or chicken (Uhe et al. 1992). Fish protein suppressed subjective appetite compared to beef or chicken by over three hours. The level of satiety produced by the fish meal also declined more slowly over time than those of the other meals. In an effort to identify regulatory mechanisms to explain this response, the authors measured plasma glucose, insulin and amino acid concentrations in response to each test meal. No differences were found, among treatments, in plasma insulin or glucose concentrations. However, differences in the rate of digestibility and
ratio of plasma amino acids were observed. Profiles of most of the plasma amino acids (measured through an indwelling catheter inserted in the antecubital before meals and every 15 minutes thereafter for the first hour and 30 minutes over the second and third hours) were similar after each of the three meals, but taurine concentrations were higher after the fish meal at 45, 60 and 90 minutes than after the chicken or beef meals. Plasma methionine levels were also higher following the fish meal at 90, 150 and 180 minutes compared to chicken and beef. A trend for lower concentrations of plasma tyrosine and histidine was seen in the fish consumers, but was not significant. The amino acid concentrations took longer to reach peak levels after the fish meal (144 minutes) compared to the beef or chicken meals (111 and 118 minutes, respectively).

The ratios of plasma tryptophan: large neutral amino acids (LNAA) and tyrosine: LNAA were calculated. These ratios are known to predict brain uptake of tryptophan and tyrosine, which exert precursor control over the neurotransmitters serotonin, and tyrosine, respectively. After a small initial increase at 15 minutes, the tryptophan: LNAA ratio declined, with the decrease after the fish meal being slower than that of the other meals (significant at 45 and 60 minutes). The tyrosine: LNAA ratio was constant and no differences were seen among treatments. The authors suggested that the slower decline in the tryptophan: LNAA ratio accounted for the greater satiety of the fish meal. The role of higher taurine and methionine plasma concentrations in satiety was undetermined.

When comparing the effect of protein source on food intake, it is crucial to design the test meals such that the protein sources are the only variables being tested; otherwise it is difficult to determine which variable is influencing the subsequent food intake. For
example, when young women were given mixed meals of equal protein content containing either mycoprotein (produced by continuous fermentation of the Fusarium graminearum mushroom) or chicken as the protein source, the former suppressed desire to eat, measured by visual analogue scales, three hours later. The mycoprotein meal also lowered energy intake compared to the chicken meal by 236 kcal over the remainder of the test day and by 288 kcal on the following day (Turnbull et al. 1993). Both test meals had similar energy values (580 kcal and 560 kcal for chicken and mycoprotein, respectively) and had the same macronutrient contents (protein 44 g, carbohydrate 81 g and fat 11 g). The difference between the meals lay both in the fiber content (mostly from the mycoprotein itself) - with 10 g in the chicken meal compared to 17 g in the mycoprotein meal - and the protein source. The authors attributed the lower food intake following mycoprotein to the higher fiber content of the meal. However, while the meals were balanced for nutrient and caloric content, the protein content of the chicken meal was higher that than the mycoprotein meal. To compensate, low-fat cottage cheese was substituted for the raspberry jelly in the dessert for the chicken meal. Thus it remains unknown whether it was the fibre, the protein source, or both that played a role in suppressing food intake.

When protein is a component in a mixed meal, the protein source was not found to be a factor in food intake suppression (Lang et al. 1998). In this study, 6 dietary protein sources (egg-albumin, casein, gelatin, soy protein, pea protein and wheat gluten) were fed in mixed macronutrient meals, and showed no effect on eating behaviour or satiating power at dinner, 8 hours later. Measurements of plasma glucose and insulin
found fluctuations of both measurements over the post-prandial period, increasing to a maximum 1-hour after lunch and then progressively decreasing until the final measurement 30 minutes prior to dinner. There were no significant differences among the protein-manipulated lunches with respect to plasma glucose and insulin concentrations, and no interaction with time and any type of lunch. Furthermore, the effect of time had a significant effect on subjective appetite, which was suppressed after lunch meals and rose steadily toward dinnertime, but no effect among the specific treatments was seen. These results might be expected, however, because the fat and fibre content were not controlled for and the protein was provided in a meal with a high caloric content (mean of 1242 kcal), which may have masked any differential effect of the proteins on satiety. Furthermore, the total protein content of the six meals varied from 61.4 g to 74.4 g, and the treatment protein in the meals ranged from 40.1 g to 47.1 g.

In a follow-up study, the protein source again did not affect food intake at dinner (Lang et al. 1999). Three protein sources (casein, gelatin and soy) were manipulated in mixed meal treatments, but each protein was given at two amounts (25 g or 50 g protein), as part of a mixed meal of 425 kcal or 860 kcal, for a total of 6 treatments, and food intake measured at dinner, 8 hours later, from a self-selecting buffet meal. The test meals were controlled for energy, macronutrients, fibre and palatability. When satiety was evaluated, using visual analogue scales, the latency for appetite to recover following lunch, corresponding to the return of hunger, was significantly increased after the higher energy meals, but was unaffected by source, except among the higher protein and energy containing 860 kcal lunches, where latency for satiety with the gelatin lunch was longer
than for the casein lunch (311 vs. 248 minutes, respectively). Manipulating the protein source in the lunches significantly affected plasma insulin over time, but had no effect on plasma glucose or glucagon. The insulin AUC following the 860 kcal lunches was higher for soy protein than for gelatin, and following the 425 kcal lunches was higher for casein protein than for gelatin. Glucose and insulin responses following the higher caloric casein-enriched meal were induced 1 to 1.5 hours later than the higher calorie soy-enriched meal. These differences in the post-prandial metabolic response may be explained by modifications in the rate of gastric emptying and by the effect of amino acids on the pancreatic endocrine responses. Differences among protein sources with respect to their metabolic responses (by 2 hours after the load) and the return of hunger (3 to 7 hours after the load) may be an indicator that food intake measurements 8 hours later was too long of a delay to detect differential effects of protein source on food intake.

2.2.3.3. Peptides and Food Intake

Peptides arising from the digestion of proteins also appear to affect food intake. Commonly prepared hydrolysed proteins differ in their effects on food intake regulation when compared to their parent intact protein. In rats, preload gavages of albumin hydrolysate suppressed food intake more than intact albumin, whereas intact whey suppressed food intake more than whey hydrolysate (Morgan 1998). Only one preliminary study of human subjects, conducted in our laboratory, has compared the effects of protein form on food intake (Rackal 1998). Young men were given 50 g preloads in a 400 mL drink of either intact whey protein or whey hydrolysate. Two hours later, both whey protein and its hydrolysed form suppressed food intake compared to
control. However, the sample size was small (only 10 subjects), leaving uncertain whether the effect of the hydrolysate is different from the intact protein in humans.

2.2.3.4. Amino Acids and Food Intake

Free amino acids are the final digestion products of protein and many studies have shown that individual amino acids suppress food intake. Many amino acids have been tested for their effect on food intake. When rats were gavaged with essential amino acids in selected groups, based on their common physiological functions in relation to their hypothesized role in food intake control, they all suppressed food intake over the first two hours. However, the degree to which the different groups suppressed food intake was not the same, indicating that food intake suppression caused by essential amino acids was not accounted for by an equal effect of its component amino acid groups (Anderson et al. 1994b). Similarly, when rats were gavaged with non-essential amino acids, food intake was suppressed in the short term only by glutamine, glycine or serine given individually, over one hour. When 6 of the 8 non-essential amino acids found in egg albumin were given together, food intake was suppressed over one, two and twelve hours following gavage (Anderson et al. 1994c).

In humans, single amino acids or amino acid mixtures suppress food intake but the quantities needed to bring about a response are large and well over the usual intake (Ryan-Harshman et al. 1987). For example, preloads of 2 and 3 g of tryptophan suppressed subsequent food intake compared to placebo capsules, but no significant
suppression was found following a 1 g tryptophan preload (Hrboticky 1986). The usual dietary intake is 1 g or less.

2.3. MECHANISMS OF PROTEIN-INDUCED SATIETY

The mechanisms that account for satiety response in protein consumption have not been defined, but include both pre- and post-absorptive elements. In the past, it has been hypothesized that amino acids and neurochemical events in the brain explain the decrease in food intake of rats after meals or protein preloads. However, these events occur relatively late in satiety and it is more likely that dietary protein-derived peptides initiate the satiety cascade, leading to the inhibition of food intake through a series of overlapping physiological processes, beginning with the peptides it releases and its very specific interactions with gut hormones (Trigazis et al. 1997). The following sections provide background on some post-absorptive and pre-absorptive mechanisms believed to be involved in protein-induced satiety.

2.3.1. POST-ABSORPTIVE SIGNALS

Post-absorptive signals occur once nutrients have been digested and absorbed from the small intestine. Upon digestion of protein into constituent peptides and amino acids, absorption in the intestine leads them into the portal circulation, resulting in alterations in plasma and brain amino acid concentrations. The two main theories of how protein modulates food intake post-absorptively are the aminostatic hypothesis and the brain neurotransmitter hypothesis.
2.3.1.1. Plasma Amino Acids: The Aminostatic Hypothesis

The aminostatic theory of food intake regulation proposed by Mellinkoff was prompted by observation of an inverse relationship between plasma amino acid concentrations and appetite in humans. He suggested the plasma amino acid pattern might serve as a signal to the appetite centre, and that the pattern may be more important than the total levels of amino acid nitrogen in regulating appetite (Mellinkoff et al. 1956). Changes in plasma amino acids, after a meal, have been associated with decreased food intake in animals fed high-protein diets, excess amino acids, or deficient or imbalanced diets (Harper et al. 1970). While extremes in amino acid content of the diet alter food consumption, the role plasma amino acids play, after a meal containing balanced protein (in amounts approximating requirements), in feeding behaviour remains uncertain (Anderson and Li 1987; Anderson et al. 1994). It is believed that fluctuations in plasma amino acid patterns are monitored by the brain to mediate feeding behaviour (Fryer et al. 1955; Mellinkoff et al. 1956; Peng et al. 1972). It is postulated that the amino acid concentration in the brain is maintained within broadly defined upper and lower limits, and that animals modify their food intake to keep the concentration within this limit (Harper et al. 1970; Peters and Harper 1987).

It is unlikely, however, that changes in brain amino acid concentrations provide an explanation for the initial satiety signals arising from protein ingestion. Appetite suppression occurs prior to changes in plasma or whole brain amino acid concentrations. For example, rats gavaged with albumin or an amino acid mixture showed no temporal association between plasma and whole brain amino acid concentrations and their effect
on food intake (Anderson et al. 1994). Similarly, microdialysis studies show that free amino acid concentrations change in several brain regions, but not until 20 or 40 minutes after rats begin to feed. The time course of these changes suggests that free amino acids in brain regions may serve as intermediary signals in the satiety cascade, but are too late to be the primary signals that develop after the rats eat protein (Choi et al. 2000).

2.3.1.2. Brain Amino Acids: The Brain Neurotransmitter Hypothesis

The brain neurotransmitter hypothesis is based on observations that some amino acids act as precursors of neurotransmitters. Tryptophan (Trp), phenylalanine (Phe), tyrosine (Tyr) and histidine (His) are presumed to be important in food intake control mechanisms because they are precursors to the neurotransmitters serotonin, catecholamines and histamine, respectively, all of which are known to be involved in the control of feeding behaviour (Anderson 1981; Anderson et al. 1984). Because they exert precursor control over neurotransmitter synthesis, these amino acids provide a link between the composition and quantity of food consumed and the brain signal regulation of food intake.

Serotonin (5-hydroxytryptamine) is a neurotransmitter involved in the regulation of food intake. Increased serotonin activity leads to food intake suppression (Blundell and Hill 1987; Luo and Li 1990). Because serotonin synthesis is partially under precursor control, dietary factors influencing tryptophan availability affect serotonin synthesis (Anderson 1988a; Anderson et al. 1984; Blundell and Hill 1987). Serotonin synthesis is dependent on Trp; consequently, fluctuations in Trp availability greatly
influence its synthesis (Teff and Young 1988). Carbohydrate had been shown to increase serotonin levels by increasing the precursor levels of Trp in the brain. The insulin rise after a carbohydrate meal raises the tryptophan levels and lowers the large neutral amino acid levels (which compete for the same carrier system into the brain as tryptophan), allowing tryptophan to be moved into the brain at an accelerated rate. Protein, on the other hand, decreases the tryptophan: large neutral amino acid ratio, and hence a depression in brain serotonin occurs (Anderson 1981). Serotonin also appeared to be involved in the regulation of food choice, where increased serotonergic activity led to a decreased preference for carbohydrates in rats given dietary choice (Li and Anderson 1983).

The catecholamines modulate food choice and meal composition primarily through a neuronal system via the hypothalamus. Tyrosine and phenylalanine are the major precursors of the catecholamines, and fluctuations in these amino acids affect the synthesis and turnover of the catecholamines in activated neurons. Rats selectively increased carbohydrate intake after microinjection of norepinephrine into the paraventricular nucleus of the hypothalamus (Leibowitz 1988). Carbohydrate however is not the only nutrient whose selection is modified by activity of the catecholaminergic neurons. When catecholaminergic tone was suppressed by clonidine, a pre-synaptic α-receptor agonist, rats increased their intake of both protein and total food (Mauron et al. 1980). Conversely, amphetamine, a central catecholaminergic agonist, decreased total food intake, with a greater effect on protein intake (Blundell et al. 1975). Intraperitoneal injections of Tyr and Phe suppressed feeding in rats (Bialik 1989; Morris et al. 1987). It
is not clear if food intake suppression or selection is regulated in normal feeding situations as a result of signals to the central nervous system by precursor effects of Tyr. The synthesis of catecholamines is influenced by Tyr availability, but there are no reports of the effect of diet-induced variations in brain Tyr on food choice. It is possible, however, that the elevated synthesis of norepinephrine that occurs after protein consumption might direct the animal to prefer carbohydrate in the next meal (Gibson and Wurtman 1978).

Evidence for histamine involvement in food intake regulation is two-fold. First, brain His and histidine decarboxylase are found in the highest concentrations in the brain hypothalamus (Schwartz et al. 1970), a region of the brain associated with food intake regulation and changes in their levels are associated with suppression of food intake. Secondly, changes in brain His and histamine are associated with food intake suppression. Decreases in brain His by feeding of a His deficient diet (Peng et al. 1972; Sanahuja et al. 1962), or decreases in histamine by injection of a brain histidine (His) decarboxylase inhibitor (Menon et al. 1971) resulted in increased food intake by rats. Intra-cerebral injections of His or histamine inhibit feeding in rats (Cohn et al. 1973), and intra-ventricular injections of histamine inhibit feeding in cats (Clineschmidt and Lotti 1973). Intraperitoneal injections of His resulted in suppressed food intake in rats and were associated with significant elevations in brain His and histamine, indicating that the action of histidine is via the synthesis of His in the brain (Sheiner et al. 1985). Intragastric injections and intraperitonal injections of histidine both suppressed food intake in rats, but histidine consistently reduced food intake with sensitivity to the
intraperitoneal route being much greater than the intragastric route of delivery.

Furthermore, when histidine decarboxylase was blocked, the effect of His on food intake was partially reversed, supporting the hypothesis that His regulates food intake, at least in part through its precursor control of histamine (Vaziri et al. 1997).

The role of individual amino acids arising from protein ingestion in food intake regulation in normal feeding is uncertain. Only large amounts of the single free amino acids suppress food intake (Li and Anderson 1983). These large amounts are unrepresentative of amino acids acquired during a meal (Bialik et al. 1989; Fernstrom 1987; Morris et al. 1987), suggesting that other metabolic events must occur in response to food intake in order for amino acids to play a role.

2.3.1.3. Post-Absorptive Hormonal Response

The peripheral hormonal response to food and its effect on satiety and food intake are very complex. Two of the most commonly studied hormones stimulated by protein ingestion, glucagon and insulin, are discussed here.

The pancreatic hormone glucagon inhibits food intake in many species, including humans, and is thought to play a physiological role in the regulation of food intake. The mechanism by which glucagon is believed to suppress food intake is via glucosensitive cells in the liver (Ritter et al. 1986), as well as in the vagal nerve (Geary and Smith 1983). Furthermore, there is evidence for a thermogenic effect of glucagon (Billington et al. 1991).
The role that insulin plays in satiety, either directly or indirectly, has also been investigated extensively. As some amino acids such as arginine, leucine, phenylalanine and leucine in combination with glutamine have a strong insulinotropic effect on incubated β cells of the pancreas (van Loon et al. 2000), protein-induced insulin release may play a role in food intake suppression.

It has been hypothesized that fluctuations in peripheral insulin levels correspond to satiety in humans. A number of studies have observed enhanced satiety, based on VAS, when modest and sustained increases in insulin were experimentally achieved (VanderWeele et al. 1985; 1994; Woods et al. 1986). However, it is not clear whether it is the glucose uptake in the cells induced by increased insulin levels or the insulin itself that is responsible for the increased satiety. Acute administration of insulin leading to hypoglycemia, stimulates food intake in a variety of species, including humans (Grossman 1986). However, insulin is not necessarily the factor generating satiety, because the hyperphagia effect induced by the insulin is abolished when supplementary glucose is administered. Complicating an interpretation of the role of insulin is the observation that when chronically administered in low doses and in the presence of either normo- or hypo-glycaemia, insulin reduces food intake in experimental animals (Hirschberg 1998).

In some human studies, lower insulin concentrations have been associated with greater post-prandial satiety (Holt et al. 1992; Holt and Brand Miller 1995; Lavin and
Read 1995). In others, higher serum insulin concentrations are associated with increased satiety (Speechly and Buffenstein 1999; Speechly et al. 1999).

Although the precise relationship between insulin and the regulation of food intake is undetermined, there are several reasons for expecting it to play a major role. First, it has been suggested that physiological levels of insulin represent a sensor of peripheral metabolic status, which is supported by the well-known clinical finding of a positive correlation between insulin levels and the degree of adiposity (Hirschberg 1998). Second, peripheral insulin affects the brain mechanisms involved in feeding behaviour and energy balance (Campfield 1997). Insulin enters the brain from circulation where it acts on insulin receptors expressed by the brain neurons involved in energy intake. Based on direct administration of insulin into the brain, it can be suggested that peripheral insulin may play an important regulatory role. Administration of insulin into the brain reduces energy intake, whereas a deficiency of insulin increases energy intake (Schwartz et al. 2000). Central administration of insulin inhibits neuropeptide Y mRNA expression in the arcuate nucleus of the hypothalamus and increases corticotropin releasing hormone mRNA expression in the paraventricular nucleus of the hypothalamus. Thus central administration of insulin alters the gene expression of two major neuropeptides that are important in food intake and body energy regulation (Campfield 1997).

As hormonal responses such as insulin and glucagon have been implicated in modulating food intake regulation, it is of interest to note of a recent study which compared relationships among the effects of whey, a “fast protein”, and casein, a “slow protein”, on absorption rates, hormonal response and protein turnover in humans (Boirie
et al. 1997). Following a meal of 30 g of each protein consumed by healthy young men, insulin levels increased similarly after both meals, with no difference in plasma insulin at 0, 40 and 300 minutes after the meal. However, there was a difference in the kinetics of protein turnover. Slowly absorbed casein promoted post-prandial protein deposition by an inhibition of protein breakdown without excessive increases in amino acid concentration, whereas whey protein, which was quickly absorbed, stimulated protein synthesis and also oxidation. The authors suggested that the speed of amino acid absorption after protein ingestion had a major impact on the post-prandial metabolic response to a single meal of protein and hormonal response was not an explanation. However, Fruhbeck (1998) argued that the hormonal response should not be ruled out when explaining the differences in the effects of the two sources on protein kinetics. While the insulin concentrations were not different between treatments, the insulin to glucagon ratio may have played a role. The proportion of amino acids known to stimulate insulin release was the same in both proteins; however, a higher ratio of branched-chain amino acids occurred in whey protein, which led it to have a synergistic effect with insulin on protein metabolism. Furthermore, the glucagon-stimulating amino acids were lower in casein than in whey. The observations that the protein source is a factor affecting protein turnover and that the mechanism may be related to hormonal responses suggest that it is worth exploring the role of these post-absorptive hormonal responses in satiety that is induced by specific protein sources.
2.3.2. PRE-ABSORPTIVE SIGNALS- THE ROLE OF THE GUT

The presence of protein in the gastrointestinal tract is the stimulus for pre-absorptive signals of satiety. Some of these signals are most likely transmitted by the vagus nerve to the brain (Reidelberger 1994; Ritter et al. 1994). There is also evidence for the presence of mechanoreceptors, osmoreceptors and chemoreceptors in the gut that may influence food intake (Blundell 1991; Li and Anderson 1983). Finally, gastrointestinal hormones involved in the regulation of feeding are also released by protein in the small intestine (Bray 1992; Kissileff and van Itallie 1982; Lee et al. 1994; Liddle et al. 1986).

2.3.2.1. Mechanoreceptors, Osmoreceptors and Chemoreceptors

The arrival of food causes mechanoreceptors that line the smooth muscle wall of the proximal gastrointestinal to send satiety signals via the vagus to the brain (Nicholl et al. 1985). Gastric distension in animals inhibits food intake (Deutsch et al. 1978). Similarly, in humans, distending a balloon in the stomach initially reduces food consumption (Pasquali et al. 1990), but the effect decreases as the stomach adapts to the presence of the balloon (Ramhamadany et al. 1989). To date, no studies have compared the effects of macronutrient specific induction of mechanoreceptors on satiety. One study induced gastric distension in young men and infused subjects with 1.5 kcal/min of protein, lipid, carbohydrate or saline, and found that more pain, nausea and bloating was caused by the lipid than any of the other treatments. The authors attributed the findings
to a greater distal gastric volume with the lipid infusion (Ladabaum et al. 2001), however the effect of the macronutrient infusions on satiety was not addressed.

While gastric distension may suppress short-term food intake, it is not the only factor that accounts for the sensation of satiety. Satiety can last up to four hours after a moderate sized meal at which time the stomach contains very little food; thus if gastric distension alone induced satiety, it would be expected that satiety would cease as soon as the stomach was emptied and distension was relieved (Thompson and Malagelada 1981).

Osmotic pressure changes in the gut are also detected by osmotic receptors. Hypertonic solutions suppress short-term feeding (Harper and Boyle 1976) and it is hypothesised that the osmotic pressure exerted by food particles in the stomach draws water from the body into the gut, reducing plasma water and temporarily inducing dehydration. Osmolarity alone does not account for the more satiating effects of a higher protein diet. In monkeys, a 15% protein diet compared to a 50% protein diet fed orally, with the diets controlled for osmolarity by simultaneous intragastric infusion, the higher protein diet still suppressed food intake (Hannah et al. 1990). However, whether protein has a greater effect than fat or carbohydrate on suppressing appetite due to osmolarity alone has yet to be investigated.

Protein digestion leads to signals to the brain via the vagus nerve through chemoreceptors in the small intestinal wall (Read et al. 1994). Endocrine cells dispersed in the gut epithelium perform chemoreception; they are bipolar cells extending an apical
process to the gut lumen. Infusions of amino acids into the gut increase firing rates in the 
vagus via contact with chemoreceptors (Jeanningros, 1982).

Peptide products of protein digestion also play a role in food intake regulation. 
Peptides derived from milk protein and wheat gluten exhibit opiate-like activity at the 
level of the gut (Brantl and Teschemacher 1979; Morley 1982). Digestion products of 
 Dietary protein that exhibit opioid-like activity are termed exorphins, because they are 
analogous to exogenously derived opiate-like materials. These products delay gastric 
emptying. Infusion of casein lowered the amplitude and frequency of small intestinal 
contractions compared to soy when given intagastrically to the canine small bowel. Pre-
treatment with naloxone blocked the inhibitory effects of casein, suggesting that casein 
peptides stimulate opioid receptors (Defilippi et al. 1995). Similarly, infusing active 
 opioid-like peptides, such as bovine casein digest into guinea pig ileum, abolishes 
electrically simulated contractions. However, administration of (-)naloxone, an opiate 
antagonist, blocks the inhibition of contractions by the casein peptides (Brantl and 
Teschemacher 1979).

There is evidence that the digestion products of the protein, and not the intact 
protein itself, are inducing the opiate-like effects in the gut. In dogs, intragastric 
instillation of digested gluten elicits a greater peripheral vein insulin and glucagon 
response than an equal amount of undigested gluten. When the digested gluten is given 
in conjunction with naloxone, the insulin response is lowered, suggesting that the 
hormonal response is secondary to the activation of the opiate receptor (Morley 1982). 
Another study found that casomorphin peptides could be isolated from the duodenal
chyme of minipigs after feeding with casein, whereas no casomorphin-like peptides could be detected after a casein meal in the gastric chyme.

Peptide products derived from two milk protein (casein and whey) sources were found to induce different physiologic responses pre-absorptively, where ingestion of the whey protein was recovered rapidly in the upper intestine, mostly in the form of the intact protein, whereas casein was slowly recovered in the jejunum, mainly in the form of degraded peptides (Boirie et al. 1997). Most likely, the difference between the two proteins can be attributed to the clotting and/or precipitation of the casein (unlike the soluble whey) in the acidic media of the stomach, giving it longer exposure to gastric peptic hydrolysis and the slow emptying from the stomach of degraded peptides that are subsequently hydrolyzed by pancreatic proteases (Mahe et al. 1996). These two milk proteins illustrate that different protein sources are broken down at different rates to different peptides and emphasise the importance of protein source in determining physiological responses.

2.3.2.2. Gastrointestinal Hormones

Many hormones are released upon the entry of food into the small intestine. Such peptide hormones as cholecystokinin, glucagon-like peptide-1, cerulein, bombesin, gastrin, secretin, glucagon, insulin, somatostatin, neurotensin, substance P and pancreatic polypeptide are known to contribute to the process of satiation (Bray 2000). This section focuses on two hormones released by the arrival of protein in the gut, which had been studied extensively, cholecystokinin and glucagon like peptide-1.
Cholecystokinin is the most studied gut hormone known to suppress food intake. CCK is a polypeptide released from the duodenal mucosa upon arrival of food. Many studies show that both endogenous and exogenous CCK suppress food intake in many species of animals, including rabbits (Bernstein et al. 1976), mice (Moran and McHugh 1982), rats (Moran et al. 1986), and humans (Hill et al. 1990; Smith and Gibbs 1994; Stacher et al. 1982). Protein arrival in the intestine causes CCK release, which acts on the CCKA-receptors on the vagus nerve, providing sensory information to the brain and resulting in meal termination.

Intestinal release of CCK mRNA is modified by diet. Of the three macronutrients, protein is the most effective in releasing CCK. CCK mRNA expression declines in the intestine when rats are fasting or given low protein diets. In contrast, when rats are fed high protein diets, CCK mRNA expression increases (Kanayama and Liddle 1991).

In humans, proteins, peptides, amino acids and fats administered intraduodenally stimulate CCK release (Go et al. 1970; Isaacs et al. 1987; Liddle et al. 1985). Overall, fat and protein are the strongest secretagogues of CCK release. Of the free amino acids tested in humans, Trp and Phe are the most potent CCK secretagogues (Konturek et al. 1973). Carbohydrates in the form of glucose and starch are weak secretagogues of CCK in humans (Liddle et al. 1985).

In rats, protein is the strongest stimulant of CCK release, while fats do so to a lesser extent and carbohydrates and amino acids do not (Lewis and Williams 1990;
Liddle et al. 1986; Schneeman et al. 1977). This variation indicates that differences exist among species, for nutrient induced CCK release.

Intact protein stimulates CCK release more than protein hydrolysates. It has been hypothesized that this occurs because the intact protein can stimulate CCK release by protecting CCK releasing peptides (CCK-RP) from proteolytic inactivation in the intestinal lumen (Herzig et al. 1996). CCK-RP is secreted from CCK-RP cells into the proximal small intestine and inactivated by trypsin released from the pancreas. Postprandially, when food enters the duodenum, partly digested protein from the stomach binds to trypsin, thereby competing with CCK-RP. This competition between the two proteins (dietary protein vs. CCK-RP) allows for CCK-RP to remain in its active form for longer period of time and therefore increases the likelihood of CCK cells releasing CCK into the blood stream (Herzig et al. 1996; Miyasaka et al. 1989). This concept may partially explain why intact dietary proteins stimulate CCK secretion in rats more than peptides, fats or carbohydrates (Liddle et al. 1986). In humans, evidence has indicated that a similar mechanism of CCK secretion may be occurring. One study found that when POTII, a proteinase inhibitor found in potatoes, was added to a test meal containing protein, CCK release in the plasma was elevated (Hill et al. 1990). It was suggested that the POTII enzyme promoted enhanced CCK release, because it inhibited trypsin and chymotrypsin degradation of CCK-RP, allowing CCK-RP to be active longer and therefore prolonging CCK release.

While CCK is the gut hormone most strongly associated with protein and food intake, glucagon-like peptide-1 (7-36) amide (GLP-1), another gut hormone has only
recently been associated with protein ingestion in humans. GLP-1 exerts a variety of actions on metabolism and behavior. It is primarily known for its regulation of carbohydrate metabolism through stimulation of glucose-dependent insulin secretion from the pancreatic β-cells, thereby accelerating glucose disposal (Drucker 1998). GLP-1 also decreases food intake in experimental animals (Peters et al. 2001) and humans (Gutzwiller et al. 1999).

Both protein and peptones (protein hydrolysates) have a transient, weak effect on GLP-1 secretion in humans (Elliott et al. 1993). Small increases in GLP-1 were found in plasma of human subjects after consuming a protein meal (375 kcal) (Elliott et al. 1993) or receiving peptone through ileal perfusion (Layer et al. 1995). However, peptones are also a stimulant to GLP-1 secretion in rats. For example, egg albumin hydrolysate (5%) evoked a sustained release of GLP-1 when perfused into the isolated rat ileum in vitro (Dumoulin et al. 1998). Peptones (egg albumin hydrolysate and meat hydrolysate, 5% each) dose-dependently stimulated GLP-1 release in the isolated vascularity perfused rat intestines (jejunoileum and colon), with 2 to 4-fold increases from baseline within 5 minutes following the initiation of infusion persisting throughout the infusion period (Cordier-Bussat 1998). In contrast, isocaloric quantities of bovine serum albumin or of an amino acid mixture had no stimulatory effect (Cordier-Bussat 1998). Similarly, perfusion of rat ileum with amino acids had no effect on GLP-1 secretion (Hermann et al. 1995). These findings imply that the L cell may require a certain structure of peptides for GLP-1 secretion. Because protein is digested by gastric acid and proteolytic enzymes in the stomach and duodenum, and amino acids are absorbed from the upper small intestine,
it is unlikely that significant quantities of proteins and peptones under natural feeding conditions reach the ileum and thus stimulate GLP-1 release from the L cell. However, under a pathological condition where digestion of protein is impaired, protein consumption may result in an increased release of GLP-1. It is not yet clear what role protein-induced release of GLP-1 plays in food intake and satiety.

2.4. SUMMARY

Although the specific mechanisms by which protein produces satiety and reduces food intake have not been defined, it is clear that protein and its breakdown products impact on food intake regulatory systems in experimental animals and in humans, at least in part by gastrointestinal hormones. Evidence that the source of protein and the composition of proteins direct physiological responses and feeding responses in experimental animals suggests that they may also occur in humans. However, the effect of protein source on feeding response in humans has received little investigation. Therefore, the objective of this research was to determine the effects of pure protein sources on subsequent food intake in humans.
CHAPTER 3.

EXPERIMENTAL OUTLINE
3.1. HYPOTHESIS

The hypothesis of this thesis is that the effect of proteins on short-term satiety and food intake in humans depends on the protein source.

3.2. OBJECTIVES

The primary objective of this thesis was to describe the effect of selected protein sources, consumed as drinks, on short-term satiety and food intake in young men. A secondary objective was to determine the effects of these protein sources on mood and memory in young men. Two objectives were addressed in 2 experiments:

1) To determine the effect of egg, whey and soy proteins on subsequent subjective appetite and food intake in young males one hour after their consumption (Experiment 1).

2) To determine the interaction between time of testing and the effect of egg and whey proteins on food intake in young males (Experiment 2).

The results of the appetite and food intake portion of both experiments are presented in the main body of the thesis. In addition, the effects of treatments on mood (experiment 1) and memory (experiment 1 and 2) were also studied concurrently with the treatment effects on food intake. However, for the purpose of clarity, these findings (which were not significant) are included in appendices 7 and 8.
CHAPTER 4.

MATERIALS AND METHODS
4.1. SUBJECTS

Healthy, non-smoking, Caucasian males aged 18-35 years with a BMI between 20-25 kg/m² (WHO 1997) were recruited by signs posted across the University of Toronto downtown campus and surrounding areas. Subjects were excluded if they were breakfast skippers, diabetics or taking any medications. Restrained eaters were also excluded upon their identification by a score of 11 or higher (Herman and Polivy 1980; Polivy and C.P. Herman et al. 1978) on the administered Eating Habits Questionnaire (Appendix 2). The study was divided into two separate experiments, for which there were separate recruitments. The University of Toronto Human Subjects Review Committee approved this study.

All subjects were required to attend a pre-screening interview, and individuals that met the initial screening requirements then completed a Baseline Information Questionnaire, signed a consent form, and were given an outline of the study (Appendix 2). Subjects were asked to maintain consistent levels of activity the day and morning before each session.

Based on power analysis for a within-subject design (Appendix 1) using results from a preliminary study investigating the effect of whey protein on food intake in young men, a sample size of 14 was estimated to be required to show a treatment response of 214 kcal (Rackal 1998). Fifteen subjects were recruited for experiment 1 and all subjects completed the required sessions. However, 2 men were dropped from the study for having irregular food consumption and activity patterns (i.e. did not to eat dinner prior to test day or had 2 consecutive workouts before arrival). The characteristics of the remaining 13 subjects are listed in Table 1. The dependent measures for these 13 subjects
are used for all analyses throughout the experiment. In experiment 1, all subjects had their fat free mass (FFM) estimated by the bioelectric impedance analysis (BIA) technique, because the goal was to administer protein preloads that were proportional to the subjects' lean body mass. Thus larger subjects would have a slightly larger preload than smaller subjects would.

The mean age for the 13 participants in the study was 22.2 years and the mean BMI was 22.1 kg/m². FFM was measured by bioelectric impedance with the Xitron4000BIS machine (Xitron Technologies, Inc., San Diego, CA, USA) set at 50 kHz single frequency. The resulting resistance value for each subject was used in conjunction with Segal's equation for lean men to estimate the subjects' FFM (Segal et al. 1988). The mean FFM for the group was 60.1 kg.

In the second experiment, 24 young men, with the same characteristics of those in experiment 1 were recruited (Table 2). All 24 men completed the study, but 2 subjects were dropped; one subject received an inadequate pizza lunch, and the other received the wrong preload on an experimental day. Subject characteristics for experiment 2 are shown in Table 2. The average BMI for the 22 participants was 22.8 kg/m² and the average age was 22.3 years. In the second experiment, subjects did not undergo BIA, but their FFM was estimated to be 86.8% of their body mass. This assumption was based on the strong correlation between body mass and FFM (r²=0.93) found in the first experiment and also because the same subject population formed the sample for this experiment.
Table 1: Experiment 1. Subject Characteristics

<table>
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<th>Subject</th>
<th>Arrival Time (AM)</th>
<th>Age (yrs)</th>
<th>Weight (kg)</th>
<th>Height (m)</th>
<th>BMI(^1) (kg/m(^2))</th>
<th>FFM(^2) (kg)</th>
<th>Protein or Sucrose(^3) (g)</th>
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<td>1.72</td>
<td>23.0</td>
<td>58.1</td>
<td>43.58</td>
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</tbody>
</table>

Mean 22.2 69.2 1.80 22.1 60.1 45.10
SEM\(^4\) 2.6 4.8 0.06 1.6 4.5 3.35

\(^1\)BMI = Body Mass Index (kg/m\(^2\))
\(^2\)FFM = Fat Free Mass (inserting resistance from bioelectric impedance into Segal Equation for Lean Men)
\(^3\)Protein or sucrose = g of protein or sucrose subject consumed in treatment preloads
\(= \text{FFM (kg) } \times 0.75 \text{ g/kg (FFM)}\)
\(^4\)SEM = standard error of the mean; n = 13
### Table 2: Experiment 2. Subject Characteristics

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Arrival Time (AM)</th>
<th>Age (yrs)</th>
<th>Weight (kg)</th>
<th>Height (m)</th>
<th>BMI $^1$</th>
<th>Protein (g)$^2$</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>31</td>
<td>66.8</td>
<td>1.72</td>
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<td>83.6</td>
<td>1.86</td>
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<td>1.86</td>
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<td>75.0</td>
<td>1.81</td>
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<td>48.75</td>
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<td>1.66</td>
<td>22.9</td>
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<td>20</td>
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<td>1.76</td>
<td>22.7</td>
<td>45.80</td>
</tr>
<tr>
<td>20</td>
<td>11</td>
<td>20</td>
<td>87.7</td>
<td>1.91</td>
<td>24.0</td>
<td>57.02</td>
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<td>20</td>
<td>68.2</td>
<td>1.75</td>
<td>22.3</td>
<td>44.32</td>
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<td>11</td>
<td>21</td>
<td>78.6</td>
<td>1.80</td>
<td>23.5</td>
<td>51.11</td>
</tr>
</tbody>
</table>

| Mean      | 22.3   | 73.2   | 1.80     | 22.8     | 47.61   |
| SEM       | 0.7    | 1.7    | 0.00     | 0.3      | 1.12    |

$^1$BMI = Body Mass Index (kg/m$^2$)

$^2$Protein = g of protein subject consumed in treatment preloads

$= 0.65$ g protein/kg body weight

$^3$SEM = standard error of the mean; n = 22
4.2. EXPERIMENTAL DESIGN

4.2.1. Experiment 1: The Effect of Sucrose and Intact Protein Sources on Appetite and Food Intake

Subjects chose to arrive at either 9:10 AM or 11:00 AM and were asked to arrive at the same time for every test session. Five treatments in the form of liquid preloads were used: 1) sweet control; 2) egg albumen; 3) whey; 4) soy protein and 5) sucrose. In a repeated measures design, each subject received each treatment given in a 400 mL solution of sugar-free Strawberry Kool-Aid (Kraft, Canada) immediately followed by 50 mL of water. The treatments were equalized in terms of sweetness based on pre-trial testing (Table 3) with the addition of the non-caloric sweetener, sucralose (Tate and Lyle Specialty Sweeteners, London, England). The egg albumen was from Egg D'Lite (Optimum Nutrition, Florida, USA). The whey protein isolate was from Ultimate Balance (Delaware, USA); and unflavoured soy protein from Swiss Herbal Remedies (Missouri, USA). Redpath sugar (Redpath Industries Ltd., Toronto, Canada) was used for the sucrose preloads. The proteins were purchased at local health food stores (egg: Sunshine Natural Foods, whey: Health Valley Natural Food, soy: The Health Shoppe) in Toronto, Canada, and the sucrose from a local grocery store (Dominion) in Toronto, Canada. The composition of each protein source is reported in Appendix 5.

Sucralose was chosen as the non-caloric sweetener because it has no effect on the nervous system, carbohydrate metabolism, blood glucose, blood fructose or insulin secretion; this sweetener can pass through the body unmetabolized (Knight 1994). The sweetness intensity of sucralose ranges from 400 to 800 times sweeter than sucrose, depending on application. The manufacturers of Splenda®, the commercial form of this
sweetener, claim that 6 mg of sucralose is equivalent in sweetness to 1 teaspoon of sucrose (4.3 g). This equation was used to equate sweetness between the different doses of sucrose in each preload; however, the intense sweetness of the sucrose preload required adjustments, so that more sucralose was added than originally estimated to produce similar perceived sweetness level among all treatments. After establishing the quantity of sucralose additions to treatments, a taste test was conducted so that subjects could rank perceived sweetness. Subjects were unable to differentiate sweetness intensities between the treatments. This pre-trial taste test for sweetness and palatability was given by having people (n=11) come to the laboratory and taste the pre-mixed, randomized drinks, one at a time. After tasting each drink, subjects rated the drinks on a scale of 1 to 5 for both sweetness (1=not sweet at all; 5=very sweet) and for palatability (1=not pleasant at all; 5=very pleasant). Between tasting of drinks, subjects consumed a small amount of water to clear their palate. The goal of preparing the drinks was focused on equalizing sweetness of drinks, as equalizing palatability was difficult (Table 3).

The treatment loads of proteins and sucrose were based on FFM and were designed to provide 0.75 g/kg FFM. However, because there was a small amount of maltodextrin added to the commercial egg protein mix, a proportional amount of maltodextrin was added to all drinks. For the sucrose treatment, maltodextrin was added so that the caloric values of all the drinks were equivalent. To equalize sweetness among treatments, 3.75 mg sucralose per kg FFM was added to the sweet control and 5 mg of sucralose per kg FFM was added to each of the protein drinks. Each drink also contained 40 mg of Kool-Aid per kg FFM. The exact composition of a 40 g treatment, which would be done for an individual of approximately 65 kg, is shown in Table 4. All drinks
were prepared the night before serving, stored in the refrigerator and served chilled to subjects the following morning. Subjects were required to consume the complete 400 mL drink followed by 50 mL of water, all within 5 minutes or less.

Table 3: Experiment 1. Pre-Experiment Trials of Drink Sweetness and Palatability$^{1,2}$

<table>
<thead>
<tr>
<th></th>
<th>Sweetness (n=11)</th>
<th>Palatability (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.6 ± 0.4</td>
<td>4.1 ± 0.3$^{a}$</td>
</tr>
<tr>
<td>Egg</td>
<td>3.8 ± 0.4</td>
<td>3.6 ± 0.4$^{a}$</td>
</tr>
<tr>
<td>Whey</td>
<td>3.8 ± 0.3</td>
<td>1.9 ± 0.2$^{b}$</td>
</tr>
<tr>
<td>Soy</td>
<td>2.7 ± 1.3</td>
<td>1.9 ± 0.3$^{b}$</td>
</tr>
<tr>
<td>Sucrose</td>
<td>3.8 ± 0.3</td>
<td>3.7 ± 0.3$^{a}$</td>
</tr>
<tr>
<td>F; p</td>
<td>1.73; 0.16</td>
<td>13.11; 0.00</td>
</tr>
</tbody>
</table>

$^{1}$Mean ± SEM  
$^{2}$Values are based on a 5-point scale rating from 0 (lowest) to 5 (highest)  
$^{3}$Means with different superscripts within a column are different (p<0.05)
Table 4: Experiment 1. Composition of Treatment Drinks For a Subject with 53 kg Fat Free Mass

<table>
<thead>
<tr>
<th>Protein (g)</th>
<th>Control</th>
<th>Egg</th>
<th>Whey</th>
<th>Soy</th>
<th>Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.44</td>
<td>0</td>
</tr>
<tr>
<td>Sugar-Free² Kool-Aid (g)</td>
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<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Maltodextrin³ (g)</td>
<td>3.14</td>
<td>3.64</td>
<td>3.64</td>
<td>2.55</td>
<td>4.07</td>
</tr>
<tr>
<td>Sucralose⁴ (g)</td>
<td>0.15</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Total Energy⁵ (kcal)</td>
<td>11</td>
<td>177</td>
<td>177</td>
<td>177</td>
<td>177</td>
</tr>
</tbody>
</table>

¹Drinks were created to provide protein intake of 0.75 g/kg FFM for each subject. The template drinks shown here assumed a subject (~53.3 kg of FFM) would have 40 g of protein in his drink. For each subject, the drink contents were adjusted according to their individual FFM.

²Sugar-Free Kool-Aid was added to all drinks proportionally to make them seem more similar.
³Maltodextrin was added to all other treatments because it was present in the commercial egg protein mix.
⁴Sucralose was added as the sweetener.
⁵Based on: protein=4 kcal/g, maltodextrin=3.74 kcal/g (sucrose=3.96 kcal/g), Kool-Aid=1.89 kcal/g and sucralose=0 kcal/g).
4.2.2. Experiment 2: The Effect of Egg and Whey Protein on Time of Testing and on Appetite and Food Intake

A two-factor within subject, repeated measures on one factor, design was used in experiment 2, where all subjects were given all treatments (repeated measures factor) and subjects arrived at either 8:30 AM or 11:00 AM for all their test sessions (non-repeated measure factor). Experiment 2 followed similar procedures to experiment 1 and was designed to measure the effect of egg and whey protein (compared to control) at the two times of the morning on the appetite and food intake. The three treatments were control, egg-albumen and whey protein (Table 6).

As in experiment 1, a pre-trial taste test was conducted to ensure that treatments were equally sweet. However, for this experiment pre-trial test, the procedure was like that of the experimental design (see below). In this pre-trial test, two controls were used with slightly different sucralose contents (Table 5). The control-1 drink had 0.15 mg sucralose (the amount used in the control in experiment 1) and the control-2 drink had 0.10 mg sucralose (a slightly lesser quantity of sucralose than that used in experiment 1). The purpose of using two controls was to try and equalize sweetness of drinks better; however, since there were no differences between the two controls in the pre-trial, the original control drink sweetness was maintained for consistency. Subjects (n=12), who matched the criteria of those recruited for the study, were required to arrive to the laboratory on test day mornings fasted, to drink the entire drink within 5 minutes, and then to rate sweetness and palatability using the same VAS that were completed in the main experiments. Again the focus of the pre-trial tests was to equalize sweetness, not palatability, of the treatments (Table 5).
For the main experiment, all drinks were prepared the night before serving, stored in the refrigerator, and served chilled to subjects the following morning. Subjects were required to consume the complete drink and the 50 mL of water, all within 5 minutes or less.

Table 5: Experiment 2. Pre-Experiment Trials of Drink Sweetness and Palatability

<table>
<thead>
<tr>
<th></th>
<th>Sweetness (n=12)</th>
<th>Palatability (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-1</td>
<td>69.9 ± 4.2</td>
<td>57.6 ± 5.9&lt;sub&gt;ab&lt;/sub&gt;</td>
</tr>
<tr>
<td>Control-2</td>
<td>68.7 ± 3.6</td>
<td>64.8 ± 5.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Egg</td>
<td>68.0 ± 4.9</td>
<td>43.8 ± 7.6&lt;sub&gt;bc&lt;/sub&gt;</td>
</tr>
<tr>
<td>Whey</td>
<td>68.7 ± 4.6</td>
<td>39.3 ± 6.9&lt;sub&gt;c&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

F; p<sup>3</sup> 0.34; 0.99  5.45; 0.00

<sup>1</sup>Mean ± SEM
<sup>2</sup>Values are based on 100 mm VAS with 0=Not at all, 100= Very
<sup>3</sup>Control-1 has the same sweetness as the control in experiment 1 and control in experiment 2, Control-2 was a trial drink, with slightly less sucralose (not used in the experiment)
<sup>a</sup>Means with different superscripts within a column are different
<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Egg</th>
<th>Whey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g)</td>
<td>0</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Sugar-Free Kool-Aid (g)</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Maltodextrin (g)</td>
<td>3.64</td>
<td>3.64</td>
<td>3.64</td>
</tr>
<tr>
<td>Sucralose (g)</td>
<td>0.15</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Total Energy (kcal)</td>
<td>17</td>
<td>177</td>
<td>177</td>
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</tbody>
</table>

1 Drinks were created to provide protein intake of 0.65 g/kg body mass. The template drinks shown here assumed a subject (~53.3 kg of FFM) would have 40 kg of protein. For each subject, the drink contents were adjusted according to their individual body mass.

2 Sugar-Free Kool-Aid was added to all drinks proportionally to make them seem more similar.

3 Maltodextrin was added to both whey and the control because it was present in the commercial the egg protein mix.

4 Sucralose was added as the sweetener.

5 Based on: protein=4 kcal/g, maltodextrin=3.74 kcal/g, Kool-Aid=1.89 kcal/g and sucralose=0 kcal/g.
4.3. EXPERIMENTAL PROCEDURE

All subjects were asked to fast for 12 hours before each visit, with the exception of water, which was allowed up to 1 hour before their arrival. Those subjects who regularly consumed coffee or tea in the morning were asked to consume one cup of either beverage at the same time before every session, to prevent withdrawal symptoms from confounding results. However, no subjects reported consuming a pre-study beverage.

When subjects arrived at the Department of Nutritional Sciences for each session, they were asked to fill out a Sleep Habits and Stress Factors Questionnaire to provide information regarding any factors that could affect appetite or memory (i.e. compliance with fasting, sleep deprivation, sickness). Individuals who scored less than 50 mm on the Physical Comfort visual analogue scales (VAS) before testing began were asked if they felt comfortable enough to continue with the test, and all reported that they were. Subjects who noted unusual stress or unusual sleep patterns (i.e. major upcoming test, or not enough sleep the previous night) on the Sleep and Stress questionnaire were asked to reschedule the session for another day (2 subjects). The participants then continued to complete baseline VAS measuring subjective Motivation to Eat (Appendix 3). Upon completion of the questionnaires, the subjects proceeded across the hall into the taste panel room, where they were provided with the 400 mL treatments in a covered opaque cup, followed by 50 mL of water. After the subjects had consumed the preload, the timer was started and subjects were asked to rate the sweetness and palatability of the drink using VAS. Subjects were then returned to the original room, where they were required to fill out VAS at repeated intervals (specific to each experiment, see below) over the next hour.
Immediately after completion of the final VAS at 60 minutes, subjects returned to the taste panel room where they were served a pizza lunch and bottled spring water (1.5 L Crystal Springs, Quebec, Canada). Four varieties of pizzas were available and subjects ranked them according to their preference on the preliminary interview day. Participants were served two pizzas of the variety they ranked first, and one each of their second and third choices of pizza per tray. Subjects were instructed to eat until they were “comfortably full” and were made aware that they would be presented with another hot tray of pizzas in 5 to 10 minutes. Two identical trays were offered to each subject (5 to 10 minutes apart) and a third to subjects who were capable of eating more. Subjects were told to knock on the door when they were comfortably full, to inform the experimenter they were finished, but also so they would not be interrupted during that time. No subject consumed all the food presented to him. Once the subjects were finished with their lunch, they rated the palatability of the pizza and completed the post meal Motivation to Eat and Physical Comfort VAS.

Both experiments followed a similar experimental procedure. The differences between them are described under their respective headings, below.

4.3.1. Experiment 1

In experiment 1, subjects filled out a VAS for Physical Comfort and Motivation to Eat every 15 minutes to 60 minutes following completion of the preload. Once the VAS were completed, each page of the questionnaire was turned over by the subject to prevent his referral to previous responses.
Subjects also completed mood and memory tests over the one-hour period between preload and pizza meal, which are described in Appendices 7 and 8, respectively.

Test days had at least one day between them, and all subjects consumed food and filled out VAS in the same room each time, but never with another subject present.

4.3.2. Experiment 2

Experiment 2 followed a similar design to the first experiment, with the following exceptions. Subjects filled out VAS for Motivation to Eat every 20 minutes, after the preload. Physical Comfort VAS was completed only at baseline and 60 minutes after treatment.

In the second experiment, subject behaviour was more extensively recorded than it was in experiment 1. First subjects were requested to keep all activities consistent prior to the days before test sessions and avoid strenuous physical activities prior to or on the day of test sessions. If moderate exercise occurred on the day prior to test sessions, the participants were then asked to repeat the same activity at the same time of day before each test session. Subjects completed a form to report all activities following their wake-up time to the time at which they came for testing. The experimenter administered a detailed questionnaire to subjects just before tests began to identify behavioural patterns. The purpose of screening the behavioural patterns was to reschedule subjects for another day when unusual incidents occurred, to prevent them having an effect on the experimental outcome.
Subjects in experiment 2 filled out a detailed food diary listing the foods and times eaten from 3:00 PM onwards, the day before their test (Appendix 4). Each food record was reassessed the morning of the test sessions to ensure portions and preparations were properly recorded, and that no items were forgotten from the list. Subjects were told at the pre-screen interview to eat the same meals the day before each test session, and to ensure that these foods were a regular part of their diet.

Subjects also completed memory tests over the one-hour period between preload and pizza meal, which are described in Appendix 8.

In the second experiment, test days were pre-set such that all subjects had each treatment administered to them once a week, at the same pre-designated arrival time.
Figure 1. Experiment 1. Test Protocol

Subjects Arrive at the Department of Nutritional Sciences

**Arrival**

Subjects fill out:
- Baseline Questionnaires
- VAS Physical Comfort
- VAS Appetite
- VAS Mood

**Injection**

Consume preload drink (within 5 minutes)

**Delivery**

- Immediate Recall Memory
- VAS Physical Comfort
- VAS Appetite
- VAS Mood

**Exit**

- VAS Physical Comfort
- VAS Appetite
- VAS Mood

**Delayed Recall Memory**

- VAS Physical Comfort
- VAS Appetite
- VAS Mood

**Ingestion**

- Immediate Recall Memory
- VAS Physical Comfort
- VAS Appetite
- VAS Mood

**Ad libitum**

Pizza Meal

$t=$time, based on the timer (which is started once subject has fully consumed his preload drink.

Data for mood and memory tests are reported in Appendices 7 and 8, respectively.
Figure 2. Experiment 2. Test Protocol

Subjects Arrive at the Department of Nutritional Sciences

Subjects fill out:
- Baseline Questionnaires
- VAS Physical Comfort
- VAS Appetite

consume preload drink (within 5 minutes)

Immediate Recall Memory
- VAS Physical Comfort
- VAS Appetite

VAS Physical Comfort
- VAS Appetite

Delayed Recall Memory

Immediate Recall Memory
- VAS Physical Comfort
- VAS Appetite

Pizza Meal (ad libitum)

t=time, based on the timer (which is started once subject has fully consumed his preload drink)
Data for memory tests are reported in Appendix 8.
4.4. DEPENDENT MEASURES

4.4.1. Food intake

The amount of food and water consumed was determined by weighing prior to presentation and again after the trays were removed. The energy consumed (kcal) was determined by converting the net weight consumed to kilocalories using the information provided by the pizza manufacturer (McCain Foods Ltd., Florenceville, New Brunswick) (Appendix 6).

Items recorded in the food diaries by each subject in experiment 2 were entered into the Nutritionist Five (Version 2.2.1, 2000, San Bruno, California, USA) to calculate the amount of energy provided by the foods recorded by each subject.

4.4.2. Subjective Appetite

Subjective appetite was assessed by a Motivation to Eat questionnaire, which consists of four VAS questions. Each VAS is a 100mm line anchored at either end with opposing statements. Subjects record an ‘X’ the line to indicate their feelings at that moment in time. The four questions comprising the Motivation to Eat questionnaire (Appendix 3) were:

1) How strong is your desire to eat? (‘Very weak’ to ‘Very strong’)
2) How hungry do you feel? (‘Not hungry at all’ to ‘As hungry as I’ve ever felt’)
3) How full do you feel? (‘Not full’ at all to ‘Very full’)
4) How much food do you think you could eat? (‘Nothing at all’ to ‘A large amount’)

4.4.3. Subjective Physical Comfort

The physical comfort questionnaire was used to assess the subject’s physical well-being. This questionnaire (Appendix 3) consisted of only one question: How well do you feel? ('Not well at all' to 'Very well').

4.4.4. Perceived Palatability

The palatability of each preload, as well as each pizza meal, was rated by the marking the line on the VAS question (Appendix 3): How pleasant have you found the beverage (food)? ('Not pleasant at all' to 'Very pleasant').

4.4.5. Perceived Sweetness

The perceived sweetness of each beverage was rated by marking the line on the VAS question (Appendix 3): How sweet have you found the drink? ('Not sweet at all' to 'Extremely sweet').

4.5. DATA ANALYSIS

Statistical analyses were facilitated by SAS version 7.1 (Statistical Analysis Systems, SAS Institute Inc., NC, USA) and SPSS version 9.0.1 (Statistical Package for Social Sciences, Inc., Chicago, IL, USA). When appropriate, the data were analyzed by one-way or two-way repeated measures ANOVA. Duncan’s post-hoc tests were performed to determine differences among treatment. The General Linear Models (GLM) procedure was used to conduct ANOVA on all data sets. Correlations between dependent measures were made, using Pearson’s Correlation Coefficient. Finally, Student’s t-tests were conducted and used where appropriate. Data are presented as mean
± standard error of the mean (SEM). The p-value of less than 0.05 was considered to indicate statistical significance.

4.5.1. Food intake

In the first experiment, treatment effects on food intake (kcal) and water (g) consumed during the pizza meal were analyzed for all subjects combined using a repeated measures one-way ANOVA. Upon determining there were an equal number of subjects in each arrival time group, a retrospective analysis was conducted to determine if there was any effect of arrival time on food intake, and if there was any interaction effect between arrival time and treatment on food intake (using the two-way ANOVA with repeated measures on one factor – treatment). Food intake of the subjects within each arrival time was analyzed independently using one-way repeated measures ANOVA. Finally, Student’s t-test was used to compare the effects of each treatment on food intake at the two arrival time groups.

The second experiment was designed to test for the effect of both treatment and arrival time on food intake. Thus the two-way ANOVA with repeated measures only on treatment was conducted. When no effect of arrival time, or arrival time by treatment interaction was found, all the subjects were pooled to determine the effects of treatment on food intake, with a repeated measures one-way ANOVA. To be consistent with the first experiment and because the sample size was determined to allow such an analysis, the effect of treatment on food intake in the two arrival groups was analyzed in each arrival time group independently, with one-way repeated measures ANOVA. The different pattern in food intake, prompted a further analysis using the t-test to determine
differences in food intake after each treatment at the two arrival times, as was done in experiment 1.

For both experiments, energy intake compensation at the test meal for each treatment was calculated using the formula:

\[(\text{Kcal consumed after control} - \text{Kcal consumed after treatment}) / \text{kcal in treatment} \times 100\]

Furthermore, in experiment 2, to determine if there were any differences in food intakes the night before the test days for each treatment, the recorded energy intake of subjects was compared among treatments by one-way repeated measures ANOVA.

4.5.2. Subjective Appetite

To assess the effect of treatments on appetite, the summary measure average appetite was calculated for each measurement time during the 60 minute test period, from the four individual questions of the Motivation to Eat VAS, according to the formula:

Average Appetite = \([Q1 + Q2 + (100-Q3) + Q4] / 4\]

The value of question 3 was subtracted from 100 because it is has extremes opposite to the other questions. Average appetite was then expressed as difference from baseline for statistical analyses. A two-way ANOVA was then used to assess the main effects of treatment and time. A two-way ANOVA was also conducted on the data expressed as change from baseline for each of the individual questions in the Motivation to Eat VAS, to confirm that the calculated average appetite accurately reflected the effect of treatments on the individual questions. For both average appetite and the individual appetite questions, if there was no overall significance found on the two-way ANOVA,
the effects of treatment on food intake for each of the time points were reported with a one-way repeated measures ANOVA.

4.5.3. Subjective Physical Comfort

For experiment 1, the effect of treatment on subjective physical comfort over time compared to baseline was determined by two-way repeated measures ANOVA. A secondary analysis using one-way repeated measures ANOVA analysis determined the effect of treatment on subjective physical comfort at each time of measurement.

In experiment 2, the effect of treatment on feelings of well-being at 60 minutes compared to baseline was analyzed using a one-way repeated measures ANOVA.

4.5.4. Perceived Palatability

The comparisons of the palatability of the preloads and of pizza as reported on the VAS were made by one-way repeated measures ANOVA.

4.5.5. Perceived Sweetness

The sweetness scores of the treatments reported on the VAS were compared by one-way repeated measures ANOVA.

4.5.6. Pre-test Questionnaires

Questions that subjects answered, each day before testing began, from the Sleep and Stress Habits Questionnaire were compared by Student’s t-test between the group that arrived earlier (9:10 / 8:30 AM) and the group that arrived later (11:00 AM). The questions included:
1. Hours of fasting prior to test (Exp. 1 & 2)

2. Hours of sleep obtained the night before the test (Exp. 1 & 2)

3. Time of wake-up on test day (Exp. 2)

4. Hours subjects were awake prior to arrival for the test (Exp. 2)

4.5.7. Correlations

Measures after all treatments within each experiment were used for correlation analysis between the following variables:

1. Food Intake and Average Appetite 0 min

2. Food Intake and Average Appetite 15 min (or 20 min Exp.2)

3. Food Intake and Average Appetite 30 min (or 40 min Exp.2)

4. Food Intake and Average Appetite 45 min

5. Food Intake and Average Appetite 60 min

6. Food Intake and Individual 60 min Appetite Questions

7. Food Intake and Drink Palatability

8. Food Intake and Drink Sweetness

9. Food Intake and Hours Fasting Before Arrival

10. Food Intake Hours of Sleep Previous Night

11. Food Intake and Physical Comfort 60 min

12. Perceived Sweetness and Average Appetite 15 min

13. Perceived Palatability and Average Appetite 15 min

14. Perceived Palatability and Physical Comfort 60 min

15. Hours Awake Before Arrival and Food Intake (Exp. 2)
CHAPTER 5.

RESULTS AND DISCUSSION
The results and discussion of experiment 1 and 2 are reported separately. First, the effect of treatments on subjective appetite and food intake for experiment 1 is presented, followed by the effects of treatments on subjective appetite and food intake in experiment 2. The results of the mood and memory tests are reported in Appendices 8 and 9, respectively.

Experiment 1:

THE EFFECT OF PROTEIN SOURCE AND SUCROSE ON SUBJECTIVE APPETITE AND FOOD INTAKE

5.1 RESULTS

5.1.1 Food and Water Intake

There was an effect of treatment \([F=7.10, p<0.00]\) on the amount of food consumed during the test meal given one hour after the preloads (Figure 3, Table 7). Whey and soy suppressed food intake from control, but egg protein and sucrose did not. There was no difference between sucrose and soy protein, or between soy and whey protein. The amount of water consumed with the pizza was not affected by treatment preload \([F=1.82, p=0.14]\) (Table 7).

The 13 subjects chose to arrive at either 9:10 AM arrival or 11:00 AM for all of their test sessions. Because the two groups were evenly split (\(n=6\) arrived at 9:10 AM and \(n=7\) arrived at 11:00 AM), the effect of arrival time, as well as treatment, on food intake was analyzed. An effect of treatment \([F=8.24, p<0.00]\), but no main effect of arrival time was found on food intake \([F=1.65, p=0.23]\). However, the interaction between time of arrival and treatment was statistically significant \([F=3.07, p=0.03]\).
Within the 9:10 AM group (n=6), there was a significant effect of treatment on food intake \([F=9.06, p<0.00]\) (Figure 4). Sucrose, whey and soy protein suppressed food intake compared to control. Whey suppressed food intake more than the sucrose, but was not different from soy protein, whereas there was no difference in food intake between soy and sucrose. Egg protein did not suppress food intake compared to control, but was not different from sucrose or soy treatments. For the 11:00 AM group (n=7), there was an effect of treatment on food intake \([F=4.61, p=0.01]\) (Figure 4). In the mean comparison, egg increased food intake compared to control, whey and soy protein. The control was not significantly different from sucrose, soy or whey, but whey protein suppressed food intake compared to control, sucrose and egg treatments.

Comparison of the mean food intake following each individual treatment between 9:10 AM and 11:00 AM found that food intake was greater after consuming egg protein at 11:00 AM compared to 9:10 AM \((t=0.32, p=0.75)\) (Table 8).

The percent energy compensation in the test meals for the egg, whey, soy and sucrose treatments, respectively, were -33.4%, 95.7%, 59.1% and 11.5% (Table 7), when the data for all subjects, ignoring time, was included.
Figure 3: Test meal food intake: calories consumed from a pizza test meal one-hour after treatments

*Treating treatments with different letters are different (p<0.05)*
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Test Meal (^2) (kcal)</th>
<th>Test Meal + Preload (^3) (kcal)</th>
<th>Water Intake (^4) (g)</th>
<th>% Compensation (^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>850.8 ± 67.5(^a)</td>
<td>1050.0 ± 66.5(^a)</td>
<td>348.9 ± 44.8</td>
<td>0</td>
</tr>
<tr>
<td>Egg</td>
<td>913.1 ± 66.9(^a)</td>
<td>1112.2 ± 64.8(^a)</td>
<td>470.0 ± 46.0</td>
<td>-33.4</td>
</tr>
<tr>
<td>Whey</td>
<td>661.8 ± 57.2(^c)</td>
<td>861.0 ± 56.1(^c)</td>
<td>409.5 ± 59.1</td>
<td>95.7</td>
</tr>
<tr>
<td>Soy</td>
<td>730.8 ± 71.7(^bc)</td>
<td>930.0 ± 70.0(^bc)</td>
<td>425.7 ± 53.9</td>
<td>59.1</td>
</tr>
<tr>
<td>Sucrose</td>
<td>824.5 ± 60.5(^ab)</td>
<td>1023.7 ± 58.3(^ab)</td>
<td>387.6 ± 48.2</td>
<td>11.5</td>
</tr>
</tbody>
</table>

\(^1\)Mean ± SEM; n = 13
\(^2\)Energy consumed at the test meal
\(^3\)Energy total of preload and test meal
\(^4\)Water intake at test meal
\(^5\)Compensation = (kcal control test meal − kcal treatment test meal)/kcal preload\(^*\)100

\(^a\)Means with different superscript letters within a column are different, p<0.05
Figure 4: Effect of treatments on energy intake from the test pizza meal at two arrival times

a) 9:10 AM group (n=6)

b) 11:00 AM group (n=7)

*Treatments with different letters are different (p<0.05)
Table 8: Experiment 1. Effect of Arrival Time on Food Intake for Individual Treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>9:10 AM (n=6)</th>
<th>11:00 AM (n=7)</th>
<th>t; p²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>875.3 ± 63.8</td>
<td>829.7 ± 117.7</td>
<td>0.32; 0.75</td>
</tr>
<tr>
<td>Egg</td>
<td>768.2 ± 74.5</td>
<td>1037.2 ± 84.3</td>
<td>-2.35; 0.04*</td>
</tr>
<tr>
<td>Whey</td>
<td>602.9 ± 37.5</td>
<td>712.3 ± 100.9</td>
<td>-0.95; 0.36</td>
</tr>
<tr>
<td>Soy</td>
<td>610.2 ± 90.8</td>
<td>834.2 ± 96.7</td>
<td>-1.67; 0.12</td>
</tr>
<tr>
<td>Sucrose</td>
<td>748.4 ± 77.7</td>
<td>889.7 ± 88.0</td>
<td>-1.18; 0.26</td>
</tr>
</tbody>
</table>

¹Mean ± SEM
²t and p values, Student’s t-test
*Means within a row are different, p<0.05
5.1.2. Average Appetite

The average appetite ratings are reported as both absolute values (Table 9) and as change from baseline (Table 10). All statistical analyses of the data for average appetite were based on the change from baseline scores (Table 10) because they compare each individual's rating of appetite questions to his own pre-treatment scores for that particular test, which minimizes the differences between subjects. An overall effect of treatment \( [F=1.37, \ p=0.26] \) and a time by treatment interaction \( [F=0.88, \ p=0.57] \) were not found. However, there was an overall effect of time \( [F=11.55, \ p<0.00] \). That is, average appetite increased with time. Although there was no significant interaction, the response to time was analyzed for each individual treatment. Appetite increased over time for each of control, sucrose and soy preloads \( [F=9.03, \ p<0.00, \ F=4.85, \ p<0.00 \text{ and } F=3.53, \ p=0.02] \), respectively], but no effect of time was present for egg or whey treatments \( [F=2.48, \ p=0.08 \text{ and } F=1.25, \ p=0.31, \text{ respectively}] \). The response to treatment at each individual time was also analyzed. No treatment effect was found (Table 10).
Table 9: Experiment 1. Effect of Treatment on Absolute Average Appetite Scores

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>Egg</th>
<th>Whey</th>
<th>Soy</th>
<th>Sucrose</th>
<th>F; p</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>67.8 ±</td>
<td>69.2 ±</td>
<td>70.7 ±</td>
<td>70.9 ±</td>
<td>66.5 ±</td>
<td>0.36;</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>3.4</td>
<td>3.7</td>
<td>3.4</td>
<td>2.9</td>
<td>0.84</td>
</tr>
<tr>
<td>15</td>
<td>60.7 ±</td>
<td>65.0 ±</td>
<td>64.9 ±</td>
<td>57.4 ±</td>
<td>59.1 ±</td>
<td>1.38;</td>
</tr>
<tr>
<td></td>
<td>3.7</td>
<td>3.0</td>
<td>3.7</td>
<td>4.0</td>
<td>3.1</td>
<td>0.26</td>
</tr>
<tr>
<td>30</td>
<td>61.8 ±</td>
<td>63.7 ±</td>
<td>62.6 ±</td>
<td>59.6 ±</td>
<td>60.9 ±</td>
<td>0.24;</td>
</tr>
<tr>
<td></td>
<td>3.9</td>
<td>3.5</td>
<td>4.4</td>
<td>4.0</td>
<td>2.5</td>
<td>0.91</td>
</tr>
<tr>
<td>45</td>
<td>64.9 ±</td>
<td>65.6 ±</td>
<td>64.5 ±</td>
<td>62.6 ±</td>
<td>66.6 ±</td>
<td>0.32;</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>3.2</td>
<td>3.3</td>
<td>3.5</td>
<td>2.8</td>
<td>0.86</td>
</tr>
<tr>
<td>60</td>
<td>68.8 ±</td>
<td>67.8 ±</td>
<td>68.5 ±</td>
<td>64.9 ±</td>
<td>69.5 ±</td>
<td>0.51;</td>
</tr>
<tr>
<td></td>
<td>3.9</td>
<td>3.6</td>
<td>3.1</td>
<td>3.5</td>
<td>3.4</td>
<td>0.73</td>
</tr>
</tbody>
</table>

1Mean ± SEM (mm); n=13

Table 10: Experiment 1. Effect of Treatment on Change from Baseline Average Appetite Scores

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>Egg</th>
<th>Whey</th>
<th>Soy</th>
<th>Sucrose</th>
<th>F; p</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>-7.2 ±</td>
<td>-4.2 ±</td>
<td>-5.8 ±</td>
<td>-13.6 ±</td>
<td>-7.4 ±</td>
<td>1.64;</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>3.7</td>
<td>1.6</td>
<td>3.8</td>
<td>3.8</td>
<td>0.18</td>
</tr>
<tr>
<td>30</td>
<td>-6.0 ±</td>
<td>-5.5 ±</td>
<td>-8.1 ±</td>
<td>-11.2 ±</td>
<td>-5.5 ±</td>
<td>0.85;</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>3.8</td>
<td>2.9</td>
<td>2.3</td>
<td>2.9</td>
<td>0.50</td>
</tr>
<tr>
<td>45</td>
<td>-2.9 ±</td>
<td>-3.7 ±</td>
<td>-6.2 ±</td>
<td>-8.3 ±</td>
<td>0.7 ±</td>
<td>1.53;</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>4.0</td>
<td>3.6</td>
<td>3.2</td>
<td>2.4</td>
<td>0.21</td>
</tr>
<tr>
<td>60</td>
<td>1.0 ±</td>
<td>-1.4 ±</td>
<td>-2.2 ±</td>
<td>-6.0 ±</td>
<td>3.1 ±</td>
<td>1.20;</td>
</tr>
<tr>
<td></td>
<td>3.8</td>
<td>4.2</td>
<td>3.6</td>
<td>3.5</td>
<td>3.7</td>
<td>0.32</td>
</tr>
</tbody>
</table>

1Mean ± SEM (mm); n=13
5.1.3. Motivation to Eat

Results from the Motivation to Eat questions are reported as absolute values (Table 12) and change from baseline values (Table 13). All statistical analyses are based on the change from baseline values using the repeated measures two-way ANOVA for each of the four questions in the Motivation to Eat questionnaire. The effect of treatment on each of the individual questions, at each time point is reported in Table 12.

For the desire to eat question, there was an overall effect of treatment [F=2.78, p=0.04], and time [F=9.31, p<0.00], but no time by treatment interaction was found [F=0.94, p=0.51]. The marginal means for the overall effect of treatment are reported in (Table 11). Soy suppressed desire compared to all the other treatments. Desire increased with time. When the individual treatments were examined for the effect of time, there was an increase in desire to eat for control and sucrose [F=5.07, p<0.00 and F=3.66, p=0.02, respectively], but not for egg, whey or soy protein [F= 1.84, p=0.16, F=1.89, p=0.15 and F=1.39, p=0.26, respectively]. Mean comparisons at each time of measurement also showed that the treatment effect was due primarily to soy protein, which at 15 minutes after preload [F=2.77, p<0.04], suppressed desire to eat compared to the control, and at 45 minutes [F=3.23, p=0.02] again suppressed desire to eat compared to control, but not to whey or egg protein. There was no effect of treatment on desire to eat at 30 minutes or 60 minutes [F=1.18, p=0.33 and F=2.20, p=0.08, respectively] (Table 13).

For the hunger question, neither an overall effect of treatment [F=1.33, p=0.27], nor treatment by time interaction [F=0.66, p=0.79] was found. However there was an increase with time [F=7.91, p=0.00]. When individual treatments were examined for their effect compared for their effect of time, there was an increase for control, soy and
sucrose \([F=2.80, p=0.05, F=4.02, p=0.01\] and \(F=3.10, p=0.04\), respectively], but no effect for egg or whey \(F=2.60, p=0.07\) and \(F=1.56, p=0.22\), respectively]. Mean comparisons at each time of measurement, showed no effect of treatment on hunger (Table 13).

For the fullness question, neither an overall effect of treatment \([F=0.75, p=0.56]\), nor treatment by time interaction \([F=0.72, p=0.73]\) was found, however there was a decrease with time \([F=9.18, p<0.00]\). When individual treatments were examined for the effect of time, there was a decrease for control, soy and sucrose \([F=3.56, p=0.02, F=3.43, p=0.03\] and \(F=3.24, p=0.03\), respectively], but none for egg and whey \([F=2.14, p=0.11\] and \(F=1.97, p=0.14\), respectively]. Mean comparisons at each time of measurement showed no effect of treatment on fullness (Table 13).

For the amount question, neither an overall effect of treatment \([F=1.22, p=0.31]\), nor treatment by time interaction \([F=1.17, p=0.31]\) was found, however there was an increase with time \([F=4.62, p=0.01]\). When individual treatments were examined for the effect of time, there was an increase after control and sucrose \([F=4.31, p=0.01\] and \(F=3.11, p=0.04\), respectively], but not for egg, whey and soy \([F=0.09, p=0.97, F=0.03, p=0.99\] and \(F=2.73, p=0.06\)]. Mean comparisons at each time of measurement showed no effect of treatment on amount (Table 13).
Table 11: Experiment 1. Effect of Treatment on Overall Desire Change from Baseline Scores (Marginal Means)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Desire</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-1.4 ± 2.0a</td>
</tr>
<tr>
<td>Egg</td>
<td>-6.3 ± 2.0a</td>
</tr>
<tr>
<td>Whey</td>
<td>-5.2 ± 2.0a</td>
</tr>
<tr>
<td>Soy</td>
<td>-14.1 ± 2.1b</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-1.9 ± 1.7a</td>
</tr>
</tbody>
</table>

F       | 14.91
P       | <0.00

1Mean ± SEM (mm); n=13
2Treatments with different letters along the column are different, p<0.05
## Table 12: Experiment 1. Effect of Treatment on Questions 1 to 4 Absolute Motivation to Eat Scores

<table>
<thead>
<tr>
<th>Question</th>
<th>Time (min)</th>
<th>Control</th>
<th>Egg</th>
<th>Whey</th>
<th>Soy</th>
<th>Sucrose</th>
<th>F; p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Desire</td>
<td>0</td>
<td>65.0 ± 5.1</td>
<td>70.5 ± 4.3</td>
<td>69.2 ± 4.6</td>
<td>72.1 ± 3.9</td>
<td>65.8 ± 3.1</td>
<td>0.67; 0.61</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>58.3 ± 4.6</td>
<td>63.8 ± 3.8</td>
<td>65.8 ± 4.4</td>
<td>54.3 ± 5.4</td>
<td>60.4 ± 3.4</td>
<td>1.41; 0.25</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>61.4 ± 4.0</td>
<td>62.5 ± 4.6</td>
<td>59.5 ± 4.9</td>
<td>58.0 ± 4.2</td>
<td>60.0 ± 3.5</td>
<td>0.26; 0.90</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>65.5 ± 4.1</td>
<td>63.6 ± 4.3</td>
<td>63.5 ± 3.7</td>
<td>57.8 ± 4.8</td>
<td>65.2 ± 3.5</td>
<td>1.01; 0.41</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>69.2 ± 4.6</td>
<td>67.2 ± 4.0</td>
<td>67.1 ± 3.7</td>
<td>61.9 ± 4.0</td>
<td>69.7 ± 3.5</td>
<td>1.15; 0.35</td>
</tr>
<tr>
<td>2-Hunger</td>
<td>0</td>
<td>66.3 ± 4.4</td>
<td>66.4 ± 4.3</td>
<td>68.5 ± 4.3</td>
<td>69.0 ± 3.8</td>
<td>60.8 ± 3.3</td>
<td>0.70; 0.59</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>58.2 ± 4.6</td>
<td>64.0 ± 3.5</td>
<td>62.1 ± 4.5</td>
<td>57.7 ± 4.2</td>
<td>55.8 ± 4.1</td>
<td>0.89; 0.48</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>60.3 ± 4.9</td>
<td>60.3 ± 4.5</td>
<td>59.7 ± 5.4</td>
<td>56.2 ± 4.6</td>
<td>57.5 ± 2.6</td>
<td>0.25; 0.91</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>62.5 ± 5.3</td>
<td>62.8 ± 4.1</td>
<td>63.7 ± 4.0</td>
<td>61.8 ± 3.9</td>
<td>63.0 ± 3.9</td>
<td>0.05; 1.00</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>66.2 ± 4.9</td>
<td>66.4 ± 4.9</td>
<td>66.8 ± 4.1</td>
<td>63.7 ± 3.8</td>
<td>67.1 ± 5.1</td>
<td>0.20; 0.94</td>
</tr>
<tr>
<td>3-Fullness</td>
<td>0</td>
<td>22.8 ± 4.2</td>
<td>27.2 ± 5.4</td>
<td>19.6 ± 3.7</td>
<td>23.5 ± 4.2</td>
<td>23.7 ± 3.5</td>
<td>0.47; 0.76</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>34.5 ± 4.8</td>
<td>32.2 ± 4.1</td>
<td>31.8 ± 4.4</td>
<td>38.9 ± 4.2</td>
<td>38.5 ± 4.5</td>
<td>0.87; 0.49</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>35.8 ± 4.4</td>
<td>31.6 ± 4.5</td>
<td>31.3 ± 5.4</td>
<td>33.3 ± 4.1</td>
<td>35.4 ± 4.4</td>
<td>0.23; 0.92</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>30.6 ± 3.9</td>
<td>27.9 ± 3.8</td>
<td>32.1 ± 5.1</td>
<td>30.6 ± 3.4</td>
<td>26.4 ± 3.8</td>
<td>0.41; 0.80</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>23.3 ± 3.8</td>
<td>26.5 ± 4.6</td>
<td>22.6 ± 3.3</td>
<td>29.0 ± 4.0</td>
<td>25.8 ± 3.9</td>
<td>0.50; 0.74</td>
</tr>
<tr>
<td>4-Amount</td>
<td>0</td>
<td>62.7 ± 4.4</td>
<td>67.1 ± 2.7</td>
<td>64.6 ± 3.8</td>
<td>66.0 ± 3.7</td>
<td>62.9 ± 2.9</td>
<td>0.41; 0.80</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>60.3 ± 4.0</td>
<td>64.4 ± 2.7</td>
<td>63.4 ± 3.6</td>
<td>56.3 ± 3.7</td>
<td>58.5 ± 2.7</td>
<td>1.36; 0.26</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>61.3 ± 4.0</td>
<td>63.7 ± 3.0</td>
<td>62.5 ± 3.4</td>
<td>58.0 ± 4.0</td>
<td>61.5 ± 2.7</td>
<td>0.47; 0.75</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>62.2 ± 3.7</td>
<td>63.6 ± 2.4</td>
<td>62.8 ± 2.8</td>
<td>61.2 ± 3.6</td>
<td>64.5 ± 2.0</td>
<td>0.31; 0.87</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>66.2 ± 4.3</td>
<td>64.0 ± 2.8</td>
<td>62.1 ± 3.0</td>
<td>63.0 ± 3.8</td>
<td>67.1 ± 3.2</td>
<td>0.60; 0.66</td>
</tr>
</tbody>
</table>

1Mean ± SEM (mm); n=13
2Questions (on a scale of 0 to 100):
   1= How strong is your desire to eat? 
   2= How hungry do you feel? 
   3= How full do you feel? 
   4= How much food do you think you could eat?
Table 13: Experiment 1. Effect of Treatment on Questions 1 to 4 Change From Baseline Motivation to Eat Scores

<table>
<thead>
<tr>
<th>Question</th>
<th>Time (min)</th>
<th>Control</th>
<th>Egg</th>
<th>Whey</th>
<th>Soy</th>
<th>Sucrose</th>
<th>F; p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Desire</td>
<td>15</td>
<td>-6.5 ± 3.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-6.8 ± 4.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-3.5 ± 2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-17.8 ± 5.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-5.4 ± 4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.77; 0.04</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>-3.6 ± 3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-8.1 ± 4.8</td>
<td>-9.7 ± 4.3</td>
<td>-14.1 ± 3.0</td>
<td>-5.8 ± 3.0</td>
<td>1.18; 0.33</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>0.5 ± 3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-6.9 ± 5.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>-5.7 ± 4.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>-14.2 ± 4.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.1 ± 2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.23; 0.02</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>4.2 ± 5.2</td>
<td>-3.3 ± 5.0</td>
<td>-2.2 ± 4.4</td>
<td>-10.2 ± 4.1</td>
<td>3.9 ± 3.4</td>
<td>2.20; 0.08</td>
</tr>
<tr>
<td>2-Hunger</td>
<td>15</td>
<td>-8.2 ± 4.0</td>
<td>-2.4 ± 4.0</td>
<td>-6.4 ± 2.6</td>
<td>-11.3 ± 3.8</td>
<td>-4.9 ± 4.2</td>
<td>1.08; 0.38</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>-6.0 ± 3.1</td>
<td>-6.1 ± 4.6</td>
<td>-8.8 ± 3.7</td>
<td>-12.8 ± 2.9</td>
<td>-3.3 ± 2.8</td>
<td>1.32; 0.28</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>-3.8 ± 4.2</td>
<td>-3.5 ± 4.7</td>
<td>-4.8 ± 4.4</td>
<td>-7.2 ± 3.3</td>
<td>3.0 ± 3.1</td>
<td>1.32; 0.29</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>-0.2 ± 4.1</td>
<td>0.0 ± 4.9</td>
<td>-1.6 ± 4.6</td>
<td>-5.3 ± 3.6</td>
<td>6.3 ± 6.0</td>
<td>1.11; 0.36</td>
</tr>
<tr>
<td>3-Fullness</td>
<td>15</td>
<td>11.6 ± 5.9</td>
<td>5.1 ± 4.2</td>
<td>12.2 ± 3.5</td>
<td>15.5 ± 4.7</td>
<td>14.8 ± 6.0</td>
<td>0.94; 0.45</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>12.9 ± 5.1</td>
<td>4.5 ± 4.7</td>
<td>11.7 ± 4.0</td>
<td>9.8 ± 3.6</td>
<td>11.7 ± 5.9</td>
<td>0.59; 0.68</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>7.8 ± 4.7</td>
<td>0.8 ± 5.2</td>
<td>12.5 ± 4.5</td>
<td>7.2 ± 4.3</td>
<td>2.3 ± 4.3</td>
<td>1.27; 0.30</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>3.5 ± 4.4</td>
<td>-0.6 ± 6.3</td>
<td>3.0 ± 3.8</td>
<td>5.5 ± 4.6</td>
<td>2.1 ± 3.9</td>
<td>0.31; 0.87</td>
</tr>
<tr>
<td>4-Amount</td>
<td>15</td>
<td>-2.4 ± 3.8</td>
<td>-2.7 ± 3.5</td>
<td>-1.2 ± 1.8</td>
<td>-9.7 ± 2.9</td>
<td>-4.4 ± 2.7</td>
<td>1.47; 0.22</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>-1.4 ± 2.6</td>
<td>-3.4 ± 3.1</td>
<td>-2.2 ± 2.6</td>
<td>-8.0 ± 2.9</td>
<td>-1.4 ± 2.7</td>
<td>1.24; 0.31</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>-0.5 ± 3.2</td>
<td>-3.5 ± 2.9</td>
<td>-1.8 ± 3.5</td>
<td>-4.8 ± 3.1</td>
<td>2.2 ± 2.6</td>
<td>1.00; 0.42</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>3.5 ± 3.5</td>
<td>-3.1 ± 3.0</td>
<td>-2.0 ± 3.8</td>
<td>-3.0 ± 3.9</td>
<td>4.2 ± 3.9</td>
<td>1.34; 0.27</td>
</tr>
</tbody>
</table>

<sup>1</sup>Mean ± SEM (mm); n=13
<sup>2</sup>Questions (on a scale of 0 to 100):
  1= How strong is your desire to eat?
  2= How hungry do you feel?
  3= How full do you feel?
  4= How much food do you think you could eat?
<sup>3</sup>Means with different superscripts within a row are different, p<0.05
5.1.4. Physical Comfort

No overall effect of treatment \( [F=2.10, p=0.10] \), time \( [F=1.02, p=0.40] \), or time by treatment interaction \( [F=0.72, p=0.73] \) was found for feelings of well-being, assessed by the physical comfort VAS. There were no effects of treatment on physical comfort at each of the measured time points (Table 14).

**Table 14: Experiment 1. Effect of Treatment on Change from Baseline Physical Comfort Scores**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.4 ± 4.8</td>
<td>3.6 ± 3.1</td>
<td>3.3 ± 3.2</td>
<td>2.8 ± 3.0</td>
</tr>
<tr>
<td>Egg</td>
<td>2.1 ± 2.2</td>
<td>1.9 ± 2.7</td>
<td>0.3 ± 2.3</td>
<td>2.2 ± 3.9</td>
</tr>
<tr>
<td>Whey</td>
<td>-2.2 ± 3.5</td>
<td>-7.2 ± 3.9</td>
<td>-3.6 ± 3.4</td>
<td>-4.5 ± 3.3</td>
</tr>
<tr>
<td>Soy</td>
<td>3.8 ± 2.7</td>
<td>1.8 ± 2.7</td>
<td>0.0 ± 2.1</td>
<td>1.2 ± 3.2</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-0.8 ± 2.7</td>
<td>-1.2 ± 2.7</td>
<td>-1.5 ± 2.7</td>
<td>-5.1 ± 1.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F</th>
<th>1.64</th>
<th>2.07</th>
<th>0.98</th>
<th>2.16</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>0.18</td>
<td>0.10</td>
<td>0.43</td>
<td>0.09</td>
</tr>
</tbody>
</table>

\(^1\text{Mean ± SEM (mm) physical comfort score; } n=13\)
\(^2\text{Scoring: Not well at all}=0; \text{Very well}=100\)
5.1.5. Palatability

There was no difference in the palatability ratings of the pizza between treatments \([F=2.09, p=0.10]\) (Table 15). The drinks, however, were rated to be different \([F=12.00, p<0.00]\), with the control being as palatable as the sucrose drinks, but more palatable than the protein drinks. The sucrose and egg protein drinks were equivalent, but both were more palatable than whey. The whey drink was not different from the egg protein drink; and all protein drinks were more palatable than the soy drink.

The pre-experiment testing of drinks also found that drinks were not equally palatable (Table 3). However, in order to study the effect of the pure protein sources on food intake, it was important to not add additional ingredients to the commercial protein mixes. Thus, it was difficult to adjust the palatability of the protein drinks.

Table 15: Experiment 1. Palatability Ratings of Beverage and Pizza after Treatment$^{1,2}$

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drink</th>
<th>Pizza</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>59.3 ± 5.5$^a$</td>
<td>67.5 ± 3.7</td>
</tr>
<tr>
<td>Egg</td>
<td>44.3 ± 6.5$^{bc}$</td>
<td>66.9 ± 4.0</td>
</tr>
<tr>
<td>Whey</td>
<td>33.9 ± 5.3$^c$</td>
<td>60.2 ± 4.5</td>
</tr>
<tr>
<td>Soy</td>
<td>17.7 ± 3.9$^d$</td>
<td>65.1 ± 4.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>57.5 ± 5.8$^{ab}$</td>
<td>60.8 ± 4.1</td>
</tr>
</tbody>
</table>

| F    | 12.00 | 2.09 |
| p    | <0.00 | 0.10 |

$^1$Mean ± SEM (mm); n=13
$^2$Scoring: Very pleasant = 0; Not pleasant at all = 100
$^a$Treatments with different letters within a column are different, \(p<0.05\)
5.1.6. **Sweetness**

Treatment differences were found in the subjective sweetness ratings of the preload drinks \( F = 6.39, p < 0.00 \) (Figure 5). All drinks were found to be equivalent, except for the soy drink, which was less sweet than all the other preloads.

![Bar chart showing perceived sweetness of treatments](image)

**Figure 5:** Perceived sweetness of treatments

\(^a\)Treatments with different letters are different \((p < 0.05)\)
5.1.7. Characteristics of Subjects in the Two Arrival Time Groups

The 9:10 AM arrival group was slightly older in age (1.9 years) than the 11:00 AM arrival group (Table 16). There were no differences in the mean BMI between the group that had a 9:10 AM arrival time and the group that had 11:00 AM arrival time.

The number of hours that subjects reported to be fasting at the arrival time was not significantly different between the two arrival time sessions.

Subjects, who had the later arrival time of 11:00 AM, on average, had a slightly longer amount of sleep (0.7 hours) than the subjects who arrived at 9:10 AM (Table 16).

Table 16: Experiment 1. Characteristics of Subjects in the Two Arrival Time Groups

<table>
<thead>
<tr>
<th></th>
<th>9:10 AM (n=6)</th>
<th>11:00 AM (n=7)</th>
<th>t; p²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23.2 ± 1.3</td>
<td>21.3 ± 0.8</td>
<td>3.07; 0.00*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.2 ± 0.7</td>
<td>22.0 ± 0.6</td>
<td>0.16; 0.88</td>
</tr>
<tr>
<td>Hours of Fasting (hours)³</td>
<td>13.7 ± 0.3</td>
<td>14.0 ± 0.3</td>
<td>-0.67; 0.50</td>
</tr>
<tr>
<td>Hours of Sleep (hours)⁴</td>
<td>6.6 ± 0.2</td>
<td>7.3 ± 0.2</td>
<td>-2.35; 0.02*</td>
</tr>
</tbody>
</table>

¹Mean ± SEM  
²t and p values, Student’s t-test  
³Hours of fasting prior to starting the test  
⁴Hours of sleep the night before the test  
*Means within a row are different, p<0.05
5.1.8. Correlations

There were no relationships between food intake and any of: average appetite at 0, 15, 30, 45 and 60 minutes, the individual Motivation to Eat questions at 60 minutes, physical comfort at 60 minutes, perceived drink sweetness or perceived drink palatability (Table 17). Neither the hours of sleep subjects had prior to test sessions nor the hours they reported to fast before the test correlated with food intake one hour after the treatments were consumed.

Furthermore, there was no relationship between average appetite at 15 minutes and perceived sweetness of drinks or perceived palatability of the drinks. No correlation was found between perceived palatability of drinks and physical comfort at 60 minutes (Table 17).
Table 17: Experiment 1. Correlations Between Dependent Measures

<table>
<thead>
<tr>
<th>Correlated variable</th>
<th></th>
<th>r ( ^1 ); p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food Intake</td>
<td>Average Appetite 0 min</td>
<td>-0.224; 0.07</td>
</tr>
<tr>
<td>Food Intake</td>
<td>Average Appetite 15 min</td>
<td>-0.093; 0.46</td>
</tr>
<tr>
<td>Food Intake</td>
<td>Average Appetite 30 min</td>
<td>-0.069; 0.46</td>
</tr>
<tr>
<td>Food Intake</td>
<td>Average Appetite 45 min</td>
<td>-0.028; 0.58</td>
</tr>
<tr>
<td>Food Intake</td>
<td>Average Appetite 60 min</td>
<td>-0.005; 0.97</td>
</tr>
<tr>
<td>Food Intake</td>
<td>Desire 60 min</td>
<td>0.076; 0.54</td>
</tr>
<tr>
<td>Food Intake</td>
<td>Hunger 60 min</td>
<td>-0.106; 0.40</td>
</tr>
<tr>
<td>Food Intake</td>
<td>Full 60 min</td>
<td>0.024; 0.85</td>
</tr>
<tr>
<td>Food Intake</td>
<td>Amount 60 min</td>
<td>0.057; 0.65</td>
</tr>
<tr>
<td>Food Intake</td>
<td>Drink Palatability</td>
<td>-0.033; 0.79</td>
</tr>
<tr>
<td>Food Intake</td>
<td>Drink Sweetness</td>
<td>0.093; 0.46</td>
</tr>
<tr>
<td>Food Intake</td>
<td>Hours of Fasting Before Arrival</td>
<td>0.074; 0.56</td>
</tr>
<tr>
<td>Food Intake</td>
<td>Hours of Sleep Previous Night</td>
<td>0.146; 0.25</td>
</tr>
<tr>
<td>Food Intake</td>
<td>Physical Comfort 60 min</td>
<td>0.144; 0.25</td>
</tr>
<tr>
<td>Perceived Sweetness</td>
<td>Average Appetite 15 min</td>
<td>0.074; 0.56</td>
</tr>
<tr>
<td>Perceived Palatability</td>
<td>Average Appetite 15 min</td>
<td>0.060; 0.64</td>
</tr>
<tr>
<td>Perceived Palatability</td>
<td>Physical Comfort 60 min</td>
<td>0.188; 0.13</td>
</tr>
</tbody>
</table>

\( ^1 \)Pearson’s Correlation Coefficient
*Significance at the 0.01 level (2-tailed)
5.2. DISCUSSION

The results of this experiment support the hypothesis that the effect of proteins on food intake depends on the protein source. Isocaloric preloads of whey and soy protein, but not of egg protein or sucrose, suppressed food intake one hour later compared to control.

Sucrose was included as a treatment in this experiment as a carbohydrate source to compare the effects on food intake of protein with a pure carbohydrate source. While many studies have compared the effect of high protein with high carbohydrate treatments on food intake and satiety, the treatments have contained significant quantities of fat, as well as protein and carbohydrate (Booth 1970; Hill and Blundell 1986; Latner and Schwartz 1999), making it difficult to assess the effect of the macronutrient alone. Based on a literature review, it was predicted that a minimum dose of sucrose necessary to lower food intake after 20 to 60 minutes is 50 g when a sample size less than 20 subjects is used (Anderson 1995). However, in a study using a similar protocol to this study and with 15 subjects, 25, 50 and 75 g of sucrose suppressed food intake at one hour, compared with an unsweetened control (water) but only the 75 g treatment suppressed food intake compared to the sweet control (Woodend 2000). In the present study the average sucrose treatment was 45.1g and only a sweet control was provided. Thus the observation that the mean energy consumption of subjects following sucrose was not less than that of the mean consumption following the sweet control was not unexpected.

Although the literature generally supports the view that protein ingestion suppresses food intake more than carbohydrate (Barkeling et al. 1990; Latner and Schwartz 1999; Ohlson and Parente Hart 1965), some studies have failed to do so (de Graaf et al. 1992). The results of the present study support the observation that protein
decreases food intake compared with pure carbohydrate sources, but also shows that the source of protein may be a factor in such comparisons. Thus some of the studies which failed to show an effect may be explained by the protein source used in the treatment, as well as by the fact that it was fed in a food containing fat and carbohydrates.

The findings of this experiment are consistent with the only report in the literature that compared the effect of relatively pure protein sources on food intake. When 50 g meals of either lean chicken, beef or fish were given to young men, the subjects rated the fish meal to be more satiating than either chicken or beef over a three-hour period using VAS (Uhe et al. 1992). While this report suggested that protein source was a factor in determining appetite suppression, food intake was not measured. Two recent studies comparing the effect of protein sources on food intake did not find protein source within a lunch meal to be a factor in determining food intake (Lang et al. 1998; 1999). However, two aspects of the design probably explain these results. First, protein contributed only ~20% of calories in the ~425 and 1242 kcal of test meals. Second, food intake was not measured until 8 hours later.

Egg protein did not suppress food intake, which was surprising. In rats, egg protein preloads suppress food intake over the first two hours of access to food cups. Because blocking CCK\textsubscript{A} receptors in rats prevents suppression of food intake by egg protein preloads (Cochi 2000; Morgan 1998; Trigazis 1997), a specific mechanism of action appears to operate at the gut level. The absence of effect of egg protein on food intake in humans may indicate that this same mechanism does not function in humans.

The young men adjusted their energy intake compared to control most precisely (96%) after the whey protein treatment (Table 7). The compensation in test meal food intake for the soy preload energy content was 59%. Thus subjects consumed more
calories in total (preload and pizza) with the soy than they did with the control treatment. Compensation after the egg preload was negative because subjects ate a greater quantity of pizza after this treatment than after the control preload. The 45 g sucrose treatment resulted in an unexpectedly low consumption of 11%. In a previous study, similar in design, 50 g sucrose preloads resulted in 44% compensation (Woodend 2000). It is difficult to explain these differences because there were only small differences in the study designs. In Woodend’s study, sucrose was given in 300 mL preloads, whereas in this study 450 mL preloads were used, raising the possibility that volume may be a factor. Furthermore, in this study, sugar-free strawberry Kool-Aid and maltodextrin were added to all preload drinks.

The failure to control adequately for both sweetness and palatability among the treatments was of concern. Sucralose was added to all the drinks proportionally (Table 4) to match the sweetness of the sucrose treatment. In the preliminary study, the drinks were reported as equally sweet \( [F=1.73, p=0.16] \), whereas in the main study, subjects rated soy to be less sweet than all the others. The discrepancy in sweetness findings of test drinks between the pretrial ratings and the main study ratings may be explained by the methods of rating employed. In the pre-trial ratings, subjects were not required to drink the whole preload before rating the sweetness of drinks. They were simply asked to taste each drink and rate it. Subjects tasted all pre-trial drinks on the same day, at any time they were available, and drank water between each sample of the test drinks. Furthermore, the pre-trial sweetness testing differed from the methods of the main test, which had subjects rate sweetness on a 100 mm scale. In this pre-trial subjects rated the drinks on a scale of 1 to 5 \( (1=\text{not sweet at all}; 5=\text{very sweet}) \), which may have reduced the sensitivity of the test for sweetness. In addition, a slightly smaller sample size
(n=11) was used in the pre-trial, which may have been a factor, because soy was rated to be nearly 1 point less sweet than the other treatments. Fortunately, there was no correlation between perceived sweetness of the treatments and food intake (Table 17), suggesting that this difference in sweetness was not a determinant of food intake one hour later.

As with sweetness, there was a difference in palatability of treatments in the main experiment. However, this was expected, as pre-trial testing also found differences [F=13.11; p<0.00]. The thick consistency of the soy treatment made it very difficult to disguise. However, there was no correlation between perceived palatability of the preloads and food intake. This is consistent with reports that “liking” of a preload does not influence food intake one hour later (Graff et al. 1999). Furthermore, although soy suppressed food intake and had a low palatability, it did not suppress food intake compared to the sucrose preload, which rated high on the palatability scale. Conversely, whey had the same consistency and palatability as the egg treatment, but these treatments had different effects on food intake.

The failure to find a consistent effect of treatment on the Motivation to Eat VAS scales and to find no correlations between average appetite and food intake was surprising. The only treatment effect obtained was at 15 minutes, when soy suppressed desire to eat compared to all other treatments, and at 45 minutes compared to control and sucrose (Table 13). This may be due to thick consistency of the soy drink. It is unclear why only the desire to eat ratings was affected by soy.

Many of the previous studies of similar design from this laboratory have found an association between treatment and appetite using VAS (Rackal 1998; Stewart et al. 1997;
Woodend 2000). Other laboratories also report VAS to be useful in tracking the effect of treatment with time on appetite (Brown 1998; Rolls et al. 1988; Uhe et al. 1992).

One possible explanation for the current results resides in the experimental design. The subjects here were required to complete 13 VAS questions (appetite and mood) every 15 minutes as well as complete 3 memory tests and a video exercise. The inclusion of the memory and mood measurements, as well as the video exercise may have contributed to the high variance in all VAS responses (Herman et al. 1999). The purpose of the video was to distract subjects from remembering words for a delayed recall memory test, by having them identify audio and visual cues from pre-recorded music videos. Subjects were not evaluated for their performance on the video section because the measurements were conducted inappropriately. Consistent grading between subjects could not be done because some subjects marked visual cues from different camera angles as appearing on screen, whereas others did not. However, as subjects were unaware of the purpose of the video task, the 30 minutes of constant focus may have affected the attentiveness needed for completing the VAS sections. Taken together, the high multiplicity of tasks each person was required to complete may have interfered with the subjects' attention to the VAS on subjective appetite.

An unexpected finding arising from this study was that arrival time modifies the effect of protein source on food intake. The effect of arrival time was unexpected because the time difference was set at only two hours and because the sample size was small, with an n=6 and n=7 at 9:10 AM 11:00 AM, respectively.

Although the experimental design did not include arrival time as a main factor, retrospective analyses pointed to this being a factor. First, the effect of arrival time was shown by the interaction between arrival time and treatment effects in the two-way
ANOVA \([F=3.07, p=0.03]\). To better understand the pattern of food intake following treatments, a one-way repeated measures ANOVA was also conducted to examine the effect of treatment on food intake within each group. There were also less distinct differences in the mean food intake following each treatment of the 11:00 AM group (compared to the 9:10 AM means); the only significant differences in food intake were that egg protein increased food intake compared to control and whey suppressed food intake compared to sucrose and egg protein (Figure 3).

Differences in effect of the treatments on food intake between the two arrival times were also examined by comparing food intake between the 9:10 AM group and the 11:00 AM group for each treatment (Table 8). The only difference found was that more food was consumed following an egg protein preload at 11:00 AM compared to 9:10 AM \([F=5.54, p=0.04]\). Analyses of food intake in the 11:00 AM group showed more variability in food intake following treatments, indicated by standard error of means for the treatments, compared to the variability in the 9:10 AM group food intake (Table 8).

Some aspects of the study require comment because they may have influenced the results. First, there was no standardized washout period for test days within subjects or between them. The protocol was established to have a minimum one-day and a maximum of two weeks washout between treatments. While some subjects chose to be tested once a week, some opted to have their sessions every other day, while others preferred a more random schedule. The lack of uniformity both within and between subjects for time between sessions caused difficulty in filtering out the effect of “novelty” of the pizza when food intake was measured. While treatment order did not affect food intake significantly (Table 18), the decrease in food intake between the mean caloric consumption after the first treatment and after the fifth treatment was 130 kcal. For this
reason, it seems reasonable to set a longer and fixed washout period, such as one-week. Adherence to this schedule, however, might reduce the number of treatments that are practical in a study, as some subjects expressed reticence to be committed to long-term studies.

Finally, it became clear in the analysis of the data that more detailed knowledge of subject behaviour prior to the test sessions would be of value. Subjects were told to maintain a normal diet and not participate in any unusual physical activities on days prior to test sessions. While they recorded the contents and time of their final meal the day before, the investigator did not check the data. For example, subjects may have written what time they had dinner and what they consumed, or they may have written what they had as a snack before sleeping. Inconsistencies made it difficult to identify unusual behavioural patterns, which may have affected the following day’s test. Furthermore, subject perceptions of “unusual physical activities” varied greatly; some participated in heavy exercise before some test sessions, as they considered it normal for them.

In conclusion, this experiment supported the hypothesis that, in humans, the effect of protein on food intake depends on its source. The unexpected finding that the time of morning affected the food intake response suggested a need to repeat the experiment with more focus on the effect of arrival time response and with more control over factors that might affect study outcome. Thus, as described in the section on design and procedures, the second experiment limited the number of treatments to three, including only a sweet control, egg and whey proteins. A washout period between treatments of one-week for all subjects was imposed. Subjects were asked to strictly limit to their activities and standardize them the day prior to and on test sessions. Subjects were also requested to eat the same meal (part of their normal routine) before each test session night. The
number of hours subjects slept, what time they woke up in the morning and what their specific activities were performed on the morning of test days were also recorded to ensure consistency of behaviour prior to treatments. During experiment 2, visual analogue scales were completed only 4 times over one-hour and the video task was eliminated.

Table 18: Experiment 1. Effect of Treatment Order on Food Intake

<table>
<thead>
<tr>
<th>Treatment Order</th>
<th>Energy Intake (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>829.5 ± 65.8</td>
</tr>
<tr>
<td>2nd</td>
<td>863.0 ± 54.2</td>
</tr>
<tr>
<td>3rd</td>
<td>814.8 ± 81.8</td>
</tr>
<tr>
<td>4th</td>
<td>775.5 ± 64.3</td>
</tr>
<tr>
<td>5th</td>
<td>699.4 ± 70.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.07</td>
<td>0.10</td>
</tr>
</tbody>
</table>

1Mean ± SEM (mm); n=13
Experiment 2:

THE EFFECT OF PROTEIN SOURCE AND TIME OF ARRIVAL ON FOOD INTAKE

5.3. RESULTS

5.3.1. Food and Water Intake

Twenty-two subjects completed the second experiment, with 12 subjects in the 8:30 AM group and 10 subjects in the 11:00 AM group. A two-way ANOVA with repeated measures on one factor (treatment) found that treatment affected food intake \( [F=11.41, p<0.00] \), but as in experiment 1, there was no overall effect of arrival time on food intake \( [F=1.41, p=0.25] \). In contrast to experiment 1, there was no interaction between treatment and time of arrival \( [F=2.31, p=0.11] \).

As no effect of arrival time was found in the two-way ANOVA, the data for all 22 subjects were pooled and the effect of treatment on food intake was measured by one-way repeated measures ANOVA. Treatment effect on food intake was significant when subjects were pooled \( [F=10.89, p<0.00] \). As in experiment 1, whey suppressed food intake compared to control and egg. There was no difference in food intake suppression between control and egg.

Twenty-four subjects were recruited for the second experiment (22 in the final analyses) so that there would be enough subjects in each arrival time group to determine the effect of treatments on food intake by a repeated measures one-way ANOVA for each group separately. Thus the effects of treatment on food intake in the 8:30 AM and 11:00 AM groups were once again analyzed for each group. In the 8:30 AM group (n=12),
there was a significant effect of treatment on food intake \([F=11.64, p<0.00]\). In this group, both egg and whey protein suppressed food intake compared to control, and there was no significant difference between. As in experiment 1, the pattern of effect of treatment was different for the 11:00 AM group \((n=10)\). Again a significant effect of treatment on food intake \([F=4.40, p=0.03]\) was found. There was no difference either between egg and control, or between whey protein and control, but whey protein and control, however, whey protein suppressed food intake significantly more than egg protein (Figure 6).

No differences in food intake were found between the 9:10 AM and 11:00 AM groups when each treatment was compared (Table 20). However, there was a trend for food intake to be greater following egg protein at 9:10 AM compared to 11:00 AM \([t=-1.92, p=0.07]\).

The percent compensation following preloads was 27.7% and 125.8% for the egg and whey treatments, respectively (Table 19).

The effect of treatment on the amount of water consumed with the pizza approached statistical significance \([F=3.92, p=0.06]\). The subject tended to drink less water after control than either of the protein treatments (Table 19).

To reduce variability in the food intake measures, the subjects were asked to consume the same meals and to record the amount of food consumed from 3:00 PM onwards, the day prior to each test session. Using two-way ANOVA with repeated measures on one factor (arrival time), it was found that there were no differences between treatments in calories consumed the night before \([F=0.26, p=0.77]\) and no differences in between the two arrival groups of 8:30 AM group and the 11:00 AM group in calories consumed the night before \([F=0.05, p=0.83]\); there was also no interaction \([F=1.03,\)
p=0.36] between treatment and time of arrival for calories consumed the night before test days. As with the food intake data on the treatment days, the data was pooled for all subjects. Again, there were no differences between treatments in caloric intake the days prior to testing [F=0.18, p=0.84] (Table 21).

![Energy Intake (kcal) vs Treatment](image)

**Figure 6:** Test meal food intake: calories consumed from a pizza test meal one-hour after treatments

*a*Treatments with different letters are different (p<0.05)
Table 19: Experiment 2. Effect of Treatments on Food and Water Intake

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Test Meal</th>
<th>Test Meal + Preload</th>
<th>Water Intake</th>
<th>% Compensation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(kcal)</td>
<td>(kcal)</td>
<td>(g)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1039.8 ± 72.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1057.7 ± 72.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>338.9 ± 34.9</td>
<td>-</td>
</tr>
<tr>
<td>Egg</td>
<td>988.3 ± 78.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1198.6 ± 80.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>420.5 ± 57.9</td>
<td>27.7 ± 29.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Whey</td>
<td>768.1 ± 62.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>978.4 ± 62.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>396.9 ± 44.5</td>
<td>125.7 ± 31.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

F: 10.89, p<0.00

Means with different superscript letters within a column are different. p<0.05

<sup>1</sup>Mean ± SEM; n = 22
<sup>2</sup>Energy consumed at the test meal
<sup>3</sup>Energy total of preload and test meal
<sup>4</sup>Water intake at test meal
<sup>5</sup>% compensation = (kcal control test meal − kcal treatment test meal)/kcal preload*100
Figure 7: Effect of treatments on energy intake from the test pizza meal at two arrival times

a) 8:30 AM group (n=12)

b) 11:00 AM group (n=10)

*Treatments with different letters are different (p<0.05)*
Table 20: Experiment 2. Effect of Arrival Time on Differences in Food Intake for Individual Treatments

<table>
<thead>
<tr>
<th></th>
<th>8:30 AM</th>
<th>11:00 AM</th>
<th>t; p²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=12)</td>
<td>(n=10)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1027.6 ± 60.0</td>
<td>1054.5 ± 147.5</td>
<td>-0.18; 0.86</td>
</tr>
<tr>
<td>Egg</td>
<td>859.3 ± 77.4</td>
<td>1143.2 ± 133.5</td>
<td>-1.92; 0.07*</td>
</tr>
<tr>
<td>Whey</td>
<td>710.4 ± 75.9</td>
<td>837.3 ± 101.8</td>
<td>-1.02; 0.32</td>
</tr>
</tbody>
</table>

1Mean ± SEM
2t and p values, Student’s t-test
*Means within a row are close to significance; p=0.07

Table 21: Experiment 2. Energy Intake Prior to Test Days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Energy Intake (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1202.0 ± 58.8</td>
</tr>
<tr>
<td>Egg</td>
<td>1218.2 ± 62.9</td>
</tr>
<tr>
<td>Whey</td>
<td>1214.8 ± 58.2</td>
</tr>
</tbody>
</table>

F 0.18
p 0.84

1Mean ± SEM (mm); n=22
2Calories consumed the day prior to treatment days, calculated from subjects’ food diaries
5.3.2. Average Appetite

The average appetite ratings are reported both as absolute values (Table 22) and as change from baseline values (Table 23). All statistical analyses of the data for average appetite were based on the change from baseline scores (Table 22) because they compare each individual's rating of appetite questions to his own pre-treatment scores for that particular test, which minimizes the differences between subjects. No overall effect of treatment on average appetite \([F=1.25, p=0.30]\) and no time by treatment interaction \([F=0.98, p=0.42]\). However, an overall effect of time \([F=6.21, p<0.00]\) was found, where average appetite increased over time after 20 minutes. The response to time for each individual treatment was therefore analyzed. Average appetite increased over time for control and egg \([F=4.43, p=0.02\) and \(F=4.06, p=0.02\), respectively] but no effect of time was present for whey \([F=0.38, p=0.68]\). The response to treatment at each individual time of measurement was also analyzed, but no treatment effects were found (Table 23).
Table 22: Experiment 2. Effect of Treatment on Absolute Average Appetite Scores<sup>1</sup>

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>Egg</th>
<th>Whey</th>
<th>F; p</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>64.3 ± 3.8</td>
<td>65.0 ± 3.7</td>
<td>68.3 ± 2.8</td>
<td>1.19; 0.32</td>
</tr>
<tr>
<td>20</td>
<td>60.0 ± 2.8</td>
<td>58.9 ± 3.6</td>
<td>60.4 ± 3.2</td>
<td>0.12; 0.88</td>
</tr>
<tr>
<td>40</td>
<td>62.3 ± 2.9</td>
<td>60.1 ± 3.2</td>
<td>59.7 ± 3.6</td>
<td>0.40; 0.67</td>
</tr>
<tr>
<td>60</td>
<td>67.3 ± 3.1</td>
<td>64.4 ± 3.2</td>
<td>61.3 ± 4.0</td>
<td>1.52; 0.23</td>
</tr>
</tbody>
</table>

<sup>1</sup>Mean ± SEM (mm); n=22

Table 23: Experiment 2. Effect of Treatment on Change from Baseline Average Appetite Scores<sup>1</sup>

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>Egg</th>
<th>Whey</th>
<th>F; p</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>-4.0 ± 3.7</td>
<td>-6.2 ± 3.3</td>
<td>-7.4 ± 3.2</td>
<td>0.41 0.67</td>
</tr>
<tr>
<td>40</td>
<td>-1.9 ± 4.1</td>
<td>-4.9 ± 3.4</td>
<td>-7.5 ± 3.8</td>
<td>1.03 0.37</td>
</tr>
<tr>
<td>60</td>
<td>2.7 ± 4.4</td>
<td>-0.6 ± 3.7</td>
<td>-5.9 ± 4.3</td>
<td>1.91 0.16</td>
</tr>
</tbody>
</table>

<sup>1</sup>Mean ± SEM (mm); n=22
5.3.3. Motivation to Eat

Results from the Motivation to Eat questions are reported as absolute values (Table 24) and change from baseline values (Table 25). All statistical analyses are based on the change from baseline values using the repeated measures two-way ANOVA for each of the four questions in the Motivation to Eat questionnaire. The effect of treatment on each of the individual questions, at each time point is reported in Table 24.

For the desire question, no overall effect of treatment \( F=0.93, p=0.40 \), or time by treatment interaction \( F=0.82, p=0.51 \) was found. However, there was an increase with time \( F=3.81, p=0.03 \). When individual treatments were examined for their effect with time, there was an increase after control \( F=3.78, p=0.03 \), but not after egg and whey treatments \( F=1.63, p=0.21 \) and \( F=0.16, p=0.85 \), respectively. Mean comparisons at each time of measurement showed no effect of treatment on desire to eat (Table 25).

For the hunger question, no overall effect of treatment \( F=0.26, p=0.78 \), or time by treatment interaction \( F=2.10, p=0.09 \) was found. However, there was an increase with time \( F=5.50, p=0.01 \). When individual treatments were examined for their effect of time, there was an increase after control \( F=6.18, p<0.00 \), but not after egg and whey treatments \( F=2.89, p=0.07 \) and \( F=0.13, p=0.88 \), respectively. Mean comparisons at each time of measurement showed no effect of treatment on hunger (Table 25).

For the fullness question, no overall effect of treatment \( F=2.09, p=0.14 \), or time by treatment interaction \( F=0.92, p=0.47 \) was found. However, there was a decrease with time \( F=3.62, p=0.04 \). When individual treatments were examined for their effect of time, there was a decrease after egg \( F=3.59, p=0.04 \), but not after control and whey treatments \( F=1.66, p=0.20 \) and \( F=0.41, p=0.66 \), respectively. Mean comparisons at each time of measurement showed no effect of treatment on fullness (Table 25).
For the amount question, no overall effect of treatment \( [F=1.45, p=0.24] \), or time by treatment interaction \( [F=0.04, p=0.99] \) was found. However there was an increase with time \( [F=3.42, p=0.04] \). When individual treatments were examined for their effect of time on amount, there was no change after control, egg or whey treatments \( [F=1.61, p=0.21, F=1.54, p=0.23 \) and \( F=1.88, p=0.16 \), respectively]. Mean comparisons at each time of measurement showed no effect of treatment on amount (Table 25).
Table 24: Experiment 2. Effect of Treatment on Questions 1 to 4 Absolute Motivation to Eat Scores

<table>
<thead>
<tr>
<th>Question</th>
<th>Time (min)</th>
<th>Control</th>
<th>Egg</th>
<th>Whey</th>
<th>F; p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-desire</td>
<td>0</td>
<td>63.3 ± 4.8</td>
<td>64.6 ± 4.8</td>
<td>67.8 ± 4.0</td>
<td>0.79; 0.46</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>61.0 ± 3.5</td>
<td>59.3 ± 4.2</td>
<td>61.7 ± 3.5</td>
<td>0.21; 0.81</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>61.6 ± 3.4</td>
<td>59.0 ± 3.6</td>
<td>63.0 ± 3.7</td>
<td>0.59; 0.56</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>68.1 ± 2.9</td>
<td>63.9 ± 3.7</td>
<td>63.1 ± 4.7</td>
<td>0.72; 0.49</td>
</tr>
<tr>
<td>2-hunger</td>
<td>0</td>
<td>61.5 ± 5.0</td>
<td>62.5 ± 4.7</td>
<td>61.8 ± 3.6</td>
<td>0.04; 0.96</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>57.2 ± 3.7</td>
<td>57.1 ± 4.3</td>
<td>60.2 ± 3.4</td>
<td>0.42; 0.66</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>61.5 ± 3.0</td>
<td>58.8 ± 4.0</td>
<td>59.0 ± 3.9</td>
<td>0.28; 0.76</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>67.3 ± 3.4</td>
<td>63.4 ± 3.5</td>
<td>59.9 ± 4.8</td>
<td>1.30; 0.28</td>
</tr>
<tr>
<td>3-fullness</td>
<td>0</td>
<td>27.5 ± 3.8</td>
<td>24.5 ± 3.4</td>
<td>20.0 ± 3.3</td>
<td>2.52; 0.09</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>36.3 ± 3.5</td>
<td>37.3 ± 4.3</td>
<td>34.9 ± 4.0</td>
<td>0.20; 0.82</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>34.0 ± 4.2</td>
<td>35.1 ± 3.6</td>
<td>37.0 ± 4.7</td>
<td>0.29; 0.75</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>30.8 ± 3.6</td>
<td>29.5 ± 3.0</td>
<td>34.5 ± 4.4</td>
<td>1.11; 0.34</td>
</tr>
<tr>
<td>4-amount</td>
<td>0</td>
<td>64.9 ± 4.1</td>
<td>64.0 ± 3.6</td>
<td>67.0 ± 2.6</td>
<td>0.43; 0.66</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>64.1 ± 3.2</td>
<td>62.6 ± 4.0</td>
<td>60.0 ± 4.1</td>
<td>0.75; 0.48</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>65.5 ± 3.4</td>
<td>64.3 ± 3.5</td>
<td>61.6 ± 3.4</td>
<td>0.87; 0.42</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>68.5 ± 3.2</td>
<td>66.3 ± 3.5</td>
<td>64.4 ± 3.2</td>
<td>1.01; 0.37</td>
</tr>
</tbody>
</table>

1 Mean ± SEM (mm); n=13
2 Questions (on a scale of 0 to 100):
   1= How strong is your desire to eat?
   2= How hungry do you feel?
   3= How full do you feel?
   4= How much food do you think you could eat?
Table 25:  
Experiment 2. Effect of Treatment on Questions 1 to 4 Change From Baseline Motivation to Eat Scores

<table>
<thead>
<tr>
<th>Question&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Time (min)</th>
<th>Control</th>
<th>Egg</th>
<th>Whey</th>
<th>F; p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>-2.2 ± 4.4</td>
<td>-5.3 ± 4.9</td>
<td>-6.0 ± 4.0</td>
<td>0.34; 0.71</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>-1.7 ± 4.5</td>
<td>-5.6 ± 4.4</td>
<td>-4.8 ± 4.4</td>
<td>0.50; 0.61</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>4.8 ± 5.0</td>
<td>-0.7 ± 4.8</td>
<td>-4.6 ± 4.9</td>
<td>1.66; 0.20</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>-4.3 ± 4.5</td>
<td>-5.5 ± 3.5</td>
<td>-1.6 ± 3.9</td>
<td>0.35; 0.71</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.0 ± 4.9</td>
<td>-3.8 ± 3.9</td>
<td>-2.8 ± 4.4</td>
<td>0.27; 0.76</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>5.8 ± 4.7</td>
<td>0.9 ± 4.8</td>
<td>-1.9 ± 5.1</td>
<td>0.94; 0.40</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>8.9 ± 3.7</td>
<td>12.8 ± 3.4</td>
<td>14.9 ± 4.2</td>
<td>0.75; 0.48</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>6.6 ± 4.4</td>
<td>10.6 ± 3.4</td>
<td>17.0 ± 5.0</td>
<td>2.09; 0.14</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>3.4 ± 5.0</td>
<td>5.0 ± 3.5</td>
<td>14.5 ± 4.9</td>
<td>2.61; 0.09</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>-0.8 ± 4.5</td>
<td>-1.4 ± 3.4</td>
<td>-7.0 ± 3.8</td>
<td>1.28; 0.29</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.5 ± 4.8</td>
<td>0.3 ± 3.6</td>
<td>-5.3 ± 3.7</td>
<td>1.26; 0.30</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>3.5 ± 4.5</td>
<td>2.3 ± 3.8</td>
<td>-2.6 ± 3.7</td>
<td>1.18; 0.32</td>
</tr>
</tbody>
</table>

<sup>1</sup>Mean ± SEM (mm); n=22  
<sup>2</sup>Questions (on a scale of 0 to 100):  
1= How strong is your desire to eat?  
2= How hungry do you feel?  
3= How full do you feel?  
4= How much food do you think you could eat?
5.3.4. Physical Comfort

Feelings of well-being were not different among treatments at 0 \([F=0.57, p=0.60]\) and 60 minutes \([F=1.89, p=0.16]\) (Table 26). There was no effect of treatment on the difference in the change in physical comfort scores from baseline to 60 minutes post pre-load \([F=0.44, p=0.65]\).

Table 26: Experiment 2. Effect of Treatment on Physical Comfort Scores\(^1,2\)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (min)</td>
<td>60 (min)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>70.2 ± 3.2</td>
<td>65.2 ± 3.8</td>
</tr>
<tr>
<td>Egg</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>73.6 ± 3.3</td>
<td>71.5 ± 3.4</td>
</tr>
<tr>
<td>Whey</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>74.3 ± 3.9</td>
<td>71.3 ± 3.9</td>
</tr>
<tr>
<td>F</td>
<td>0.57</td>
<td>1.89</td>
</tr>
<tr>
<td>p</td>
<td>0.57</td>
<td>0.16</td>
</tr>
</tbody>
</table>

\(^1\)Mean ± SEM (mm) physical comfort score; n=22
\(^2\)Scoring: Not well at all=0; Very well=100
5.3.5. Palatability

There was no difference in the palatability ratings of the pizza between treatments \([F=0.87, p=0.43]\) (Table 27). The drinks, however, were rated to be different in palatability \([F=15.53, p<0.00]\). The control treatment was more palatable than the egg or whey treatments, but the latter two treatments were not different from each other.

Table 27: Experiment 2. Palatability Ratings of Beverage and Pizza after Treatment\(^{1,2}\)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drink</th>
<th>Pizza</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>58.9 ± 4.8(^a)</td>
<td>74.3 ± 3.7</td>
</tr>
<tr>
<td>Egg</td>
<td>37.4 ± 4.9(^b)</td>
<td>76.8 ± 2.9</td>
</tr>
<tr>
<td>Whey</td>
<td>38.8 ± 6.0(^b)</td>
<td>73.0 ± 3.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14.91</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>&lt;0.00</td>
<td>0.43</td>
</tr>
</tbody>
</table>

\(^1\)Mean ± SEM (mm); \(n=22\)

\(^2\)Scoring: Very pleasant = 0; Not pleasant at all = 100

\(^a\)Treatments with different letters along a column are different, \(p<0.05\)
5.3.6. Sweetness

There was no overall effect of treatment on perceived sweetness \([F=3.29, p=0.05]\) (Figure 8), as found in the pre-experiment trials (Table 5).

![Figure 8: Perceived sweetness of treatments](image-url)
5.3.7. Characteristics of Subjects in the Two Arrival Time Groups

There were no significant differences in the mean age and BMI between the groups arriving at 8:30 AM or at 11:00 AM arrival (Table 28).

The number of hours that subjects reported fasting prior to the arrival time was different. The subjects in the 8:30 AM arrival time group fasted one hour longer than the subjects in the 11:00 AM group.

Subjects who had the later arrival time of 11:00 AM, on average, reported to have slept for a longer time (1.1 hours) than the subjects who had the earlier arrival time of 8:30 AM. However, the amount of time subjects reported to be awake before arriving for their test sessions was not different between the two arrival time groups (Table 28).

Table 28: Experiment 2. Characteristics of Subjects in the Two Arrival Time Groups 1

<table>
<thead>
<tr>
<th></th>
<th>8:30 AM (n=12)</th>
<th>11:00 AM (n=10)</th>
<th>t; p^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22.5 ± 1.0</td>
<td>22.1 ± 1.0</td>
<td>0.28; 0.78</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>22.6 ± 0.5</td>
<td>23.1 ± 0.3</td>
<td>-0.86; 0.40</td>
</tr>
<tr>
<td>Hours of Fasting (hours)^3</td>
<td>12.8 ± 0.1</td>
<td>13.9 ± 0.4</td>
<td>-2.92; 0.01*</td>
</tr>
<tr>
<td>Hours of Sleep (hours)^4</td>
<td>7.0 ± 0.2</td>
<td>8.1 ± 0.1</td>
<td>-5.26; 0.00*</td>
</tr>
<tr>
<td>Hours Awake (hours)^5</td>
<td>1.7 ± 0.1</td>
<td>1.9 ± 0.2</td>
<td>-0.93; 0.36</td>
</tr>
</tbody>
</table>

1Mean ± SEM
2t and p values, Student’s t-test
3Hours of fasting prior to starting the test
4Hours of sleep the night before the test
5Hours awake prior to arrival for the test
*Means within a row are different, p<0.05
5.3.8. Correlations

There was no relationship between average appetite at 0 minutes and food intake (Table 29). There was a positive correlation between food intake and average appetite at 20, 40 and 60 minutes and hunger at 60 minutes, amount at 60 minutes and a negative correlation with full at 60 minutes. There was no relationship between desire, perceived drink sweetness or physical comfort at 60 minutes and food intake; there was also no correlation between perceived palatability of the drinks and physical comfort at 60 minutes.

There was a positive correlation between average appetite at 20 minutes and food intake measured one hour after the preload was consumed. There were no relationships between the perceived sweetness of the drinks or perceived palatability of the drinks and average appetite scored at 20 minutes. However, there was a positive correlation between drink palatability and food intake (Table 29).

No relationships were found between the numbers of hours subjects were fasted before testing, the numbers of hours subjects slept before testing or the number of hours subjects were awake on test days before arrival and food intake.
Table 29: Experiment 2. Correlations Between Dependent Measures

<table>
<thead>
<tr>
<th>Correlated variable</th>
<th>r (^1); p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food Intake Average Appetite 0 min</td>
<td>0.177; 0.16</td>
</tr>
<tr>
<td>Food Intake Average Appetite 20 min</td>
<td>0.303; 0.01*</td>
</tr>
<tr>
<td>Food Intake Average Appetite 40 min</td>
<td>0.305; 0.01*</td>
</tr>
<tr>
<td>Food Intake Average Appetite 60 min</td>
<td>0.317; 0.01*</td>
</tr>
<tr>
<td>Food Intake Desire 60 min</td>
<td>0.184; 0.14</td>
</tr>
<tr>
<td>Food Intake Hunger 60 min</td>
<td>0.294; 0.02*</td>
</tr>
<tr>
<td>Food Intake Full 60 min</td>
<td>-0.305; 0.01*</td>
</tr>
<tr>
<td>Food Intake Amount 60 min</td>
<td>0.387; 0.00*</td>
</tr>
<tr>
<td>Food Intake Drink Palatability</td>
<td>0.275; 0.03*</td>
</tr>
<tr>
<td>Food Intake Drink Sweetness</td>
<td>0.143; 0.25</td>
</tr>
<tr>
<td>Food Intake Hours of Fasting Before Arrival</td>
<td>0.009; 0.94</td>
</tr>
<tr>
<td>Food Intake Hours of Sleep Previous Night</td>
<td>0.022; 0.86</td>
</tr>
<tr>
<td>Food Intake Hours Awake Before Arrival</td>
<td>0.187; 0.13</td>
</tr>
<tr>
<td>Food Intake Physical Comfort 60 min</td>
<td>-0.003; 0.98</td>
</tr>
<tr>
<td>Perceived Sweetness Average Appetite 20 min</td>
<td>0.225; 0.07</td>
</tr>
<tr>
<td>Perceived Palatability Average Appetite 20 min</td>
<td>0.098; 0.43</td>
</tr>
<tr>
<td>Perceived Palatability Physical Comfort 60 min</td>
<td>0.026; 0.84</td>
</tr>
</tbody>
</table>

\(^1\)Pearson's Correlation Coefficient  
*Significance at the 0.01 level (2-tailed)
5.4. DISCUSSION

This experiment provided further evidence to experiment 1 that the effect of protein on food intake depends on the protein source, and that time of measurement is a factor in determining the feeding response.

A two-way ANOVA, with repeated measures on one factor (arrival time) found, neither a treatment by arrival time interaction, nor an effect of arrival time itself on food intake. There was however a treatment effect, which allowed the pooling of both groups to determine the effects of protein source on food intake independent of arrival time. Thus a one-way repeated measures ANOVA was used to determine the effects of egg and whey protein on food intake, compared to the control, in all 22 subjects combined.

Whey protein suppressed food intake one hour after preloads were consumed, whereas isocaloric preloads of egg protein had no effect on subsequent food intake, when the total sample was included in the analyses (Figure 6). As in experiment 1, the young men adjusted their energy intake compared to control more precisely with the whey protein (compensation of 126%) than for egg (28%). Thus subjects consumed more calories in total (preload and pizza) with the egg than they did with the control treatment. Although percent compensation was not identical between experiments, the differences in percent compensation were relatively small and not surprising given the measures employed.

As in experiment 1, neither sweetness nor palatability of drinks were factors influencing results. Sucralose was added to all drinks in the same proportion (Table 5) so that all drinks would be as sweet as in the first experiment. In experiment 2, subjects deemed the perceived sweetness of all 3 drinks equal. However, once again, there was no
correlation between perceived sweetness of treatments and food intake, suggesting that this difference in sweetness was not a determinant of food intake one hour later.

The effect of the proteins on food intake was not explained by palatability of the preloads or by the physical comfort of subjects. While there was a direct correlation between drink palatability and food intake, both protein treatments were equally less palatable than the control. Thus the lower palatability of whey protein does not explain the decreased food intake with whey protein, as egg protein was equally unpalatable, but had no effect on food intake. Similarly, the results were not explained by physical comfort scores, as there were no differences in subjects' subjective physical comfort between treatments and no direct correlation between physical comfort and food intake.

Similar to experiment 1, no effect of treatment on either the Average Appetite or the Motivation to Eat VAS was seen. However, different from experiment 1, direct correlations between average appetite, all the individual appetite questions, except desire, and food intake were found (Table 29). The strong correlation between the VAS for appetite and food intake, independent of treatment, is consistent with previous associations found between subjective appetite and food intake (Brown 1998; Rolls et al. 1988; Stewart et al. 1997). Why the first experiment did not find these relationships is unclear. As discussed, the subjects had fewer tasks to complete over the one-hour time period, which may have improved their focus on the VAS for Motivation to Eat. More likely, a larger sample size of 22 subjects versus 13 in experiment 1, accounted for the increased statistical power. Because the correlations obtained explained less than 15% of the variation in food intake, a large sample size is required to obtain statistical significance.
A major objective of experiment 2 was to determine the effect of time of treatment on the response in food intake, and it was disappointing that there was no effect of arrival time on food intake nor treatment by arrival time interaction effect on food intake, as was found in experiment 1. Thus it would appear that time of arrival is not a major factor in determining feeding response. Nevertheless, further analyses provided some support for time of arrival being a consideration. Using one-way repeated measures ANOVA, as was done in experiment 1, it was found that in the 8:30 AM group (n=12) both egg and whey protein suppressed food intake compared to control, and the two proteins were not different from each other. In the 11:00 AM group, no significant differences were found between the control and either protein (n=10). However, whey suppressed food intake more than egg protein. Therefore, the effect of time on the pattern of food intake response to protein source was the same in experiments 1 and 2.

The Student’s t-tests were again performed, comparing food intake between the 8:30 AM group and the 11:00 AM group for each treatment, to better understand the differences individual treatments had on food intake on the feeding response between the two arrival times. While there were no differences between any of the two arrival times, there was a trend for increased food intake following egg protein at 11:00 AM compared to the intake following egg at 8:30 AM (Table 20). This pattern in food intake was similar to that seen in experiment 1, where t-tests found only egg to have an effect on the feeding responses between the two arrival times (Table 8).

The difference in arrival time on food intake response to treatment was not explained by caloric intake prior to test days. Subjects consumed the same meals before their test days, and no differences were calculated in caloric intake before tests between
treatments or between arrival time groups. Subjects also reported no differences in the amount of time (~2 hours) they were awake before arrival in the test facility.

Overall, the data derived from a closer recording of information suggested that these were not other major factors influencing determination of the effects of the treatments. However, more attention to behavioural patterns allowed for rescheduling sessions when irregularities occurred, thus allowing for more control over variables, other than the treatment, and their effect on food intake.

In summary, it was found that over the short-term period of one-hour, the feeding response to egg and whey preloads differed, but these responses were also influenced by the time of morning at which the preloads were consumed.
CHAPTER 6.

GENERAL DISCUSSION
6. GENERAL DISCUSSION

The results of this study support the hypothesis that the effect of protein on food intake in humans is affected by the protein source. As well, the time of morning was shown to be a factor in determining the feeding response to proteins.

This is the first study to systematically compare almost pure protein sources on food intake in humans. When all subjects were included in both experiments, the pattern of food intake was the same. Whey protein suppressed food intake compared to control, and egg protein did not.

The particular proteins tested in this study (egg-albumen, whey and soy) were chosen for several reasons. First, they were available in almost pure forms in commercially sold products. These made the proteins easy to obtain and practical to study. Second, the sources included both animal and plant proteins that are commonly consumed, both naturally and in supplemental form, in the diet. And finally, the proteins selected have been tested as pure protein sources for their effect on food intake in animals (Morgan 1998; Trigazis 1997).

The proteins were given based on estimated fat-free mass (FFM) because the initial plan for this study was to determine the effects of pure protein and carbohydrate sources on food intake in lean men and then examine their effects in obese men. Thus treatment doses were based on FFM so that the effects of fat mass and its metabolic consequence on the relationships between protein load, FFM and food intake could be examined. However, experiment 2 put a priority on confirming the results of experiment 1.
The dose at which the proteins were selected was based on a previous preliminary study that found 50 g of whey sufficient to suppress food intake in young men (Rackal 1998). In experiment 1, the protein dose was determined to be 0.75 g/kg FFM, for each subject, based on the assumption that the average 70 kg male, with a body fat of 15%, would receive approximately 45 g of protein in their preload. To be consistent with the Rackal study, a slightly higher dose would have been desired. However, the soy protein treatment limited the quantity of protein that could be mixed in the drink, due to its high viscosity. In experiment 2, although the soy treatment was removed, the treatments were given to be consistent with experiment 1. A dose response experiment is needed to determine what amount egg protein suppresses food intake at one hour. Clearly, the amount of whey protein was sufficient to suppress food intake and whether or not a smaller quantity would be effective remains to be determined.

Measuring the effects of protein preloads on food intake at one hour may be been inappropriate for expression of an effect of egg protein. One hour was chosen to be consistent with our studies of carbohydrates and fat using similar protocols, so that comparisons could be made (Catherine 2000; Woodend 2000). Furthermore, in a previous study, 50 g of whey protein suppressed food intake in young men 2 hours later (Rackal 1998), and showed that its effect on subjective appetite was strongest up to one hour following preloads. Why neither whey nor egg protein showed any effect on appetite measured by the VAS is not clear, but may be of guidance on testing for a more appropriate time of measurement of food intake after egg albumen.

The suppression of food intake induced by whey protein was expected. Both a preliminary study in humans (Rackal 1998) and animal studies (Morgan 1998) have shown whey protein preloads to suppress food intake over the short-term. However, a
weaker effect of egg-albumen on food intake appears also in animal studies. More surprisingly, however, was the finding in this study that egg protein did not suppress food intake when all subjects were included in the analyses for both experiments. Numerous animal studies have shown that egg protein is not as effective as whey in suppressing food intake over the short term (Morgan 1998), but it is effective compared to controls and to cornstarch (Cochi 2001; Morgan 1998; Trigazis 1997). In the short-term, egg-albumen works, at least in part, via a neurocrine mechanism through CCKA receptors to suppress food intake (Trigazis et al. 1997). Thus it seems possible that egg-albumen alone is able to signal satiety in humans. Furthermore, in experiment 2, when the results for subjects arriving at 8:30 AM were analyzed, egg protein suppressed food intake compared to the control. It should not be unexpected that differences in feeding response to protein source occur in humans. The significance of protein source in regulating other aspects of metabolism in humans has been identified recently. Whey is a “fast” protein and casein is a “slow” protein in affecting protein synthesis. Whey protein is digested and absorbed from the gut more efficiently than casein and has a pronounced increase in protein synthesis, but no change in protein breakdown. Casein, however, is digested more slowly and its whole-body metabolic response is a slight increase in protein synthesis, but a marked inhibition in protein breakdown (Boirie et al. 1997). Whether or not egg-albumen or soy proteins also have characteristics of “fast” or “slow” proteins is unknown. It would have been helpful to measure the plasma amino acid concentrations of subjects both prior to and during the test session to determine if the appearance rate of any particular amino acids, or the ratio of amino acids played a role in the different responses to protein source. While recent studies demonstrated no temporal association between plasma and whole brain amino acid concentrations and their effect on food
intake (Anderson et al. 1994), measuring plasma amino acid concentrations would help to describe the rate of protein digestion and absorption. If the rate of protein absorption, however, is involved in the short-term suppression of food intake, then it would be expected that "fast" proteins suppression of food intake would be seen sooner than the "slower" proteins.

There may be, however, other physiological explanations for the differences in feeding responses to protein sources over the short-term. For example, the hormonal responses are involved in food intake regulation and these are affected by protein source. For example, casein, gelatin and soy, when incorporated into mixed meals (435 kcal) at lunch, had different effects on insulin and glucose responses. The AUC was higher for insulin was higher for the casein protein lunch than it was for the gelatin protein lunch, and the peak responses for both glucose and insulin occurred 1 to 1½ hours later following the soy-enriched meal than the casein meal (Lang et al. 1999). It is difficult to associate these physiological values with food intake, because the study measured food intake much later than the differences occurring in hormonal responses. Fruhbeck (1998) also noted differences in hormonal response to protein source, where the insulin to glucagon ratio over 3 hours was different between whey, a fast protein, and casein, a slow protein. While both milk proteins have the same percentage of amino acids that stimulate insulin secretion, the higher proportion of branched chain amino acids in whey protein leads it to have a synergistic effect with insulin on protein metabolism.

This study provides preliminary evidence that a difference in testing time of only 2 to 2½ hours may affect the feeding response to treatments. Unfortunately, the results of the two experiments were not totally conclusive. In experiment 1, an interaction effect between arrival time and treatment was found. In experiment 2, this interaction was not
evident. However, in both experiments, food intake following egg protein at the later sessions was greater than intake following egg protein in the later sessions, whereas there was no difference in food intake following whey protein between arrival time groups, in both experiments.

In both experiments, whey protein suppressed food intake both when all subjects were included in the analyses and in the earlier sessions. In contrast, it did not suppress food intake compared to control during the later 11:00 AM sessions. Egg had no effect on food intake when data for the two times was pooled and was not different from the control in the earlier times, in both experiments. However, at 11:00 AM in experiment 1, food intake was greater after the egg preload, but not in experiment 2.

An explanation for the effect of arrival time was not derived from records of the behaviour of the subjects prior to testing. An attempt for monitoring the behaviour more clearly was made in experiment 2, as external factors may have played a role in food intake regulation on test days, such as strenuous physical activity, unusual diets and lack of sleep. The data has demonstrated that there was no difference in the hours of fasting between the two arrival time groups in the first experiment, but that in the second experiment the later group had fasted for approximately one hour longer than the group that started the testing earlier. In both experiments, subjects in the 11:00 AM group slept slightly longer than those in the earlier group. In experiment 2, it was noted that while subjects who arrived at 11:00 AM had slept longer than those who arrived at 8:30 AM, all subjects had been awake for approximately 2 hours before arriving for their respective test sessions.

A criticism of the study may be the use of different subjects for the 9:10 / 8:30 AM sessions and the 11:00 AM sessions. It may be possible that the design could be
improved by having the same subjects come in for both test times. However, this was not
done for two reasons. First, that would require subjects to be tested for 6 test sessions
(control, egg, whey at 8:30 AM and 11:00 AM each). This high demand and repetition of
pizza consumption would increase the variability of the data. Secondly, in order to
confirm the effects of arrival time in the first experiment, it was necessary to repeat the
design in the second experiment.

It is of note that arrival time for the earlier session was changed from 9:10 AM to
8:30 AM, for the second experiment. While a 2 to 2 ½ hour difference of arrival time
affected feeding responses, it is unlikely that a 20 minutes change would do so, as the
responses between the earlier sessions had the same patterns in both experiments. The
small sample size may explain why the egg did not reduce food intake compared to
control in the 9:10 AM group of experiment 1, but did in the 8:30 AM group of
experiment 2. In experiment 1, at 9:10 AM (n=6), egg suppressed food intake compared
to control by 107 kcal (not significant); however, with doubling the sample size (n=12)
of the 8:30 AM group in the second experiment, the reduction in food intake following
egg compared to control was 168 kcal, and the difference was statistically significant.

To explain both the effect of protein source and time on food intake,
measurement of physiological parameters will be required. Protein source may have an
effect on satiety by several mechanisms. It has been well established that in rats, egg-
albumen suppresses food intake over the short-term, at least in part, through activation of
CCK_A receptors (Trigazis et al. 1997). In humans, it has been shown that when POTII, a
proteinase inhibitor found in potatoes, was added to a meal containing protein, plasma
CCK increased. It was suggested that the POTII enzyme increased CCK release by
inhibiting trypsin and chymotrypsin degradation of CCK-RP and allowing CCK-RP to
remain active longer to prolong CCK release (Hill et al. 1990). Animal studies have also shown that not all proteins suppress food intake over the short-term by CCK release (Morgan 1998). The extent to which protein sources suppress food intake via CCK release in humans is not known and requires further investigation.

Also of interest would be to determine the plasma amino acid concentration in subjects fed different protein sources, over the short-term. It has been shown that when animals are fed high protein meals, their ratio of plasma tryptophan to other large neutral amino acids (which compete with tryptophan for uptake into the brain) is reduced. Since serotonin synthesis is partially under precursor control (Teff and Young 1988), decreased tryptophan availability in the brain suppresses synthesis. Serotonin appears to be involved in food intake selection, where increased serotonergic activity leads to a lower preference for carbohydrates in rats (Li and Anderson 1983). Similarly, young men given an amino acid mixture preload deficient in tryptophan, created to induce reduced brain serotonin levels, consumed lower protein at a self-selected buffet 5 hours later (Young et al. 1988). If young men were given the opportunity to choose their meal from self-selected buffets and both macronutrient and caloric consumption were recorded, perhaps there would be a better indication of how the protein sources affect mechanisms regulating food intake.

The effect of time on physiological parameters would be reasonable to assume that subjects who fasted longer (i.e. the 11:00 AM group) would have a higher level of ketones and free fatty acids circulating, which may reduce their appetite. However, it was seen that there were no significant differences in mean food intake between the early arrival groups and the later arrival groups; it was only the pattern of response to the proteins that were different between the two arrival groups. There is the possibility of a
hormonal response, which fluctuates throughout the morning, being involved that may
differentiate the response of the two arrival groups. For example, it is known that
cortisol, a corticosteroid hormone, reaches its peak concentration in the morning and then
declines over the length of the day. Cortisol release is in response to both light and
circadian rhythm and has been suggested to play a role in glucose and insulin regulation
(Plat et al. 1999), which in turn have been implicated as mediators of food intake
regulation (Hirschberg 1998). Measurements of plasma levels of glucose, insulin and
cortisol may also be helpful in trying to find the mechanisms driving the different feeding
responses to proteins at different times of the morning. If there is a specific hormonal, or
other physiological, regulator of the feeding response to proteins at 8:30 AM versus
11:00 AM, this factor mostly likely interacts specifically with the egg-albumen protein,
which induced a different response at those two test times. Whey protein, on the other,
tends to demonstrate a more consistent suppressive effect on food intake at one hour.

In summary, the findings of this study are of importance because they are the first
step towards systematically evaluating the effects of pure protein sources on food intake
responses in humans; knowing that differences occur gives reason to investigate further
the physiology driving the various responses to protein-specific food intake regulation.
Finally, this study suggests that even small differences in the time of day when
measurements are made play a role in the response to treatments and therefore the design
of similar studies. The observation may also have a practical application, for example, in
determining the optimal time of day to consume protein for appetite control.

In conclusion, this study supports the hypothesis that the effect of protein on
short-term food intake in humans is affected by the protein source. As well, the time of
testing appears to be a factor in determining the feeding response.
CHAPTER 7.
SUMMARY AND CONCLUSION
7.1. SUMMARY

1. Whey and soy protein, but not egg, suppressed food intake one hour later in young men.

2. There was arrival time by treatment interaction for the feeding response in young men with egg and whey protein, indicated by an increase in food intake after egg protein treatments at 11:00 AM compared to food intake after egg protein treatments of the earlier arrival group food intake after egg.

7.2. CONCLUSION

This study supports the hypothesis that the effect of protein on short-term food intake in humans depends on the protein source. Furthermore, the time of testing appears to be a factor in determining the feeding response.
CHAPTER 8.

REFERENCES
8. REFERENCES


APPENDIX 1

SAMPLE SIZE CALCULATION
SAMPLE SIZE CALCULATION

For a within subjects design, the equation is:

\[ n = \left( \frac{(Z_{\alpha} + Z_{1-\beta})\sigma}{\Delta} \right)^2 \]

\[ \Delta = 214 \]
\[ \sigma = 291.6 \]
\[ \alpha = 0.05 \]

\[ n = 14 \text{ when } \beta = 0.20 \]

(from Julia Rackal, unpublished)
APPENDIX 2

SCREENING QUESTIONNAIRES:
BASELINE INFORMATION QUESTIONNAIRE
FOOD ACCEPTABILITY LIST
EATING HABIT QUESTIONNAIRE
OUTLINE OF PARTICIPANT'S ROLE
CONSENT FORM
BASELINE INFORMATION QUESTIONNAIRE
(please print all answers)

NAME: ____________________________ AGE: ________________

ADDRESS: ____________________________________________________________

PHONE: ( ) ________________________

HEIGHT: _______ WEIGHT: _________ BMI (kg/m²) __________

PARTICIPATION IN ATHLETICS/ EXERCISE:
Activity: ____________________________

How often? _ ________________________ How long (hours)? ________________

Do you usually eat breakfast? YES____ NO____
    If YES, how many times/week? ________
    If YES, what do you usually eat for breakfast? ____________________________

HEALTH STATUS

Do you have diabetes? YES_____ NO_____
Do you have any other major diseases? YES_____ NO_____ 
    If YES, please specify_________________________________________________
Are you taking any medications? YES_____ NO_____ 
    If YES, please specify_________________________________________________
Are you on a special diet? YES_____ NO_____ 
    If YES, please specify_________________________________________________
Have you recently gained or lost weight? YES    NO

If YES, please specify________________________________________

Do you smoke? YES    NO

How many alcoholic beverages do you consume per day? ______ per week? ______

FOOD ACCEPTABILITY LIST

At all 5 sessions, you will receive a protein or carbohydrate beverage.

Please indicate whether you will be able to drink the beverage provided:

YES    NO

At the end of each of the 5 sessions, you will be provided with pizza. To provide you with a meal that you will enjoy, please rank the following pizzas according to your personal preference (i.e. 1, 2, 3) in the spaces provided. If you do NOT like a particular type of pizza, then do NOT rank it, but place an “X” in the space provided.

Pepperoni (cheese, pepperoni):______________________________

Deluxe (cheese, pepperoni, peppers, mushrooms):____________

Three Cheese (mozzarella, cheddar, parmesan):______________

Deli Lovers (cheese, pepperoni, salami, bacon):_____________
EATING HABITS QUESTIONNAIRE

Choose the appropriate answer to best describe your personal situation.

1. How often are you dieting?

   Never ____ rarely ____ sometimes ____ often ____ always ____

2. What is the maximum amount of weight (in pounds) that you have ever lost within one month?

   1 - 4 ____ 5 - 9 ____ 10 - 14 ____ 15 - 19 ____ 20+ ____

3. What is your maximum weight gain within one week?

   0 - 1 ____ 1.1 - 2 ____ 2.1 - 3 ____ 3.1 - 5 ____ 5.1+ ____

4. In a typical week, how much does your weight fluctuate?

   0 - 1 ____ 1.1 - 2 ____ 2.1 - 3 ____ 3.1 - 5 ____ 5.1+ ____

5. Would a weight fluctuation of 5lbs affect the way you live your life?

   Not at all _____ slightly ____ moderately ____ very much ____

6. Do you eat sensibly in front of others and splurge alone?

   Never ____ rarely ____ often ____ always ____

7. Do you give too much time and thought to food?

   Never ____ rarely ____ often ____ always ____

8. Do you have feelings of guilt after overeating?

   Never ____ rarely ____ often ____ always ____

9. How conscious are you of what you are eating?

   Not at all ____ slightly ____ moderately ____ extremely ____

10. How many pounds over your desired weight were you at your maximum weight?

    0-1 ____ 2 - 5 ____ 6 - 10 ____ 11 - 20 ____ 21+ ____
INITIAL SCREENING INTERVIEW:

Each participant will provide the interviewer with basic information (anthropometric, health status) and answer questionnaires pertaining to food habits, in addition to completing a food acceptability list. Each subject will undergo bioelectric impedance analysis to determine lean body mass.

SESSIONS: 5 in total

NIGHT BEFORE EACH SESSION:

Fast from 9:00 PM, except for water. No water should be consumed for one hour prior to arrival. If subject is a regular morning coffee/tea drinker, one serving of it should be consumed one hour prior to arrival.

SCHEDULE FOR EACH SESSION:

8:30 Participants arrive at Dept. of Nutritional Sciences, Rm. 331 (Fitzgerald Bldg). Participants are expected to stay within the department for the duration of the experiment (approx. 8:30 to 10:00 am).

8:35 Participant will complete sleep and stress, appetite, mood and physical comfort questionnaires.

8:45 Participant will be given a sweet beverage and will be asked to rate its palatability and sweetness.

8:50 Participant will complete physical comfort, appetite and mood questionnaires every 15 minutes over one-hour. Memory tests will be given at 15 min, 45 min and 60 min.

9:50 Unlimited pizza lunch is served, participant will eat until comfortably full. After lunch, participant will complete appetite and palatability questionnaires.

• note: starting time is just an example, subjects may choose to begin at 7:00, 8:30 or 10:00 AM and should arrive at the same time for each session

If you have any questions about this study, please contact:

Sandy Tecimer Investigator (416) 978-3700
Dr. G. Harvey Anderson Principal Investigator (416) 978-1832
EFFECT OF MACRONUTRIENTS ON APPETITE AND MEMORY

Outline of Participant’s Role

INITIAL SCREENING INTERVIEW:

Each participant will provide the interviewer with basic information (anthropometric, health status) and answer questionnaires pertaining to food habits, in addition to completing a food acceptability list. Furthermore, each subject will be trained in assessing food portions, so that a food diary can be properly completed and discussed prior to each session. Subjects may choose to follow one of 2 scheduled test times at either 8:30 am or 11am and must then arrive at all 3 sessions at the chosen time. Each session is set to be one week apart.

SESSIONS: 3 in total

PRIOR TO EACH SESSION:
Fast for 12 hours prior to arrival, except for water. No water should be consumed for one hour prior to arrival. If subject is a regular morning coffee/tea drinker, one serving of it should be consumed one hour prior to arrival. Subjects will refrain from strenuous physical activity and alcohol consumption for 24 hours prior to each test session.

SCHEDULE FOR EACH SESSION (i.e. a 9am arrival):

8:30  Participants arrive at Dept. of Nutritional Sciences, Rm. 331 (Fitzgerald Bldg). Participants are expected to stay within the department for the duration of the experiment (approx. 8:30 to 10:30 am).

8:35  Participant will complete sleep and stress, appetite and physical comfort questionnaires. Subject will then go over the previous day’s food diary with the interviewer.

9:00  Participant will be given a sweet beverage and will be asked to rate its palatability and sweetness.

9:05  Participant will complete an appetite questionnaire every 20 minutes over 1 hour. Memory tests will be given at 15 min and 45 min. Physical comfort will be assessed at 60 min.

10:05 An unlimited pizza lunch is served, participant will eat until comfortably full. After lunch, participant will complete physical comfort, appetite and palatability questionnaires.

If you have any questions about this study, please contact:
Sandy Tecimer Investigator (416) 978-3700
Dr.G.Harvey Anderson Principal Investigator (416) 978-1832
MACRONUTRIENTS, APPETITE AND MEMORY

Consent Form

The purpose of this study is to determine the effects of macronutrients on subjective appetite and its relationship with memory. I have been fully informed of what is expected of me as a participant in this research project, and I have been provided with a typewritten copy of these expectations as outlined in the attachment to this consent form.

I am aware that my participation will not involve any health risk to me, that my personal information will remain confidential and that my name will not appear in any published document.

I understand that for the purposes of this research project it is hoped that I will complete all 3 sessions. I will be compensated $20.00 per session. However, should I choose to withdraw at anytime throughout the study, I may do so without prejudice and will receive the prorated portion of the $60.00 total payment. If I should complete all 3 sessions, I will receive a bonus amount of $15.00, resulting in a $75.00 payment overall. Upon completion of the study, a summary of results will be available for me to pick up from the Department of Nutritional Sciences.

I hereby agree and give my authorized consent to participate in the study.

DATE: __________________________

PARTICIPANT NAME: ____________________________________________

PARTICIPANT SIGNATURE: ________________________________

WITNESS SIGNATURE: ________________________________________
APPENDIX 3

STUDY DAY QUESTIONNAIRES:

SLEEP AND STRESS FACTORS QUESTIONNAIRE

VAS - MOTIVATION TO EAT

VAS - PHYSICAL COMFORT

VAS - PALATABILITY

VAS - PERCEIVED SWEETNESS
EXPERIMENT #2
SLEEP HABITS AND STRESS FACTORS QUESTIONNAIRE

NAME: ______________________
SESSION: ___________________

DATE: ______________________

1. Did you have a normal night’s sleep last night?
   Yes ____ No ____

2. How many hours of sleep did you have?
   ______________________

3. What time did you go to bed last night?
   ____________

4. What time did you wake up this morning?
   __________

5. Recount your activities since waking
   Time   Activity
   _____   __________________
   _____   __________________
   _____   __________________
   _____   __________________
   _____   __________________
   _____   __________________

6. Are you experiencing any feelings of illness or discomfort, other than those from hunger?
   Today Yes ____ No ____
   Past 24 hours Yes ____ No ____
   If Yes, please describe briefly:
   ______________________

   Today Yes ____ No ____
   Past 24 hours Yes ____ No ____
   If Yes, please describe briefly:
   ______________________

8. Have you been involved in any physical activity, unusual to your normal routine, within the past 24 hours?
   Yes ____ No ____
   If Yes, please describe briefly:
   ______________________

9. Have you had anything to eat or drink, other than water, for the past 11-12 hours?
   Yes ____ No ____
   If Yes, please describe briefly:
   ______________________
Visual Analogue Scale
Motivation to Eat

DATE: ______________________
NAME: ______________________

These questions relate to your "motivation to eat" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings.

1. How strong is your desire to eat?

Very WEAK ___________________________________________ Very STRONG

2. How hungry do you feel?

NOT Hungry ___________________________________________ As hungry
at all as I have ever felt

3. How full do you feel?

NOT Full ___________________________________________ VERY
at all Full

4. How much food do you think you could eat?

NOTHING at all ___________________________________________ A LARGE
at all amount
Visual Analogue Scale
Physical Comfort

DATE: ______________________
NAME: ______________________

This question relates to your "comfort level" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings.

How well do you feel?

NOT __________________________ Very well
Well __________________________
at all
Visual Analogue Scale
Palatability

DATE: __________________________
NAME: __________________________

This question relates to the palatability of the beverage you just consumed. Please rate the pleasantness of the beverage by placing a small “x” across the horizontal line at the point which best reflects your present feelings.

How pleasant have you found the beverage?

NOT ___________________________ Very pleasant
at all ___________________________ pleasant
Visual Analogue Scale
Sweetness

DATE: ______________________
NAME: ______________________

Please rate the level of sweetness by placing a small “x” across the horizontal line at the point which best reflects your present feelings.

How sweet have you found the beverage?

NOT sweet _______________________________ Extremely sweet
at all
APPENDIX 4

FOOD DIARY:
INSTRUCTIONS
PRE-SESSION MEAL
RECORDING SHEET
INSTRUCTIONS FOR THE FOOD RECORDS

Please record all food consumed after 3 PM on the day prior to each test session. It is important to maintain your usual eating habits.

1. Foods and beverages should be recorded as soon as you consume them. Record both the time and quantity of food and drink consumed.

2. A complete description of the food most accompany your assessment.
   i.e. baked / boiled / deep-fried etc.
   raw / steamed / canned etc.
   McDonald’s / Kellogg’s / Lean Cuisine etc.

3. Please record the amount of food or beverage eaten and do so accurately.
   i.e. 1 slice of white bread
   1 cup of Kellog’s corn flakes
   2 heaping teaspoons of brown sugar
   2 creamers

   Meat portions are often difficult to estimate. If you are having difficulty, measure the item’s dimensions.
   i.e. roast beef:  5 ½” x 4 ½” x ¼”

   Also, 1 deck of playing cards is the same size as 3 ounces of cooked meat.

4. For combination dishes, please detail the individual ingredients.

<table>
<thead>
<tr>
<th>CORRECT</th>
<th>INCORRECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 medium bagel</td>
<td>1 cheese sandwich</td>
</tr>
<tr>
<td>1 tsp. margarine</td>
<td></td>
</tr>
<tr>
<td>2 oz. cheddar cheese</td>
<td></td>
</tr>
<tr>
<td>1 ½ cups pasta</td>
<td>spaghetti dinner</td>
</tr>
<tr>
<td>2/3 cups tomato sauce</td>
<td></td>
</tr>
<tr>
<td>2 oz. hamburger</td>
<td></td>
</tr>
<tr>
<td>1 tbsp. parmesan cheese</td>
<td></td>
</tr>
</tbody>
</table>
PRE SESSION MEAL

Name: ________________

Current Session #: ________________

Next date you must record food intake after 3pm: ________________

Next week you will have your ______ experimental session.

Make sure the day prior to your test session _____________, the foods you ate after 3pm and the time you ate them are consistent with the foods you ate yesterday.

You indicated eating the following foods at the following times yesterday:

Please be sure to do the same thing next week, the day before your test session.

You will be required to write down everything you consumed after 3pm, prior to your test day on the accompanying sheets, just as you did last week.

Do NOT forget to fast for 12 hours before arriving the night before your next test session.

See you next week!
<table>
<thead>
<tr>
<th>TIME</th>
<th>FOOD/ BEVERAGE</th>
<th>QUANTITY</th>
<th>MAIN INGREDIENTS</th>
<th>PREPARATION/ BRAND NAME</th>
<th>ADDITIONAL DETAILS</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:30 am</td>
<td>strawberry yogurt</td>
<td>500g</td>
<td></td>
<td>Astro</td>
<td>low-fat 1%, fruit on bottom</td>
</tr>
<tr>
<td>12:15 pm</td>
<td>pasta salad</td>
<td>4 cups</td>
<td>macaroni pasta</td>
<td>boiled in salt, barilla brand</td>
<td>enriched flower</td>
</tr>
<tr>
<td></td>
<td>carrots</td>
<td>1 large</td>
<td>peeled, boiled in salt</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>green pepper</td>
<td>half</td>
<td>raw</td>
<td></td>
<td>fresh</td>
</tr>
<tr>
<td></td>
<td>Italian salad dressing</td>
<td>3 tblsp</td>
<td>Kraft</td>
<td></td>
<td>fat-free</td>
</tr>
</tbody>
</table>
APPENDIX 5

COMPOSITION OF PROTEIN POWDERS
## COMPOSITION OF COMMERCIAL PROTEIN POWDERS

<table>
<thead>
<tr>
<th>Amino Acids (mg)</th>
<th>EGG¹</th>
<th>SOY²</th>
<th>WHEY³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per 40 g Serving</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>2241.4</td>
<td>1715.6</td>
<td>2030.8</td>
</tr>
<tr>
<td>Valine</td>
<td>3103.4</td>
<td>1986.5</td>
<td>2320.9</td>
</tr>
<tr>
<td>Leucine</td>
<td>3534.5</td>
<td>3250.6</td>
<td>3952.9</td>
</tr>
<tr>
<td>Arginine</td>
<td>2120.7</td>
<td>3024.8</td>
<td>616.5</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>4310.3</td>
<td>4605.0</td>
<td>4605.6</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>5069.0</td>
<td>7584.7</td>
<td>7144.2</td>
</tr>
<tr>
<td>Glycine</td>
<td>1293.1</td>
<td>1670.4</td>
<td>725.3</td>
</tr>
<tr>
<td>Histidine</td>
<td>862.1</td>
<td>1038.4</td>
<td>652.8</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>2413.8</td>
<td>1941.3</td>
<td>2466.0</td>
</tr>
<tr>
<td>Lysine</td>
<td>2758.6</td>
<td>2483.1</td>
<td>3445.1</td>
</tr>
<tr>
<td>Proline</td>
<td>1327.6</td>
<td>2031.6</td>
<td>2284.7</td>
</tr>
<tr>
<td>Serine</td>
<td>2586.2</td>
<td>2076.7</td>
<td>1922.0</td>
</tr>
<tr>
<td>Threonine</td>
<td>1551.7</td>
<td>1489.8</td>
<td>3010.0</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>517.2</td>
<td>496.6</td>
<td>725.3</td>
</tr>
<tr>
<td>Methionine</td>
<td>1379.3</td>
<td>541.8</td>
<td>1813.2 meth&amp;cystein⁴</td>
</tr>
<tr>
<td>Cystine</td>
<td>1137.9</td>
<td>496.6</td>
<td>0.0</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2413.8</td>
<td>2076.7</td>
<td>2284.7 phenylalanin&amp;tyr⁵</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1379.3</td>
<td>1489.8</td>
<td>0.0</td>
</tr>
</tbody>
</table>

### Nutrients

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>EGG¹</th>
<th>SOY²</th>
<th>WHEY³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>0.0</td>
<td>0.0</td>
<td>266.4</td>
</tr>
<tr>
<td>Sodium</td>
<td>371.4</td>
<td>1.20%(532.8)</td>
<td>111.0</td>
</tr>
<tr>
<td>Potassium</td>
<td>445.7</td>
<td>0.20%(88.8)</td>
<td>133.2</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>2.9</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0</td>
<td>0.4</td>
<td>0</td>
</tr>
</tbody>
</table>

¹Egg D'Lite (Optimum Nutrition, Florida)
²Soy Protein (Swiss Herbal Remedies, Missouri)
³Whey Protein (Ultimate Balance, Delaware)
⁴Methionine and Cysteine were listed together on product label
⁵Phenylalanine and Tyrosine were listed together on product label
APPENDIX 6

PIZZA COMPOSITION
# PIZZA COMPOSITION

<table>
<thead>
<tr>
<th>Nutritional Information Per 100g</th>
<th>Pepperoni</th>
<th>Deluxe</th>
<th>Three Cheese</th>
<th>Deli Lovers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g)</td>
<td>9.8</td>
<td>8.8</td>
<td>11.5</td>
<td>10.2</td>
</tr>
<tr>
<td>Total fat (g)</td>
<td>7.5</td>
<td>6.1</td>
<td>7.8</td>
<td>9.0</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>26.5</td>
<td>25.7</td>
<td>28.1</td>
<td>25.9</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>212.7</td>
<td>191.7</td>
<td>229.2</td>
<td>225.0</td>
</tr>
</tbody>
</table>

1McCain Foods: Deep and Delicious, 5" pizza
APPENDIX 7

MOOD
INTRODUCTION

The role of macronutrients in the regulation of mood has received considerable investigation for two main reasons. One, food ingestion is associated not only with satiety but also subjective feelings i.e. sleepiness, well-being. Second, this interest in nutrients as mood modulators was increased by the discovery that dietary constituents have the ability to alter formation of neurotransmitters. The synthesis and release of several neurotransmitters i.e. acetylcholine, serotonin and the catecholamines have been shown to be dependent on the availability of macronutrient-derived precursors (Wurtman et al. 1980). These neurotransmitters affect many behaviours, thus it is likely to believe that food ingestion may alter mood (Wurtman 1983).

Dietary protein modulation of mood may depend on either pre- or post-absorptive mechanisms. Pre-absorptively, proteins, such as egg-albumen, have been shown to stimulate CCK release in the gut (Bray 1992), which in turn may have a role in modulating mood. CCK is a gastrointestinal hormone, which is released in response to the presence of protein or fat in the lumen. Intravenous infusions of CCK induced sleepiness in human (Fara et al. 1969) independent of insulin and therefore may account for the post-prandial decline in alertness sometimes observed after fat ingestion. CCK has also been investigated for its role in anxiety in both rodents and humans, which is mediated by the CCKB receptors (Dauge and Lena 1998).

Post-absorptively, proteins may act through their effect on brain serotonin levels to modify mood (Young et al. 1985). Tryptophan is a precursor to serotonin, which is known to modulate mood. Protein consumption results in a decrease in the ratio of tryptophan to large neutral amino acids (LNAA). Because tryptophan competes with
LNAA for entry into the brain, as the ratio decreases, tryptophan enters the brain less readily. In normal young men, tryptophan depletion has been shown to lower mood in normal men over a 5-hour period (Young et al. 1985).

The role that specific macronutrients, and in particular protein, play in modulating mood (and memory, Appendix 8) was the secondary objective of this thesis. The effect of treatments on mood over the one-hour testing period was conducted concurrently with the appetite and food intake testing in experiment 1.

HYPOTHESIS
The effect of protein on mood in young men depends on protein source.

OBJECTIVE
To determine the effect of commercial egg, whey and soy protein, as well as sucrose, on mood in young men.

METHODS AND MATERIALS

Subjects
These tests were done in conjunction with the appetite and food intake testing, thus the subjects are the ones identified in Table 1 of the thesis. Briefly, 13 young men completed the study. The mean age was 22.2 years and the mean BMI was 22.1 kg/m².
Methods

As described in section 4.3, subjects arrived at the Department of Nutritional Sciences after an overnight fast and were given Sleep and Stress Questionnaires to complete. Subjects then consumed 400 mL control, egg, whey, soy or sucrose preloads followed by 50 mL of water, within 5 minutes. Over the next 60 minutes, in addition to the appetite monitoring, subjects were required to complete a VAS to detect changes in affective state (feelings, mood) and level of vigor (alertness, vigilance). The VAS chosen was shown to be a valid, reliable and sensitive measure of mood and takes less than a minute to complete (Monk 1989). These VAS questions consisted of 8 unipolar ratings, 4 of which were primarily concerned with subjective activation (questions 2,3,5,7) and 4 of which were based on affective state (questions 1,4,6,8). The ratings of these four scales were summed algebraically to produce a single global value of vigour (GV) and affective state (GA). An example of the VAS page for mood is found at the end of this Appendix and the 8 scale questions were given in the order presented here:

1) How alert do you feel?
2) How sad do you feel?
3) How tense do you feel?
4) How much of an effort is it to do anything?
5) How happy do you feel?
6) How weary do you feel?
7) How calm do you feel?
8) How sleepy do you feel?
As with the Motivation to Eat questions, Mood VAS were turned over as soon as subjects filled them out, to prevent them from referring to previous ratings.

**Statistical Methods**

All means were compared using two-way repeated measures ANOVA, for time (15, 30, 45 and 60 minutes) and for treatment (control, egg, whey, soy and sucrose). If there was no overall effect of treatment or time, a retrospective one-way repeated measures was used to analyze the effect of treatment at each testing time point (15, 30, 45 and 60 minutes). Significance was determined by p-values < 0.05; a Duncan’s post-hoc analysis was used to compare different means.

**RESULTS**

The mood data is reported as change from baseline values for the GV and GA values (Table A-1 and A-2), as well as for the findings from individual questions (Table A-3). All statistical analyses of the mood data were based on change from baseline values, because they compare each individual’s rating of appetite questions to his own pre-treatment scores for that particular test, which minimizes the differences between subjects.

For GV, there was no effect of treatment \([F=1.68, p=0.17]\) and no time by treatment interaction \([F=0.73, p=0.74]\). However, there was a decrease in GV with time \([F=3.98, p=0.02]\). When individual treatments were examined for the effect of time, there was a decrease after soy \([F=2.87, p=0.05]\), but no effect after control, egg, whey or sucrose treatments \([F=0.88, p=0.46, F=0.36, p=0.78, F=1.46, p=0.24\) and \(F=0.88, p=0.46,\)
respectively]. Mean comparisons at each time of measurement showed no effect of treatment on GV (Table A-1).

For GA, there was no effect of treatment \([F=0.87, p=0.49]\) or time \([F=0.14, p=0.93]\) and no time by treatment interaction \([F=0.95, p=0.50]\) were found. There were also no effects of time for any of the individual treatments examined. Mean comparisons at each time of measurement showed no effect of treatment on GA (Table A-2).

**Table A-1: Experiment 1. Effect of Treatment on Change from Baseline Global Vigour Scores**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.3 ± 3.9</td>
<td>5.8 ± 1.9</td>
<td>8.2 ± 2.2</td>
<td>0.3 ± 4.4</td>
</tr>
<tr>
<td>Egg</td>
<td>-0.2 ± 3.2</td>
<td>-3.2 ± 2.4</td>
<td>-2.6 ± 2.9</td>
<td>-2.2 ± 3.5</td>
</tr>
<tr>
<td>Whey</td>
<td>2.7 ± 3.5</td>
<td>0.7 ± 3.6</td>
<td>1.7 ± 3.7</td>
<td>-2.7 ± 3.5</td>
</tr>
<tr>
<td>Soy</td>
<td>4.8 ± 4.5</td>
<td>2.2 ± 4.5</td>
<td>-0.8 ± 5.1</td>
<td>-2.8 ± 5.3</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-1.3 ± 2.0</td>
<td>-2.1 ± 2.5</td>
<td>-3.9 ± 2.3</td>
<td>-4.8 ± 3.6</td>
</tr>
</tbody>
</table>

---

<table>
<thead>
<tr>
<th></th>
<th>1.75</th>
<th>1.66</th>
<th>2.43</th>
<th>0.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>0.15</td>
<td>0.17</td>
<td>0.06</td>
<td>0.91</td>
</tr>
</tbody>
</table>
**Table A-2:** Experiment 1. Effect of Treatment on Change from Baseline Global Affect Scores

<table>
<thead>
<tr>
<th>Treatment</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.0 ± 3.3</td>
<td>-1.1 ± 2.7</td>
<td>-0.6 ± 2.3</td>
<td>-1.4 ± 2.2</td>
</tr>
<tr>
<td>Egg</td>
<td>3.3 ± 3.2</td>
<td>3.6 ± 2.6</td>
<td>4.0 ± 2.9</td>
<td>4.6 ± 2.9</td>
</tr>
<tr>
<td>Whey</td>
<td>-3.2 ± 2.2</td>
<td>-0.7 ± 2.5</td>
<td>1.2 ± 1.5</td>
<td>-1.2 ± 1.6</td>
</tr>
<tr>
<td>Soy</td>
<td>4.2 ± 3.1</td>
<td>1.6 ± 2.7</td>
<td>-0.0 ± 3.3</td>
<td>1.1 ± 2.4</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.4 ± 2.4</td>
<td>1.6 ± 3.0</td>
<td>1.2 ± 2.3</td>
<td>-0.5 ± 2.8</td>
</tr>
</tbody>
</table>

| F         | 1.20 | 0.61 | 0.48 | 1.32 |
| p         | 0.32 | 0.66 | 0.75 | 0.28 |

*Mean ± SEM (mm) mood score; n=13

For the alert question, there was no effect of treatment [F=0.78, p=0.55] or time by treatment interaction [F=1.09, p=0.37]. However, there was a decrease in alertness with time [F=5.76, p<0.00]. When individual treatments were examined for the effect of time, there was a decrease after soy and sucrose [F=3.84, p=0.02 and F=4.35, p=0.01, respectively], but not after control, egg and whey treatments [F=1.53, p=0.22, F=1.57, p=0.21 and F=1.27, p=0.30, respectively]. Mean comparisons at each time of measurement showed no effect of treatment on alertness (Table A-3).

For the sad question, there was no effect of treatment [F=1.18, p=0.33] or time [F=0.16, p=0.93], and no time by treatment interaction was found [F=1.26, p=0.24]. When individual treatments were examined for the effect of time, no effects after any of the treatments were found. Mean comparisons at each time of measurement showed no effect of treatment on sadness (Table A-3).
For the tense question, there was no effect of treatment \([F=1.43, p=0.24]\) or time by treatment interaction \([F=0.53, p=0.66]\), and no time by treatment interaction was found \([F=0.58, p=0.86]\). When individual treatments were examined for their effect of time, no effects after any of the treatments were found. Mean comparisons at each time of measurement showed no effect of treatment on tension (Table A-3).

For the effort question, there was no effect of treatment \([F=1.00, p=0.42]\) or time by treatment interaction \([F=0.98, p=0.47]\); however, there was an increase in effort with time \([F=4.58, p<0.00]\). When individual treatments were examined for the effect of time, there was an increase after control and whey \([F=3.66, p=0.02\) and \(F=2.94, p=0.05\), respectively], but not after egg, soy and sucrose treatments \([F=1.08, p=0.37, F=2.14, p=0.11\) and \(F=1.35, p=0.27\), respectively]. Mean comparisons at each time of measurement showed no effect of treatment on effort (Table A-3).

For the happy question, there was no effect of treatment \([F=0.68, p=0.61]\) or time by treatment interaction \([F=0.66, p=0.79]\), and no effect of time was found \([F=0.33, p=0.81]\). When individual treatments were examined for their effect of time, no effects after any of the treatments were found. Mean comparisons at each time of measurement showed no effect of treatment on happiness (Table A-3).

For the weary question, there was no effect of treatment \([F=1.55, p=0.20]\) or time by treatment interaction \([F=0.89, p=0.56]\), and no effect of time was found \([F=0.89, p=0.56]\). When individual treatments were examined for their effect of time, no effects after any of the treatments were found. Mean comparisons at each time of measurement showed no effect of treatment on weariness at any time point except at 45 minutes, when weariness was greater after egg and sucrose treatments than after control (Table A-3).
For the calm question, there was no effect of treatment \([F=0.29, p=0.88]\) or time \([F=0.11, p=0.95]\); however, there was a treatment by time interaction \([F=1.98, p=0.03]\). When individual treatments were examined for their effect of time, no effects after any of the treatments were found. Mean comparisons at each time of measurement showed no effect of treatment on calmness (Table A-3).

For the sleepy question, there was no effect of treatment \([F=1.12, p=0.36]\) or time by treatment interaction \([F=0.68, p=0.76]\), and there was no effect of time \([F=1.50, p=0.23]\). When individual treatments were examined for their effect of time, no effects after any of the treatments were found. Mean comparisons at each time of measurement showed no effect of treatment on sleepiness (Table A-3).
### Table A-3: Experiment 1. Effect of Treatment on Questions 1 to 8 Change from Baseline Mood Scores

<table>
<thead>
<tr>
<th>Question</th>
<th>Time (min)</th>
<th>Control</th>
<th>Egg</th>
<th>Whey</th>
<th>Soy</th>
<th>Sucrose</th>
<th>F; p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Alert</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>8.8 ±</td>
<td>2.3 ±</td>
<td>-1.8 ±</td>
<td>6.2 ±</td>
<td>-0.3 ±</td>
<td>0.74;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.3</td>
<td>5.3</td>
<td>4.7</td>
<td>6.6</td>
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<td>0.57</td>
<td></td>
</tr>
<tr>
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<td>-3.6 ±</td>
<td>4.4 ±</td>
<td>-1.8 ±</td>
<td>0.50;</td>
<td></td>
</tr>
<tr>
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<td>5.4</td>
<td>4.3</td>
<td>5.9</td>
<td>6.5</td>
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</tr>
<tr>
<td>45</td>
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<td>-4.8 ±</td>
<td>-3.9 ±</td>
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<td>0.87;</td>
<td></td>
</tr>
<tr>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>-3.7 ±</td>
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</tr>
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<tr>
<td>5. Happy</td>
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</tr>
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<td>3.7</td>
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</tbody>
</table>

1Mean ± SEM (mm); n=13

2Questions phrase: How (adjective) do you feel?

Scoring: Very little = 0; Very much = 100
Table A-3:  Experiment 1. Effect of Treatment on Questions 1 to 8 Change from Baseline Mood Scores\(^1\) (Cont’d)

<table>
<thead>
<tr>
<th>Question(^2)</th>
<th>Time (min)</th>
<th>Control</th>
<th>Sucrose</th>
<th>Whey</th>
<th>Soy</th>
<th>Egg</th>
<th>F; p</th>
</tr>
</thead>
<tbody>
<tr>
<td>6. Weary</td>
<td>15</td>
<td>-8.9 ±</td>
<td>1.3 ±</td>
<td>-5.0 ±</td>
<td>-4.8 ±</td>
<td>4.0 ±</td>
<td>1.52;</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>4.8</td>
<td>3.4</td>
<td>4.5</td>
<td>4.4</td>
<td>4.4</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>-7.8 ±</td>
<td>2.7 ±</td>
<td>-5.8 ±</td>
<td>-3.6 ±</td>
<td>1.7 ±</td>
<td>1.03;</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>-10.8 ±</td>
<td>4.0 ±</td>
<td>-5.8 ±</td>
<td>-3.2 ±</td>
<td>7.7 ±</td>
<td>3.65;</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>-1.9 ±</td>
<td>0.4 ±</td>
<td>-0.8 ±</td>
<td>-1.5 ±</td>
<td>2.7 ±</td>
<td>0.14;</td>
</tr>
<tr>
<td></td>
<td>5.2</td>
<td>3.9</td>
<td>5.8</td>
<td>6.2</td>
<td>3.8</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>7. Calm</td>
<td>15</td>
<td>1.9 ±</td>
<td>1.5 ±</td>
<td>-7.6 ±</td>
<td>6.7 ±</td>
<td>2.3 ±</td>
<td>1.06;</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>4.5</td>
<td>4.9</td>
<td>6.3</td>
<td>5.3</td>
<td>4.6</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>-5.0 ±</td>
<td>0.1 ±</td>
<td>7.0 ±</td>
<td>-2.2 ±</td>
<td>0.9 ±</td>
<td>0.83;</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>-3.8 ±</td>
<td>-2.1 ±</td>
<td>7.6 ±</td>
<td>3.8 ±</td>
<td>-0.1 ±</td>
<td>1.02;</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>-1.4 ±</td>
<td>-0.4 ±</td>
<td>2.3 ±</td>
<td>4.5 ±</td>
<td>0.6 ±</td>
<td>0.32;</td>
</tr>
<tr>
<td></td>
<td>3.1</td>
<td>4.9</td>
<td>5.0</td>
<td>3.7</td>
<td>4.1</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>8. Sleepy</td>
<td>15</td>
<td>-10.8 ±</td>
<td>3.2 ±</td>
<td>-0.8 ±</td>
<td>-9.1 ±</td>
<td>-1.4 ±</td>
<td>1.47;</td>
</tr>
<tr>
<td></td>
<td>5.9</td>
<td>2.4</td>
<td>4.2</td>
<td>4.8</td>
<td>7.6</td>
<td>4.3</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>-5.8 ±</td>
<td>4.2 ±</td>
<td>2.9 ±</td>
<td>-2.5 ±</td>
<td>5.8 ±</td>
<td>0.99;</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>-7.7 ±</td>
<td>-0.9 ±</td>
<td>2.5 ±</td>
<td>1.0 ±</td>
<td>-0.3 ±</td>
<td>0.89;</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>-5.0 ±</td>
<td>1.8 ±</td>
<td>4.3 ±</td>
<td>3.1 ±</td>
<td>2.0 ±</td>
<td>0.51;</td>
</tr>
<tr>
<td></td>
<td>6.4</td>
<td>6.7</td>
<td>6.6</td>
<td>6.4</td>
<td>3.9</td>
<td>0.73</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Mean ± SEM (mm); n=13

\(^2\)Questions phrase: How (adjective) do you feel?

Scoring: Very little = 0; Very much = 100

\(^a\)Means with different superscripts within a row are different, p<0.05
DISCUSSION

The results of this experiment do not support the hypothesis that the effect of protein on mood depends on protein source. No significant effects of treatment on mood were detected in this experiment. However, some changes in mood over time were detected. There was a decrease in GV and alertness, as well as an increase in effort.

The effects of treatment on mood are consistent with other studies that found no effect of 40 g or 100 g sucrose preloads on mood in healthy young adults (38 men; 31 women) (Brody and Wolitzky 1983). Mood was not affected in young adults (31 men; 29 women) 30 or 60 minutes after 40 g sucrose preloads (Reid and Hammersley 1995).

In the present study, only one component of vigor, the question pertaining to weariness was affected by treatment. Only at 45 minutes, subjects were wearier after egg and sucrose preloads than after the control (Table A-3).

There are three main factors that may have interfered with our ability to detect changes in mood following treatments in our young male subjects. First, it is difficult to detect mood fluctuations that are very subtle in normal population (Kolata 1982). Second, the large variation in mood scores between and within subjects requires larger sample sizes to observe a significant effect. Finally, the time of measurement used in this study may have been too short to detect changes in mood alteration induced by treatments. It has been suggested that food-induced mood alterations occur at least 2 hours post preload (Lieberman et al. 1986; Spring et al. 1989) coinciding with peak serotonin and CCK levels (Wells et al. 1997). Because the primary dependent measures of the study were to determine the effect of treatment on appetite and food intake, the experimental design was appropriate for that goal, but not ideal for the study of mood.
While other groups have found that amino acids preloads in young men can alter mood (Young et al. 1985), the current study found no such effect. Future studies would require larger sample sizes, more subtle tools to measure the effects of treatment on mood and possibly a longer time frame of observation to determine if protein or specific sources of protein alter mood in young adults.
Visual Analogue Scale
Mood

DATE: ______________________
NAME: ______________________

These questions relate to your "mood state" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings.

5. How alert do you feel?
very little ________________________________ very much

6. How sad do you feel?
very little ________________________________ very much

7. How tense do you feel?
very little ________________________________ very much

8. How much of an effort is it to do anything?
very little ________________________________ very much

9. How happy do you feel?
very little ________________________________ very much
Visual Analogue Scale
Mood (continued)

10. How weary do you feel?

very little ___________________________ very much

11. How calm do you feel?

very little ___________________________ very much

12. How sleepy do you feel?

very little ___________________________ very much
APPENDIX 8

MEMORY
INTRODUCTION

The effect of protein on memory is poorly defined. Most work in this area has been focused on glucose, which has been implicated as a key modulator of memory. The effects of glucose on memory have generally been restricted to declarative memory (Craft et al. 1994) and are seen more clearly in those with pre-existing memory deficits, such as the elderly or those with Alzheimer's (Azari 1991). Recent work has found that preloads of glucose and fat may improve memory in young men over the short term (Catherine 2000; Woodend 2000).

The mechanism by which protein affects memory may be through CCK. It has been hypothesized that CCK release modulates memory via direct contact with CCK$_B$ and CCK$_A$ receptors.

CCK$_B$ receptors are present in the hippocampus and frontal cortex of the brain (Dauge and Lena 1998). Activation of these CCK$_B$ receptors with agonists has been shown, in rats, to enhance performance on a variety of memory tests (Lena et al. 1999; Taghzouti et al. 1999).

CCK$_A$ receptors are located primarily in the periphery (Dauge and Lena 1998) and may also have a role in the influence of CCK on memory. Rats that lack this receptor show an impairment of memory (Nomoto et al. 1999). The effects of CCK's interaction with CCK$_A$ are likely mediated via vagal afferent nerves. Memory enhancement, through stimulation of this receptor with CCK-8 infusions, is abolished in vagotimized rats (Flood et al. 1992).

The vagus nerve itself has been implicated to influence memory regardless of the source of stimulation. Many peripheral compounds can stimulate the vagus nerve to send
messages to the brain. Electrical stimulation of the vagus nerve modulates memory storage in rodents (Clark et al. 1998). This suggests that any substance with the ability to activate the vagus nerve may also possess the potential to modulate memory through this mechanism.

Investigators have also suggested that elevated insulin, in response to hyperglycemia, could be a possible mediator of the memory enhancing effects of a glucose beverage (Craft et al. 1993). Insulin could potentially improve memory as a result of its interaction with receptors in the brain stimulating increased glucose utilization in the hippocampus (Craft et al. 1994). It is unknown if an elevated insulin response to some amino acids has any effect on memory.

The influence of nutrient intake on memory modulation was also the secondary objective of this thesis. The testing of memory was done concurrently with the appetite testing in both experiments 1 and 2 to determine the effects of proteins on immediate and delayed recall.

**HYPOTHESIS**

The effect of protein on memory in young men depends on protein source.

**OBJECTIVE**

To determine the effect of commercial egg, whey and soy protein on short-term memory in young men.
METHODS AND MATERIALS

Subjects

These tests were done in conjunction with the appetite and food intake testing, thus the subjects are the ones identified in Tables 1 and 2 of the thesis. Briefly, in experiment 1, 13 young men completed the study (Table 1). The mean age was 22.2 years and the mean BMI was 22.1 kg/m². In experiment 2, 22 young men completed the study (Table 2). The mean age was 22.3 years, and the mean BMI was 22.8 22.1 kg/m².

Methods

As described in section 4.3, subjects arrived at the Department of Nutritional Sciences after an overnight fast and were given Sleep and Stress Questionnaires to complete. Subjects then consumed 400 mL control, egg, whey, soy or sucrose preloads, followed by 50 mL of water, within 5 minutes. Over the next 60 minutes, in addition to the appetite monitoring, subjects were also given memory tests.

The memory tests for experiment 1 and 2 were conducted at t = 15 minutes and t = 60 minutes to test for immediate recall. Delayed recall (of the words from 15 minutes) was measured at t = 45 minutes. Between the t = 15-minute immediate recall and t = 45 minutes delayed recall tests, subjects were given a video to watch and were asked to mark down certain audio and visual cues on paper to keep them distracted (experiment 1) or simply watched movie videos that they chose from the available selection (experiment 2).

Experiment 1

In experiment 1, immediate memory recall tests consisted of 10 different sets of 20 flashcards and each flash card had a word written on it. The words were selected from
a book used to devise such memory tests (Thorndike and Lorge, 1944). The 20 flashcards were presented to the subject in 1-second intervals; once all 20 words were presented, subjects provided a written recall. This procedure was repeated 3 times with the same set of flashcards, each recall written on a different page. The delayed recall memory test required subjects to write down as many words as they could remember from the t = 15 minutes word list and the t = 60 minutes immediate recall used a new set of flashcards. Each time a subject completed his written recall, the page was removed from him to prevent influence on further memory trials. The words used in the memory test are found in Figure A-1 and an example of the page subjects were asked to record words they recalled on is found in Figure A-2.

Experiment 2

In experiment 2, word list memory tests were replaced with paragraph memory tests. The standard 3 Wechsler Memory paragraphs were used and 3 other modified versions (the Morris revision, the Kaplan revision) were also used (Kaplan et al. 2000). In total, 6 paragraphs were pre-recorded on an audiocassette. At t = 15 minutes after the preload was consumed, 1 story would be played for the subject. Subjects were then asked to repeat back the story in as much detail as they could remember; this was the first immediate recall test. At t = 45 minutes after the preload, subjects were asked to recall as much as they could remember from the story they heard at t = 15 minutes; this was the delayed recall test. At t = 60 minutes, a different pre-recorded story was played for the subjects, and he was then asked to repeat it back in as much detail as he could remember; this was the second immediate recall test in the experiment. All recalls were audio recorded and transcribed by the experimenter; they were scored according to the
instructions of the Wechsler Memory Scale (Wechsler 1997). The scoring system is based on exact words, as well as specific concepts subjects are required to remember. There was a maximum score of 25 points for each story. All 6 stories used in this experiment and an example of the scoring system used are shown in Figure A-3 and Table A-6, respectively.

**Data Analysis**

In experiment 1, memory performance was based on the number of words that were correctly recalled from the flashcard presentations. In experiment 2, memory performance was judged based on the number of points a subject scored when he was asked to repeat as much of the story as he could remember. Treatment effects on memory performance were evaluated by one-way ANOVA. Significance was determined by p-values < 0.05; a Duncan's post-hoc analysis was used to compare different means.

**Experiment 1**

The total (sum of the scores on each of the three trials) t = 15 minutes and t = 60 minutes immediate recall values were analyzed separately for treatment effect. The difference in total scores at t = 15 minutes and t = 60 minutes after each treatment drink was calculated to test for any change in performance between these time points. Scores for each of the three trials at the two time points were also analyzed.

On the assumption that the memory score after the control treatment was representative of each subject's baseline performance, the memory score after control treatment was subtracted from the other treatment memory scores separately at t = 15 minutes and t = 60 minutes. The t = 45 minutes delayed recall was analyzed by
comparing the total number of words recalled across all 5 treatments. Subtracting the control treatment values from the treatment values also assessed the delayed recall.

**Experiment 2**

In experiment 2, the $t = 15$ minutes and $t = 60$ minutes scores were analyzed separately for a treatment effect. The difference between the paragraph scores at $t = 15$ minutes and $t = 60$ minutes after each preload was also calculated to test for a change in performance between these two time points. Again, assuming that the memory score after the control treatment represents a subjects’ baseline performance, the control scores were subtracted from the corresponding treatment scores at both $t = 15$ minutes and $t = 60$ minutes. The difference would reflect any change in performance due to the treatment.

**RESULTS**

**Experiment 1**

The effect of the three proteins and sucrose on memory was tested for immediate recall at $t = 15$ minutes and $t = 60$ minutes, and a delayed recall test was done at $t = 45$ minutes (Table A-4). At $t = 15$ minutes, no significant effect of treatment was found for the first two trials of immediate recall, but the third trial indicated that memory scores were higher after soy compared to whey and egg, but not compared to sucrose or control; only whey suppressed memory compared to control and egg, but did not differ from sucrose or egg ($F=3.11, p=0.02$). No significant effect of treatment on memory was seen for the total number of words remembered, or for difference from control.
The delayed recall test, counting the number of words remembered from $t = 15$ minutes indicates no significant effect at $t = 45$ minutes. However when expressed as the difference from control, there was a trend for enhanced delayed recall after soy compared to control more than the other treatments ($F=2.70, p=0.06$) (Table A-4). The second immediate recall test was carried out at $t = 60$ minutes and showed no significant effect of treatment on memory for any of the measurements taken.
### Table A-4: Experiment 1.
Memory Scores 15 minutes, 60 minutes post preload and 45 min delayed recall

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>Egg</th>
<th>Whey</th>
<th>Soy</th>
<th>Sucrose</th>
<th>F; p</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st trial</td>
<td>7.6 ± 0.7</td>
<td>7.3 ± 0.6</td>
<td>7.2 ± 0.5</td>
<td>7.5 ± 0.5</td>
<td>7.8 ± 0.7</td>
<td>0.21; 0.93</td>
</tr>
<tr>
<td>2nd trial</td>
<td>11.5 ± 0.6</td>
<td>11.5 ± 0.7</td>
<td>11.3 ± 0.8</td>
<td>11.5 ± 0.8</td>
<td>12.0 ± 0.9</td>
<td>0.19; 0.94</td>
</tr>
<tr>
<td>3rd trial</td>
<td>14.4 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.1 ± 0.7&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>12.4 ± 0.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.0 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.5 ± 0.9&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>3.11; 0.02</td>
</tr>
<tr>
<td>Total</td>
<td>33.5 ± 1.8</td>
<td>31.8 ± 1.7</td>
<td>30.8 ± 1.7</td>
<td>34.0 ± 1.9</td>
<td>33.3 ± 2.4</td>
<td>0.82; 0.52</td>
</tr>
<tr>
<td>Change from Control&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0</td>
<td>-1.6 ± 2.0</td>
<td>-2.6 ± 2.1</td>
<td>0.5 ± 2.2</td>
<td>-0.2 ± 2.6</td>
<td>1.11; 0.36</td>
</tr>
<tr>
<td>45 min Recall Score</td>
<td>9.7 ± 0.7</td>
<td>8.8 ± 1.1</td>
<td>9.5 ± 1.1</td>
<td>10.5 ± 1.0</td>
<td>10.2 ± 1.0</td>
<td>1.43; 0.24</td>
</tr>
<tr>
<td>Change from Control&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0</td>
<td>-0.9± 0.9</td>
<td>-0.2 ± 1.0</td>
<td>0.8 ± 1.0</td>
<td>0.5 ± 0.9</td>
<td>2.70; 0.06</td>
</tr>
<tr>
<td>60 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st trial</td>
<td>6.4 ± 0.5</td>
<td>7.2 ± 0.4</td>
<td>6.2 ± 0.6</td>
<td>6.8 ± 0.6</td>
<td>7.5 ± 0.7</td>
<td>1.19; 0.32</td>
</tr>
<tr>
<td>2nd trial</td>
<td>10.7 ± 1.0</td>
<td>11.4 ± 0.8</td>
<td>10.3 ± 0.7</td>
<td>11.2 ± 0.8</td>
<td>12.5 ± 1.0</td>
<td>1.44; 0.23</td>
</tr>
<tr>
<td>3rd trial</td>
<td>13.7 ± 0.8</td>
<td>14.0 ± 0.7</td>
<td>13.8 ± 1.0</td>
<td>14.5 ± 0.9</td>
<td>14.8 ± 1.0</td>
<td>0.68; 0.61</td>
</tr>
<tr>
<td>Total</td>
<td>30.8 ± 2.1</td>
<td>32.5 ± 1.8</td>
<td>30.2 ± 2.2</td>
<td>32.5 ± 2.2</td>
<td>34.8 ± 2.6</td>
<td>1.26; 0.30</td>
</tr>
<tr>
<td>Change from Control&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0</td>
<td>1.8 ± 2.4</td>
<td>-0.5 ± 1.7</td>
<td>1.7 ± 2.6</td>
<td>4.0 ± 2.3</td>
<td>1.38; 0.27</td>
</tr>
</tbody>
</table>

<sup>1</sup>Mean ± SEM (mm) mood score; n=13
<sup>2</sup>Change from Control = (total treatment score – total control scores)
<sup>a</sup>Values with different letters along the same row are significantly different
Experiment 2

The analyses conducted on the $t = 15$ minutes and $t = 60$ minutes immediate recall scores, as well as the $t = 45$ minutes delayed recalls did not reveal a treatment effect of egg or whey on memory (Table A-5).

Table A-5: Experiment 2. 15 minutes, 60 minutes post preload and 45 min delayed recall

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>Egg</th>
<th>Whey</th>
<th>F; p^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>13.5 ±</td>
<td>14.5 ±</td>
<td>13.6 ±</td>
<td>0.65;</td>
</tr>
<tr>
<td></td>
<td>0.9</td>
<td>0.7</td>
<td>0.7</td>
<td>0.53</td>
</tr>
<tr>
<td>Change from Control^2</td>
<td>0</td>
<td>1.0 ±</td>
<td>0.2 ±</td>
<td>1.03;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9</td>
<td>0.0</td>
<td>0.32</td>
</tr>
<tr>
<td>45 min</td>
<td>11.6 ±</td>
<td>12.6 ±</td>
<td>12.0 ±</td>
<td>0.52;</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0.9</td>
<td>0.8</td>
<td>0.60</td>
</tr>
<tr>
<td>Change from Control^2</td>
<td>0</td>
<td>1.0 ±</td>
<td>0.4 ±</td>
<td>0.52;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>1.1</td>
<td>0.48</td>
</tr>
<tr>
<td>Index of Forgetting^3</td>
<td>1.8 ±</td>
<td>1.8 ±</td>
<td>1.6 ±</td>
<td>0.96;</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>0.4</td>
<td>0.5</td>
<td>0.91</td>
</tr>
<tr>
<td>60 min</td>
<td>13.2 ±</td>
<td>13.6 ±</td>
<td>14.0 ±</td>
<td>0.43;</td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>0.9</td>
<td>0.7</td>
<td>0.65</td>
</tr>
<tr>
<td>Change from Control^2</td>
<td>0.0</td>
<td>0.4 ±</td>
<td>0.8 ±</td>
<td>0.18;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.7</td>
<td>0.9</td>
<td>0.67</td>
</tr>
</tbody>
</table>

^1Mean ± SEM (number of points scored); n=22
^2Change from Control = (total treatment score – total control scores)
^3Index of forgetting = (45 min recall score – score at 15 min)
Figure A-1: Experiment 1. Word Lists For Memory Testing.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>COTTAGE</td>
<td>BARREL</td>
<td>CLOSET</td>
<td>DEALER</td>
<td>FLOWER</td>
</tr>
<tr>
<td>2</td>
<td>RIDER</td>
<td>SCHOOL</td>
<td>SERIES</td>
<td>CITY</td>
<td>WILLOW</td>
</tr>
<tr>
<td>3</td>
<td>FRONTIER</td>
<td>BASKET</td>
<td>SPANIARD</td>
<td>MARBLE</td>
<td>SERVICE</td>
</tr>
<tr>
<td>4</td>
<td>HAMMER</td>
<td>WEATHER</td>
<td>CULTURE</td>
<td>REFUGE</td>
<td>STOCKING</td>
</tr>
<tr>
<td>5</td>
<td>HUSBAND</td>
<td>OLIVE</td>
<td>INCOME</td>
<td>DARKNESS</td>
<td>LEATHER</td>
</tr>
<tr>
<td>6</td>
<td>HERALD</td>
<td>SYSTEM</td>
<td>WIDSOM</td>
<td>WEAPON</td>
<td>MATTER</td>
</tr>
<tr>
<td>7</td>
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<td>MOTION</td>
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<td>DECAY</td>
<td>SLUMBER</td>
<td>SURPRISE</td>
<td>FACTOR</td>
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</table>
Figure A-2: Experiment 1. Example of page on which subjects were asked to record the words they recalled from the memory test.

DATE: ___________________
NAME: ___________________

Please write down as many words as you can remember.
Figure A-3: Experiment 2. Memory Paragraphs

STORY 1 (Wechsler Memory Scale – Revised (WMS-R))

Anna Thompson of South Boston, employed as a cook in a school cafeteria, reported at the City Hall Station that she had been held up on State Street the night before and robbed of fifty-six dollars. She had four small children, the rent was due, and they had not eaten for two days. The police, touched by the woman’s story, took up a collection for her.

STORY 2 (WMS-R)

Robert Miller was driving a ten-ton truck down a highway at night in the Mississippi Delta, carrying eggs to Nashville, when his axle broke. His truck skidded off the road, into a ditch. He was thrown against the dashboard and was badly shaken. There was no traffic and he doubted that help would come. Just then his two-way radio buzzed. He quickly answered, “This is Grasshopper”.

STORY 3 (WMS-R)

At 6:00 on Monday evening, Joe Garcia of San Francisco was watching television as he dressed to go out. A weather bulletin interrupted the program to warn that thunderstorms would move into the area within the next two to three hours and remain until morning. The announcer said the storm could bring hail and up to 4 inches of rain and cause the temperature to drop by 15 degrees. Joe decided to stay home. He took off his coat and sat down to watch old movies.

STORY 4 (MORRIS-REVISION)

At eleven o’clock on Saturday morning, Rick Ventura of Fort Lauderdale was preparing his breakfast when he heard someone knock. A red-headed woman appeared at his apartment to announce that a tow truck would take away his sports car within three or four minutes unless he moved it. The neighbour warned that he would receive a ticket and a twenty-dollar fine because he had parked the car in the driveway for two hours. Rick looked at his wristwatch, jumped up and hurried out of the apartment.

STORY 5 (KAPLAN-REVISION)

A teacher named Cathy Davis was walking her bull terrier in Johnson Park, in a suburb near Vancouver, when a tall man tried to take her orange purse. Her dog bit the man with its powerful jaws. He realized that his right knee was bleeding and began to flee. The dog chased the man into the thorny bushes. Suddenly, a police officer arrived. He said to the woman, you should thank Shaggy.

STORY 6 (KAPLAN-REVISION)

David Simpson of Quebec City, a skilled astronaut in the Canadian aeronautics program travelled on the Space Shuttle to the Russian space station on a three-month voyage last March. During the flight back to Earth, an asteroid hit the aircraft, causing damage to one wing. The pilot was forced to land in the sea, where the crew was rescued by the Air Force.
**Table A-6: Experiment 2. Example of Logical Memory Scoring Criteria for Paragraphs**

<table>
<thead>
<tr>
<th>Text for Story 1</th>
<th>General Rule</th>
<th>Examples of Alternatives 1-Point Responses</th>
<th>Examples of 0-Point Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anna</td>
<td>&quot;Anna&quot; or variant of the name</td>
<td>Ann; Annie; Annette</td>
<td>Angela; Allison</td>
</tr>
<tr>
<td>Thompson, of South</td>
<td>&quot;Thompson&quot; is required</td>
<td>from South; who lived in South; who came from the South</td>
<td>Thomkins; Thomas</td>
</tr>
<tr>
<td>Boston,</td>
<td>&quot;Boston&quot; (in any context)</td>
<td>who worked in Boston; on a trip to Boston</td>
<td></td>
</tr>
<tr>
<td>Employed⁴</td>
<td>An indication that she held a job</td>
<td>worked; had a job as; who earned a living as</td>
<td>who wanted to be; employed a cook</td>
</tr>
<tr>
<td>as a cook⁴</td>
<td>&quot;Cook&quot; or some form of the word is required</td>
<td>who cooked</td>
<td>as a waitress; in the kitchen</td>
</tr>
<tr>
<td>in a school</td>
<td>&quot;School&quot; is required</td>
<td>at a high school; by a school</td>
<td>in a hospital; at a company</td>
</tr>
<tr>
<td>cafeteria,</td>
<td>&quot;Cafeteria&quot; is required</td>
<td></td>
<td>lunchroom; dining hall; diner; restaurant; kitchen</td>
</tr>
<tr>
<td>reported</td>
<td>Indication that a formal statement was made to someone in authority (in any context)</td>
<td>filed a complaint; said to the police; made a statement; notified the police; called the police; told the police</td>
<td>said; told how</td>
</tr>
<tr>
<td>at the City Hall</td>
<td>&quot;City Hall&quot; (in any context)</td>
<td>went to City Hall; called City Hall</td>
<td></td>
</tr>
<tr>
<td>Station</td>
<td>&quot;Station&quot; (in any context), or a word or phrase denoting a police station</td>
<td>police station; train station; stationhouse; police headquarters; precinct house; police department</td>
<td>office; building</td>
</tr>
<tr>
<td>that she had been held up</td>
<td>An indication that she had been held up (i.e., gun point or knife)</td>
<td>that someone held her up; that she was in a stick up</td>
<td>that she was beaten; that she was attacked; that she was robbed; she got mugged</td>
</tr>
<tr>
<td>on State Street</td>
<td>&quot;State Street&quot; (in any context)</td>
<td>she lived on State Street; on her way to State Street</td>
<td>on some street; State Avenue</td>
</tr>
<tr>
<td>the night before</td>
<td>Indication that the hold up occurred the previous night</td>
<td>last night; the previous night</td>
<td>at night; one night; yesterday; the day before</td>
</tr>
<tr>
<td>and robbed</td>
<td>Indication that a robbery took place</td>
<td>was robbed; her money was stolen; they took her money; someone took her purse</td>
<td>lost her money; somebody took her things</td>
</tr>
<tr>
<td>Text for Story 1</td>
<td>General Rule</td>
<td>Examples of Alternatives 1-Point Responses</td>
<td>Examples of 0-Point Responses</td>
</tr>
<tr>
<td>-----------------</td>
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<td>-----------------------------</td>
</tr>
<tr>
<td>of fifty-six dollars.</td>
<td>Indication that an amount of money greater than $49 but less than $60 was taken from her</td>
<td>fifty-some dollars; fifty-five dollars; about fifty dollars</td>
<td>sixty-five dollars; a lot of money; the police collected fifty-six dollars for her</td>
</tr>
<tr>
<td>She had four</td>
<td>“Four” is required together with an indication that the children were hers</td>
<td>she was the mother of four</td>
<td>she had two; she had some; there were some</td>
</tr>
<tr>
<td>small children,</td>
<td>“Children” or a synonym is required</td>
<td>little children; kids; small kids; young children</td>
<td>babies; girls; sons; small boys</td>
</tr>
<tr>
<td>the rent was due,</td>
<td>A phrase indicating that the rent was due</td>
<td>she had not paid the rent; she owed for the rent; the landlord had to be paid; she needed the money for rent</td>
<td>she owed money; she needed money; there was no money</td>
</tr>
<tr>
<td>and they had not eaten</td>
<td>Indication that her children, or the family, were without food</td>
<td>they had gone without food; they were hungry; there was no food; her kids had nothing to eat; she couldn’t feed her family</td>
<td>there wasn’t much food; they had only a little food; she had not eaten; didn’t have money to buy food</td>
</tr>
<tr>
<td>for two days.</td>
<td>“Two days” is required, or a phrase meaning about two days</td>
<td>for a couple of days; for one or two days; for two or three days</td>
<td>for days; for several days; for three days</td>
</tr>
<tr>
<td>The police,</td>
<td>A word or phrase signifying one or more members of the police department (in any context)</td>
<td>the cops; the policeman; the detectives; the police officer; the (where police is clearly meant)</td>
<td>they (unspecified); some people; her neighbours; somebody</td>
</tr>
<tr>
<td>touched by the woman’s story,</td>
<td>An indication that her story evoked sympathy</td>
<td>were touched; felt sorry for the woman; wanted to help her; were sympathetic; were impressed by her story (implying emotional reaction)</td>
<td>listened to her story; helped her; believed her</td>
</tr>
<tr>
<td>took up a collection</td>
<td>A phrase indicating that money was collected</td>
<td>chipped in; collected money; donated; collected some food</td>
<td>gave her some money; found some money</td>
</tr>
<tr>
<td>for her.</td>
<td>An indication that the money was collected for her or her children</td>
<td>and gave it to her; for her children; for her family; for them; to help her out</td>
<td>as a gift; to make things better; for food</td>
</tr>
</tbody>
</table>

1From Logical Memory Subset of the Wechsler Memory Scale-Revised (Wechsler 1997).
2“Anna Thompson, a cook in a ...” gets credit for “employed” and “as a cook”.
DISCUSSION

The results of these two experiments do not confirm the hypothesis that protein source has an effect on memory. However, in experiment 1, the memory tests in this subject population indicated that the soy treatment might enhance memory when compared to the control, as seen at the third trial of 15 minute immediate recall and the 45 minute delayed recall test, when expressed as a difference from control. In experiment 2, paragraph memory tests were used to improve the sensitivity of memory test (Craft et al. 1993), because subjects had high performance level at baseline testing. However, in experiment 2 no effect of treatment was found on memory at any of the time points, despite the change in testing paradigm.

Unfortunately the soy protein was not included in experiment 2 (for reasons relevant to the food intake experiment) and thus the effect of soy on memory from the experiment 1 could not be confirmed. Soy protein has received interest for its memory enhancing effects in women, because of its phytoestrogen content; estrogen has been linked with cholinergic and serotonergic activity in the brain (Pan et al. 2000).

The lack of any consistent finding in memory enhancement may again be due to the subject population, which is young, healthy and has good baseline memory function. No other data in the literature, to date, examines the effects of protein on memory. The effect of sucrose on memory documented in the literature is inconsistent. It has been shown that glucose enhances memory in elderly subjects (Gold 1992; Manning et al. 1990; Parsons and Gold 1992), those with Alzheimer's (Craft et al. 1992) and older subjects with pre-existing memory defects (Anderson 1998). The effect of glucose on memory improvement is less established in younger or healthier subjects. It has
generally been established that subjects with good baseline memory values do not improve memory with glucose preloads over the short-term (Azari 1991; Hall et al. 1989). While one study, using a protocol very similar to this study, found a general improvement in immediate recall with young male subjects (Catherine 2000), the study used a 75 g preload of sucrose. The dose of sucrose used in this study was an average of 45.1 g, just over half the amount used to find memory improvement with glucose; this may indicate that below a certain threshold value, sucrose does not improve memory in such a young healthy population.

Although we did not measure blood constituents in this study, we expect that some proteins to increase plasma CCK. CCK has been implicated in regulating memory in rats (Dauge and Lena 1998). Thus the prospect of more research into the area of protein and memory is an exciting one. This study found no effects of treatment on memory in young men. A study designed primarily to determine the effect of treatment on memory in such a young, healthy and educated population may require a design more suited for detecting such effects. For example, future studies may use a larger sample size, and more difficult tests to determine the effect of treatment on memory. Furthermore, a longer time between immediate and delayed recall may be effective in making the recall test more difficult and may more readily distinguish the effects of protein sources on memory.