EFFECTS OF THE COMBINATION OF PROTEIN AND FIBER IN WHOLE FOODS ON SATIETY, BLOOD GLUCOSE RESPONSE AND FOOD INTAKE IN HUMANS

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Abstract

The obesity epidemic continues worldwide and is considered a major health concern. A shift back to consumption of whole foods may be an important part of the solution in the current rising obesity trend. Both protein and fiber have satiating properties, but few studies have examined whether their impact on different biological mechanisms work additively to more strongly enhance the overall satiety potential of a meal.

In the first study, the objective was to determine the effects of a high protein meal (beef) compared to a moderate protein, high fiber meal (beans) on subjective appetite and energy intake at a subsequent meal. We hypothesized that a moderate protein, high fiber meal containing beans would be as satiating as a high protein meal containing beef. Twenty-eight adults, 14 men and 14 women participated in this randomized, controlled study in which subjects consumed two test lunches including a "meatloaf" made from either beef or beans. The primary outcome was to observe satiety ratings using visual analogue scales to assess hunger, satiety, fullness, and prospective food intake. Secondary outcomes included: food intake at the subsequent meal offered in the form of snacks, gastrointestinal tolerance, and palatability of the meals. No difference between the beef and bean was observed for appetite ratings over 3 hours, food intake at the subsequent meal, or sum of GI score. Gas and bloating were reported more often after the bean meal than the beef meal. The conclusion to this first study was that a beef-based meal with high protein and a bean-based meal with moderate protein and high fiber

produced similar satiety, while the bean-based meal resulting in higher, yet moderate, gas and bloating.

In the second study, we examined the effect of egg alone and in combination with whole grains compared to a refined ready-to-eat cereal on satiety and food intake in human subjects. We hypothesized that breakfast meals containing eggs, both high protein with white toast and moderate protein with whole grain toast containing fiber, would result in increased satiety ratings compared to an isocaloric standard refined cereal breakfast. Forty-eight adults, 24 men and 24 women, participated in this randomized, cross-over study. We designed whole food diets, controlled for macronutrients. The primary outcome was to observe satiety ratings using visual analogue scales to assess hunger, satiety, fullness, and prospective food intake. Secondary outcomes included: post-prandial blood glucose response, food intake at the subsequent meal offered in the form of an ad libitum pizza lunch, gastrointestinal tolerance, and palatability of the meals. No difference was observed between the cereal and egg + whole grain toast breakfasts for AUC for all satiety ratings however the egg + white bread breakfast was significantly improved for all 4 satiety ratings. Lunch intake was significantly reduced in both egg breakfasts compared to the cereal breakfast. No difference was observed for the sum GI score between the egg + white toast, egg + whole grain toast and cereal breakfasts, however gas and bloating was significantly higher for the egg + white toast breakfast compared to the cereal meal. Food intake at the subsequent meal was reduced for both egg breakfasts compared to the cereal breakfast.

The results from these studies support the hypothesis that protein and fiber contained within whole foods results in greater satiety than refined carbohydrate foods. Protein, with and without fiber, produced the greatest satiety outcomes suggesting that the incorporation of high protein foods into the diet, specifically for breakfast, may result in greater feelings of satiety that could lead to decreased food intake and weight loss over time.

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Chapter One

LITERATURE REVIEW

Satiety Overview

Satiety and satiation are often times used interchangeably but are two distinct actions in terms of appetite regulation. Satiety can be defined as the processes that suppress the urge to eat and inhibit further eating during the postprandial period until the next eating occasion [1]. Satiety, therefore, impacts timing of next meal, consumption at the next meal or both. Satiation is the processes that lead to satisfaction of appetite occurring during the meal and ends with the cessation of the meal. Satiation, therefore, determines the size of the meal or eating occasion. The breakdown of satiation and satiety periods is shown below (figure. 1-1) and also illustrates the various inputs that feed back to the brain to illicit a response.

Measurements of Satiety

The standard for measuring satiety subjectively utilizes a visual analog scale (VAS). Both the use of multi-point equilateral ratings and 100 mm lines anchored with opposing ends have been validated. Satiety can be measured subjectively in this way as it asks the subject questions relating to hunger, fullness, desire to eat, satisfaction and has them place a mark on the line with the left end anchoring the “not at all” and the right anchoring “the most I’ve ever been”. These marks are then measured and a value can be assigned to each question for each subject. The use of VAS for satiety has been validated and is typically the good standard for satiety scoring [2-3]. The objective measurement of satiety has a number of biomarkers associated but one alone cannot verify/explain how much, how often and why a person eats.

The landscape of satiety is complex and includes multiple time points including time and size of last meal, nutrient content, caloric density, liking of food, and activity level. In addition to controlling for all of the former points, the cognitive aspect of eating is one that a researcher has the least control over including social eating, emotional eating, and convenience/availability of foods. In order to control for some of this variability, researchers implement techniques such as randomizing the treatments, using overnight fasting prior to arrival, and having each subject serve as their own control with a crossover design.

Mechanisms of Satiety

The mechanisms involved in satiety are present along the entire length of the gastrointestinal tract and include: chewing and saliva production, nutrient absorption time, gastric distention and stretch, gut hormone release, ileal brake, transit time and fermentation. Gastric distention senses the volume of the meal as in enters the gastric cavity and can be relayed back to the brain via input from stretch mechanosensors [4-6]. The release of gut hormones active in satiety are released with a number of these mechanistic actions and impact satiety cooperatively including: nutrient sensing, taste receptors, and fermentation [7-8].

The regulation of appetite control occurs within the brain with the hypothalamus controlling the majority. This control system includes the lateral hypothalamic area, the ventromedial hypothalamic nucleus and the arcuate nucleus. This system has direct communication with peripheral compounds originating within the gut that contribute to food intake and satiety via an incomplete barrier at the median eminence [9]. A number of the peripheral compounds originate from the gastrointestinal tract. Multiple gut hormone genes and bioactive peptides are expressed or produced in the intestines [10]. These peptides are responsible for modulating appetite, food intake and digestion rate via chemical and mechanical stimuli.

Most of the gut peptides are able to alter food intake meal by meal. The gut hormones include: Glucagon-like petide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), peptide YY (3-36), pancreatic polypeptide, oxyntomodulin, ghrelin, cholecystokinin, glucagon and amylin. All but ghrelin and GLP-2 decrease food intake while as ghrelin levels increase, food intake increases. GLP-2 does not have a direct effect on food intake. Peptide YY (3-36), GLP-1, GLP-2 and oxyntomodulin are secreted in the L cells of the distal small intestine and large intestine and could potentially be modulated by actions within, specifically fermentation of low-digestible and non-digestible carbohydrates. Peptide YY has also exhibited effects on increased energy expenditure [11] and levels correlate with body weight inversely [12]. Oxyntomodulin inversely reduces food intake dependent on levels and is also expressed via the proglucagon gene. Ghrelin is the only gut hormone that increases food intake. It is secreted in the stomach and has action on the growth hormone secretagogue (GHS) receptor in the stomach but expression of GHS receptor also occurs in the hypothalamic arcuate nucleus which is likely the site of its orexigenic effects. Ghrelin levels fall prior to a meal and decrease rapidly following a meal [13].

GLP-1 (7-36) originates from the proglucagon molecule and is released from the enteroendocrine L cells in the distal gut, predominantly in the ileum and colon. GLP-1fluctuates dependent on meal state and is co-secreted with peptide YY in the L cells. GLP-1 levels rise after a meal (and potentially also in the anticipation of a meal) and fall in the fasted state. GLP-1 inversely reduces food intake, delays gastric emptying and blunts glucagon release [14]. GLP-1 levels increase following food intake and remain elevated for 5 to 120 minutes in the bloodstream postprandial [15-17].

No difference in GLP-1 levels were found between a 10% protein compared to a 25% protein meal but this may be due to the 10% levels ability to reach the threshold protein level to illicit a response that may not be intensified by adding additional protein [18]. Carbohydrates seem to have the greatest impact on GLP-1 release. The nutrient sensing of glucose molecules has been linked to carbohydrate digestion sensing via the Tas sweet receptors that stimulate GLP-1 release and by knocking out Tas, the GLP-1 secretion was decreased compared to the characteristic increase typically seen with a high carbohydrate diet [19]. Protein can also elicit GLP-1 release but the peak of release is typically blunted and recovers more quickly than carbohydrate stimulation [20]. Glutamine, however, has demonstrated to have a potent effect on GLP-1 which could suggest that specific peptides can be more efficient than others in terms of GLP-1 release [21].

Cholecystokinin (CCK) is produced by enteroendocrine K cells located in the duodenum and jejunum along the gastrointestinal mucosa. Release of CCK is stimulated by the presence of nutrients in the lumen, specifically protein and fat. CCK is directly linked to inhibiting food intake related to its action within the arcuate nucleus via the nucleus of the solitary tract as well as contributes to additional physiological responses that also reduce food intake and enhance satiety. Multiple studies have been linked to reduced food intake associated with CCK levels [22-25] while CCK antagonists have shown to increase food intake and reduce satiety [26].

An increase is CCK, as well as GLP-1, has been associated with decreased gastric emptying rates which could impact overall satiety [27]. It has been demonstrated that bitter compounds elicit an increased CCK response via the bitterness receptors along the gastrointestinal tract [5]. The CCK increase was also associated with decreased gastric emptying time but also provides circulating CCK to act on other satiety targets as well.

Glucose Response and Satiety

The glycemic index (GI) of a food is the ranking of a carbohydrate on a scale of 0 t o100 according to the extent to which they raise blood sugar levels over a 2 hour period after eating (i.e. their immediate effect on blood sugar levels) compared to glucose [28]. The rating provides a ranking for various foods but this value is arbitrary in that it does not take into account the amount of the specific food consumed. The glycemic load accounts for serving size of the food by taking the GI of a food multiplied by the grams of available carbohydrate in the serving. The glycemic response (GR) is then the effect that the carbohydrate-containing food has on blood glucose concentration during the digestion process.

The rate at which dietary carbohydrates are digested and absorbed results in variation in postprandial glycemic responses with the potential to impact satiety and appetite. Carbohydrates that are digested and absorbed more slowly, in turn resulting in a gradual rise in blood glucose, have been proposed to suppress appetite [29-30] but results are inconsistent [31] and the rate and extent of carbohydrate digestibility may only partially explain the mechanisms.

Variation in glycogen stores have also been suggested as an appetite modulator. The glucostatic and glycogenostatic theories hypothesize that low blood glucose and depletion of glycogen stores leads to increased hunger and energy intake [32-33] Additionally, when macronutrient intake is altered, glycogen stores can be replenished through alternative pathways and do not necessarily promote an increase in energy intake or carbohydrate cravings [34]. A study involving a high fat diet compared to a low-fat diet concluded that regulation of neither fat nor carbohydrate was tightly regulated therefore does not suggest that the glucostatic and glycogenostatic models are the main point of food intake control [35]. A review of this concept suggests while some evidence supports this concept, it is limited to date [36].

The autonomic nervous system is thought to stimulate early release of GLP-1 prior to nutrients reaching the distal small intestine [37]. Glucose sensing occurs all along the gastrointestinal tract and while GLP-1 secreting cells are present mainly in the L-cells of the ileum, GLP-1 release can be triggered earlier via actions of neurons contained in the autonomic and enteric nervous system involving glucose sensing units such as SGLT3 [38]. Glucose signalling has been equated to leptin signalling in terms of its ability to regulate food intake and that the current obesity epidemic could be related to reduced neuroendocrine sensitivity resulting in less effective glucose signal and therefore higher energy intake [39]. This is therefore relevant to glycemic index and load of food in that a steady, blunted release of glucose may be beneficial in terms of preventing desensitization of glucose signalling due to high blood glucose peaks observed with refined carbohydrate foods containing rapidly available glucose.

Low GI foods, by virtue of their slow digestion and absorption, produce gradual rises in blood glucose and insulin levels, and may thus have potential benefits for health including improved glucose tolerance for individuals with diabetes or high-risk of diabetes, weight management, improved lipid parameters, and decreased fat mass. Low GI diets have not only been shown to improve both glucose and lipid levels in people with diabetes (type 1 and type 2) [40-41] but also the potential to decrease the risk of developing type 2 diabetes [42]. High GI foods have also been shown to decrease plasma glucose, increase hunger ratings, and stimulate areas associated with reward and craving in the brain [43].

The ability to elicit a lower blood glucose response following a first, low GI meal and also a standardized second meal has been termed the “first-meal” and “second-meal effect”, respectively [44]. Glycemic index of a food is one mechanism at play for this effect but colonic fermentation and delayed gastric emptying, linked to low GI foods that are slowly digestible or "lenta" containing fermentable fiber and resistant starches, have also exhibited this effect [45-47]. The various mechanisms, however, do not impact all aspects of glycemic control and food intake equally.

Dietary Fiber

Dietary fiber is an important nutrient in the diet that offers many benefits for optimal health including cardiovascular health, gut health and weight management [48]. Current fiber intake is well below the recommended intake of 25 grams per day for women and 38 grams per day for men [49]. Dietary fiber is broadly defined by the American Association of Cereal Chemists (2001) is as follows:

"Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plants substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation."

As stated in the definition above, fiber is contained within plants. Plant foods inherently contain fiber and when we consume plant foods, one of the many benefits includes fiber consumption. The classification of fibers is not as simple as soluble versus insoluble as many foods contain a mixture of both. The range of fibers contained in foods and isolated from plants is large and includes viscous, non-viscous, bulking, fermentable, non-fermentable, and functional (when isolated and added back to food items as an ingredient). My research will be focusing on whole food diets so I will focus on the fiber contained in various plant forms in the natural state in which we eat them, specifically whole grains and legumes, and only briefly on fruits and vegetables and the isolated plant fibers used for fortification of food products.

Fiber and Satiety

Fiber intake has been greatly associated with lower body weight in animal models as well as in humans [50-52]. Multiple mechanisms have been postulated around how fiber could be modulating body weight [53]. The proposed mechanisms include: displacement of higher energy density foods; increased chewing leading to greater secretion of saliva and gastric juices leading to greater distention; delayed gastric emptying; delayed nutrient uptake and/or digestion; and fermentation [54]. All of the proposed mechanisms also include modulating various gut hormones which elicit a physiological action [55-59].

Fruits and Vegetables

Fruits and vegetables are typically grouped together in recommendations of daily intake. While they are each distinct in flavor, texture and nutrients, they are both typically high in water and fiber and low in fat and calories [60]. All of the mentioned characteristics provide fruits and vegetables with the capability of contributing to satiety, reduced food intake and weight management. Multiple satiety/satiation studies have been conducted on various fruits and vegetables [61-66]. The addition of boiled carrots in 3 different doses (100, 200 and 300 grams) demonstrated that satiety following the meal increased with the portion size of carrots added but the difference was significant only at the 200 and 300 gram dose [61]. These results validate the author's suggestion around dose effect from a previous study in which only 96-164 grams (4.4 grams fiber) of vegetables were added to a meal and no impact on satiety was noted [62]. Gustafsson et al also continued by investigating the impact of processing conditions of spinach on satiety and found that the fiber and water content positively correlated with satiety while processing conditions did not [63].

The benefit of whole fruits and vegetable on satiety has also been demonstrated. Apples in different forms (apple, applesauce and apple juice with and without added fiber) were consumed prior to a meal [64]. Fullness ratings were: apple>applesauce>apple juices>control with the apple group food intake reduced by 15%. The addition of pectin (4.8 grams) to the juice was added to match the fiber content of the apple and applesauce but did not have the same satiety impact. This was also observed with grapes versus grape juice and oranges versus orange juice with the same results [65]. The addition of pectin to orange juice at higher doses (5, 10, 15, 20 grams) did have a significant impact on satiety when compared to a standard orange juice but was not compared against a whole orange [66]. These results highlight that the best form for fruits and vegetables in terms of satiety is the whole food with the inherent water and fiber intact within the native structure. The greater impact could be due to the density of the food, longer mastication time involved, low energy density, but most likely it is due to a combination of all of the above.

Whole Grains

Whole grains consist of a variety of cereals including: wheat, rice, maize, oats, rye, barley, triticale, sorghum, and millet. The composition of a whole grain is similar in all containing a hard, protective hull encapsulating the inner endosperm, bran and germ. The endosperm is made mostly of starch and storage proteins and is all that remains following the milling and refinement process. The bran and germ are the layers that comprise the many components that are beneficial to health. These components include: fiber, trace minerals, phenolic compounds, phytate, lignin, plant stanols and sterols, vitamins and minerals [67]. The milling of grains produces a product that is highly concentrated in rapidly digestible starches and comprises the bulk of grains that are eaten today.

Many studies have been conducted to compare the impact of whole grains versus refined grains on satiety and food intake. Sustained satiety was observed during a 3 week trial comparing whole grain rye to refined wheat at breakfast [68]. The whole grain rye porridge resulted in higher satiety ratings, lower hunger and desire to eat during the 4 hours postprandial when compared to the refined wheat breakfast. The rye breakfast did not differ in transit time to the wheat but did show high levels of breathe hydrogen highlighting colonic fermentation was occurring. Multiple factors contribute to whole grains being more satiating than refined grains including: fiber content, low energy density, and an intact structure results in slower digestion rates.

The intact structure of grains has been shown to have a benefit on appetite, satiety, and energy intake [69-71]. The comparison of whole and milled rye kernel yielded no difference to compared to one another in bread form while the whole kernel in porridge form yielded significant increases in satiety and decreases in hunger when compared to the milled rye kernel porridge [69]. The authors conclude this could be directly linked to structure as the nutritional compositions of the two were identical. This could also be due to the fact that the whole kernel has a higher water holding capacity and therefore the water content could be greater providing a more filling breakfast. In addition, whole rye kernels resulted in higher satiety and lower energy intake at subsequent meal when compared with white wheat bread [70]. Also, the high content of indigestible carbohydrate in the breakfast product was related to improved satiety and higher breath hydrogen, both of which correlated with lower energy intake at the subsequent meal.

This was demonstrated again with the mean satiety scores of whole kernel wheat and whole wheat meal bread were significantly higher than a reference white wheat bread, with the whole kernel bread showing the greatest impact on satiety [71] while no significant difference in gastric emptying rate between the breads was observed. Interestingly, a significant increase in the antral cross-sectional area was observed with the whole kernel bread, and the whole meal wheat trending upward from control though not significant, at 15 min post-ingestion suggesting that although the gastric emptying rate did not differ between any of the test meals, it leaves the potential that gastric distention was sensed via the mechanosensors that could be playing a role in the increased satiety [72].

Legumes

Legumes are rich in a number of nutrients including protein, complex carbohydrates, dietary fiber with a significant level of vitamins and minerals [73]. The content of slowly digestible carbohydrates, high fiber, protein and moderate energy density provide a number of positive attributes for a more satiating diet and an aid in weight management [74].

The fiber content in legumes is predominantly insoluble (wide range of 1/3- 3/4, with the remaining being soluble) and typically ranges from 15%-32%. The blend of soluble and insoluble fiber provides a range of positive effects such as fecal bulking, fermentable substrate, and viscous fiber that can slow gastric distention and emptying. In addition to the dietary fiber content, legumes also contain oligosaccharides that are highly fermentable. Specifically, the oligosaccharides are α-galactosides (α-1,6 linked galatosyl groups attached to a sucrose molecule but are generally referred to as raffinose (1 group), stachynose (2 groups), and verbascose (3 groups). This has, historically, provided legumes with a negative association but the fermentation is beneficial and yields short chain fatty acids (SCFA) and may therefore not only be considered prebiotic [75] but can also stimulate the release of gut hormones related to satiety such a GLP-1 [76].

In assessing legume intake and satiety impact, one study found bean puree to be more satiating than potato puree with less desire to eat and hunger at 180 and 240 minutes post-prandial [77]. In contrast, another study found a variety of beans to be equal in satiety potential as white bread but more than water [78]. Legumes were used as a high-unavailable carbohydrate meal in comparison to a low-unavailable carbohydrate meal of readily digestible carbohydrate (white rice, yogurt and potatoes) and displayed significantly lower hunger and higher fullness [79].

Legume intake could also be useful in weight management over time. Consuming legumes for 3 weeks did not lead to weight loss; the control group actually lost significantly more weight than the legume group although 3 weeks on each diet may not be adequate to assess changes in body weight [80]. When consuming an energy deficient diet, pulses have been shown to have a significantly greater weight loss after 8 weeks compared to a control diet, -7.8 kg compared to -5.3 kg, respectively [81]. In the context of a low glycemic index diet, the intake of legumes in combination with whole grain bread resulted in 0.6 reduction in body mass index (BMI) and 1.5 kg weight loss compared to no change with a high glycemic control diet [82].

A study comparing the intake of rice and beans to lean meat for 8 weeks revealed a greater weight loss at 4 weeks in the bean group [83]. In contrast, an 8 week intervention found the legume meal to result in significant weight loss equal to the meat, egg and lean dairy group when compared to a fatty fish diet and control diet [84]. Both animal and plant proteins consumed at higher levels in the diet have been linked to increased satiety, decreased food intake and increased weight loss [85, 86]. The evidence suggests that legumes unique nutrients may also lead to reduced appetite, reduced food intake and possibly over time lead to weight loss.

Various legumes have been found to have differing effects on glycemic response at initial and second meal, insulin, appetite, and food intake [87-89]. One study found lentil and yellow pea to decrease food intake with specific time points having lower appetite ratings but no impact on glycemic response at first or second meal [87] while another found lentil to decrease glycemic response at both first and second meal and flatten insulin levels [88]. Chickpea did lower glycemic response over a 5 hour period with no difference in food intake compared to a high carbohydrate control [87]. These observed variances may be due to differences in the ratio of slowly digestible carbohydrates and resistant starch among various legumes [47]. Isolated protein and fiber from yellow peas were fed to assess which component is responsible for the second meal effect and found that protein had the greatest impact but effects were lessened [89]. This suggests that intact legumes have a greater impact than the isolated components.

Fiber fortification

Fiber fortification is highly prevalent in most food products today. The recommendation to increase fiber intake to 38 and 25 grams daily for men and women, respectively, has made fiber enriched products very popular with consumers. The positive health attributes from fiber intake is well established including diabetes, cardiovascular disease, weight management and even certain cancers [90-96]. While some fiber used for fortification is used solely for a specific functionality (i.e. hydrocolloids in beverages), the fiber used for fortification is typically classified as functional fiber. The fiber is being added to a product to increase the grams of fiber in the food product. Typically used fibers include dextrins, resistant starch, oligofructose/inulin, β-glucan, bran, pectin, aleurone, wheat germ, and wheat bran. These products are essentially refined foods with added refined fibers. And while positive benefits have been demonstrated for the addition of some functional fibers, they typically only target one point in the satiety cascade, where a whole food fiber would likely target multiple sites of action, pre- and post-absorption. It has been shown that this may not be the best approach if we want to deliver all the added benefits of fiber as they are naturally found in plant products as the whole has been shown to be greater than its sum of parts [97, 98].

Protein

Protein is an essential macronutrient for all mammals, including humans. The human body requires protein as a source of essential amino acids as building blocks of cells and serves as the major structural component of all cells in the body. The primary structure of protein begins with the peptide bond that links the carboxyl group from one amino acid to the amino group of the adjacent amino acid.

A linear polymer of amino acids forms the polypeptide. When consumed, proteins need to be broken down to their basic structure, peptides and single amino acids. This process begins in the stomach with pepsin followed by trypsin, chymotrypsin and elastase in the duodenum and upper ileum in which the polypeptides are hydrolyzed into peptide chains of two to six amino acids and single amino acids when they are then absorbed. The digestion and absorption of protein involves operating energy-requiring post-prandial processes.

Current FDA recommendations for protein vary with age and gender. These include: 56 grams/day for adult males, 46 grams/day for adult females, and 13-19 grams/day for children. This is based on the current recommendation of 0.8 grams/kg body weight for adults, and 1.2 grams/kg body weight for children to account for growth. These recommendations are based on adequate intakes that retain lean body mass but current thought is shifting that diets rich in protein, higher than the recommended levels, may aid in weight management [99].

Protein and Satiety

Protein requires the most energy input into their processes, 20-30%, while fat and carbohydrate are much less at 0-3% and 5-10%, respectively [100] and is believed to be the reason that protein has demonstrated to be the most satiating macronutrient [101], although the mechanism(s) behind this have yet to be elucidated. One recurring mechanism is protein produces the largest thermogenesis value during digestion and absorption compared to fat or carbohydrate. Multiple studies have shown a greater output of thermic energy following protein consumption compared to fat or carbohydrate. In a 15% protein versus a 30% protein meal, the 30% expended 34 kj/hour more than the 15% protein meal [102].

Amino acids can also be divided by their metabolic fate. Ketogenic amino acids can form ketone bodies, therefore consumption of ketogenic amino acids results in higher levels of ketone bodies. β-hydroxybutyrate is an important ketone body that has been shown to reduce food intake [103]. Glucogenic amino acids can be converted to glucose via gluconeogenesis. Leucine and lysine are amino acids that have been linked to increased satiety effects, both of which are ketogenic amino acids, and found in whey which may be a mechanism of satiety observed for whey [104].

Protein Source

While protein is shown to induce larger effects on thermogenesis, it also depends on the source of the protein. The comparison of pork and soy meal to a high carbohydrate meal found pork to have a greater thermic expenditure of 3.9% over the carbohydrate meal compared to only 1.9% for the soy meal [105]. While thermogenesis alone can impact energy expenditure, and perhaps over a long period of time lead to weight loss, this may only partially explain why protein has been shown to yield higher satiety scores.

Studies have shown an increase in satiety when partially replacing fat or carbohydrate with increased protein, both at a single meal and over a 24 hour period [27, 106-107]. A high protein breakfast compared to a high carbohydrate breakfast found no difference in satiety ratings or subsequent food intake but did show a decreased gastric emptying rate most likely due to stronger impact of GLP-1 and CCK release in the high protein treatment [27]. High protein lunch provided more satisfaction, less pre-dinner hunger, and less pre-dinner excitement compared to a high carbohydrate lunch. 31%

more calories were consumed at dinner with the high carbohydrate lunch than the high protein lunch [106]. A diet of 30% protein lead to significant increases in diet-induced thermogenesis, sleeping metabolic rate, GLP-1 concentration and satiety and decreases in hunger when compared to 10% protein diet [107].

Satiety studies have been inconclusive on the differences of animal protein compared to vegetable protein in terms of satiety. Legumes contain 17-40% protein, higher than the 7-13% typically found in whole grains [73]. A study comparing the intake of rice and beans to lean meat for 8 weeks revealed a greater weight loss at 4 weeks (not at 8 weeks, possibly due to large drop-out rate) in the bean group [108].

In contrast, another 8 week intervention found the legume meal to result in significant weight loss (-8.3 kg +/- 2.9%) which was equal to the meat, egg and lean dairy group (-8.4 kg +/- 1.2%) compared to a fatty fish diet and a control diet [109]. The mechanism for protein-induced satiety has not yet been fully elucidated so the difference between animal protein and vegetable protein leads to a number of confounding variables that obscure results. Independent of full explanations regarding what mechanisms are at play, both animal and plant proteins consumed at higher levels in the diet have been linked to increased satiety, decreased food intake and increased weight loss [104, 110].

Protein Quality

Most proteins are eaten as intact but food processing can cause hydrolysation prior to consumption. All proteins develop bitter peptides when hydrolyzed. Food ingredient producers are working to discover ways in which to decrease this bitterness so they are still pleasant to consume in a heat treated product [111]. Bitterness of a protein hydrolysate has been linked to hydrophobicity and therefore a high content of Leucine, Proline, Phenylalanine, Tyrosine, Isoleucine and Tryptophan residues. It is thought that plant proteins are inherently more bitter than animal proteins due to the inclusion of these residues but also, in an intact form, due to the presence of other components such as fiber, trace minerals, phenolic compounds, phytate, lignin, plant stanols and sterols, vitamins and minerals. With the discovery of taste receptors also present along the gastrointestinal tract, the development of bitter hydrolysates during protein digestion could play a role in proteins ability to induce satiety. The investigation of plant protein is then indeed interesting to consider as not only do plants containing protein also contain fiber which impacts satiety mechanisms, the additional bitter component introduces another mechanism in which protein may play a role.

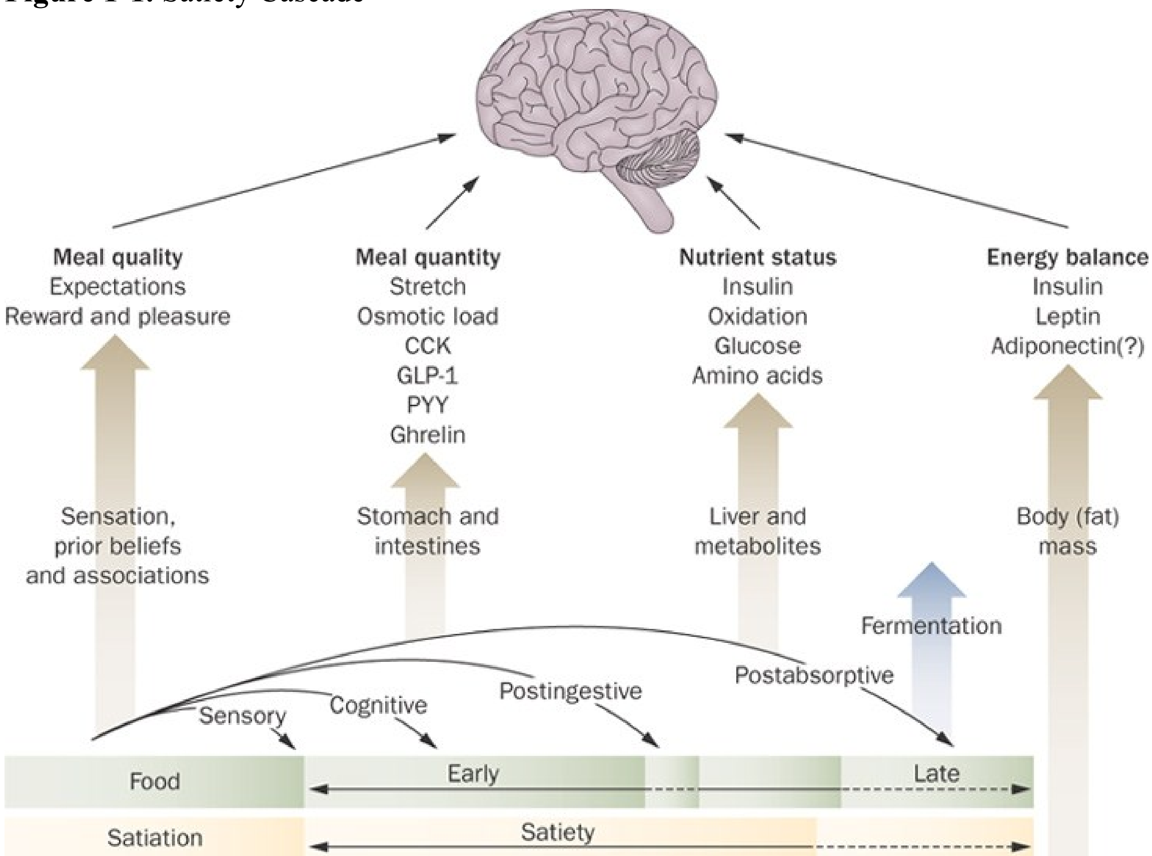
Specific individual amino acids have been shown to elicit stronger satiety signals than others. Glutamate/glutamine has been shown to increase satiety when given orally [112] and preliminary data with beef show that total amino acid concentration in plasma is negatively associated with hunger but also that threonine and alanine are negatively correlated to hunger, histidine and lysine are positively correlated with fullness and tryptophan and threonine are negatively correlated with prospective food consumption [113].

Proteins are given a Protein Digestibility Corrected Amino Acid Score (PDCAAS). This score relates to the limiting amino acid concentrations but also to the bioavailability that is corrected for via true fecal digestibility [114]. Animal-derived proteins tend to have a higher PDCAAS than plant proteins and while the content of essential versus non-essential amino acids may not be relevant to the satiety potential of a protein, the digestibility, and therefore availability, indeed does have a strong impact on a proteins satiety inducing potential.

Protein and Fiber Combinations on Satiety

High protein, high fiber snack bars eaten twice a day between meals resulted in reduced subsequent meal intake [115]. In obese dogs, a high protein diet with the addition of high fiber resulted in greater weight loss than a high protein, mid-level fiber diet [116]. The addition of lupin kernel flour, which is naturally high in protein and fiber, to bread products resulted in higher satiety scores and reduced energy intake acutely [117] but did not find changes to body weight over time. The concept of combining the two most satiating macronutrients would suggest an additive or even synergistic effect as each macronutrient and food form exerts satiety effects by independent mechanisms. While a limited number of studies have tested this combination out, none have tested this within a whole food diet.

Figure 1-1. Satiety Cascade



The diagram illustrates how psychological and physiological stimuli arising from the consumption of a food modulate the effects of that food on appetite sensations and the pattern of eating. Blundell 2010. [118]

Chapter Two

THE EFFECTS OF A BEEF-BASED MEAL COMPARED TO A CALORIE MATCHED BEAN-BASED MEAL ON APPETITE AND FOOD INTAKE

Summary

Protein and fiber have strong satiety-inducing potential. Beef is a high quality, protein-rich food. Beans contain moderate levels of protein as well as fiber. The objective of this study was to determine the effects of a high protein meal (beef) compared to a moderate protein, high fiber meal (beans) on subjective appetite and energy intake at a subsequent meal. Twenty-eight adults, 14 men (ages 24±5y, BMI 23±2 kg/m^2) and 14 women (ages 25±5y, BMI 22±2 kg/m^2) consumed two test lunches including a "meatloaf" made from either beef or beans. The beef meal provided 26 grams of protein and 3 grams of fiber while the bean meal provided 17 grams of protein and 12 grams of fiber. An ad libitum snack was given 3 hours after the test meal. Visual analogue scales were used to assess hunger, satiety, fullness, and prospective food intake. Gastrointestinal (GI) tolerance was assessed over 24 hours. No difference between the beef and bean was observed for appetite ratings over 3 hours, food intake at the subsequent meal (632±75 kcal vs. 611±75 kcal, respectively), or sum of GI score (2.2±0.5 vs. 2.9±0.5, respectively). Gas and bloating were reported more often after the bean meal than the beef meal (2.0±0.4 vs. 1.3±0.4, p-value 0.057). A beef-based meal with high protein and a bean-based meal with moderate protein and high fiber produced similar satiety, while the bean-based meal resulting in higher, yet moderate, gas and bloating.

Introduction

Protein is considered the most satiating macronutrient [119] with fiber exhibiting effective satiety induction as well [120, 121]. The combination of protein and fiber could provide a dual mechanistic action that may put forth greater satiety impact than either on their own. The satiety impact of combining protein and fiber has not been investigated fully in humans within a meal. Consuming a high satiating, whole food meal containing immediate satiation and sustained satiety mechanisms could potentially result in reduced caloric intake at the next meals, which if routine, could result in improved weight management [122]. Beef is a typically consumed food item that contains a high amount of high quality protein. In recent years, the introduction of meat substitutes and alternatives has provided additional options for meal choices for more than just those seeking vegetarian options. These products typically consist of plant based ingredients such as protein isolates, whole legumes, whole grains and vegetables providing a good source of protein with the addition of intrinsic fiber. Beef does not typically induce any adverse gastrointestinal symptoms when consumed in customarily consumed quantities but beans have been linked to increases in bloating and flatulence due to the content of soluble fiber and oligosaccharides providing fermentable substrate resulting in gas production [75]. This study investigated whether a meal containing beef, a high quality and quantity protein food, has more satiety-inducing potential than a bean based meal containing a moderate protein level with high fiber content. Our hypothesis was that the bean based meal would exert similar satiety potential compared to the beef based meal based on the action of differing satiety mechanisms from the difference in nutrient composition, but in addition, the bean meal may exert greater gastrointestinal symptoms than the beef meal.

Methods and Materials

Experimental Design

This study was a randomized, repeated measures design with individual subjects serving as their own control. A total of 28 subjects were enrolled in this study and consisted of 2 test visits for each subject: beef (protein) and bean (protein + fiber) meals totaling 56 test visits. Subjects were randomized to receive one of two treatments following a standardized breakfast. Visits were separated with a 1 week wash-out period. The use of 100 mm visual analog scale to assess hunger, fullness, desire to eat and prospective food intake provided individual physiological impact and emotional state of hunger/satiety following treatment.

Subjects

Subjects were recruited via flyers placed around the University of Minnesota campuses. Interested individuals were screened via a phone interview. Screening included health status and history, dietary intake and eating habits questionnaires. Subjects were eligible to participate if inclusion and exclusion criteria were met. This research was reviewed and approved by the University of Minnesota Institutional Review Board Human Subjects Committee.

Prior to any procedures the study coordinator obtained signatures on informed consent (Appendix A). Following acceptance into the study, each subject received instructions for the day before study visits. In the 24 hours prior to each visit, subjects followed a low-fiber, lead-in diet, which prohibited the use of fiber supplements and alcohol. The same diet was consumed prior to the second treatment of the study for that subject. Subjects collected 24-hour food records from lunch the day before the study until they arrived for lunch at the testing center. Women were only scheduled during the follicular phase of their menstrual cycle.

Inclusion Criteria

Subjects were screened and enrolled in the study if inclusion criteria were met. We included subjects that were male or female between 18 to 65 years of age. They were of healthy weight (BMI $\geq$18 and $\leq$27 kg/m^2); non-dieting (stable weight for last 3 months); non-smoking; not taking medications; non-vegetarian; and typically consume breakfast.

Exclusion criteria

Subjects will be excluded if they had: distaste for beans; current smoker; restrained eating habits (score greater than 11) (Appendix B); weight change of more than10 pounds in the past 3 months; any history of disease or significant past medical history including diabetes, cancer, kidney/liver disease, gluten intolerance, ulcerative colitis, diverticulitis, or Crohn's disease; take medications regularly; taken antibiotics in the past 3 months; are vegetarian; high fiber intake (more than 15g per day); do not normally eat breakfast or lunch; are pregnant or lactating; or if they have irregular menstrual cycles; participated in a dietary intervention study within the last month.

Test Meals

Subjects received 2 isocaloric meals with 400 mL of water over 2 visits. The 2 meals were formulated to be matched in weight, calories, total fat (Table 2-2). The beef meal contained 26 grams of protein and 3 grams of fiber and the bean meal contained 17 grams of protein and 12 grams of fiber. These meals were formulated and prepared using commercially available foods. They were prepared in a food grade test kitchen and packaged in a ready to eat meal tray, flash frozen using a commercial Servolift Eastern Corporation Irinox blast freezer. The meals were then transferred to a subzero freezer until the visit day. The meals were heated in the microwave prior to the visits.

Study Visits

Fasted subjects (approximately 4 hours since breakfast) arrived at the University of Minnesota St. Paul campus between 11:45 a.m. and 12:00 p.m. The initial visit and subsequent visit included baseline anthropometrics prior to test meal. The test meals were served for lunch. All visits were held in a quiet room, which allowed subjects to read, use laptops, work quietly, or listen to music. Visits were scheduled at least 1 week apart, ensuring alignment with the follicular phase of the female subjects.

Subjects were given instructions for completing the computerized visual analog scale (VAS) system and completed their baseline appetite assessment. The test meal was served for lunch with 400 ml water and subjects were instructed to consume the meal within 15 min. Appetite sensations were rated by VAS at 15, 30, 45, 60, 90, 120, and 180 min after baseline.

Subjects were then given a tray of snack foods of varying size and flavor (Table 2-3) and 1 liter of water. Subjects were told to eat until comfortably satisfied. After 30 min, the remaining snack foods and water were weighed, and energy intakes were calculated. Subjects were instructed to keep a detailed food record for the remainder of the day and breakfast the next morning.

Food records were analyzed with the Nutrition Data System for Research (NDSR, version 2012, Nutrition Coordinating Center, Minneapolis program) for determination of energy, carbohydrate, fat, protein, and fiber intake.

Study Outcomes

Subjective Satiety Scores (Visual Analog Scales)

Visual Analog Scales (VAS) were used to assess subjective satiety ratings consisting of a 100 mm validated scale [3] and served as the primary outcome of this study. The questions asked subjects to rate their feelings for four satiety related endpoints: Hunger (How hungry do you feel? 0 mm-I am not hungry at all, 100 mm- I have never been more hungry; Satisfaction (How satisfied do you feel? 0 mm- I am completely empty, 100 mm- I cannot eat another bite; Fullness (How full do you feel? 0

mm- I am not at all full, 100 mm- I am totally full; Prospective food intake (How much do you think you could eat? 0 mm- Nothing at all, 100 mm- a lot (Appendix C).

Food Intake

The food intake of the subsequent meal was measured via total kcals of an assortment of snacks consumed 3.5 hours following the lunch meal. The snack assortments consisted of typical snacks found in a vending machine ranging from sweet to savory/salty. All of the snacks provided on the tray contained a total of 940 kcals (Table 2-3). Subjects were told to consume as little or as much of as many of the snacks provided until they were comfortably full over a 30 minute period. The snacks were then weighed to determine how much of each was consumed to calculate total calories consumed.

The subjects were also instructed to record their food intake for the remainder of the day including as much detail about the food item as possible as well as the approximate serving of each food. A portion guide information sheet on serving sizes was provided (Appendix D). Dietary records were analyzed with the Nutrition Data System for Research (NDSR, version 2012, Nutrition Coordinating Center, Minneapolis program) for determination of energy, carbohydrate, fat, protein, and fiber intake.

Gastrointestinal Tolerance

The gastrointestinal tolerance of the meals was also assessed. Gastrointestinal tolerance was measured by subjective scales, previously used in our laboratory [123].

Specific questions included gas or bloating, nausea, flatulence, diarrhea/loose stools, constipation, gastrointestinal cramping and gastrointestinal rumbling to assess subjective gastrointestinal tolerance on a 4-point Likert scale. The scale used a 0-3 rating with 1- none, 2- mild, 3- moderate and 4- severe (Appendix E).

Palatability

Visual Analog scales were also used to assess the palatability of the meals. The questions asked subjects to rate their opinion of the visual appearance, smell, taste and overall pleasantness of the meals with 0 mm indicating good and 100 mm indicating bad, and aftertaste that was rated as 0 mm indicating much and 100 mm indicating none (Appendix F).

Statistics

The sample size for the study (n=28) was calculated based on expected changes in satiety seen in previous studies in our lab with fiber treatments. The subjects were divided into 14 women and 14 men blocks and the repeating sequences were divided evenly for each.

Subjects were randomized according to a Williams's design that balances treatments over visits and subjects (Appendix G). Other parameters are compared among treatments using a mixed effects linear model with a random subject effect (Proc Mixed). This procedure calculates treatment means, standard errors, and statistical differences among means. Carryover and interaction terms will be tested. Statistical significance is achieved at $p < 0.05$.

In analyzing the data, a strong interaction between treatments and treatment order was found, specifically among those assigned to eat the bean meal second. Due to this unexpected treatment order effect, we analyzed and present results based on the first visit only, n=14 for each group.

Sample Size and Power Calculations

Sample size was based on power calculations (80% power with $\alpha=0.05$) calculated from the differences in visual analog scale (VAS) scores. A change on the VAS of 10 mm is considered clinically significant. The sample size of 28 is based on literature for clinical research and considers both the average expected difference observed as well as the standard deviation for VAS ratings [3]. The chosen sample size is elevated to encompass the expected difference in subsequent food intake as well. Our primary outcome for this study remains satiety impact outcomes from the VAS data.

Results

Subject Demographics

Twenty-eight subjects (14 men and 14 women) completed both visits. Mean age ± SEM was 23.7 ± 5.3 and 24.9 ± 5.2 for males and females, respectively. Mean BMI ± SEM was 23.3 ± 1.8 and 21.7 ± 2.4 for males and females, respectively. The restrained eating scores for men were 6.2 ± 0.8 and for women were 9.6 ± 0.9 (Table 2-1). A statistically significant difference in hunger was found for the bean meal treatment but only for those in the beef-bean meal treatment order.

Subjective Satiety Scores

The area under the curve (AUC) for the satiety scores (fullness, hunger, satisfaction and prospective food consumption) for baseline through 180 minutes for the two treatments is shown in table 2-3.

There were no significant differences for AUC for any of the satiety measures. There was a general trend for each of the four means at each time point to be improved (lower for hunger and prospective food consumption and higher for satisfaction and fullness) with the bean meal compared to the beef mean but only significant at the 15 minute time point for hunger.

Food Intake

No difference in mean food intake for the remainder of the day, 632 ± 75 kcal for the beef meal and 611 ± 75 kcal (mean ± SEM) (Figure 2-6) or snack intake at subsequent meal, 612 ± 58 for the beef meal and 665 ± 49 for the bean meal (mean ± SEM) (Figure 2-5), was found.

Gastrointestinal Tolerance

There was no significant difference in the sum of gastrointestinal symptoms overall but reported occurrences was higher with the bean compared to the beef meal (2.9

± 0.5 compared to 2.2 ± 0.5, respectively). Gas and bloating were also reported more often with the bean than the beef meal (2.0 ± 0.4 compared to 1.3 ± 0.4, respectively) though not statistically significant (Figure 2-7).

Palatability

No difference in palatability was observed between the two meals. There were slight differences in the perception of the meals between males and females but no difference within gender (Figure 2-8). Overall, the two test meals were equally palatable.

Discussion

The current research on satiety and food intake has focused on the satiation and satiety potential of a meal with the thought that increased satiety will result in reduced food intake at subsequent meal(s) throughout the day, leading to a reduction in total energy consumption throughout the day. These reductions, over time, could equate to weight loss and/or management over time. The use of a set of validated question VAS is the best tool to capture these subjective feelings of satiety but the multitude of factors at play regarding food intake need to be considered and controlled for. These factors include the emotional, hedonic, reward and social aspects of eating, controlled in the cortex and limbic systems, which have the ability to override the hypothalamic nutrient-sensing control of food intake [124].

The findings of this study support the hypothesis that the satiety impact of a high quality protein beef meal compared to a lower protein bean meal with high fiber content are similar. No significant differences were found between the two test meals for any of the satiety AUC ratings. A significant difference was found at 15 minutes following the meal for both hunger and fullness ratings but was not significant for the additional time points. The question of whether a stronger feeling of fullness or reduced hunger at individual time points impacts overall satiety has yet to be determined. Also, the findings of no difference in subsequent meal energy intake or energy intake for the remainder of the day suggest no long-term satiety differences between the two meals.

Differences in protein and fiber content have shown varying results in overall satiety ratings but higher ratings of fullness have been associated with reduced energy intake for the remainder of the day [125, 126].

The presence of protein in foods has been linked to satiety effects as well as weight management [103, 107, 127]. Protein source has also been thought to impact satiety potential, suggesting that protein from animal sources are more satiating than plant-based proteins although studies have been inconclusive [73, 108, 128]. The individual amino acid content of proteins varies depending on source as does the label of complete or incomplete protein, referring to whether all essential amino acids are present. To date, studies have been inconclusive on the role of individual amino acids on satiety mechanisms in humans but some have shown variation for each amino acid in energy expenditure during amino acid catabolism [129]. Beef protein is a high quality protein containing all of the limiting amino acids needed in the human body although legumes has the highest content of lysine and threonine compared to other plant-sourced proteins [130], which have been linked to increased satiety ratings [116, 131].

The intake of fiber, intrinsic or supplemented, has been greatly associated with lower body weight in both animals and humans [50-52]. The mechanisms of action for fiber on satiety for the substitution of beans in this study include: displacement of higher energy dense foods, delayed gastric emptying due to the intact structure of the fiber-containing whole legumes, delayed nutrient uptake or absorption and fermentation [53, 54].

Other studies have shown that the strongest predictor for satiety potential is portion size (energy density) [125]. In this study, the portion size, as well as the meal presentation, was equal. The meals were matched for portion size and energy content. This could further explain why no differences were found between the two meals. The reduction in protein content for the bean meal could be supplemented with satiety action coming from the increased fiber content. Fiber has been shown to elicit satiety signals as well as result in decreased body weight [120, 132]. The substitution of the beef for beans led to a reduction in protein being substituted with fiber in the formulation. This substitution did lead to an increase in carbohydrate in the bean meal however the additional 12 grams of carbohydrate was coming from the intrinsic fiber in the beans.

There were no differences found in the palatability of the two test meals however we did observe a strong interaction for the treatment order for the baseline hunger scores. This interaction was isolated only to the beef-bean sequence order. This interaction exhibited hunger ratings at baseline to be inconsistent and correlated with treatment order. The hunger ratings were reduced as well as scattered at baseline for those subjects receiving the bean meal second. These baseline hunger ratings were not associated with the palatability of the meal as these VAS ratings were recorded prior to the meal being served.

During the screening and consent process, it was disclosed that the participants would be consuming products using commercially available ingredients consisting of bean and beef based meat-loaf style meals. In disclosing this information, it is possible that some individuals who had received the beef meat loaf meal for the first visit then anticipated the consumption of a less familiar, bean-based meat loaf meal and therefore rated their hunger as less or unsure of how to rate. This phenomenon could be explained by the term food neophobia [133]. Food neophobia can be described as the inclination to avoid new foods and has been thought to be a biological mechanism of protection against the ingestion of toxins via new and/or unfamiliar foods.

The outcomes of this study suggest no differences between the beef and bean-based meals, however the power of the study was reduced following the treatment order interaction resulting in a reduction in sample size of 50% as well as the inability to use the cross-over design to further reduce individual variability. Differences therefore may exist that we were unable to observe due to lack of power. In order to more fully investigate these potential differences, a study incorporating a cross-over design is ideal. Also, there is the need to consider the potential interaction of food neophobia. This could potentially be corrected using commonly consumed foods that would not lead to preconceived anxiety regarding new food consumption or by acclimating the subjects to the new foods to be consumed during the study prior to the actual start date.

The higher occurrence of gastrointestinal symptoms reported is consistent with typical legumes consumption. The soluble fiber, specifically α-galactosides, of legumes has been linked to increased gas and bloating but this gas production can be viewed as beneficial with the production of fermentation products as well as stimulation of gut hormone release [75, 139].

Conclusion

In conclusion, this study suggests no difference in satiety ratings or subsequent food intake following two test meals of varying protein and fiber content. We did find that foods in food forms uncommon with usual consumption may lead to undesirable interactions resulting in the lack of power to detect actual differences. Additionally, the consumption of a bean-based meal results in a higher incident of gastrointestinal symptoms than a beef-based meal. Further studies are needed to confirm if true differences do exist between these two meal types, specifically with greater sample sizes that correct for the potential of treatment order interactions.

Acknowledgements

The Minnesota Beef Council provided the grant funding to support this research. We thank Karin Schaefer for her support throughout the research process. We would also like to thank the research assistants and the participants for completing the study visits.

Table 2-1: Subject Demographic Characteristics*

	Men (n=14)	**Women (n=14)**
Age (yrs)	23.7 ± 5.3	24.9 ± 5.2
BMI (kg/m^2)	23.3 ± 1.8	21.7 ± 2.4
Weight (kg)	76.2 ± 7.9	60.0 ± 9.8
Restrained Eating Score	6.2 ± 0.8	9.6 ± 0.9

*Baseline values presented as mean ± SE

Table 2-2: Meal nutrition facts

	Beef Meal	**Bean Meal**
Weight (g)	233	233
Kcals	440	440
Carbohydrate (g)	56	65
Fiber (g)	3	12
Protein (g)	26	17
Fat (g)	13	13

Table 2-3: Snack nutrition facts

Snack	Serving Size	Calories	Protein	Fat	Carbs
Oreo Cookies	57 g	270	2 g	11 g	41 g
Beef Stick	26 g	110	6 g	9 g	2 g
Sun Chips	42.5 g	210	3 g	10 g	29 g
Crunchy Granola Bar	42 g	190	4 g	6 g	29 g
Trail Mix	30 g	160	5 g	10 g	12 g
Total Offered	197.5 g	940	20 g	46 g	113 g

Table 2-4: Summary of Results

	Beef Meal	**Bean Meal**	**p-value***
Fullness[1]	141 ± 15	170 ± 15	0.21
Hunger[1]	121 ± 11	104 ± 11	0.30
Satisfaction[1]	150 ± 13	168 ± 13	0.35
Prospective Consumption[1]	147 ± 14	127 ± 14	0.32
Snack Intake (kcal)[2]	612 ± 58	665 ± 49	0.33
Dinner food intake (kcal)[2]	632 ± 75	611 ± 75	0.84
Visual Appeal[3]	0.43 ± 0.06	0.51 ± 0.06	0.38
Smell[3]	0.32 ± 0.04	0.35 ± 0.04	0.63
Taste[3]	0.28 ± 0.05	0.39 ± 0.05	0.15
Aftertaste[3]	0.70 ± 0.07	0.63 ± 0.07	0.49
Pleasantness[3]	0.28 ± 0.05	0.41 ± 0.05	0.09
GI Tolerance-total sum[4]	2.2 ± 0.5	2.9 ± 0.5	0.17
GI Tolerance- gas sum[4]	1.3 ± 0.4	2.0 ± 0.4	0.06
GI Tolerance- diarrhea sum[4]	0.3 ± 0.2	0.4 ± 0.2	0.27
[1]Mean AUC ± SEM, [2]Mean Total Calories ± SEM			
[3]Mean ± SEM, scale of 0-1, [4]Mean sum of scores ± SEM			
*p-value indicated for t-test			

Figure 2-1: Hunger ratings over time (n=14 for each treatment). *p<0.05

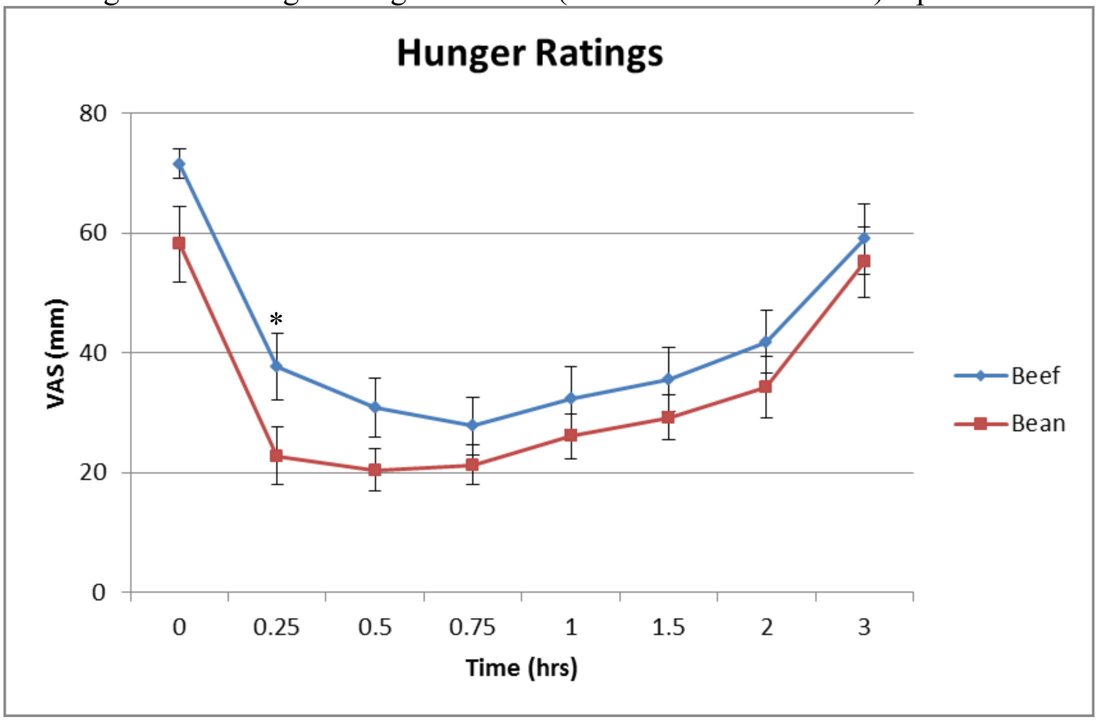


Figure 2-2: Fullness rating over time (n=14 for each treatment). *p<0.05

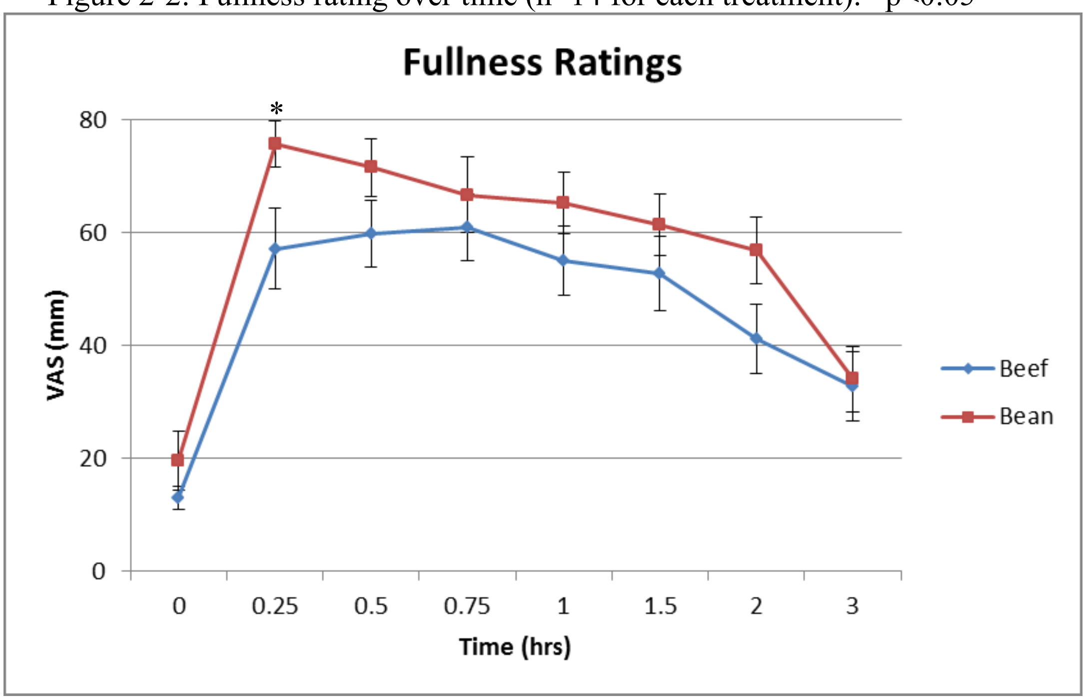


Figure 2-3: Satisfaction rating over time (n=14 for each treatment)

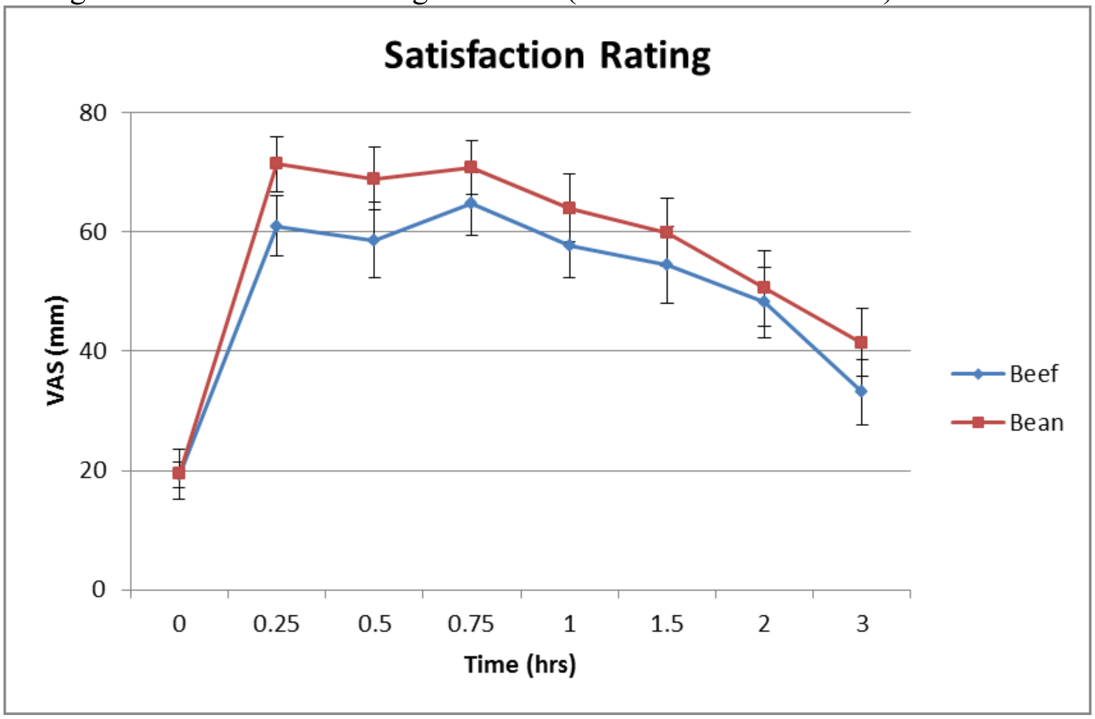


Figure 2-4: Prospective Food Intake over time (n=14 for each treatment)

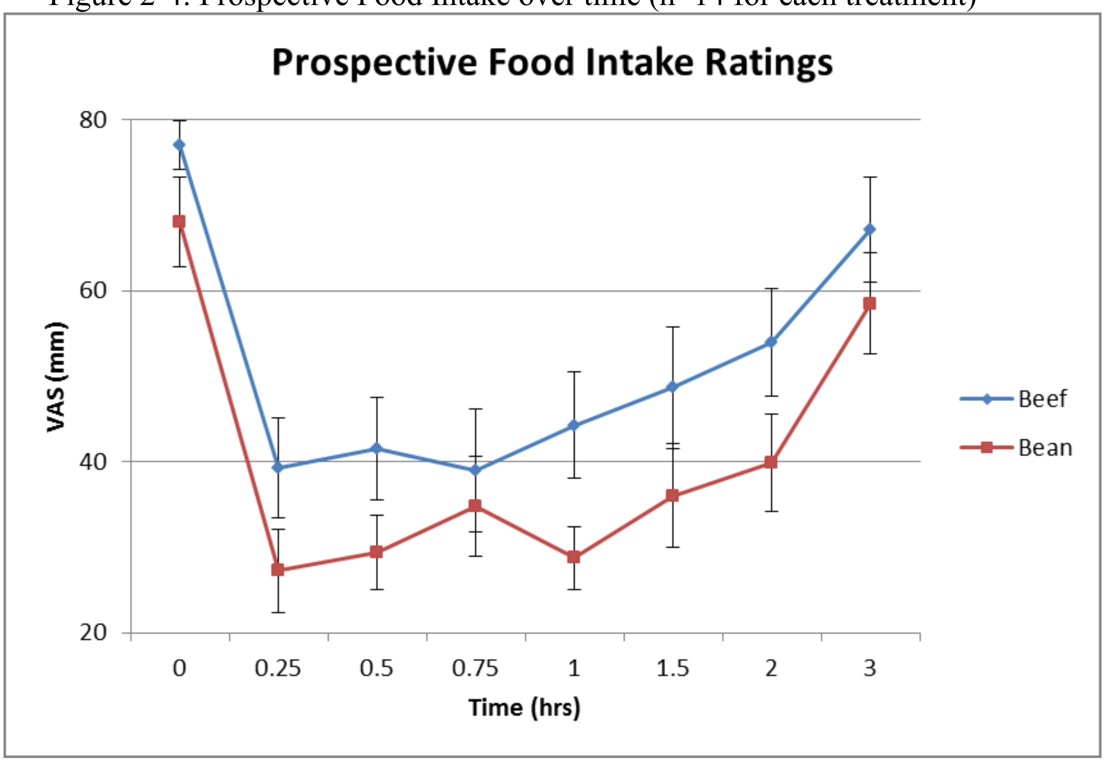
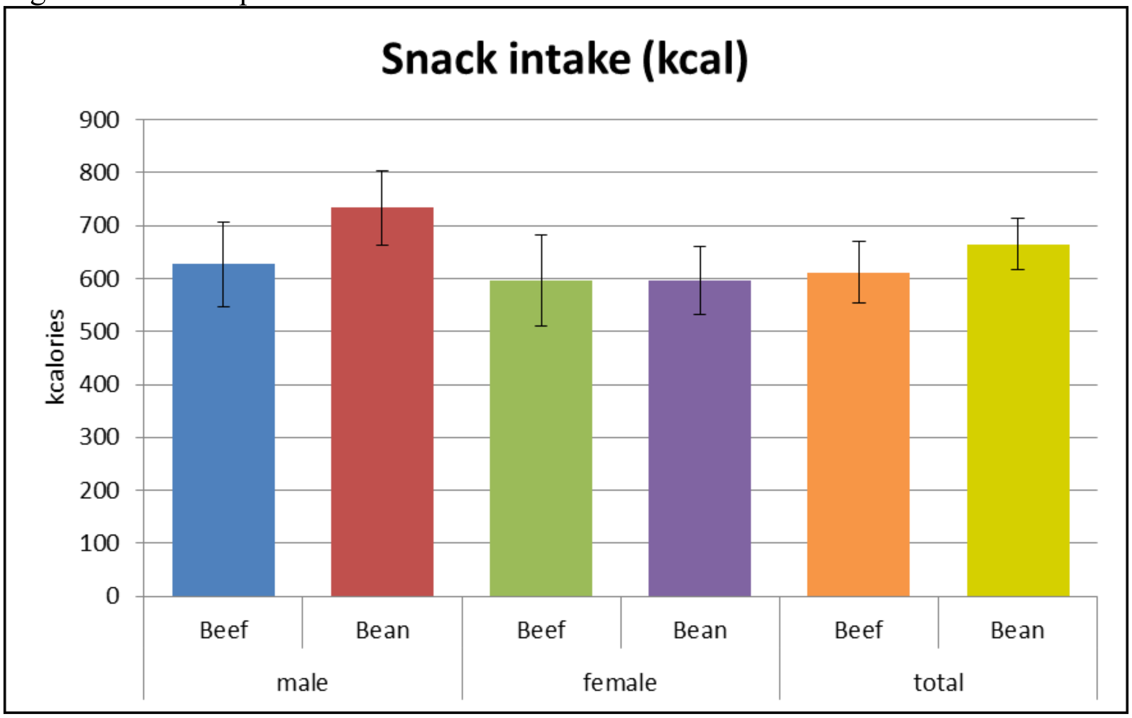
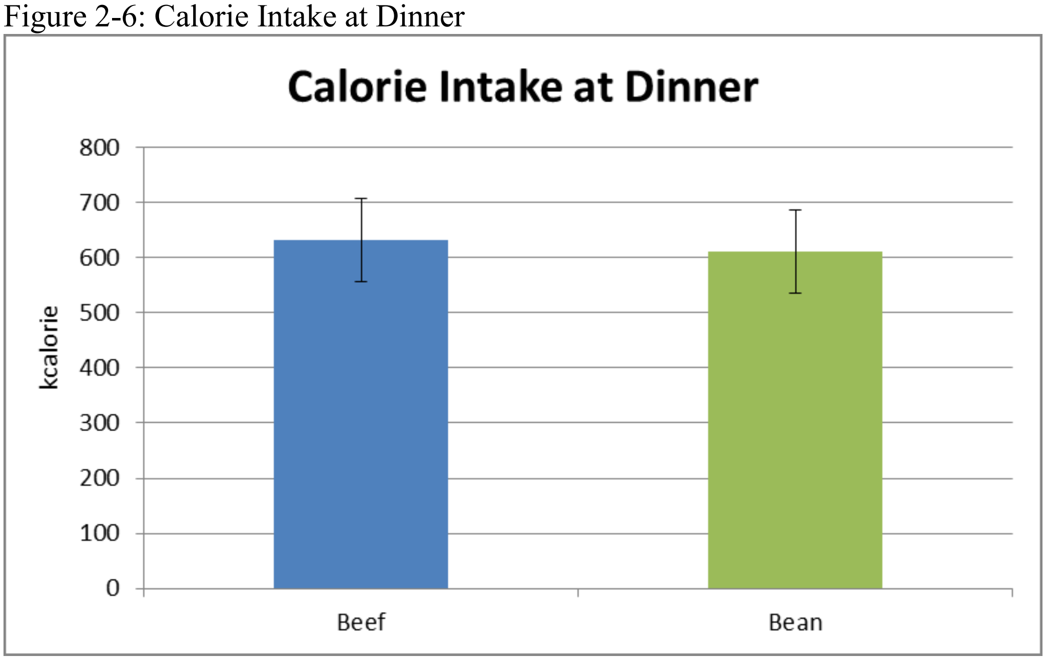


Figure 2-5: Subsequent Meal Intake as Snack



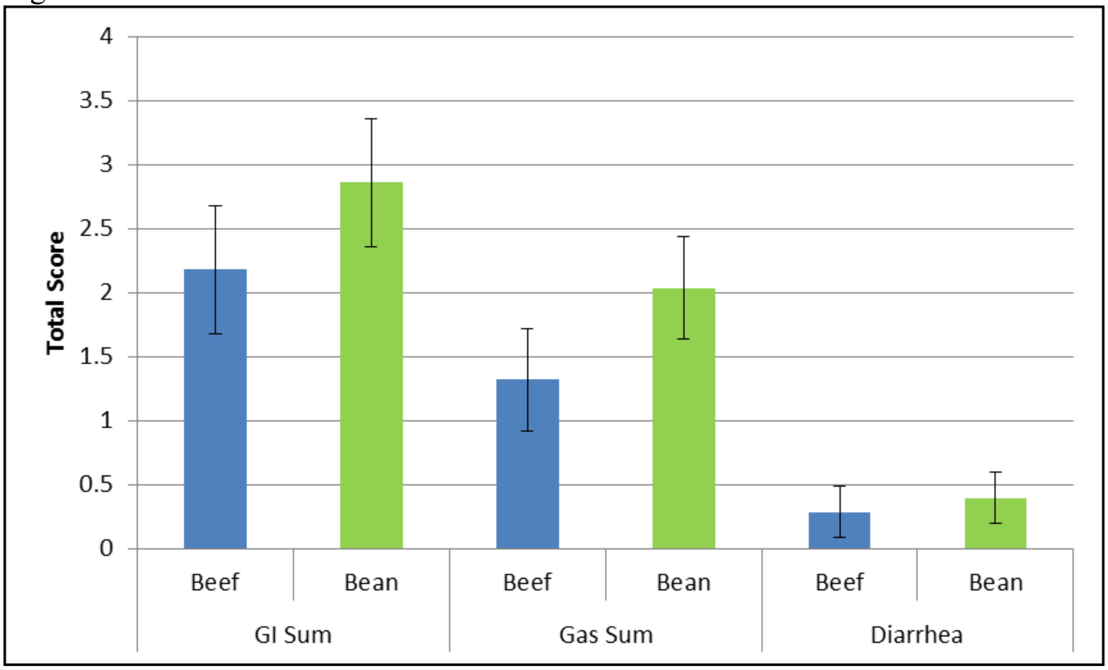
No significant differences noted within groups or treatments. p-values are as follows: within males 0.28, within females 0.98, total 0.33.

Figure 2-6: Calorie Intake at Dinner



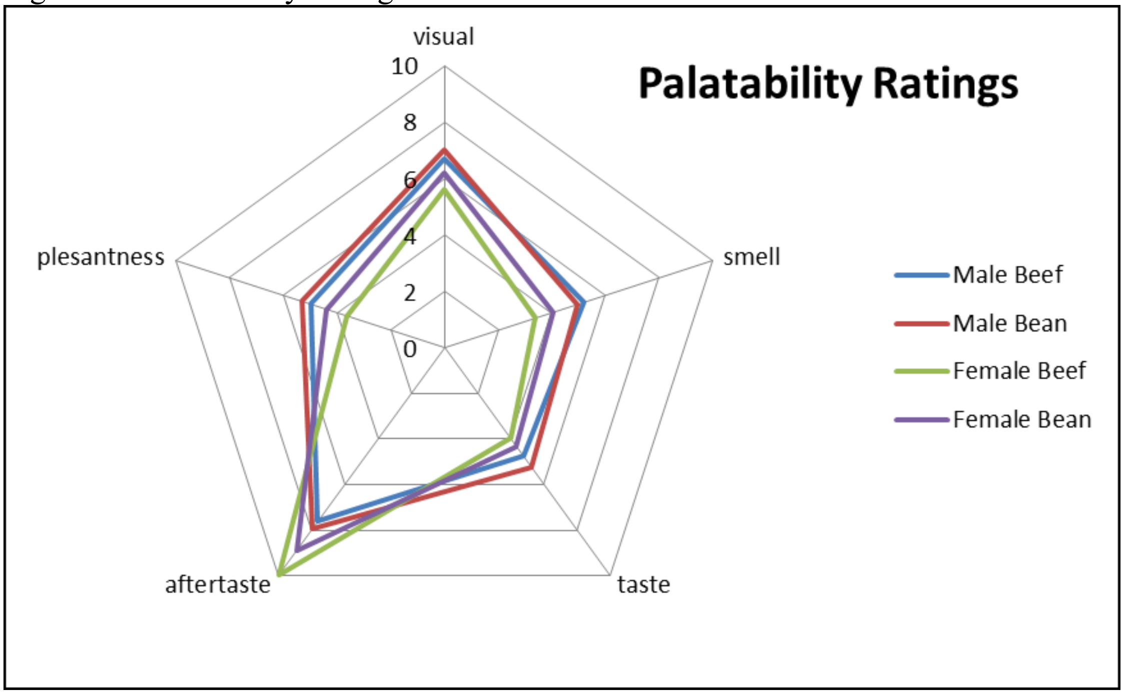
No significant differences noted. p-value 0.33.

Figure 2-7: Gastrointestinal Tolerance Scores



No significant differences noted. p-values as follows: GI Sum 0.17, Gas Sum 0.06, Diarrhea
sum 0.27.

Figure 2-8: Palatability Ratings



Chapter Three

THE EFFECTS OF THE COMBINATION OF EGG AND WHOLE GRAINS ON APPETITE, BLOOD GLUCOSE RESPONSE AND FOOD INTAKE

Summary

The obesity epidemic continues worldwide and is considered a major health concern. A shift back to consumption of whole foods may be an important part of the solution in the current rising obesity trend. Both protein and fiber have satiating properties, but few studies have examined whether their impact on different biological mechanisms work additively to more strongly enhance the overall satiety potential of a meal. A breakfast containing eggs and whole grain products combined, imparting high quality protein and fiber, may lead to higher overall satiety and result in decreased food intake at subsequent meals.

The purpose of this study was to examine the effect of egg alone and in combination with whole grains compared to a refined ready-to-eat cereal on satiety and food intake in human subjects. We designed whole food diets, controlled for macronutrients, to use in this randomized, controlled, crossover study. Forty-eight adults, 24 men (ages 24 ± 1y, BMI 23 ± 0.5 kg/m^2) and 24 women (ages 23 ± 1y, BMI 22 ± 0.5 kg/m^2) consumed three test meals for breakfast including: eggs with white toast; eggs with whole grain toast; and rice cereal with white toast. All of the breakfast meals contained 390 kcals. The eggs and white toast breakfast provided 30 grams of protein and 1 grams of fiber, the eggs and whole grain toast provided 20 grams of protein and 7 grams of fiber, and the cereal breakfast provided 10 grams of protein and 1 gram of fiber. An ad libitum lunch was served 3.5 hours after the breakfast test meal. Visual analogue scales were used to assess hunger, satiety, fullness, and prospective food intake. Blood

was drawn to measure glucose response. Gastrointestinal (GI) tolerance was assessed over 24 hours.

No difference was observed between the cereal and egg + whole grain toast breakfasts for AUC for all satiety ratings however the egg + white bread breakfast was significantly improved for all 4 satiety ratings. Lunch intake was significantly reduced in both egg breakfasts compared to the cereal breakfast (932 ± 45 kcals for the egg + whole grain toast breakfast, 866 ± 45 kcals for the egg + white toast breakfast compared to 1001 ± 45 kcals for the cereal meal, $p<0.05$). No difference was observed for the sum GI score between the egg + white toast, egg + whole grain toast and cereal breakfasts (2.8 ± 0.7, 3.0 ± 0.7 and 3.8 ± 0.7, respectively), however gas and bloating was significantly higher for the egg + white toast breakfast compared to the cereal meal (2.0 ± 0.4 vs. 1.2 ± 0.4, p-value 0.03). An egg based breakfast with high protein produced the highest satiety ratings when compared to a moderate protein and fiber egg based meal and rice cereal meal. However, food intake at the subsequent meal was reduced for both egg breakfasts compared to the cereal breakfast. Egg based breakfast meals with high protein and low/moderate fiber produced greater satiety and reduced food intake compared to a cereal-based breakfast with low protein and fiber.

Introduction

The need for obesity/weight management is great and foods that are commonplace in the diet that have a positive impact are the focus of many food companies. The increase in obesity has been linked to dietary changes favoring refined, processed foods and decreased consumption of whole food products [134]. Industry strategies toward reducing obesity rates have included functional foods and pharmaceuticals. In contrast, a shift back to the consumption of whole foods may be an important part of the solution.

Satiety is the postprandial state responsible for the timing and intake of the next meal. The concept of satiety is inherently acute as meal effect typically lasts until the subsequent meal. There is potential for longer lasting satiety effects due to braking mechanisms along the gastrointestinal tract which slow transit time and colonic fermentation. Overtime, the satiety effect of a meal could result in decreased daily caloric intake resulting in weight management and loss.

Protein is thought to be the most satiating macronutrient [122]. In addition, eggs have been shown to enhance satiety and reduce caloric intake at subsequent meals [135, 136]. Protein degradation rates vary by source resulting in variation in amino acid profusion along the gastrointestinal tract. Chemoreceptors in the small intestine are able to detect intestinal amino acids and cause enteroendocrine cells to release gut hormones linked to satiety [137]. The presence of amino acids along the gastrointestinal tract as well as in the plasma is able to initiate a cascade of effects triggering gut hormone release and the ileal brake mechanism slowing gastric emptying and digestion [137]. This results in increased feelings of satiety and potentially decreased food intake at the next meal.

Fiber has been shown to enhance satiety and feelings of fullness published from our lab [120, 121]. Whole grain wheat has resulted in decreased body fat compared to refined wheat [132]. Fiber can enhance satiety via multiple mechanisms including increased viscosity and bulking resulting in decreased transit time and fermentation products resulting in hormonal feedback signaling satiety in the brain. An inverse relationship has been found for body weight and intake of whole grain, high fiber foods and, conversely, a positive association for body weight and refined grain intake [138]. As most people do not meet the recommended daily intake for dietary fiber, increasing fiber intake can play a role in the increasing obesity trends of the population.

High fiber foods can cause gastrointestinal discomfort. Inulin is a readily fermented fiber and at doses of 10 grams per day can cause significant differences in gastrointestinal tolerance [123]. Other research from our laboratory has found that breath hydrogen, a measure of gut fermentation, is linked to higher intakes of dietary fiber [139]. It is generally accepted that higher protein foods are well digested, but information on tolerance of satiating foods is needed prior to incorporation of these ingredients into foods giving typically consumed, whole foods an advantage.

The combination of protein and fiber could provide a dual mechanistic action that may put forth greater satiety impact than either on their own. The satiety impact of combining protein and fiber has not been investigated fully in humans within a meal. High protein, high fiber snack bars eaten twice a day between meals resulted in reduced subsequent meal intake [115]. In obese dogs, a high protein diet with the addition of high fiber resulted in greater weight loss than a high protein, mid-level fiber diet [116]. The addition of lupin kernel flour, which is naturally high in protein and fiber, to bread products resulted in higher satiety scores and reduced energy intake acutely [117] but did not find changes to body weight over time. The concept of combining the two most satiating macronutrients would suggest an additive or even synergistic effect as each macronutrient and food form exerts satiety effects by independent mechanisms. While a limited number of studies have tested this combination out, none have tested this within a whole food diet.

Consuming a high satiating, whole food breakfast containing immediate and sustained satiety mechanisms could potentially result in reduced caloric intake at the next meals, which if routine could result in improved weight management.

This study intended to show that egg is an important whole food useful for weight management. The focus of this study was that the combination of egg protein and fiber derived from intact, whole foods for breakfast will provide a more satiating effect sustained throughout the day when compared to refined, isolated food products. We hypothesized that the high-protein egg breakfast will be the most satiating. The ranking of satiety scores expected is: egg, low fiber $\geq$ egg, high fiber > no egg, low fiber. The results for plasma glucose should also correlate with available carbohydrate for each meal as well as variations seen in satiety scores between test meals.

The emphasis will be: whole eggs, imparting complete protein, fat, vitamins and minerals, in combination with naturally occurring fiber, provides superior satisfaction and may result in increased feeling of satiety and decreased energy consumption at subsequent meals.

Methods and Materials

Experimental Design

This study was a randomized, repeated measures design with individual subjects serving as their own control. A total of 48 subjects were enrolled in this study and consisted of 3 test visits for each subject: egg + white toast, egg + whole grain toast, and cereal + white toast breakfast meals totaling 144 test visits. Subjects were randomized to receive one of three treatments following an overnight fast. Visits were separated with a 1 week wash-out period. The use of 100 mm visual analog scale to assess hunger, fullness, desire to eat and prospective food intake provided individual physiological impact and emotional state of hunger/satiety following treatment.

Subjects

Subjects were recruited via flyers placed around the University of Minnesota campuses. Interested individuals were screened via a phone interview. Screening included health status and history, dietary intake and eating habits questionnaires. Subjects were eligible to participate if inclusion and exclusion criteria were met. This research was reviewed and approved by the University of Minnesota Institutional Review Board Human Subjects Committee.

Prior to any procedures the study coordinator obtained signatures on informed consent (Appendix H). Following acceptance into the study, each subject received instructions for the day before study visits. In the 24 hours prior to each visit, subjects followed a low-fiber, lead-in diet, which prohibited the use of fiber supplements and alcohol. The same diet was consumed prior to the subsequent treatments of the study for that subject. Subjects collected 24-hour food records following lunch the day of the study after leaving the testing center. Women were only scheduled during the follicular phase of their menstrual cycle.

Inclusion Criteria

Subjects were screened and enrolled in the study if inclusion criteria were met. We included subjects that were male or female between 18 to 65 years of age. They were of healthy weight (BMI $\geq$18 and $\leq$27 kg/m^2); non-dieting (stable weight for last 3 months); non-smoking; not taking medications; non-vegetarian; and typically consume breakfast; able to give blood via a finger stick.

Exclusion criteria

Subjects will be excluded if they had: distaste for eggs; current smoker; restrained eating habits (score greater than 11) (Appendix B); weight change of more than10 pounds in the past 3 months; any history of disease or significant past medical history including diabetes, cancer, kidney/liver disease, gluten intolerance, ulcerative colitis, diverticulitis, or Crohn’s disease; take medications regularly; taken antibiotics in the past 3 months; are vegetarian; high fiber intake (more than 15g per day); do not normally eat breakfast or lunch; are pregnant or lactating; or if they have irregular menstrual cycles; participated in a dietary intervention study within the last month.

Test Meal Composition

Subjects received 3 isocaloric meals over 3 visits. The 3 meals were formulated to be matched in calories and total fat (Table 3-2). The cereal meal consisted of rice crisp cereal, white toast, whole milk, and margarine, weighed 260 grams, and contained 10 grams of protein and 1 gram of fiber. The egg + whole grain toast meal consisted of 1.5 egg patties, whole grain toast, skim milk, and margarine, weighed 306 grams and contained 20 grams of protein and 7 grams of fiber. The egg + white toast meal consisted of 4 egg patties, white toast, and skim milk, weighed 370 grams and contained 30 grams of protein and 1 gram of fiber (Table 3-3). These meals were formulated and prepared using commercially available foods. They were prepared in a food grade test kitchen and packaged in ready to use single serve packages. The eggs were heated in the microwave and the toast in a toaster oven at each of the visits.

Study Visits

Fasted subjects consumed 390 kcal breakfast meals. Visual analogue scales (VAS) were used to assess hunger, satiety, fullness, and prospective food intake at baseline, 15, 30, 45, 60, 90, 120, 180 and 210 minutes after breakfast. Blood samples will be collected to determine plasma glucose levels at baseline, 30, 60, 120 and 180 minutes. An ad libitum lunch was provided at the test site 3.5 hours after the breakfast meal, measured by weight (grams) and calorie intake. Gastrointestinal tolerance will be assessed at 180 minutes after breakfast and over the next 24 hours after the visit using questionnaires. 24 hour food intake will be recorded by food diary. This study was designed to provide data on the importance of whole foods on satiety. Additionally, the design will help determine if high quality egg protein alone or in combination with fiber is more effective in enhancing satiety compared to a standard American-style breakfast cereal. Increased satiety at meals could, over time, result in decreased body weight and could be important tool is the obesity fight for children and adults.

The 3 breakfast treatments are: refined grain cereal/no egg, refined grain toast + eggs, whole grain toast + egg. Macronutrient levels are described in table 3-2 and the composition of the meals outlined in table 3-3.

Prior to any study procedures, the study coordinator obtained signatures on informed consent. Following acceptance into the study, each subject received instructions for the day before study visits. In the 24 hours prior to each visit, subjects were instructed to follow a low-fiber, lead-in diet, which prohibited the use of fiber supplements and alcohol. Subjects will be required to maintain their body weight and activity level throughout the study period; specifically, to avoid excessive exercise 24 h before each visit.

Fasted subjects arrived at the lab site on the University of Minnesota campus between 7:45 and 8:15 am on the scheduled day. The initial visit and all subsequent visits included baseline anthropometrics prior to test meal. All visits were held in a quiet room, which allowed subjects to read, use laptops, work quietly, or listen to music. Visits were scheduled at least 1 week apart.

Upon arrival at the lab site, anthropometric baseline measures were assessed. Subjects were given instructions for completing the computerized VAS and completed their baseline appetite assessment. Immediately following, fasting blood samples will be drawn to evaluate baseline plasma glucose levels. Subjects then consumed one of the 3 treatment breakfasts. The test meal was consumed within 15 min. Appetite sensations were rated by VAS at 15, 30, 45, 60, 90, 120, 180 and 210 min after baseline. Blood samples will be drawn baseline, 30, 60, 120, 180 minutes. Subjects were then given an ad libitum pizza lunch and 400 mL of water. Subjects were told to eat until comfortably satisfied. After 30 min, the remaining lunch was weighed, and energy intakes were calculated. Subjects were instructed to keep a detailed food record for the remainder of the day.

Blood samples were drawn via a finger stick. Plasma glucose was measured using a One Touch Ultra 2 handheld blood glucose meter.

Gastrointestinal tolerance was measured by subjective 4-point Likert scales. Typical questions include bloating, flatulence, stomach noises, and other subjective measures of gastrointestinal tolerance.

Food records were analyzed with the Nutrition Data System for Research (NDSR, version 2012, Nutrition Coordinating Center, Minneapolis) program for determination of energy, carbohydrate, fat, protein, and fiber intake.

Study Outcomes

Subjective Satiety Scores (Visual Analog Scales)

Visual Analog Scales (VAS) were used to assess subjective satiety ratings consisting of a 100 mm validated scale [3] and served as the primary outcome of this study. The questions asked subjects to rate their feelings for four satiety related endpoints: Hunger (How hungry do you feel? 0 mm-I am not hungry at all, 100 mm- I have never been more hungry; Satisfaction (How satisfied do you feel? 0 mm- I am completely empty, 100 mm- I cannot eat another bite; Fullness (How full do you feel? 0 mm- I am not at all full, 100 mm- I am totally full; Prospective food intake (How much do you think you could eat? 0 mm- Nothing at all, 100 mm- a lot (Appendix C).

Glucose Response

Blood samples were drawn via a finger stick using the One Touch lancet. Plasma glucose was measured using a One Touch Ultra 2 handheld blood glucose meter. Subjects were instructed to administer a finger stick using the provided lancet. A drop of blood was then collected on the test strip inserted into the glucose monitor. The blood glucose value was then recorded on the computer program following the satiety VAS questions. The finger stick was not administered until all of the subjective satiety and palatability questions were complete to prevent variation in subjective responses due to anxiety related to the finger stick. Plasma glucose was recorded at baseline, 30, 60, 120, 180 minutes and the AUC was determined for each breakfast meal.

Food Intake

The food intake for the subsequent meal was measured via total kcals and weight (grams) of an ad libitum pizza lunch meal consumed 3.5 hours following the breakfast meal. The pizza lunch meal contained a total of 1860 kcals (Figure 3-1). Subjects were told to consume as little or as much of the pizza provided until they were comfortably full over a 30 minute period. The leftover pizza was then weighed to determine how much was consumed to calculate total calories consumed.

The subjects were also instructed to record their food intake for the remainder of the day including as much detail about the food item as possible as well as the approximate serving of each food. A portion guide information sheet on serving sizes was provided (Appendix D). Dietary records were analyzed with the Nutrition Data System for Research (NDSR, version 2012, Nutrition Coordinating Center, Minneapolis program) for determination of energy, carbohydrate, fat, protein, and fiber intake.

Gastrointestinal Tolerance

The gastrointestinal tolerance of the meals was also assessed. Gastrointestinal tolerance was measured by subjective scales, previously used in our laboratory [123]. Specific questions included gas or bloating, nausea, flatulence, diarrhea/loose stools, constipation, gastrointestinal cramping and gastrointestinal rumbling to assess subjective gastrointestinal tolerance on a 4-point Likert scale. The scale used a 0-3 rating with 1- none, 2- mild, 3- moderate and 4- severe (Appendix E).

Palatability

Visual Analog scales were also used to assess the palatability of the meals. The questions asked subjects to rate their opinion of the visual appearance, smell, taste and overall pleasantness of the meals with 0 mm indicating good and 100 mm indicating bad, and aftertaste that was rated as 0 mm indicating much and 100 mm indicating none (Appendix F).

Statistics

The study was a 3-treatment, 3-period crossover, with subjects randomized in equal numbers to each of the 6 sequences of treatments; randomization will be stratified by sex.

Holt et al [125] compared VAS fullness after breakfasts of low protein (croissant, 8 g) and high protein (egg + bacon, 22 g), and the effect size (difference/standard deviation) for fullness AUC was 0.4. In this study, the high protein breakfast has 30 grams of protein, and the low-protein breakfast has 10 g, so the difference is 20 grams compared to 14 grams in Holt [125]. For this study, 48 participants will give 80% power to detect an effect size of at least 0.41 in fullness AUC.

Repeated measurements were summarized by area under the curve (AUC), computed by the trapezoid rule. Breakfast treatments were compared using a mixed effects linear model (Proc Mixed) with fixed effects for sex, period, and treatment, with a random intercept for participant to model the correlation between repeated measurements from the same subject. Carryover and treatment-period interaction terms were tested.

All treatments were compared with each other. Statistical significance is achieved at $p < 0.05$.

Subjects were randomized according to a Williams's design that balances treatments over visits and subjects. Other parameters are compared among treatments using a mixed effects linear model with a random subject effect (Proc Mixed). This procedure calculated treatment means, standard errors, and statistical differences among means.

Results

Subject Demographics

Forty-eight subjects (24 men and 24 women) completed all 3 visits. Mean age ± SEM was 24 ± 1 and 23 ± 1 for males and females, respectively. Mean BMI ± SEM was 23 ± 0.5 and 22 ± 0.5 for males and females, respectively. The restrained eating scores for men were 6 ± 0.5 and for women were 6 ± 0.5 (Table 3-1). No differences were noted for gender, age, BMI, restrained eating scores between treatment groups. No differences in baseline satiety ratings or fasting glucose were observed.

Subjective Satiety Scores

No difference in baseline satiety ratings for hunger, satisfaction, fullness or prospective food consumption was observed for the egg breakfasts compared to the cereal breakfast. A significant difference was observed at baseline for satisfaction between the 2 egg breakfast meals but was slight with a 4 point difference in VAS score (18.3 ± 2 vs.

22.5 ± 2 for the egg + whole grain toast and egg + white toast, respectively). No difference was observed between the cereal and egg + whole grain toast breakfasts for AUC for all satiety ratings. The egg + white toast breakfast, however, was significantly improved for all 4 AUC for the satiety ratings compared to both the cereal and egg + whole grain toast breakfasts (figures 3-2 hunger, 3-3 satisfaction, 3-4 fullness, and 3-5 prospective food intake). The peak mean score for both fullness and satisfaction was also significantly increased for the egg + white toast breakfast compared to both the cereal and egg + whole grain toast breakfasts. These data are shown in Table 3-4.

Food Intake

The food intake at the subsequent meal following breakfast was significantly reduced for the two egg breakfasts compared to the cereal breakfast, with the lowest intake for the egg + white toast breakfast (Figure 3-7). No differences were observed between the three breakfast meals for food intake for the rest of the day (Table 3-5, Figure 3-8 and 3-9)

Glucose Response

The AUC for glucose was lowest for the egg + white toast breakfast and highest for the cereal breakfast with both egg breakfasts significantly lower compared to the cereal breakfast. Mean glucose peaks were significantly higher for the cereal breakfast compared to the two egg breakfasts. These data are shown in Table 3-4.

Gastrointestinal Tolerance

No differences were observed for the total sum of GI symptoms or for diarrhea between the 3 treatments. The egg + white toast breakfast, however, was higher in gas and bloating compared to the cereal and egg + whole grain toast breakfasts. These data are shown in Table 3-4.

Palatability

The visual appeal, pleasantness and taste scores were different between all 3 treatments with the cereal breakfast having the best scores, the egg + whole grain toast intermediate scores and the egg + white toast breakfast having the least desirable scores. Aroma scores were no different between the cereal and egg + whole grain breakfasts but were significantly less desirable for the egg + white toast breakfast. No difference in aftertaste was observed between the 3 treatments.

Discussion

The purpose of this study was to investigate the satiety and blood glucose responses for 3 typical breakfast meals containing varying levels of protein, fiber and refined carbohydrates. The hypothesis of the study was that the two egg meals would perform similarly in terms of satiety ratings compared to the cereal breakfast and that the egg + whole grain toast breakfast would perform better than the egg + white toast breakfast in terms of glucose response. While some research has shown higher protein levels to be more satiating than lower protein levels [18], there is potentially a threshold in which higher amounts do not continue to elicit a stronger satiety response [140] and that the combination of protein at a moderate level and fiber could elicit similar or stronger effects than the high protein alone.

The results of this study showed that the high protein level contained in the egg + white toast breakfast was more effective at eliciting a satiety response than the moderate protein level with fiber in the egg + whole grain toast breakfast. The cereal breakfast and the egg + whole grain toast breakfast exhibited the least satiety response with no difference found between the two. This was an unexpected outcome as the moderate protein level combined with fiber was expected to produce greater satiety ratings than the refined cereal breakfast but also to be comparable to the high protein egg + white toast breakfast. The combination of protein and fiber has been shown to enhance satiety by initiating multiple satiety mechanisms along the satiety cascade. The consumption of a high protein, high fiber snack bar resulted in increased satiety compared to a high fat, high sugar snack bar containing less protein and fiber than present in the egg + whole grain toast breakfast (10 and 4 grams, respectively) [115].

The breakfast meals used in this study were formulated using whole foods. To achieve 3 isocaloric meals, the weight of the meals were not equal. The portion size of the meals was in the order egg + white toast > egg + whole grain toast > cereal with weights of 370 grams, 306 grams and 260 grams, respectively. This may have also played a role in terms of satiety impact as the egg + white toast meal showed the greatest satiety response and was also the highest in weight indicating that the greater feelings of fullness and less hunger may be due to the intake of greater portion size.

The decrease in food intake at the subsequent meal was significant for both the egg breakfasts. This supports our hypothesis that the higher protein containing meals would lead to a decrease in food intake following the meal. This could also be attributed to the portion size of the breakfasts as well. The cereal meal, while containing the lowest level of protein and fiber of the three treatments, it also contained the smallest portion size. Additional studies have shown that portion size is also a significant factor in addition to macronutrient composition and kcals when considering satiety ratings and subsequent food intake [126]. The difference in weight between the meals could have been compensated for at the next meal in terms of intake with no additional compensation for the remainder of the day as no significant differences in overall kcals consumed was observed. However, multiple studies have shown the impact of protein [141-145] and fiber [146, 147] for breakfast in terms of increased satiety throughout the day as well as the consideration of protein and fiber intrinsically contained within whole, intact foods having a greater impact suggesting food form should also be considered [146-148].

The role of a lower glucose response following a meal has been hypothesized to increase satiety [126, 149]. The presence of protein has been shown to reduce glucose response by slowing gastric emptying and nutrient absorption [150-152]. Fiber has also exhibited blunted glucose response. It is suggested that this is due to some viscous fibers ability to inhibit rapid glucose absorption [153], fiber replacement of rapidly absorbed glucose, as well as the fermentation impact of fiber that can potentially reduce glucose response at the subsequent meal [45]. Other studies have suggested that meals that produce a lower glycemic response can help in the control of food intake via reduced cravings and reward stimulation [154].

The results of this study highlight the high glycemic response for refined cereal breakfasts. The cereal breakfast produced the highest AUC and highest peak for glucose response. The two egg breakfasts produced similar responses despite the large difference in total carbohydrate although 7 grams of the egg + whole grain toast total carbohydrate is due to the fiber content. The three meals differed in carbohydrate content with the cereal containing the highest and the egg + white toast breakfast with the lowest. Overall, we observed a reduced glycemic response for the egg breakfasts but to accurately observe this effect, the glycemic response should be considered against the total available carbohydrate in each meal as the egg + white toast meal has less than half the amount of carbohydrate than the cereal meal.

The palatability of a meal typically enhances the subjective feelings of satisfaction and sense of reward post-prandial. Interestingly, the highest ratings of satiety were on the egg + white toast breakfast which received the least palatable scores. The egg + white toast breakfast contained a large amount of eggs in the form of egg patties. Although participants were screened for the distaste for eggs, they were not screened on food form of eggs. The egg patties were found to not be visually appealing or the taste to be considered good. The same egg patties were used in the egg + whole grain toast breakfast but half the amount was served for that meal and palatability scores for that meal was improved but still scored lower than the cereal meal. The additional factors beyond homeostatic control, nutrient sensing and balance of food intake have been studied in depth and it is thought that these hedonic, sensory aspects of food can frequently override the hypothalamic control of intake [155, 156].

Overall, all of the test meals were well tolerated. The occurrence of all GI symptoms was higher with the egg breakfasts and highest for the egg + white toast breakfast that contained the higher portion of eggs. Eggs are typically well tolerated. We did observe a significant, yet modest, increase in gas and bloating for the meal containing the equivalent of four eggs.

Conclusion

In conclusion, this study suggests an egg breakfast containing a high level of protein is more satiating than an egg breakfast with moderate protein level with fiber or a cereal breakfast. However, although the subjective satiety scores suggest the two egg breakfasts are different in satiety potential, food intake was reduced in both of the egg-containing meals. We did find that foods in food forms uncommon with usual consumption may lead to reduced palatability and liking scores that may interfere with the research objectives. Additionally, the portion size of the meal may also lend to satiety effects that need to be considered. Further studies should include treatment sets with matched portion size and carbohydrate load. Overall, this study has demonstrated that egg-containing breakfast meals contribute to higher satiety ratings when compared to a typical cereal breakfast and result in the reduction of food intake at the subsequent meal. Incorporation of eggs in the breakfast meal could over time lead to reductions in body weight and aid in weight management.

By conducting this study, we have a better understanding of how typically consumed food combinations are able to induce satiety. The outcomes of this study will provide governmental agencies and commodity businesses a platform to discuss how common foods can be used to combat obesity and better manage weight.

Acknowledgements

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Table 3-1. Subject Demographic Characteristics*

	Men (n=24)	**Women (n=24)**
Age (yrs)	24 ± 1	23 ± 1
BMI (kg/m^2)	23 ± 0.5	22 ± 0.5
Restrained Eating Score	6 ± 0.5	6 ± 0.5

*Baseline values presented as mean ± SE

Table 3-2. Meal Macronutrient Content

Test Meals Nutrients	**Cereal**	**Egg/WG**	**Egg/White**
Weight (g)	260	306	370
Kcal	390	390	390
Carbohydrate (g)	58	42	25
Fiber (g)	1	7	1
Protein (g)	10	20	30
Fat (g)	15	15	15

Table 3-3: Meal Composition

Cereal	serv (g)	calories	fat	sat fat	CHO	sugar	fiber	protein
rice crispies	45.00	164	0.6	0.2	38.8	3.4	0.3	3.0
milk, whole	182.00	109	5.9	3.6	9.1	9.1	0.0	5.5
white toast	20.00	53	0.6	0.1	9.8	1.0	0.5	1.8
margarine	13.00	65	7.4	1.9	0.0	0.0	0.0	0.0
total	**260.0**	**391**	**14.5**	**5.8**	**57.7**	**13.6**	**0.9**	**10.3**
Egg+white tst	serv (g)	calories	fat	sat fat	CHO	sugar	fiber	protein
white toast	26.00	69	0.8	0.2	12.8	1.3	0.7	2.4
milk, skim	150.00	51	0.1	0.1	7.4	7.6	0.0	5.1
egg patty	194.00	271	13.5	6.8	4.5	0.0	0.0	22.6
total	**370**	**391**	**14.5**	**7.0**	**24.7**	**9.0**	**0.7**	**30.0**
Egg+WG tst	serv (g)	calories	fat	sat fat	CHO	sugar	fiber	protein
Whole grain tst	80.00	200	4.3	0.0	32.9	7.1	7.1	7.1
milk, skim	150.00	51	0.1	0.1	7.4	7.6	0.0	5.1
egg patty	66.90	93	4.7	2.3	1.6	0.0	0.0	7.8
margarine	9.43	47	5.4	1.3	0.0	0.0	0.0	0.0
total	**306.33**	**391**	**14.5**	**3.8**	**41.9**	**14.8**	**7.1**	**20.0**

Figure 3-1: Pizza Lunch Nutrition Facts

DiGiorno 4 Cheese Pizza	Whole Pizza	
NUTRITIONAL INFO	798	g
Serving Size 1/6 Pizza (133g)	1860	kcals
Servings per Container 6		
Amount Per Serving		
Calories 310	Calories from Fat 90	
	% Daily Value*	
Total Fat 10g	15%	
Saturated Fat 5g	25%	
Trans Fat 0g		
Cholesterol 25mg	8%	
Sodium 870mg	36%	
Total Carbohydrates 38g	13%	
Dietary Fiber 2g	8%	
Sugars 6g		
Protein 16g		

Table 3-4: Summary of Results

	Cereal	Egg/ WW	Egg/ White	p-value*[‡]
Fullness[1]	142 ± 8[a]	147 ± 8[a]	195 ± 8[b]	<0.0001
Hunger[1]	171 ± 8[a]	169 ± 8[a]	124 ± 8[b]	<0.0001
Satisfaction[1]	150 ± 8[a]	154 ± 8[a]	199 ± 8[b]	<0.0001
Prospective Consumption[1]	194 ± 8[a]	194 ± 8[a]	150 ± 8[b]	<0.0001
Fullness Peak[2]	66 ± 2.5[a]	65 ± 2.5[a]	80 ± 2.5[b]	<0.0001
Satisfaction Peak[2]	66 ± 2.5[a]	65 ± 2.5[a]	79 ± 2.5[b]	<0.0001
Blood Glucose[1]	32925 ± 470[a]	31397 ± 470[b]	30915 ± 470[b]	0.002
Blood Glucose Peak[2]	143 ± 3[a]	125 ± 3[b]	124 ± 3[b]	<0.0001
Lunch Intake (g)[3]	430 ± 19[a]	400 ± 19[b]	372 ± 19[b]	0.0006
Lunch Intake (kcal)	1001 ± 45[a]	932 ± 45[b]	866 ± 45[b]	0.0006
Visual Appeal[4]	34 ± 3[a]	49 ± 3[b]	69 ± 3[c]	0.0005
Smell[4]	35 ± 2[a]	38 ± 2[a]	49 ± 2[b]	0.0001
Taste[4]	31 ± 3[a]	41 ± 3[b]	59 ± 3[c]	<0.005
Aftertaste[4]	61 ± 4	59 ± 4	58 ± 4	n.s.
Pleasantness[4]	31 ± 3[a]	40 ± 3[b]	62 ± 3[c]	<0.01
GI Tolerance-total sum[5]	2.8 ± 0.7	3.0 ± 0.7	3.8 ± 0.7	n.s.
GI Tolerance- gas sum[5]	1.2 ± 0.4[a]	1.5 ± 0.4[a]	2.0 ± 0.4[b]	0.03
GI Tolerance- diarrhea sum[5]	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	n.s.
[1]Mean AUC ± SEM				
[2]Peak Mean ± SEM, [3]Mean Total Calories (or grams) ± SEM				
[4]Mean ± SEM, scale of 0-1, [5]Mean sum of scores ± SEM				
*p-value indicated for overall F-test, [‡]p-value for values with different letters				

Table 3-5: Dietary Intake Record over 24 hours

	Cereal		Egg/ WW		Egg/ White	
Gender	Male	Female	Male	Female	Male	Female
Kcal	1511 ± 135	1219 ± 135	1792 ± 135	1087 ± 135	1562 ± 138	1260 ± 138
Carbohydrate (g)	184 ± 16	150 ± 16	209 ± 16	134 ± 16	170 ± 16	160 ± 16
Fat (g)	55 ± 6	47 ± 6	64 ± 6	39 ± 6	60 ± 6	46 ± 6
Protein (g)	58 ± 6	40 ± 6	65 ± 6	38 ± 6	67 ± 6	44 ± 6
Fiber (g)	13 ± 2	12 ± 2	13 ± 2	12 ± 2	12 ± 2	12 ± 2

*no significant differences in intake between treatments

Figure 3-2: Hunger Ratings over time

Figures 3-3: Satisfaction Ratings over time

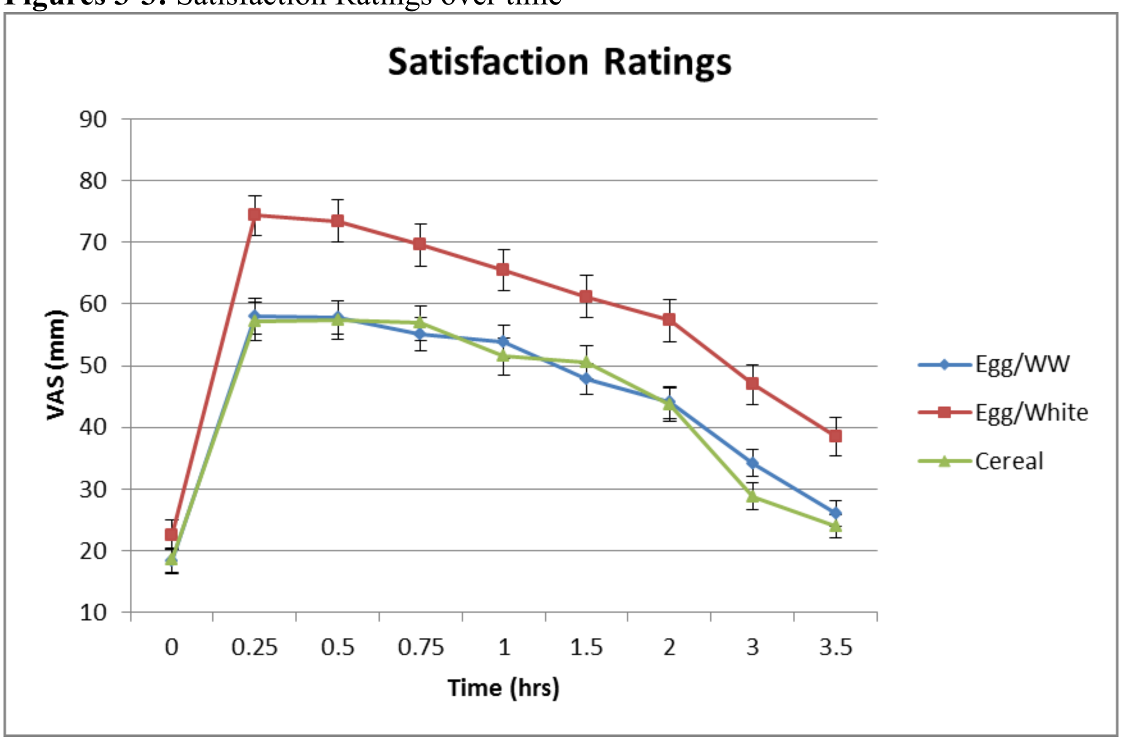


Figure 3-4: Fullness Ratings over time

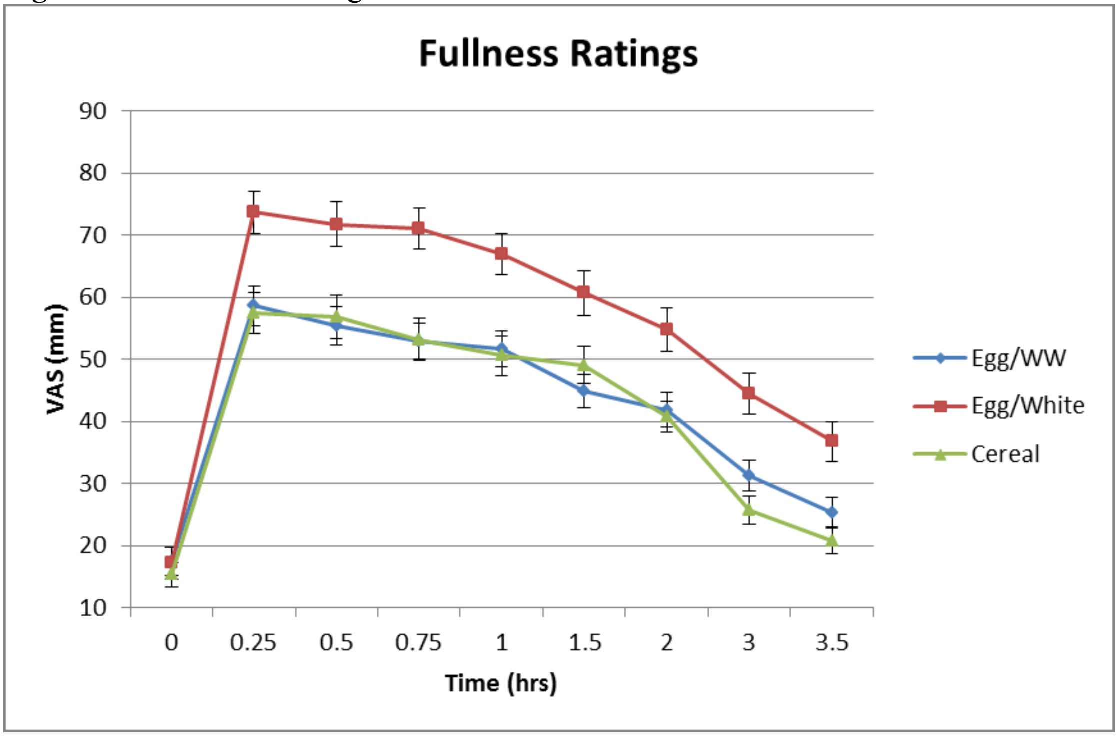


Figure 3-5: Prospective Food Consumption over time

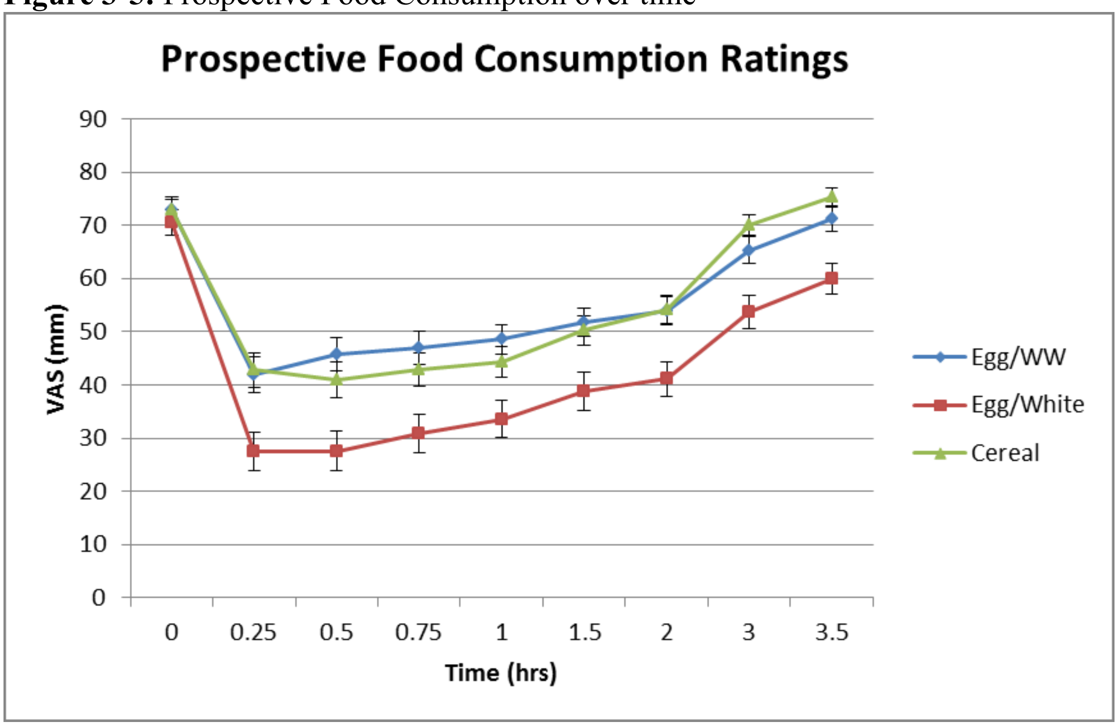


Figure 3-6: Blood glucose response over time

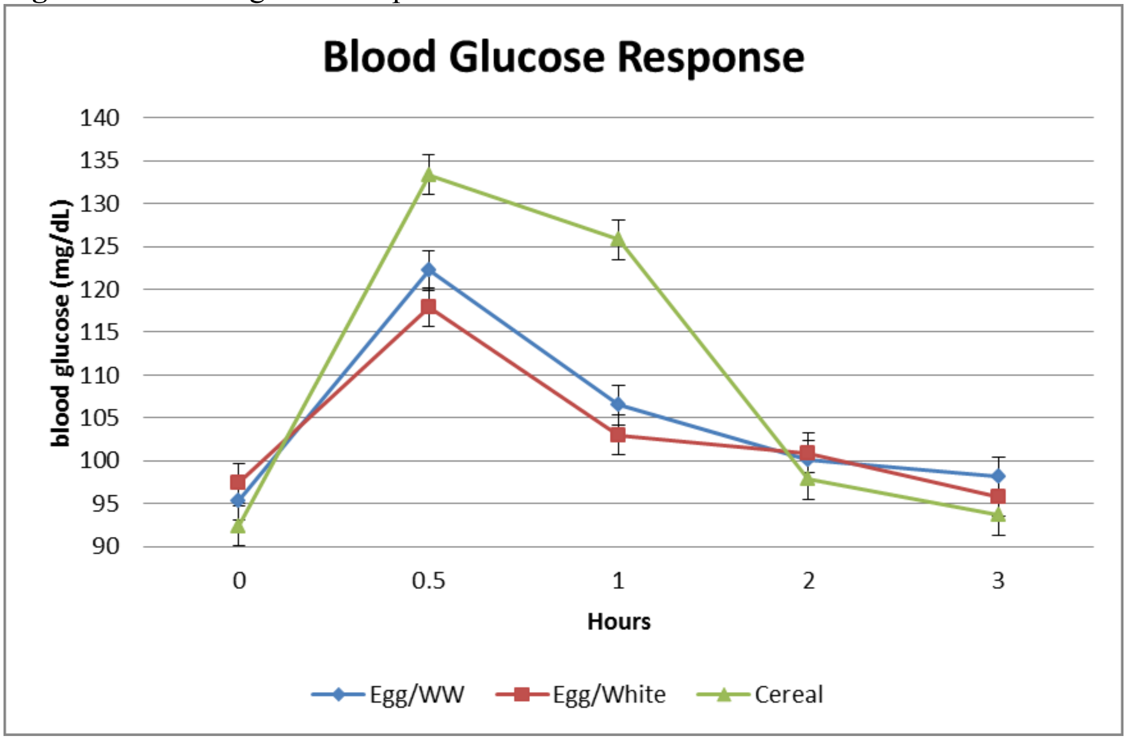


Figure 3-7: Subsequent Meal Intake at ad libitum pizza lunch

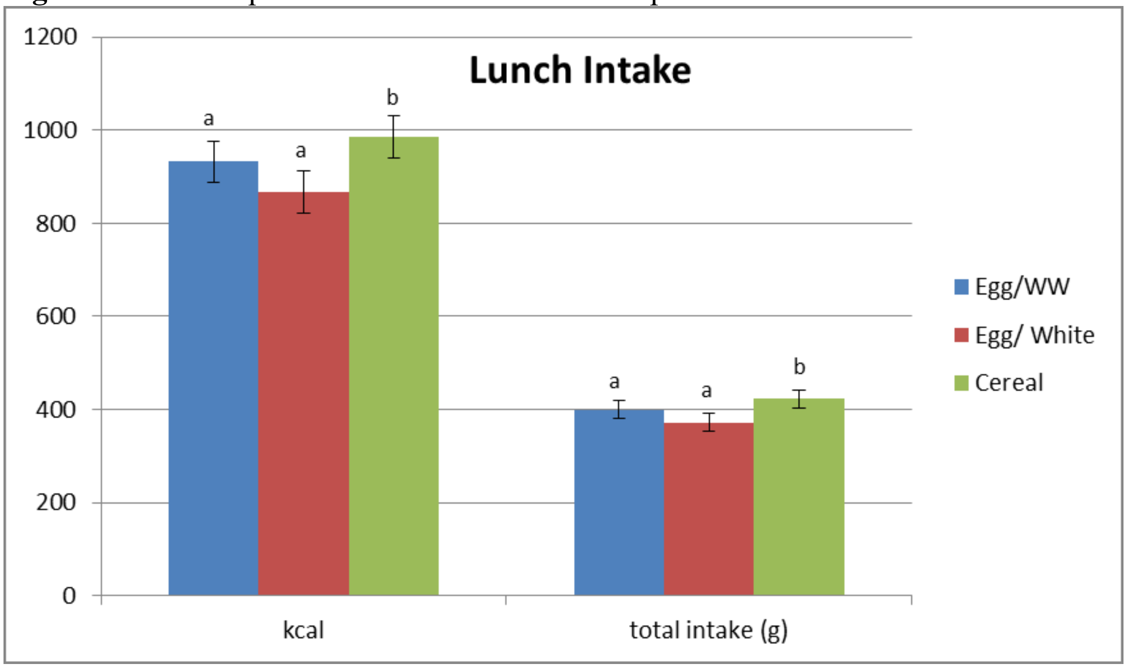
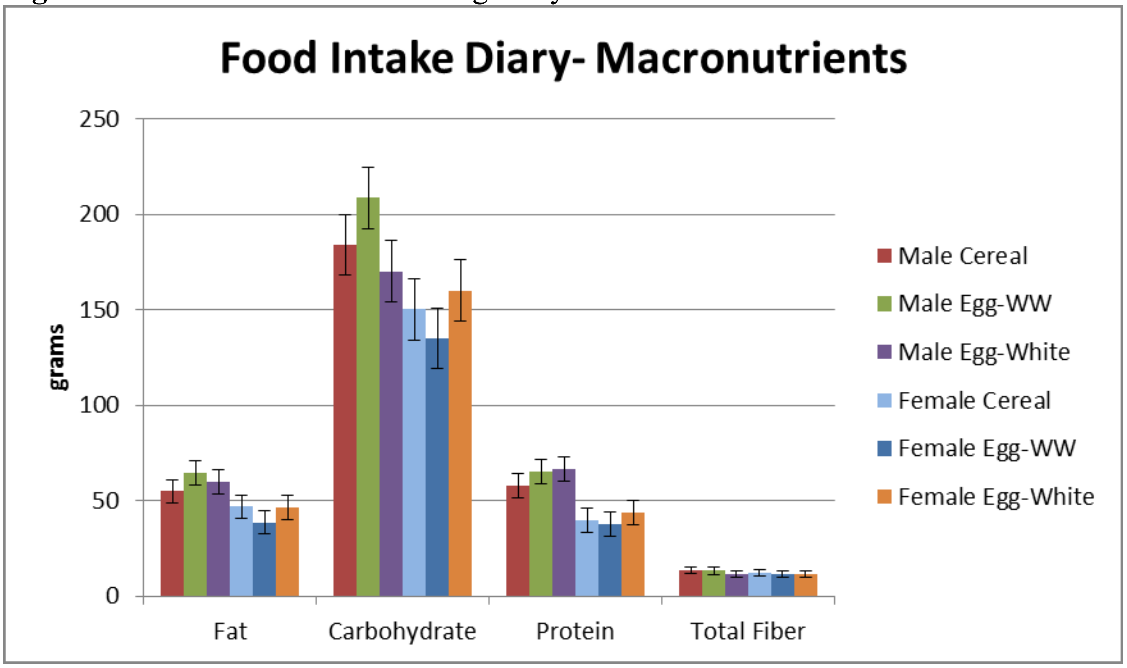
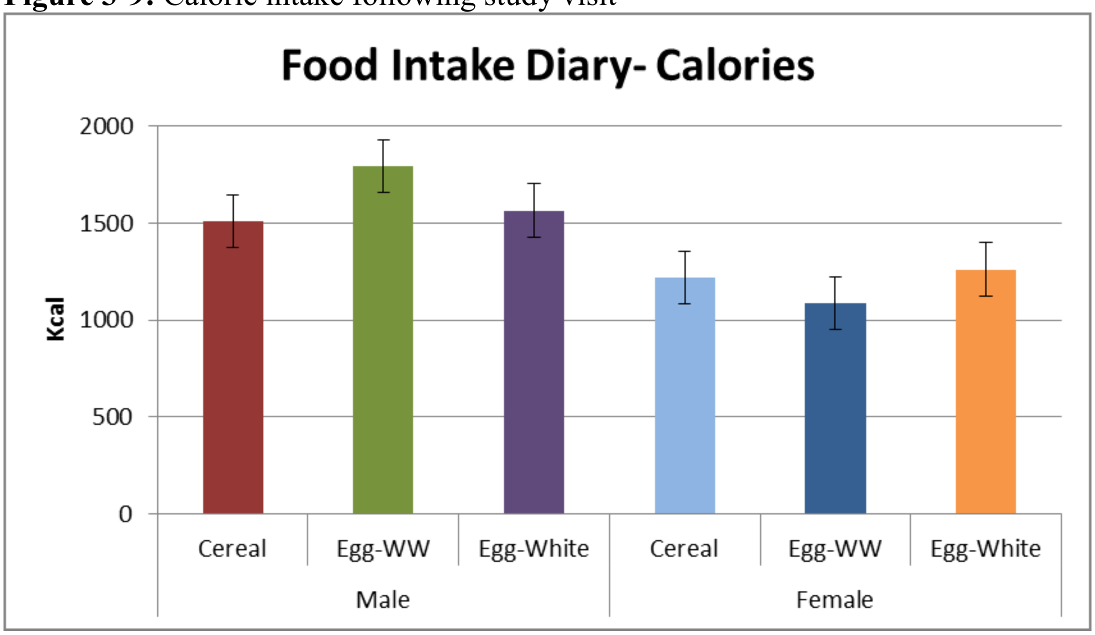


Figure 3-8: Nutrient intake following study visit



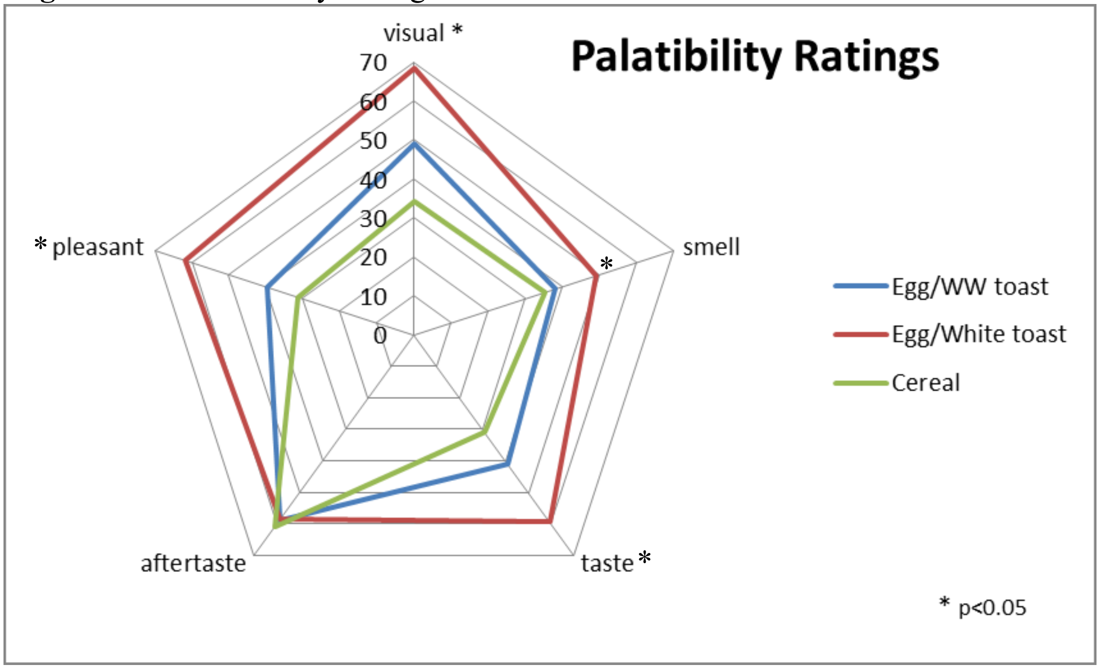
*no significant differences in intake between treatments

Figure 3-9: Calorie intake following study visit



*no significant differences in intake between treatments

Figure 3-10: Palatability Ratings of Treatment Meals



*values contained in Table 3-4.

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Appendix A: Beef Study Consent Form

SATIETY RESPONSE TO BEEF AND BEANS STUDY

CONSENT FORM

Please read this document and ask any questions you may have before agreeing to be in the study.

Joanne Slavin, Ph.D., RD in the Department of Food Science and Nutrition is conducting this study. The Department of Food Science and Nutrition at the University of Minnesota is in the College of Food, Agricultural and Natural Resource Sciences. The study is sponsored by Minnesota Beef Council.

Description and Purpose of the Study

You are being asked to participate in a study of beef and beans on effects on hunger and fullness. The meals you will consume are food products typically consumed and are safe to consume.

Approximately 28 subjects will participate in this study. The study consists of two four hour visits. Both visits are necessary to complete the study itself. The study will be conducted at McNeal Hall at the University of Minnesota. You are selected for this study because you are a man or woman in good health.

At each visit, you will consume a different meatloaf lunch. You will also be given snack selection to consume.

You will complete a survey on gastrointestinal responses associated with these meals and a 24-hour food record.

Study Procedures

At both visits, you will be given a beef or bean meal. You will also be asked to complete a survey about your level of hunger at baseline and for 3 hours after the meal.

You will be given a folder with a gastrointestinal survey and food record. The next day, subjects will drop the complete surveys at McNeal Hall 152.

You will be scheduled for your next visit at least 1 week later. This cycle will be repeated 1 time for a total of 2 study visits.

Risks Associated With the Study

The foods used in this study are provided in amounts commonly taken in foods. The beef and beans used in this study are already foods available in the United States. There are no known side effects of beef in the amounts used in this study. Beans may cause intestinal gas and loose stools, although the amounts of beans given in this study are below amounts known to cause any noticeable gastrointestinal symptoms.

Benefits Associated with the Study

There is no guarantee that you will receive any benefit by participating in this study.

Research Related Injury

In the event that this research activity results in an injury, treatment will be available including first aid, emergency treatment, and follow-up care as needed. Care for such injuries will be billed in the ordinary manner, to you or your insurance company. If you think you have suffered a research-related injury and that you may be eligible for reimbursement of some medical care costs, let the study researchers know right away.

Compensation for Participation

Study related visits, procedures, and the food for the study will be provided at no cost to you.

Successful completion of each treatments diet record and tolerance questionnaires results in payment of $50

A total of $50.00 for each completed treatment and scheduled visit, if you do not complete the whole study.

$200.00 if you complete the whole study

Confidentiality and Document Review

The results of this research study may be presented at meetings or in publications, so absolute confidentiality cannot be guaranteed. However, your identity will not be disclosed in these presentations. Data will be kept for 1 year after the study is reported in the literature.

Alternative Treatment

The alternative is to not participate in this study. You may consume beef/beans without participating in this study.

Voluntary Nature of Participation

Your decision whether or not to be in this study will not affect your current or future relations with the University of Minnesota. If you decide to be in this study, you are free to withdraw your consent and to stop participation at any time. Withdrawing your consent and stopping participation will not affect your relationship with the University of Minnesota.

New Information

If, during the course of this research study, there are significant new findings discovered that might influence your willingness to continue, the researchers will inform you of those findings.

Contacts and Questions

You may ask any questions you have now.

If you have any questions or concerns regarding the study and would like to talk to someone other than the researchers, you are encouraged to contact the Research Subjects' Advocate Line, D528 Mayo, 420 Delaware St SE, Minneapolis, MN 55455; 612-625-1650.

You will be given a copy of this form to keep for your records.

Statement of Consent:

I have read the above information. I have asked questions and have received answers. I consent to participate in the study.

Signature________________________Date ___________

Signature of Investigator or Person Obtaining Consent

Signature________________________ Date ___________

Appendix B: Eating Habits Questionnaire

Now I'm going to give you a short questionnaire about your eating patterns. Please respond with the answer that applies to you on most eating occasions.		Score
1. When I have eaten my quota of calories, I am usually good about not eating any more	T (+1) F	
2. I deliberately take small helpings as a means of controlling my weight	T (+1) F	
3. Life is too short to worry about dieting	T F (+1)	
4. I have a pretty good idea of the number of calories in common food.	T (+1) F	
5. While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it	T (+1) F	
6. I enjoy eating too much to spoil it by counting calories or watching my weight	T F (+1)	
7. I often stop eating when I am not really full as a conscious means of limiting the amount that I eat	T (+1) F	
8. I consciously hold back at meals in order not to gain weight	T (+1) F	
9. I eat anything I want, any time I want	T F (+1)	
10. I count calories as a conscious means of controlling my weight	T (+1) F	
11. I do not eat some foods because they make me fat	T (+1) F	
12. I pay a great deal of attention to changes in my figure	T (+1) F	
13. How often are you dieting in a conscious effort to control your weight? Rarely Sometimes Usually (+1) Always (+1)		
14. Would a weight fluctuation of 5 lbs. affect the way you live your life? Not at all Slightly Moderately (+1) Very much (+1)		
15. Do your feelings of guilt about overeating help you to control your food intake? Never Rarely Often (+1) Always (+1)		
16. How conscious are you of what you are eating? Not at all Slightly Moderately (+1) Extremely (+1)		
17. How frequently do you avoid "stocking up" on tempting foods? Almost never Seldom Usually (+1) Almost always (+1)		

18. How likely are you to shop for low calorie foods? Unlikely Slightly unlikely Moderately likely (+1) Very likely (+1)	
19. How likely are you to consciously eat slowly in order to cut down on how much you eat? Unlikely Slightly likely Moderately likely (+1) Very likely (+1)	
20. How likely are you to consciously eat less than you want? Unlikely Slightly likely Moderately likely (+1) Very likely (+1)	
21. On a scale of 0 to 5, where 0 means no restraint in eating and 5 means total restraint what number would you give yourself? (0) Eat whatever you want, whenever you want it (1) Usually eat whatever you want, whenever you want it (2) Often eat whatever you want, whenever you want it (3) Often limit food intake but often "give in" (+1) (4) Usually limit food intake, rarely "give in" (+1) (5) Constantly limiting food intake, never "giving in" (+1)	
TOTAL SCORE *Exclude if score 11 or higher*	

Appendix C: Visual Analog Scale for Subjective Satiety Ratings (100 mm)

Satiety Questions

How hungry do you feel?

I am not hungry at all	______________________________	I have never been more hungry

How satisfied do you feel?

I am completely empty	______________________________	I cannot eat another bite

How full do you feel?

Not at all full	______________________________	Totally Full

How much do you think you can eat?

Nothing at all	______________________________	A lot

Appendix D: Food Portion Reference Guide

Appendix E: Gastrointestinal Symptoms Questionnaire

1. Gas or bloating	☐ None	☐ Mild	☐ Moderate	☐ Severe
2. Nausea	☐ None	☐ Mild	☐ Moderate	☐ Severe
3. Flatulence	☐ None	☐ Mild	☐ Moderate	☐ Severe
4. Diarrhea or loose stools	☐ None	☐ Mild	☐ Moderate	☐ Severe
5. Constipation	☐ None	☐ Mild	☐ Moderate	☐ Severe
6. Gastrointestinal cramping	☐ None	☐ Mild	☐ Moderate	☐ Severe
7. Gastrointestinal rumbling	☐ None	☐ Mild	☐ Moderate	☐ Severe

Appendix F: Palatability Questionnaire (100 mm)

Visual Appeal

Good ____________________ Bad

Smell

Good ____________________ Bad

Taste

Good ____________________ Bad

Aftertaste

Much ____________________ None

Overall Pleasantness

Good ____________________ Bad

Appendix G: Beef Study Randomization Schedule

Women

1 Bean - Beef
2 Bean - Beef
3 Beef - Bean
4 Beef - Bean
5 Bean - Beef
6 Bean - Beef
7 Beef - Bean
8 Beef - Bean
9 Bean - Beef
10 Beef - Bean
11 Bean - Beef
12 Beef - Bean
13 Bean - Beef
14 Beef - Bean

Men

15 Bean - Beef
16 Beef - Bean
17 Bean - Beef
18 Bean - Beef
19 Beef - Bean
20 Beef - Bean
21 Bean - Beef
22 Beef - Bean
23 Beef - Bean
24 Bean - Beef
25 Beef - Bean
26 Beef - Bean
27 Bean - Beef
28 Bean - Beef

Appendix H: Egg Study Consent Form

SATIETY RESPONSE TO EGGS STUDY

CONSENT FORM

Please read this document and ask any questions you may have before agreeing to be in the study.

Joanne Slavin, Ph.D., RD in the Department of Food Science and Nutrition is conducting this study. The Department of Food Science and Nutrition at the University of Minnesota is in the College of Food, Agricultural and Natural Resource Sciences. The study is sponsored by Egg Nutrition Center.

Description and Purpose of the Study

You are being asked to participate in a study of eggs effects on hunger and fullness. The meals you will consume are food products typically consumed and are safe to consume.

Approximately 48 subjects will participate in this study. The study consists of three four hour visits. All 3 visits are necessary to complete the study itself. The study will be conducted at McNeal Hall at the University of Minnesota. You are selected for this study because you are a man or woman in good health.

At each visit, you will consume a different breakfast meal. You will also be given a lunch to consume. You will complete a survey on gastrointestinal responses associated with these meals and a 24-hour food record.

Study Procedures

At all visits, you will be given a breakfast meal. You will also be asked to complete a survey about your level of hunger at baseline and for 4 hours after the meal. Throughout the 4 hours you will be asked to periodically use a hand held device to administer a finger stick to assess blood levels.

You will be given a folder with a gastrointestinal survey and food record. Subjects will bring the folder to each visit and after the 3rd and final visit will return the completed folder to McNeal Hall 152.

You will be scheduled for your next visit at least 1 week later. This cycle will be repeated 2 times for a total of 3 study visits.

Risks Associated With the Study

The foods used in this study are provided in amounts commonly taken in foods. The breakfast foods used in this study are already foods available in the United States. There are no known side effects in the amounts used in this study. Blood meters used in this study are typically used multiple times daily in an in home setting. Fingertip soreness and minimal bleeding may occur at stick point.

Benefits Associated with the Study

There is no guarantee that you will receive any benefit by participating in this study.

Research Related Injury

In the event that this research activity results in an injury, treatment will be available including first aid, emergency treatment, and follow-up care as needed. Care for such injuries will be billed in the ordinary manner, to you or your insurance company. If you think you have suffered a research-related injury and that you may be eligible for reimbursement of some medical care costs, let the study researchers know right away.

Compensation for Participation

Study related visits, procedures, and the food for the study will be provided at no cost to you.

Successful completion of each treatments diet record and tolerance questionnaires results in payment of $25 and the finger sticks for each treatment results in a payment of $25.

A total of $25 for each completed treatment and scheduled visit, if you do not complete the whole study.

<u>Total compensation: $300.00 if you complete the entire study (All 3 visits).</u> Completion of only 1 visit: $75, completion of 2 visits: $150, completion of all 3 visits, diet records and tolerance forms: $300.

Confidentiality and Document Review

The results of this research study may be presented at meetings or in publications, so absolute confidentiality cannot be guaranteed. However, your identity will not be disclosed in these presentations. Data will be kept for 1 year after the study is reported in the literature.

Alternative Treatment

The alternative is to not participate in this study. You may consume breakfast without participating in this study.

Voluntary Nature of Participation

Your decision whether or not to be in this study will not affect your current or future relations with the University of Minnesota. If you decide to be in this study, you are free to withdraw your consent and to stop participation at any time. Withdrawing your consent and stopping participation will not affect your relationship with the University of Minnesota.

New Information

If, during the course of this research study, there are significant new findings discovered that might influence your willingness to continue, the researchers will inform you of those findings.

Contacts and Questions

You may ask any questions you have now.

If you have any questions or concerns regarding the study and would like to talk to someone other than the researchers, you are encouraged to contact the Research Subjects' Advocate Line, D528 Mayo, 420 Delaware St SE, Minneapolis, MN 55455; 612-625-1650.

You will be given a copy of this form to keep for your records.

Statement of Consent:

I have read the above information. I have asked questions and have received answers. I consent to participate in the study.

Signature_________________________Date ___________

Signature of Investigator or Person Obtaining Consent

Signature_________________________ Date ___________

Appendix I: Egg Study Randomization Schedule

Women ID	visit 1	visit 2	visit 3	Men ID			
1	581	207	893	1	893	581	207
2	207	893	581	2	581	207	893
3	893	581	207	3	207	893	581
4	893	207	581	4	207	581	893
5	581	893	207	5	893	207	581
6	207	581	893	6	581	893	207
7	581	207	893	7	893	581	207
8	207	893	581	8	581	207	893
9	893	581	207	9	207	893	581
10	893	207	581	10	207	581	893
11	581	893	207	11	893	207	581
12	207	581	893	12	581	893	207
13	581	207	893	13	893	581	207
14	207	893	581	14	581	207	893
15	893	581	207	15	207	893	581
16	893	207	581	16	207	581	893
17	581	893	207	17	893	207	581
18	207	581	893	18	581	893	207
19	581	207	893	19	893	581	207
20	207	893	581	20	581	207	893
21	893	581	207	21	207	893	581
22	893	207	581	22	207	581	893
23	581	893	207	23	893	207	581
24	207	581	893	24	581	893	207

Treatment Codes:
893 = cereal
581 = egg/ white toast
207 = egg/ whole grain toast