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Although dietary protein increases acute satiety relative to carbohydrate, the influence of protein source and level has not been clearly described. The objective of the present study was to assess acute satiety, postprandial insulin response, and post-meal behavior using different protein sources and levels. Rats were given a breakfast-like meal approximating 20% of total daily intake and containing either 35% or 20% of calories from egg white or wheat gluten. Subsequent ad-libitum chow diet intake, plasma insulin levels, and post-meal behavior were monitored. When fed a normal protein level (20%) there was no effect of protein source on intake of chow during the test period. However, the response to the higher protein diets was determined by protein source. Animals fed the 35% wheat gluten diet consumed more than the control group, while those fed the 35% egg white diet ate less than the control group. When fed at 20% of daily intake none of the diets were completely satiating as all animals consumed food during the initial period of the test period. However, it was during this period that the differences between diets were most apparent. All diets induced the behavioral satiety sequence of feeding, grooming, and resting after both breakfast and during the test period. There tended to be a lower insulin response to the higher protein diets, with the response to egg white being lower than wheat gluten. These results support the hypothesis that both protein level and source affect acute satiety in rat.

EFFECTS OF BREAKFAST DIETARY PROTEIN SOURCE AND LEVEL ON SATIETY

by

Edward Markus

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CHAPTER I

LITERATURE REVIEW

It was assumed throughout the 1800's that all proteins were of equal quality, and that while protein was required for health, meat protein could be replaced with an equal quantity of protein from beans (Carpenter 2003). This assumption was challenged by an experiment in which dogs were fed diets containing digested meat proteins. Those fed meat proteins which had been enzymatically digested by trypsin and pepsin remained healthy, while those given acid hydrosylates of the same protein did not. In 1902, a team at Cambridge isolated the amino acid tryptophan from an enzymatic digest and showed that it was destroyed by acidic conditions. Finally, in 1909 a researcher discovered that dogs could remain healthy on a diet of acid hydrosylates of protein if they were given supplemental tryptophan.

Proteins are made up of building blocks called amino acids, which are used in the synthesis of bodily tissues and the production of nitrogenous compounds such as hormones, immune mediators, and neurotransmitters (Tessari 2006). Amino acids are divided into three categories: essential, nonessential, and conditional. Essential amino acids cannot be produced by the body in sufficient quantities and must be supplied through food. Nonessential amino acids can be made in the body from essential amino acids or the breakdown of protein. Conditional amino acids are only essential during specific circumstances such as illness and stress. Humans require 20 different amino

acids, nine of which are considered essential. Protein is also the body's primary source of nitrogen.

Proteins are ranked in quality by their digestibility and amino acid content. Higher quality proteins provide all of the 20 essential amino acids in amounts necessary for synthesis of protein and maintenance of nitrogen balance. Digestibility is measured as the difference in dietary nitrogen intake in the protein and the nitrogen excreted in the feces. The current gold standard for protein quality is the Protein Digestibility Corrected Amino Acid Score (PDCAAS), which ranks proteins on a scale of 0 (worst) to 1 (best). The highest possible score, 1.00, indicates that after digestion of the protein, it provides 100 percent or more of the essential amino acids per gram of protein. Using the PDCAAS, plant protein sources tend to rank lower than animal protein foods because they contain lower amounts of the essential amino acids lysine and methionine.

Protein is constantly lost from the body through mechanisms such as protein degradation, shedding of skin cells, hair growth, and secretions in the gut. For this reason, humans must consume enough protein through their diet to avoid loss of body protein mass. Nitrogen balance (defined as the difference between nitrogen intake and excretion) is a highly regulated function. Inadequate consumption of total protein or of essential amino acids can result in edema, irritability, anorexia, ulcerating dermatoses, and an enlarged liver with fatty infiltrates. Because the body does not have a dedicated reserve of available amino acids, protein intake must occur frequently in order to preserve protein balance. The current estimate of average protein requirements for adults is 0.66 grams per

kilogram of body weight per day, which is believed to provide adequate nitrogen intake to balance output of nitrogenous compounds from the body.

Protein is a nutrient of particular concern during an animal's growth period. Early researchers noted that rats fed a diet containing 18 percent gliaden, a protein isolated from wheat, grew far less than those given a diet containing casein protein (Carpenter 2003). Protein is also critical for brain development. Protein energy malnutrition in children is associated with cerebral atrophy and appears to have negative effects on both white and grey matter (Gunston et al. 1992). Chronic protein-energy malnutrition affects the ongoing development of higher cognitive processes (Kar et al. 2008). Malnourished children perform poorly on tests of working memory, attention, learning and memory, and visuospatial ability except on tests of motor speed and coordination. Mechanistically, protein-energy malnutrition in fetal and early neonatal animal models reduces neuronal RNA and DNA content and alters fatty acid profile. These changes result in reduced protein synthesis, hypomyelination, and lower neuronal number. These effects subsequently induce alterations in structural proteins, growth factor concentrations, neurotransmitter production, and ultimately brain size. Ultrastructural changes include decreases in dendritic arbor complexity and synapse number. The hippocampus and cerebral cortex appear to be particularly vulnerable to the effects of protein-energy malnutrition.

Protein and Feeding Behavior

Energy homeostasis is one of the most highly studied and poorly understood topics in modern nutrition research. Feelings of hunger and satiety are the sensations

commonly associated with regulation of food intake. Satiety is the sensation of gastric fullness that reduces desire for food consumption during subsequent meals (Abou-Samra et al. 2011, Benelam 2009).

Peripheral Signals of Protein Intake

Oral Sensing

Protein sensing may begin in the oral cavity. Umami, which is one of the five basic taste sensations, is mainly triggered by free glutamate and has been proposed as the primary taste associated with proteins. Several umami receptors, such as metabotropic glutamate receptors and G-coupled taste receptors, can be found in the taste buds on the tongue (Palmer 2012). However, these receptors also respond to other compounds such as aspartate and 5-mononucleotides, raising questions about their specificity for protein detection. Additionally, glutamate is available in some foods which are not good sources of protein, such as tomatoes (de Araujo, 2003).

Since there does not appear to be a designated "protein taste" system, oral sensing of protein content may depend on learned associations between taste, texture, other food characteristics, and post-oral signals. This hypothesis is supported by studies which suggest that direct infusion of protein into the gut may be sufficient to establish a learned preference for protein (Miller et al. 1985). Rats given ad libitum access to a high protein (44% protein) diet and a protein-free carbohydrate diet initially select their intake without consideration for nutrient content. Over a period of about seven days, the rats gradually develop a stable protein/total intake ratio. In contrast, partially trigeminally deafferented rats (who thus had impaired somatosensory input from the oral cavity) given access to the

same diets did not acquire a stable protein/total intake ratio during 5 weeks of treatment. Their selections remained unpredictable and their protein ratio varied over the entire possible range during the period of observation. Although they were still sensitive to nutritional need and could distinguish between the different diets, the trigeminal input impairment prevented them from making rapid, fine adjustments of selection pattern in response to their metabolic state.

Protein Sensing in the Gut

Several mechanisms of protein detection within the post-oral gastrointestinal system have been suggested. The stomach has a glutamate-sensing system which may have a negative regulatory effect on gastric functions (Nakamura et al. 2011). Luminal application of glutamate at concentrations equivalent to those in food in the rat stomach increases activity in the afferent fibers of the gastric branch of the vagus nerve, which is connected with satiety (Uneyama et al. 2006). Infusion of glutamate into the stomach, duodenum, or portal vein increases activity in the vagal gastric, celiac, and hepatic nerves (Niijima, 2000). It has been shown that duodenal enterocytes and entero-endocrine cells express nutrient-specific receptors that are able to detect glutamate and other amino acids (Chaudhari, 2000 and Blackshaw, 2011).

Infusion of protein into the duodenum activates vagal afferent fibers in the rat, which are associated with satiety (Tome et al. 2009). Detection of protein in the duodenal lumen also induces release of cholecystokinin (CCK) by the entero-endocrine cells, and duodenal CCK results in an increase in the firing rate of the vagus nerve (Faipoux et al. 2008). Interestingly, the increase in activation of neural pathways related to satiety was

not suppressed when rats were given high-protein breakfast meals for several consecutive days. Furthermore, some types of protein (such as wheat) are stronger stimulators of CCK and GLP-1 release in human duodenal tissue than others, such as egg protein (Geraedts et al. 2010).

The ileal brake has also been proposed as a potential mechanism for protein sensing. The ileal brake occurs when undigested nutrients reach the ileum, and results in a combination of effects that influence digestive processes and ingestive behavior (Maljaars 2008). Activation of the ileal brake has been shown to reduce food intake and increase satiety levels. In human and animal studies, local nutrient infusion, particularly ileal protein infusion, activates the ileal brake (Meyer et al. 1998). Peptide YY (PYY), which is secreted in the ileal mucosa, is an important mediator of this process (Moran and Dailey, 2011). A study of normal-weight and obese human subjects revealed that PYY levels were highest after consuming a high-protein meal (Batterham et al. 2006). This condition also resulted in the highest satiety among study participants. It also appears to be a relatively long-lasting signal, with elevated plasma PYY levels occurring up to 6 hours after meal termination. In mice, long-term high protein diet feeding increased plasma PYY levels and reduced food intake and adiposity. Additionally, PYY-null mice were selectively resistant to the satiety-inducing and weight-reducing effects of a high-protein diet.

Other intestinal mediators have been implicated in protein-induced satiety as well. Dietary protein strongly stimulates secretion of glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) in the small intestine (Hall et al. 2003 and Bowen et

al. 2006) . GLP-1 and GIP are hypothesized to act synergistically with CCK to promote insulin secretion (Rehfeld, 2011).

Post Absorptive Protein Sensing

An increase in energy expenditure and the production of glucose through gluconeogenesis have been suggested as post-absorptive signals for protein and amino acids. Gluconeogenesis, or de novo synthesis of glucose, occurs when glucose availability is reduced during fasting or with a low or carbohydrate-free diet. In humans, a high-protein diet increased gluconeogenesis and energy expenditure while decreasing appetite compared with a low protein diet, and these effects were found to be independent of each other (Veldhorst 2009). There are no significant changes in plasma insulin levels between the two diets. The main gluconeogenic organ is the liver (Azzout et al. 1984). When rats are fed a high-protein diet, the activity of hepatic phosphoenolpyruvate carboxykinase (PEPCK), an enzyme involved in gluconeogenesis, is increased. This effect is seen in diets with and without carbohydrates, suggesting that it is the role of protein in the diet that is able to stimulate hepatic gluconeogenesis (Peret et al. 1975). Interestingly, PEPCK, which controls the conversion of oxaloacetate to phosphoenolpyruvate at the beginning of the gluconeogenic process, is up-regulated in both the fasting and fed state (Giffin et al. 1993). In contrast, glucose-6-phosphatase, which controls the final step of gluconeogenesis, is up-regulated in the fasted state and down-regulated in the fed state (Khan et al. 1985). These results suggest that a high-protein diet stimulates gluconeogenesis, but that in a fed state the newly synthesized glucose-6-phosphate is channeled towards glycogen synthesis, while in the fasted state it

is converted into glucose and released from the hepatocyte (Westerterp-Plantenga 2007). The control of PEPCK and G6Pase activity in the liver may have a profound impact on hepatic metabolism and glucose homeostasis (Trinh et al. 1998), and that the satiating effect of high protein feeding might be related to improvement of glucose homeostasis through the alteration of hepatic gluconeogenesis and subsequent glucose metabolism.

It has been demonstrated that protein stimulates diet-induced thermogenesis to a greater extent than other macronutrients (Westerterp-Plantenga, 2008). This may be due to in part to the high ATP costs of postprandial protein synthesis (Tessari et al. 2003). Amino acid oxidation may also play a role, especially when amino acids are given in excess of protein deposition. Elderly women whose diets were changed from 10 to 20% of energy from protein resulted in a 63% (with soy protein) to 95% (with animal protein) increase in protein oxidation (Pannemans et al. 1998). The subjects on the animal protein diet had greater absorptive protein oxidation, flux, and synthesis, potentially due to the greater availability of essential amino acids. Furthermore, it has been shown that meat protein increases 24-hour energy expenditure in humans to a greater extent than vegetable protein (Mikkelsen et al. 2000). However, it has never been shown that ingestion of food proteins induces satiety directly through an increase in postprandial thermogenesis (Davidenko et al. 2013). Furthermore, central mechanisms linking postprandial satiety with increased thermogenesis have yet to be demonstrated.

It has been hypothesized that elevated concentrations of plasma amino acids that cannot be used for protein synthesis may serve as a satiety signal and result in depressed food intake (Mellinkoff et al. 1956 and Harper and Peters, 1989). Mellinkoff and

colleagues demonstrated in 1956 that elevated plasma amino acid levels decrease hunger in humans independently of blood glucose levels. In rats, a high-protein diet causes rapid changes in amino acid levels in parts of the hypothalamus, while a protein-free high carbohydrate diet does not (Choi et al. 2001). It is proposed that elevated plasma amino acid levels are detected directly by the hypothalamus. Additionally, variations in free amino acid and plasma hormone concentrations influence the central nervous system.

Another potential mechanism for protein-induced satiety is the role of certain amino acids as satiety-related neurotransmitter precursors, although this hypothesis is somewhat controversial. Tryptophan, an amino acid precursor of the satiety-inhibition hormone serotonin, was first used to demonstrate this effect (Nieuwenhuizen et al. 2009 and Veldhorst et al. 2009). Brain levels of tyrosine and phenylalanine directly influence dopamine secretion, and histidine is a precursor or histamine (Fernstrom and Fernstrom, 2007). However, researchers were not able to demonstrate any effect of a histidine or tyrosine-supplemented diet on food intake in rats (Bassil et al. 2007). In contrast, another study reported that dietary histidine supplementation decreased food intake and adipose tissue in rats (Kasaoka et al. 2004), and central histamine has been suggested as a possible mediator in the depression of food intake (Mercer, 1997).

Central Control of Protein Intake

Central Homeostatic Regulation

Dietary protein may affect central energy intake regulation indirectly by triggering peripheral signal pathways or directly due to fluctuating free amino acid levels in the brain (Davidenko et al. 2013).

The brain areas and neuronal populations associated with protein-induced satiety are not fully understood. However, afferent pathways of the vagal nerve appear to have a role, as demonstrated by studies which show that introduction of protein into the duodenum results in activation of vagal afferent fibers (Darcel et al. 2005). A group of neurons called the nucleus of the solitary tract (NTS) mediates afferent vagal signals, and activation of two neuronal populations within the NTS can lead to anorexia. The first are the noradrenergic/adrenergic neurons, which when lesioned attenuate CCK-induced anorexia when lesioned (Rinaman et al. 2003). High protein feeding induces increasesof noradrenergic and adranergic neurons involved with CCK-related satiety as compared with a normal protein diet (Faipoux et al. 2008).

The second neuronal population within the NTS that can lead to anorexia is a group of neurons which express GLP-1 and are involved in both satiety and aversion-induced anorexia (Rinaman, 1999 and Faipoux et al. 2008). The GLP-1 pathway arising from the nucleus tractus solitarious (NTS), which is activated during nonphysiological anorexia, was not activated by high protein meals (Faipoux et al. 2008). This observation may explain the lack of aversive behavior observed with a high-protein diet. Neuronal circuits in the NTS can be reorganized in the short term or long term by dietary changes (Nefti et al. 2009). Additional animal studies suggest that protein increases sensitivity of the NTS to anorexic hormones, such as CCK.

In addition to afferent signals from the vagus nerve, adiposity and satiety signals are regulated in part by a key structure called the hypothalamic arcuate nucleus. Food intake is influenced through the arcuate nucleus by a balance between two primary

neuronal circuits that include anabolic and catabolic neurons. Protein intake affects both of these neuron types, inhibiting anabolic neurons and activating catabolic pathways (Faipoux et al. 2008 and Kinzig et al. 2007).

Potential intracellular pathways of influencing satiety involve the antagonist kinases AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) (Davidenko et al. 2014). High-protein diets increase hypothalamic ATP level, leading to inhibition of AMPK and subsequent activation of mTOR (Ropelle et al 2008). These kinases are present in neuropeptide Y (NPY) and pro-opiomelanocortin (POMC) neurons in the arcuate nucleus that have a major role in hypothalamic energy sensing. The mTOR pathway appears to be particularly responsive to plasma leucine levels, and knockdown of central mTOR reverses the anorectic effects of a high-protein diet in rats. Rats who consumed food supplemented with leucine had decreased food intake and weight gain than control animals. Furthermore, rats on a high protein diet had similar reduction in food intake, fat mass, and increased CSF leucine compared with rats on a leucine-supplemented control diet. Leucine administration in the brain also suppresses 24-hour food intake (Morrison et al. 2007). However, an exact mechanism of leucine activation of the mTOR pathway has yet to be fully elucidated.

Leucine and other branched-chain amino acids (BCAAs) have been considered signals of dietary protein intake. Increasing BCAAs in the diet has a similar effect on food intake as a high-protein diet (Newgard et al. 2009). Furthermore, BCAA levels in the plasma directly reflect dietary protein intake (Harper and Peters, 1985). It's worth noting that rats given the choice between a diet with or without supplemental BCAAs

selected almost exclusively the diet without BCAA (Anderson et al. 1990). This suggests that BCAA supplementation may have a pharmacological, rather than dietary, effect on appetite.

One of the most supported arguments for central control of protein intake is the ability of animals to detect and avoid an imbalance in dietary amino acid profile (Davidenko et al. 2013). A deficiency of one or more indispensible amino acids slows protein synthesis within the organism, and the corresponding transfer RNAs (which usually interact with the ribosomes) become uncharged and accumulate in the cytoplasm (Hao et al. 2005). In the anterior piriform cortex (APC), this accumulation activates general control nonderepressible 2 (GCN2) kinase and provokes conditioned learned aversion to a diet deficient in indispensible amino acids. Deactivation of GCN2 kinase (Maurin et al. 2005) and local injection of deficient amino acids into the APC (Russell et al. 2003) have been found to suppress this aversive effect.

The Role of Reward Centers in Protein Regulation

Research has examined the impact of protein intake on the reward system. Energy composition and protein content appear to have an effect on the reward value of food in addition to taste, smell, and appearance (McArthur et al. 1993). Researchers have demonstrated a flavor-conditioned preference for protein in cases of food and protein deprivation among rodents (DiBattista and Mercier. 1999). Flavors associated with protein-rich food were preferred after a low-protein preload in humans (Gibson et al. 1995). These data are consistent with observations of enhanced protein appetite among humans on a protein-restricted diet (Griffioen-Roose et al. 2012).

The relationship between a meal's macronutrient composition and its subsequent neuronal activation has been examined in humans and animals through magnetic resonance imaging. A study of breakfast-skipping overweight adolescent girls demonstrated that brain regions associated with reward (amygdala, hippocampus, anterior cingulate and parahippocampus) showed decreased activations when breakfast was added to the diet (Leidy et al. 2011). This decrease in activation was accentuated when the test subjects were offered breakfast with a higher protein content. Dietary protein is also thought to stimulate satiety centers (such as the arcuate nucleus and NTS) that further interact with the activity of reward mechanisms and food intake (Journel et al. 2012).

Effect of Feeding Pattern on Protein Metabolism

The same protein given with different feeding patterns may alter protein metabolism. A protein feeding pattern that combines meals rich and low in protein during the day may improve protein anabolism by inducing higher protein anabolism during postprandial periods and lower protein catabolism during post-absorptive periods (Pacy et al 1994). Giving elderly women 80% of their daily protein intake during a single mid-day meal was more effective at promoting whole-body protein retention than providing the same amount of protein over four different meals (Arnal et al. 1999). Young women showed greater nitrogen balance when protein intake was spread evenly over three meals than when protein was spread over two meals with an additional meal containing no protein (Leverton and Gram, 1949). However, the same conditions in young men resulted in no significant changes in nitrogen balance (Taylor et al. 1973).Whole-body leucine balance was greater with a diet given over 24 hours than with three discrete meals in

young men (El Khoury et al. 1995). In young women, protein balance was not improved when total daily protein intake was given during one meal as opposed to when it was spread over four meals (Arnal et al. 1999).

Slow and Fast Dietary Proteins and Their Effects on Satiety and Postprandial Metabolism

Slow and Fast Proteins

Fast and slow proteins were first described in 1997, when researchers discovered that ingested whey protein induced a dramatic but short-lived increase in plasma amino acids. In contrast, casein induced a prolonged plateau of increased plasma amino acid levels (Boirie et al. 1997). The difference in digestion and absorption rate between whey and casein is thought to be due to rate of gastric emptying (He et al. 2013). Whey protein stays soluble in the stomach and enters the intestine rapidly. Casein clots in the stomach, slowing its passage to the intestine and likely resulting in a more gradual release of amino acids (Boutrou et al. 2013).

Gelatin induces a sharp and rapid rise in plasma amino acids, similar to that from whey protein (Gannon et al. 1988). Cottage cheese, soy, and turkey induced a moderate increase in plasma amino acids similar to casein. Egg white, beef, and fish gave a slow and mild increase. It is unlikely that the mild increase in plasma amino acids seen with egg white was due to rapid liver metabolism since plasma urea nitrogen levels also did not increase. In another study, potato protein isolates induce a slower increase in plasma amino acid levels than whey protein and casein (He et al. 2013). Soy protein is more rapidly digested than total milk protein, but slower than whey and faster than casein (Bos

et al. 2003 and Farnfield et al. 2009). Fast proteins are still more rapidly absorbed and induce greater aminoacidemia than slow proteins when carbohydrate and fat are added to the meal, although the difference in absorption rates is slightly less dramatic (Dangin et al. 2003). This finding is relevant given that non-protein energy sources are known to decrease the rate of gastric emptying and could have suppressed the differences in digestion rates between fast and slow proteins (Calbet and MacLean, 1997).

The Effects of Protein Digestion Rate on Postprandial Metabolism

The amino acid availability from a dietary protein depends on the quantity and quality of the protein (Dangin et al. 2001). Whey protein (fast) stimulates postprandial protein synthesis to a greater extent than casein (slow) in healthy young men (Boirie et al. 1997). Ingestion of whey inhibited whole-body protein breakdown but ingestion of casein did not. Casein had a stronger effect than whey at inducing protein deposition. While the total nitrogen content and amino acid composition of the proteins were different in this study, the results suggest that speed of protein digestion and amino acid absorption has a major effect on whole-body protein metabolism. A study comparing the digestion rates of whole proteins to free amino acids in identical proportions found that protein digestion rate affected resulting aminoacidemia, leucine flux, and oxidation regardless of amino acid composition (Dangin et al. 2001).

An important aspect that influences concentrations and rate of appearance of amino acids in postprandial blood is extraction and conversion of amino acids in the splanchnic bed (gut and liver) (Felig, 1975). Compartment modeling predicts that eight hours after a meal, dietary nitrogen is composed of 28% free amino acids and 72%

protein. Approximately 30% of total nitrogen is recovered in the splanchnic bed versus 70% in the peripheral area (Fouillet et al. 2000). Interestingly, the source of dietary protein influences the distribution of postprandial protein and amino acids (Fouillet et al. 2002). Even though there is similar splanchnic retention of both soy and milk protein, soy protein had a greater proportion of nitrogen as protein and a lower proportion as free amino acids. Milk protein induced a greater uptake of peripheral nitrogen, but the amino acid portion of this retention was lower. In contrast, soy protein had a larger proportion of peripheral nitrogen in the form of nitrogen. As a consequence, the predicted protein synthesis efficiency of dietary nitrogen was significantly higher for soy protein in the splanchnic bed and significantly higher for milk protein in the peripheral area.

BCAAs selectively escape hepatic uptake and are therefore the primary amino acids excreted from the splanchnic bed after protein ingestion (Wahren et al. 1976). A marked increase was found in arterial and portal plasma concentration of most non-branched chain amino acids in patients undergoing major liver resection (van de Poll et al. 2008). Arterial plasma concentrations of BCAAs remained unchanged for these patients, suggesting a complex role for the liver in amino acid metabolism.

The digestion kinetics of dietary protein influences catabolic and anabolic activities of protein in the liver (Fouillet et al. 2002). Soy protein (fast) was more rapidly absorbed than milk protein (slow) and was associated with higher deamination and greater incorporation into protein in the splanchnic bed during the early postprandial phase. Rapidly absorbed protein transiently induces a greater enhancement of both whole-body protein synthesis and dietary amino acid oxidation compared with a more

slowly-absorbed protein (Boirie et al. 1997). In healthy young men and women, the lower postprandial retention of soy protein than milk protein appeared to be due to the more rapid appearance of dietary amino acids in the blood rather than differences in amino acid composition between the proteins (Bos et al. 2003). Thus, postprandial kinetics of dietary proteins is suggested as the main determinant of their metabolism after soy and milk protein ingestion.

A sharp rise in aminoacidemia results in increased deamination rates, presumably to prevent excessive plasma amino acid concentrations (Lacroix et al. 2006). These results suggest that digestion of fast protein may be too rapid to sustain the anabolic postprandial amino acid requirement in young adults.

Effect of Age and Protein Digestion Rate on Protein Metabolism

The effects of dietary protein digestion rate on postprandial protein metabolism may be age dependent (Koopman et al. 2006 and 2009). Age attenuates the difference in digestion rates between fast and slow protein (Dangin et al. 2002). In the elderly, the digestion of whey protein tended to be slower and the digestion of casein faster than in young subjects.

Fast and slow dietary proteins also influence protein turnover differently in young and elderly subjects (Dangin et al. 2002). In young adults, slowly-digested protein (casein) induces a greater protein gain than rapidly-digested protein (whey). In the elderly, protein gain is greater with rapidly-digested protein than slowly-digested protein (Dangin et al. 2003). In older men, whey protein stimulates postprandial muscle protein accretion more effectively than casein and casein hydrosylate (Pennings et al. 2011).

There are several reasons why age may alter the digestion rate of protein. The slowing of whey protein might be explained by the age-related slowing of gastric emptying rate (Cook et al. 1997). The enhanced speed of whey protein digestion could be due to the age-related decrease in gastric acid secretions, which could decrease the amount clotting that whey undergoes in the stomach and cause it to remain in a more rapidly-digested liquid form (Achour et al. 2001).

Effect of Protein Type on Glucose Metabolism

Dietary proteins and amino acids modulate both glucose metabolism and insulin sensitivity. Increased amino acid availability following protein ingestion may have indirect (hormone-mediated) and direct (substrate-mediated) effects on glucose metabolism (Krebs, 2005). Amino acids participate in recycling of glucose carbon via the substrate-mediated glucose-alanine cycle as well as de novo synthesis of glucose via gluconeogenesis. Although all amino acids are potentially glucogenic, alanine, serine, threonine and glycine give rise to significant amounts of glucose in the perfused liver (Exton 1972). When ingested alone, protein can increase both circulating glucagon and insulin concentrations without affecting circulating glucose concentration (Westphal et al. 1990). Beef protein stimulates insulin secretion as strongly as glucose in type 2 diabetic subjects (Nuttall et al. 1984). Protein and glucose synergistically increase plasma insulin concentration and ultimately result in lower plasma glucose concentration (Gannon and Nutall, 2010).

Effects of Digestion Rate of Different Proteins on Glucose Metabolism

Dietary proteins vary in their ability to induce release of insulin and glucagon (Tremblay et al. 2007). This may be due to the rate of appearance of amino acids in the plasma, which is influenced by rate of protein digestion. Some amino acids, such as leucine, phenylalanine, and tyrosine, are regarded as insulinetropic (van Loon et al. 2000). Branched-chain amino acids, which include leucine, isoleucine, and valine, are thought to be particularly potent insulin secretagogues (Nair and Short. 2005). It has also been suggested that the presence of protein or of digestion products of protein in the intestine, rather than the total amino acid concentration in circulation, results in an increase in incretin hormones that stimulates insulin secretion (Nuttall et al. 1985).

Different types of proteins also appear to affect glucose metabolism differently. In type 2 diabetics, the insulin and glucose responses to glucose ingested with various types of dietary proteins were compared (Gannon et al. 1988). Protein and glucose synergistically stimulated insulin secretion, and glucagon secretion was increased by protein. Gelatin and cottage cheese induced the strongest insulin response, while egg white induced the weakest insulin response. Gelatin and beef protein stimulated the strongest glucagon response, while egg white stimulated the lowest. In general, the insulin responses correlated with the level of plasma amino acid. This suggests that rate of digestion of the various proteins could have an effect on their insulinetropic properties.

This hypothesis is further supported by experiments which examined the degree of insulin and glucagon response to proteins at various levels of fractionation. Glucose, pea, and whey peptide hydrosylates induced a faster increase in plasma amino acids than

a solution containing complete milk proteins (Calbet and MacLean, 2002). The insulin response was particularly related with increases in plasma leucine, isoleucine, valine, phenylalanine, and arginine. Morifuji and colleagues (2010) reported that dietary protein hydrosylates were absorbed rapidly and the resulting rise in plasma amino acids stimulated a greater insulin response compared with nonhydrolyzed protein. Food proteins may also differ in their capacity to stimulate insulin release, possibly by differently affecting the early release of incretin hormones and insulinotropic amino acids (Nilsson et al. 2004). For example, milk proteins are insulinetropic, but the predominating insulin secretagogue is in the whey fraction.

CHAPTER II

INTRODUCTION

Satiety is defined as the satisfied feeling of being full after eating (National Institutes of Health, 2013). Ingestion of food gradually results in satiation in rats and other mammals (Halford et al. 1998). In many satiety studies, a reduction of food intake is measured as the primary dependent variable (Ishii et al. 2003). While the most common explanation of diet-induced anorexia is direct action of natural processes of appetite regulation, appetite can also be reduced indirectly as a consequence of nausea, distress, ataxia, pain, sedation, or hyperactivity (Blundell et al. 1985). Feeding behavior also depends on food characteristics (Johnson and Collier, 2001). Rats generally prefer foods which are sweet and avoid those which are sour or bitter. During food consumption, rat behavior gradually transitions from feeding to nonfeeding activities (such as grooming) and eventually shifts to resting. This structure is highly predictable and is referred to as the behavioral satiety sequence (Halford et al. 1998).

High-protein meals have been repeatedly shown to increase satiety (Westerterp-Plantenga et al. 2009). A topic which has received much less attention and which has had much more complex results is the effect of different protein sources on satiety. For instance, whey protein suppressed food intake more than egg albumin and soy protein at a pizza meal 60 minutes after preload (Anderson et al. 2004). The effect of source is modified by many factors including form (solid vs. liquid), dose, duration to next meal,

and the presence or absence of other macronutrients (Luhovyy et al. 2007). A liquid whey protein and carbohydrate preload resulted in lower intake of an ad libitum buffet meal 90 minutes after administration, compared with a preload containing the same amount of casein and carbohydrate (Hall et al. 2003). In contrast, there were no differences in consumption of a buffet meal three hours after whey, casein, gluten, or soy preloads as beverages containing carbohydrate (Bowen et al. 2006).

Traditionally, discussions of differences in intake induced by different protein sources have centered around differences in amino acid composition. However, recent evidence has suggested that the relationship between protein source and satiety may be more complex. Gelatin induced a sharp and rapid rise in plasma amino acids, similar to that from whey protein (Gannon et al. 1988). Cottage cheese, soy, and turkey induced a moderate increase similar to casein. Egg white, beef, and fish gave a slow and mild increase. It is unlikely that the mild increase in plasma amino acids seen with egg white was due to rapid liver metabolism since plasma urea nitrogen levels also did not increase. These results and others suggest that differences in digestion rates may have an impact on differences in satiety between different protein sources in addition to amino acid composition.

Different types of proteins also appear to affect glucose metabolism differently. In type 2 diabetics, the insulin and glucose responses to glucose ingested with various types of dietary proteins were compared (Gannon et al. 1988). Protein and glucose synergistically stimulated insulin secretion, and glucagon secretion was increased by protein. Gelatin and cottage cheese induced the strongest insulin response, while egg

white induced the weakest insulin response. Gelatin and beef protein stimulated the strongest glucagon response, while egg white stimulated the lowest. In general, the insulin responses correlated with the level of plasma amino acid. This suggests that rate of digestion of the various proteins could have an effect on their insulinetropic properties.

Protein source may have an effect on subsequent meal size and satiety. However, few studies have been able to compare the effects of different types of protein on satiety and analyze potential mechanisms with consistent methodology. Even less research has looked at the effects of protein source given as a breakfast meal. The objective of the current study was to examine differences between a breakfast meal of egg white and wheat gluten on second meal consumption, satiety, and postprandial metabolism. This was accomplished through feeding trials, behavioral analysis, and measurement of plasma insulin.

CHAPTER III

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (125 g; Charles River Laboratories) were housed individually in plexiglass cages (30 x 30 x 38 cm) in a temperature-controlled room and given ad libitum access to water. Upon arrival to our research facility, animals were acclimated to a reversed 12 hour light:dark cycle (lights off at 2200) and to a meal-feeding schedule. Acclimation to powdered diet and feeding schedule was gradual and occurred over 3-5 days. Rats were monitored daily and body weights were measured on an electronic balance at regular intervals (daily during testing periods and 7-10 days following surgeries) All animal studies were approved by the University of North Carolina at Greensboro's Institutional Animal Care and Use Committee (IACUC).

Feeding Schedule

The feeding protocol consisted of a 30-minute breakfast-like meal one hour into the dark cycle (1100 hr) and a five-hour ad libitum period at the end of the dark period (1600-2100 hrs). The breakfast meal approximated 20% of total daily intake by weight unless otherwise specified. Rats were trained to feeding schedule (Figure 1) for several days before testing and were randomly assigned on a daily basis to receive one of the test diets for breakfast only. Powdered rodent chow was used during the ad libitum period.

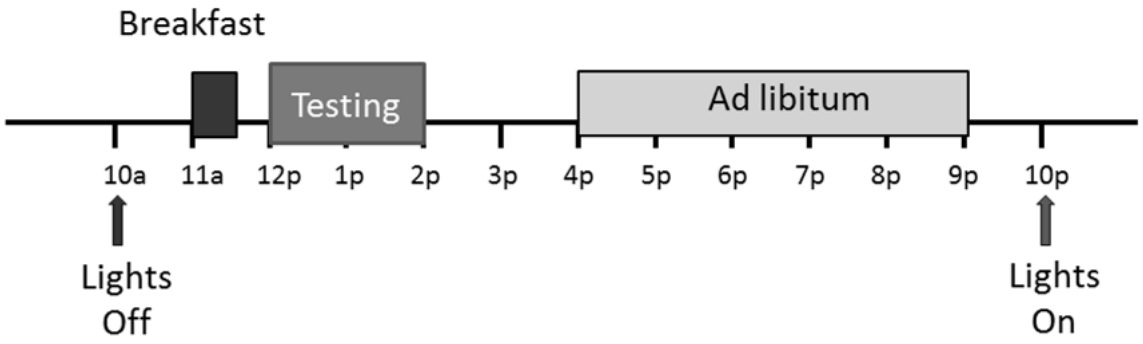

Figure 1. Animal Meal and Light Schedule.

Diet Preparation

Isocaloric diets were based on AIN-93G and contained either 20% or 35% of calories from egg white protein or wheat gluten protein (Tables 1-3). For the higher protein levels, carbohydrate level was adjusted. The diets met all nutrient requirements of rats. Appropriate mineral mixes were used to compensate for differences in mineral content of wheat gluten powder and egg white powder. All diet components were purchased from Dyets, Inc. (Bethlehem, PA) with the exception of egg white powder and maltodextrin which were purchased from Harlan (Indianapolis, IN). All diets were isocaloric and had the same percentage kilocalories from fat.

Table 1

Test Diet Composition

	Ad libitum		Egg white (20%)		Egg white (35%)		Wheat (20%)		Wheat (35%)	
	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%
Protein	20	19%	20	20%	35	35%	20	20%	35	35%
Carbohydrate	68	61%	68	61%	50	45%	68	60%	51	44%
Fat	9	20%	9	20%	9	20%	9	20%	9	21%
	g	kcal	g	kcal	g	kcal	g	kcal	g	kcal
Egg white powder	123	426	246	851	430	1488				
Wheat gluten powder	131	481					262	962	460	1688
Corn starch	372	1488	377	1508	261	1046	367	1468	243	970
Maltodextrin	120	480	122	486	84	337	118	474	78	313
Sucrose	100	400	101	405	70	281	99	395	65	261
Cellulose	53.5	0	53.5	0	53.5	0	53.5	0	53.5	0
Soybean Oil	90	810	90	810	90	810	90	810	90	810
Mineral mix (EW)	17.5	28	35	56	35	56				
Mineral mix (WG)	17.5	13					35	26	35	26
Vitamin mix	10		10		10		10		10	
Choline bitartrate	2.5		2.5		2.5		2.5		2.5	
L-Lysine	1.9	8					3.9	16	6.8	27
L-Threonine	0.5	2					1.0	4	1.8	7
	1039	4135	1037	4117	1037	4018	1042	4153	1046	4103
kcal/g		4.0		4.0		3.9		4.0		3.9

Table 2

Test Diet Amino Acid Composition

	Mg consumed in 4 g breakfast meal					Consumed	Total consumed (mg/d)				
	Basal	20%EW	35%EW	20%WG	35%WG	16 g chow	Basal	20%EW	35%EW	20%WG	35%WG
Arg	37	38	67	36	63	240	277	278	307	276	303
Lys	40	41	71	39	68	224	264	265	295	263	292
Asp	50	58	102	40	70	224	274	282	326	264	294
Glu	178	92	161	265	463	544	722	636	705	809	1007
His	18	15	27	20	35	64	82	79	91	84	99
Pro	56	25	43	87	153	256	312	281	299	343	409
Met	18	26	45	10	17	64	82	90	109	74	81
Cys	18	16	28	20	35	48	66	64	76	68	83
Thr	29	30	52	27	41	112	141	142	164	139	153
Ala	36	42	74	29	58	176	212	218	250	205	234
Gly	29	25	43	33	57	128	157	153	171	161	185
Ser	43	49	85	37	64	176	219	225	261	213	240
Leu	56	57	99	55	97	288	344	345	387	343	385
Ile	34	40	70	28	49	128	162	168	198	156	177
Val	42	50	87	34	60	144	186	194	231	178	204
Tyr	20	26	46	14	24	96	116	122	142	110	120
Phe	40	42	73	38	66	160	200	202	233	198	226
Trp	10	10	18	9	15	32	42	42	50	41	47
BCAA	133	146	256	118	206	560	693	706	816	678	766
LNAA	202	225	393	178	311	848	1050	1073	1241	1026	1159
EAA	294	278	486	307	538	1296	1590	1574	1782	1603	1834

Note. EW = egg white; WG = wheat gluten.

Table 3

Test Diet Mineral Composition

	mg/kg protein			mg/kg					
	EW	WG		Basal	20%EW	35%EW	20%WG	35%WG	Teklad 2018
Ca	890	1420	Ca	6111.4	6342.3	6506.0	6252.6	6533.8	10000
P	890	2600	P	7801.8	8386.8	8550.6	7898.0	8412.8	7000
Na	12380	290	Na	2009.8	3065.4	5343.3	1030.3	1087.7	2000
K	11160	1000	K	4071.7	4183.2	6236.6	4222.2	4420.2	6000
S	17020		S	2550.1	4186.9	7318.6	913.4	913.4	
Cl	10570	435	Cl	2101.2	2600.2	4545.1	1716.2	1802.4	4000
Zn	1.6	8.5	Zn	30.2	30.4	30.7	32.3	33.9	70
Fe	2.4	52	Fe	47.0	47.3	47.7	60.3	70.6	200
Cu	1.7	1.8	Cu	5.6	5.8	6.1	5.8	6.2	15
I	0.05		I	0.2	0.2	0.2	0.2	0.2	6
F	1.6		F	1.1	1.3	1.6	0.9	0.9	
Mg	720	250	Mg	545.4	681.1	813.6	475.2	524.7	2000
Mn	0.5	29.4	Mn						100

Note. EW = egg white; WG = wheat gluten.

Jugular Vein Catheterization Procedure

Catheters were implanted in the right external jugular vein of each rat. Animals were induced with isoflurane and injected along the top of the head with 0.25 percent Bupivacaine solution. A small (<3 cm) incision was made to the neck to the right of the trachea and immediated anterior to the clavicle. A small segment of the jugular vein was located through blunt dissection and a small orifice was made in the vein using a sterile hypodermic needle. A sterile silastic catheter filled with heparinized saline was placed into the vein until reaching the right atrium, then gently pulled back from the atrium for placement in the sinus of the superior vena cava. Two silk sutures were placed under the catheter and vein. The lower suture was tied around the catheter to prevent leakage and the upper to tie off the jugular vein. The catheter was tunneled underneath the skin and externalized through the incision on the top of the head. The neck incision was closed with sterile wound staples. Patency of the catheter was maintained by filling it with a 90 percent glycerol solution (v/v) containing 500 U heparin/mL. The head incision site was then gently opened using small retractors and the tissue gently scraped aside. The skull was lightly debrided using the edge of the scalpel blade. Gelfoam was used as necessary to keep the incision dry and reduce bleeding. The end of the venous catheter was fixed in position with dental acrylic cement and stainless steel screws anchored to the skull. Rats were maintained on isothermal pads to regulate temperature until completely recovered from anesthetic. Carprofen (5 mg/kg body weight) was administered on the day of surgery and two days afterward. Animals were evaluated daily for at least 7 days post-surgery.

Blood Sampling

Rats were given 5-7 days of ad libitum food access after surgery in order to regain lost weight and recover from the operation. Catheter patency was maintained by flushing catheters with heparinized 0.9% saline solution and filling them with 90% (v/v) glycerol solution containing 500 U/mL heparin. The flushing protocol was performed daily for 3 days following surgery and every third day afterwards. After all animals had fully recovered, the feeding schedule resumed and the blood sampling protocol was initiated. Blood samples were collected from the jugular vein (via the catheter) and placed into heparinized microtubes. Approximately 250 microliters of whole blood were collected at each time point. A total of 9 samples were taken every 30 minutes, with the first sample taken 30 minutes before the test diet was given and the final sample taken 210 minutes (3.5 hours) after the test diet was given. Each sample was briefly centrifuged for 10-15 seconds at 6000 rpm and 100 microliters of plasma were harvested. Blood volume was maintained by resuspending harvested red blood cells in a volume of 0.9% sterile saline solution equal to the amount of plasma removed at each time point. The resuspended red blood cells were readministered to each animal through the jugular vein at the time of sampling.

HomeCageScan Software

Default behavioral settings were used. Cages were calibrated before each trial. Size of drink spout and food zone were slightly exaggerated to ensure accurate behavior recognition. Behaviors were divided into eating, grooming, resting, and active categories

(Table 4). Behavioral satiety sequence was defined by the peak of each behavior and exact sequence was eating, active, grooming, resting.

Statistical Analysis

Data were analyzed using SPSS 20 (IBM Corp., Armonk, NY) and Prism (GraphPad Software, Inc., La Jolla, CA). Two-way ANOVA with main effects protein level and source was used to test the effect of diet on cumulative consumption. Two-way repeated measures ANOVA was used in feeding trials where multiple time points were measured, as well as the insulin trial. Differences with P-values < 0.05 were considered statistically significant.

Table 4

HomeCageScan Software Behavior Groupings

Behavior group	Behaviors
Eating	Eat zone 1, eat zone 2, eat zone 3
Grooming	Groom
Resting	Stretch body, stationary, sleep, settling, remain low
Active	Come down, rear up, turn, come down from partially reared, come down to partially reared, rear up from partially reared, rear up to partially reared, walk left, walk right, jump, repetitive jumping, circle, dig, forage, arousal, awaken, sniff, remain rear up, remain partially reared, walk slowly

For the behavioral trial, a 3-way repeated measures ANOVA was run for each dependent variable (eating, grooming, resting, and active) across 9 time points. The between-subjects effects were protein source, protein level, and whether or not the animal received a second meal. The repeated measures were run for within each condition and individual rat identification numbers were used as covariates in the analysis. To assess changes over time, the continuous record for each behavioral category was subdivided into 10-minute (for the first 30 minutes) and 30-minute (for the remaining 150 minutes) time bins.

Study 1

Experiment 1A

Rats were housed in hanging wire cages. Rats were given 5 grams of 1 of 3 test diets during the breakfast period: 35% egg protein, 35% wheat protein, or basal diet) and spillage was recorded. Rats were given ad libitum access to ground chow from 1200 to 1400. Consumption was measured every 30 minutes and spillage was quantified by placing a sheet of paper under each rat during each 30-minute time interval.

Experiment 1B

Rats were given 5 grams of 35% wheat gluten diet, 35% egg protein diet, or basal diet. Blood samples were taken from rats at 30-minute intervals using procedure described above. Plasma samples were frozen at -81 degrees Celsius until analysis. After thawing, samples were analyzed using Rat Insulin ELISA Kit from CrystalChem, Inc. (Downer's Grove, IL).

Study 2

Experiment 2A

Rats were housed in hanging wire cages. The study was a two by two factorial design with main effects being protein source (egg white vs. wheat gluten) and protein amount (18 or 32 percent of kilocalories). Rats were given 4 grams (approximating 20% of total daily intake) of one of the 4 diets for the breakfast period and spillage was recorded. Rats were given ad libitum access to ground chow from 1200 to 1400. Consumption was measured every 30 minutes and spillage was quantified by placing a sheet of paper under each rat during each 30-minute time interval.

Experiment 2B

Rats were placed in shoeboxes and given 5 grams (approximating 20% of total daily intake) of 35% egg protein, 35% wheat protein, or basal diet as breakfast test meal. No second meal was given. Behavior was monitored using HomeCageScan 3.0 software (Cleversys, Inc., Reston, VA) from 1100 to 1400.

Experiment 2C

Rats were placed in standard cages and given 4 grams (approximating 20% of total daily intake) 35% egg protein, 35% wheat protein, or basal diet as breakfast test meal. Behavior was monitored using HomeCageScan 3.0 software (Cleversys, Inc., Reston, VA) from 1100 to 1400. Recording software briefly paused for 3-5 minutes while ground chow second meal was added. Rats were given ad-libitum access to second meal from 1200 to 1400. The trials were repeated using a second set of larger rats, which each received 5 grams of test diet, and the data were combined for analysis.

CHAPTER IV

RESULTS

Experiment 1A

Eight animals were included in analysis. Animals that received a breakfast of wheat gluten group consumed 43% more ground chow (Figure 2) during the test period than those fed egg white ($F_{(2, 21)} = 3.903$, $P = 0.0362$). Differences in consumption between the test diets and basal diet were not statistically significant. Mean test period consumption +/- standard error of the mean was 7.3 +/- 0.7 for the basal diet, 9.5 +/- 0.9 for the wheat gluten diet, and 6.6 +/- 0.9 for the egg white diet.

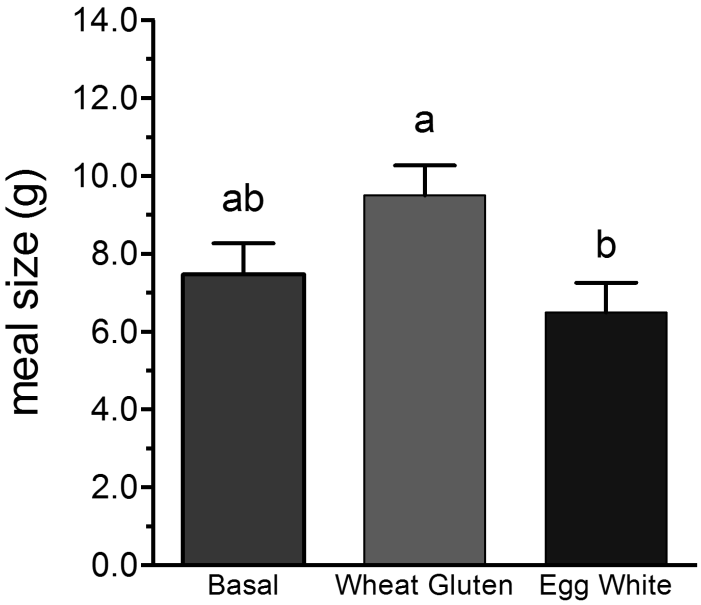

Figure 2. Total Amount of Food Consumed during Test Period in First Study. Bars with different superscripts are different ($P < .05$).

The consumption pattern during the test period was affect by both diet ($F_{(2, 21)}$ = 3.818, P = 0.0385) and time ($F_{(3, 63)}$ = 32.19, P < 0.0001; Figures 3 and 4). Animals in all groups consumed the most during the first 30 minute time period, and the wheat gluten group consumed more than the egg white group during this time period. The wheat gluten group also consumed significantly more during the fourth time period.

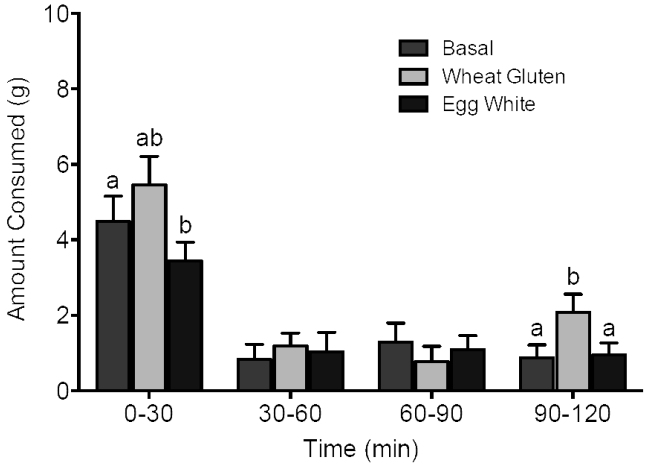

Figure 3. Amount of Food Consumed during Test Period in First Study, by Time Interval. Bars with different superscripts are different (P < 0.05).

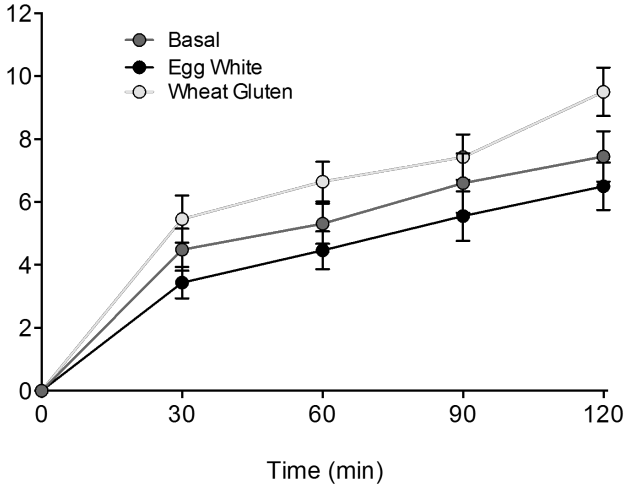

Figure 4. Cumulative Amount of Food Consumed during Test Period in First Study, by Time Interval. Bars with different superscripts are different (P < 0.05).

Experiment 1B

Three sets of samples were analyzed for basal diet, 5 sets of samples were analyzed for wheat gluten diet, and 7 sets of samples were analyzed for egg white diet. Insulin response was increased by all breakfast diets. Those receiving the basal diet had the largest response, which was 95% greater ($F_{(2, 12)} = 4.606$, $P = 0.0328$) than those fed egg white (Figures 5 and 6). Animals consuming the basal diet also had a greater insulin response on average than the gluten group, although this effect was not statistically significant. Area under the curve for insulin response +/- SEM was 580 +/- 129 for the basal diet, 432 +/- 62 for the wheat gluten diet, and 298 +/- 35 for the egg white diet. When examining time points individually, there was a significant effect of diet ($F_{(2, 116)} = 6.489$, $P = 0.0021$) and time ($F_{(8, 116)} = 9.604$, $P < 0.0001$) on the amount of insulin produced.

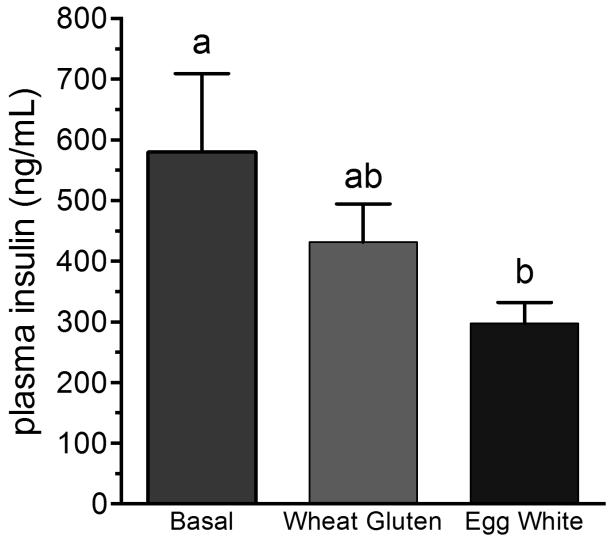

Figure 5. Area under the Curve for Plasma Insulin Response, by Diet. Bars with different superscripts are different ($P < 0.05$).

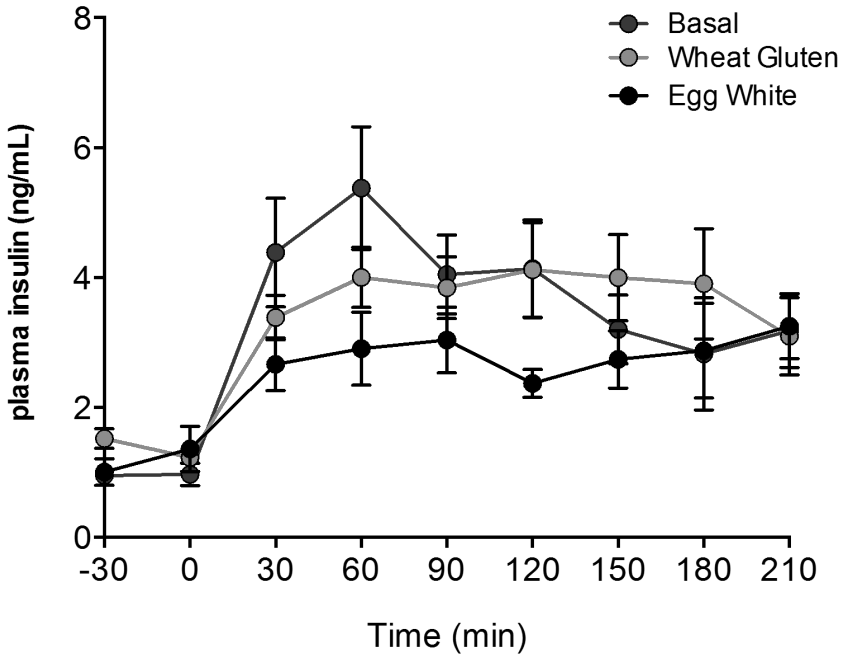

Figure 6. Plasma Insulin Response by Time Interval. Bars with Different Superscripts Are Different ($P < 0.05$).

Experiment 2A

Nine animals were included in analysis. Animals in the 35% protein wheat gluten group consumed 82% more chow ($F_{(1, 32)} = 12.48$, $P = 0.0013$) than animals in the high-protein egg white group (Figure 7). There was not a significant difference in consumption between protein sources in the 20% group. However, there was a significant interaction between protein source and protein level ($F_{(1, 32)} = 7.209$, $P = 0.0114$) because the magnitude of difference in consumption between the two protein sources was much higher at the high-protein level than the low-protein level. Mean total consumption +/- SEM for each group was 6.5 +/- 0.9 for 20% wheat gluten, 6.0 +/- 0.5 for 20% egg white, 8.2 +/- 1.1 for 35% wheat gluten, and 4.5 +/- 0.4 for 35% egg white.

Figure 7. Total Amount of Food Consumed during Test Period in Second Study. Bars with different superscripts are different ($P < 0.05$).

As in the first study, intake of chow during the test period was affected by both diet ($F_{(3, 28)} = 5.402$, $P = 0.0046$) and time ($F_{(3, 84)} = 114.3$, $P < 0.0001$; Figure 8). The high-protein wheat gluten group consumed significantly more than the high-protein egg white group during the first time interval. There was not a statistically significant difference in consumption between protein source in the low-protein diet groups. The animals consumed the largest amount of every diet during the first time interval (Figure 9).

Diet ($F_{(3, 28)} = 4.843$, $P = 0.0077$), time ($F_{(4, 112)} = 202.0$, $P < 0.0001$), and subjects ($F_{(28, 112)} = 9.850$, $P = <0.0001$) affected consumption when the data were measured at each time point. There was also a statistically significant interaction between time and diet ($F_{(12, 112)} = 4.054$, $P < 0.0001$). An examination of means revealed that although the

ranking of the diets remained the same throughout the test period in terms of cumulative consumption, the magnitude of difference between them changed somewhat.

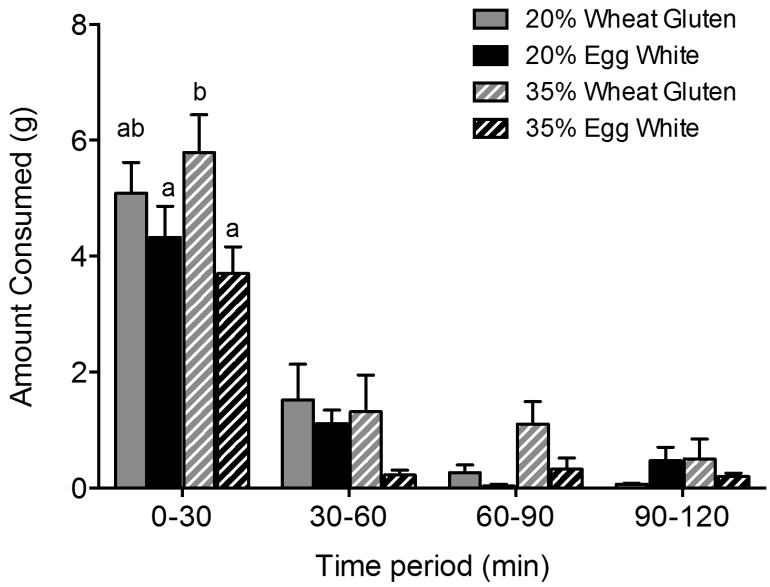

Figure 8. Amount of Food Consumed During Test Period in First Study, by Time Interval. Bars with different superscripts are different ($P < 0.05$).

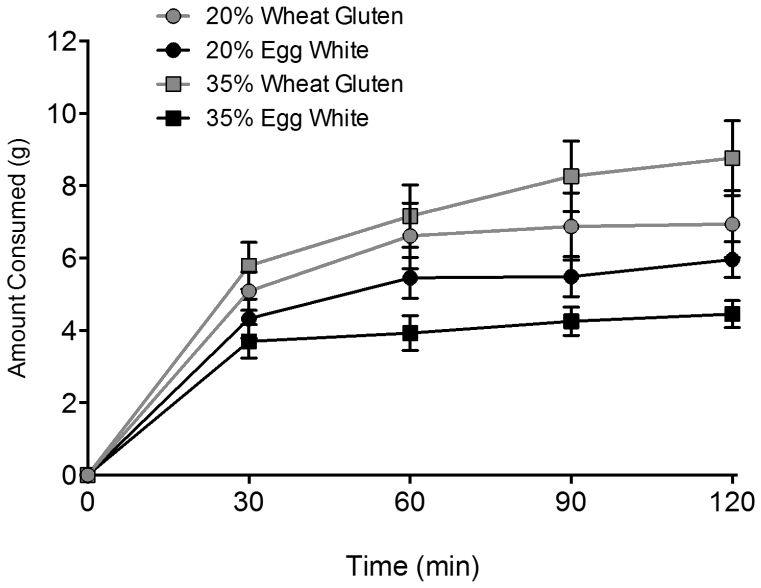

Figure 9. Cumulative Amount of Food Consumed during Test Period in Second Study, by Time Interval. Bars with different superscripts are different ($P < 0.05$).

Experiment 2B

Several of our data points did not display homogeneity of variance as assessed by Levene's Test of Homogeneity of Variances. However, our group decided to run the analysis of variance on the unaltered data set in order to preserve the integrity of the data. Protein level ($F_{(1,87)} = 4.049$, $P = 0.047$) was the only variable that had a statistically significant impact on the eating behavior (Figures 10 and 11). Protein source, protein level, and presence or absence of a second meal did not have a statistically significant effect for grooming. Protein level ($F_{(1,87)} = 8.133$, $P = 0.005$) and presence or absence of second meal ($F_{(1,87)} = 11.139$, $P = 0.001$) made a statistically significant difference in the amount of time spent resting. There was also a significant interaction between level*source ($F_{(1,87)} = 8.377$, $P = 0.005$), source*second meal ($F_{(1,87)} = 11.221$, $P = 0.001$), and source*level*second meal ($F_{(1,87)} = 3.983$, $P = 0.049$). An examination of the means revealed that the low gluten group has a precipitous increase in resting behavior following the breakfast meal, but that resting behavior soon declined and that this group actually had decreased resting behavior compared with the other diets for the rest of the measurement period. Protein source ($F_{(1,87)} = 15.585$, $P = 0.000$) and presence or absence of a second meal ($F_{(1,87)} = 7.272$, $P = 0.008$) impacted the time that the animals spent in the resting behavior, and there was a significant interaction between level*second meal ($F_{(1,87)} = 6.529$, $P = 0.012$) and source*level*second meal ($F_{(1,87)} = 5.135$, $P = 0.026$). Examination of means revealed that this was due to the rapid increase and subsequent decrease in activity seen in the gluten diets.

All diets met the subjective criteria of the behavioral satiety sequence (Figure 12). The rats progressed from the initial phase of eating, through peaks of active and grooming behavior, and to an eventual phase of predominantly resting behavior.

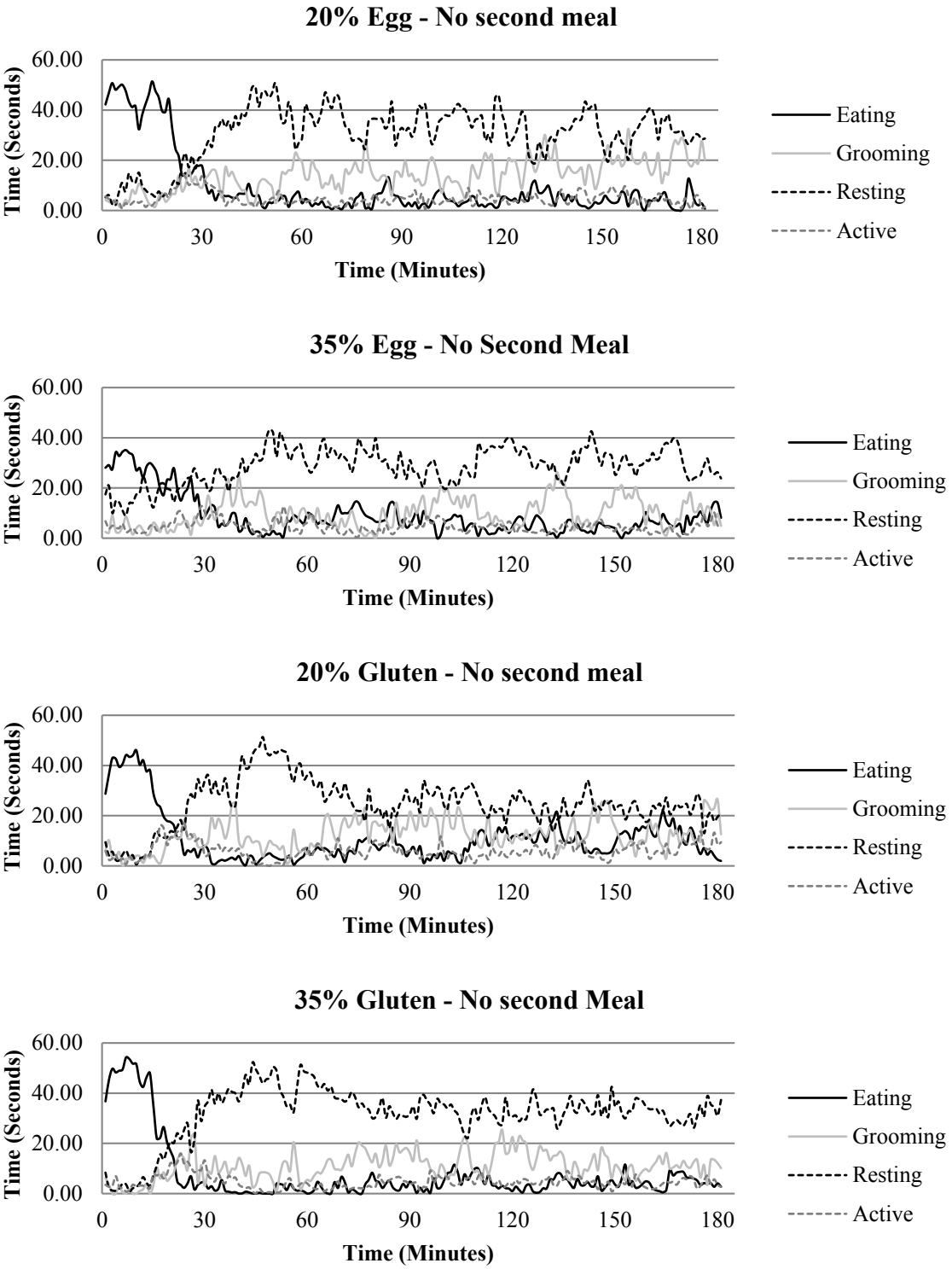

Figure 10. Comparison of Diet by Behavior, without a Second Meal.

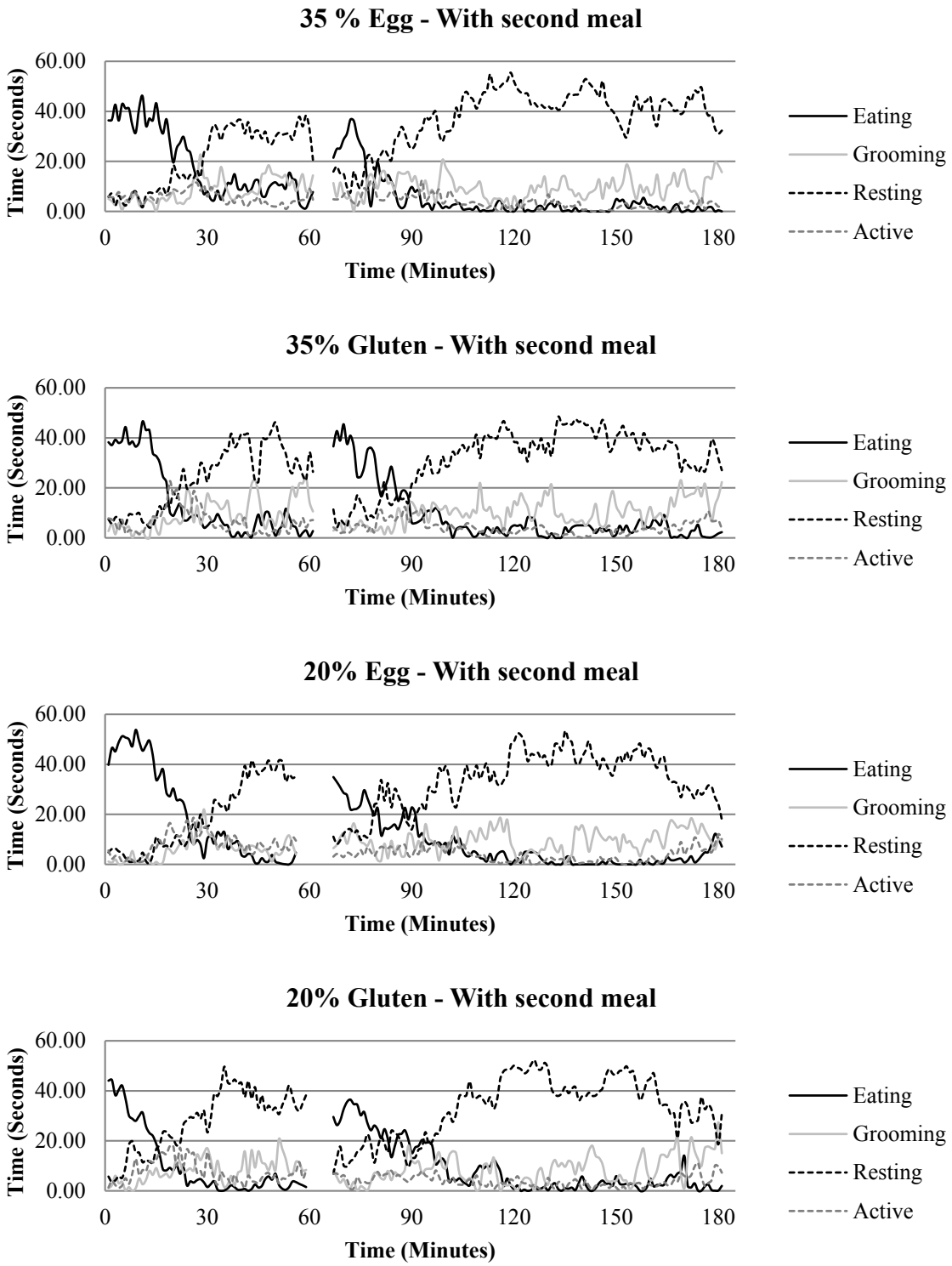

Figure 11. Comparison of Diet by Behavior, with a Second Meal.

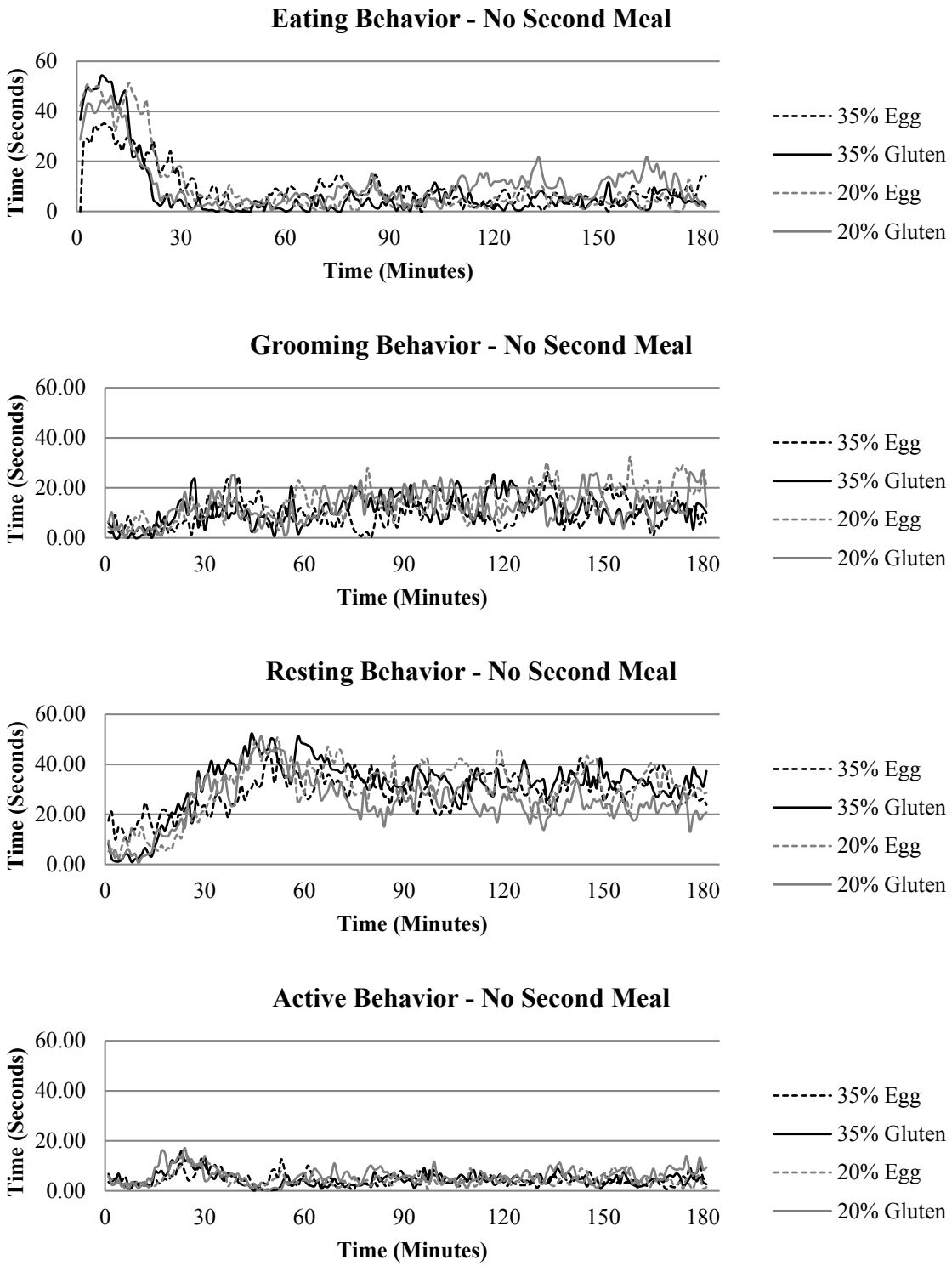

Figure 12. Comparison of Behavior by Diet, without a Second Meal.

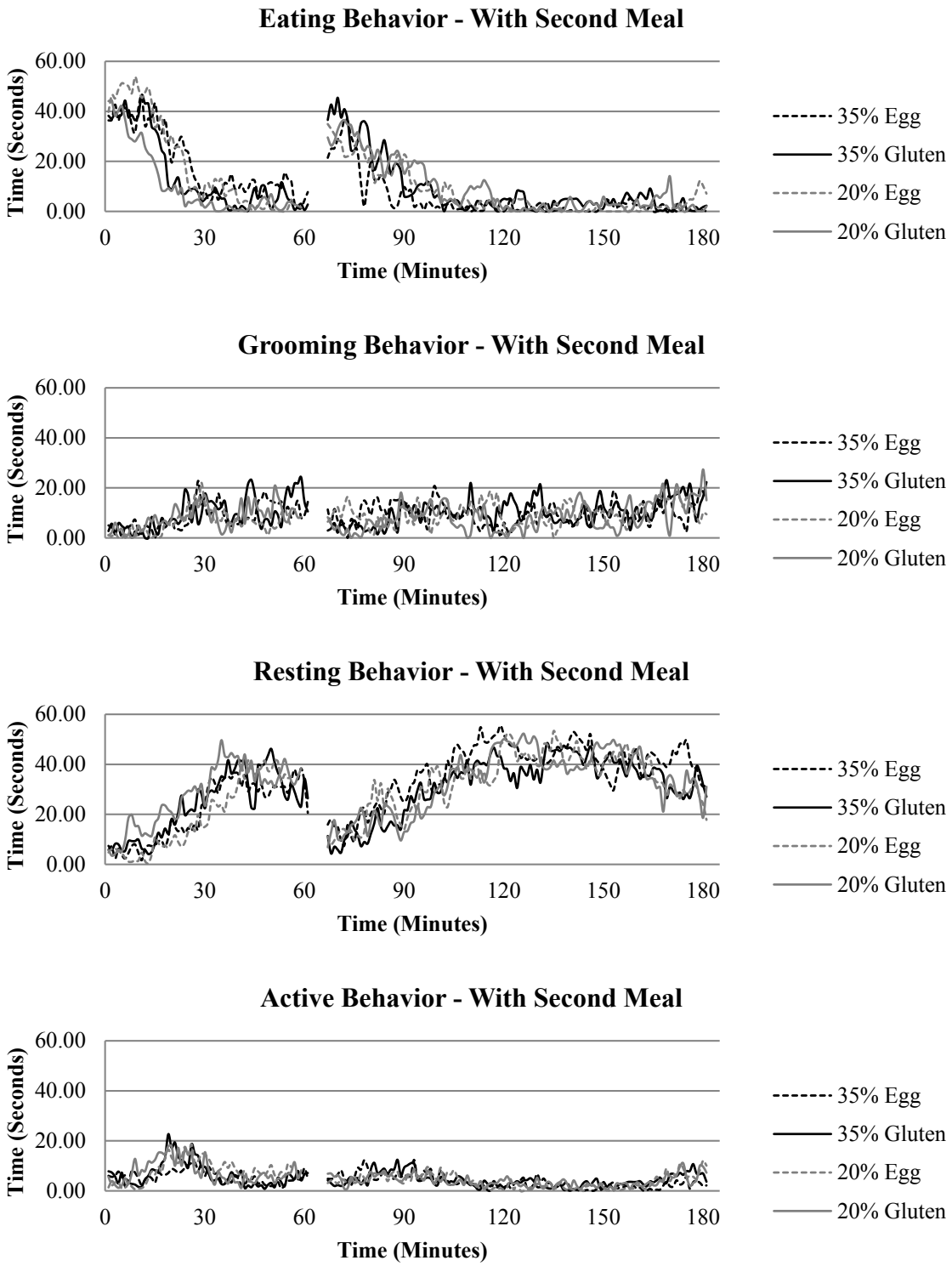

Figure 13. Comparison of Behavior by Diet, with a Second Meal.

CHAPTER V

DISCUSSION

Both the level and source of dietary protein provided at breakfast influenced food intake during a subsequent meal. Egg white was more satiating than wheat gluten protein when both were provided at a higher level (35% of calories). Rats given a high-protein egg white breakfast consumed less of a subsequent chow meal than those given a control diet. Interestingly, rats given the same level of wheat gluten protein displayed reduced satiety, as the size of subsequent meals was larger. Protein source had no effect on satiety when animals were fed a normal level of protein (20% of calories). Because all animals received the same chow diet as the test meal, the effects on intake during the test period, which began 30 minutes after breakfast was removed, was due to breakfast composition. A greater apparent satiety following the higher level of egg white is consistent with observations between increased dietary protein and satiety (Westerterp-Plantenga et al. 2012).

None of our diets induced complete satiety when given as a breakfast meal in quantities approximating 20% of daily ad-libitum intake. This was evidenced by the observation that all rats consumed chow during the first 30 min of the test period. Previous research by our lab group demonstrated that when the rats are given access to chow directly after removal of the test meal, they immediately begin eating (Du K, unpublished data). However, it is difficult to determine if this is because the volume of

diet given is unable to fully induce satiety or if there is simply a delay in satiety after completion of the breakfast meal. Our time interval trials also suggest that the primary difference in consumption between diets occurs during the first 30 minutes of the test period. Further research might include a similar trial with a larger breakfast meal size, although we were unable to do so because the rats refused to completely consume the high-egg diet at 25% of ad-libitum intake. Previous research by our lab group showed that when rats were given the choice between the egg white and wheat gluten diets, they preferred the wheat gluten (Du, 2013). This suggests the rats may have an aversion to the high egg white diet. This possibility was strengthened by the observation that the rats took longer to consume the egg white diet (particularly the high egg white diet) than the wheat gluten diets during our behavioral analysis study.

Rats in the high-gluten group consumed more than rats in the other groups at the end of the testing period (2 hours after breakfast) during our time interval trials. This suggests that wheat gluten is less able to induce satiety, which may be due to differences in processing or nutrients. For instance, egg white contains approximately twice as much tyrosine as wheat gluten (3.3 g/100 g protein vs 1.7g/100 g protein for wheat gluten). Tyrosine directly stimulates dopamine activity in the brain (Fernstrom and Fernstrom, 2007), and increased dopamine levels have been connected with altered meal patterns (Clifton et al. 2000).

Protein digestion rate may also play a role in the duration of satiety. Wheat gluten might be digested so rapidly that its satiating effects have already begun to decrease by the end of the test period. Although the digestion rate of the two proteins has not been

compared directly, egg white has been shown to induce a slow and mild increase in amino acids compared with other protein types (Gannon et al. 1988). The appearance rate and composition of plasma amino acids for each of the protein sources may also be related to protein quality. Egg protein has the highest PDCAAS score (an index of protein quality) available of 100 (Schaafsma, 2000). Wheat protein has a much lower-quality rating with a PDCAAS score of 42. This is due somewhat to amino acid content (98 for egg vs 91 for wheat), but to a much greater extent due to decreased digestibility (121 for egg vs. 47 for wheat). Plasma amino acids have been shown to affect satiety (Mellinkoff et al. 1956), but whether or not this is the mechanism influencing our results remains to be seen.

Another factor that distinguishes the two diets is sulfur content. The egg white diet contained over 7 times the amount of sulfur of the wheat gluten diet (6.99 mg per g of 35% egg white diet vs 0.91 mg per g of 35% wheat gluten diet). Although there is little to no literature on the effect of sulfur on satiety, it is possible that the egg white group ate less during the test period to avoid excess sulfur consumption.

Differences in gastrointestinal hormones influencing satiety may differ between the two protein sources. Wheat protein is a stronger stimulator of CCK and GLP-1 release in human duodenal tissue than egg protein (Geraedts et al. 2010). Duodenal CCK results in an increase in the firing rate of the vagus nerve, which is strongly associated with satiey (Faipoux et al. 2008). Hormonal mechanisms are often complex, however, and further research would be needed to determine if hormonal effects are responsible for our results. The observation that egg white induced a weaker insulin response than wheat

gluten is relevant given its increased levels of tyrosine. Tyrosine is regarded as insulinotropic (van Loon et al. 2000), yet insulin response was lowest for the egg white diet. This is the opposite of the expected finding, given the higher expected bioavailability of amino acids from egg white protein. Future research might include an analysis of blood glucose levels after the test meal.

Most of the existing research comparing protein sources focuses on the milk proteins casein and whey. A limited number of studies have been conducted with non-milk protein sources, and they have provided mixed results. Several studies have reported that egg protein is not more satiating than other protein sources. For example, Anderson and colleagues (2004) reported that human consumption of an egg albumin preload (45-50 g protein) actually increased energy intake relative to control, in contrast with comparable whey and soy preloads which decreased subsequent energy intake. Abou-Samra and colleagues (2011) reported that unlike casein and pea proteins, egg white did not decrease ad libitum consumption. Interestingly, rats given a 40% egg protein diet had lower intake from 90 to 180 minutes after preload compared with casein, lactalbumin, and soy protein (Semon et al. 1987).

To our knowledge, only one other study has compared the satiating effects of egg white and wheat gluten. Lang and colleagues examined hunger, satiety, and 24-hour energy and macronutrient intake in humans following a lunch containing casein, gelatin, soy, pea, wheat gluten, or egg white protein. They found no differences between the different sources of protein. However, these results might be explained by the large meal size (4934-5441 kJ), the fact that the test meals only contained 22% of calories from

protein, or because only 65% of the protein in each meal came from the test source. Furthermore, ad-libitum consumption was not tested until 8 hours after the test diet was administered and, unlike our study, a wide variety of different foods were offered for the ad-libitum test period.

All of the test diets induced the behavioral satiety sequence in rats. It's worth noting that the eating behavior only reported whether or not an animal was present at the food cup, and did not take into account the amount which was actually consumed.

When room temperature was reduced (see appendix) the effect of protein source was not present. This suggests that there are complex and potentially overlapping mechanisms which affect post-breakfast meal consumption.

Probably the most interesting finding of our study is that the high-protein wheat gluten group consumed more than the low-protein wheat gluten group, although the results were not statistically significant. This suggests that the wheat gluten, or a component of the wheat gluten, is exerting an orexigenic effect on the animals. These findings are in contrast with previous literature, which found that a high-gluten preload decreased ad-libitum consumption comparably to whey and soy protein (Bowen et al. 2006). Furthermore, the researchers found that wheat gluten depressed plasma ghrelin (an orexigenic hormone) and increased GLP-1 and cholecystokinin similarly to the other proteins tested. The decreased digestibility of wheat gluten also seems to be an unlikely explanation for our results, given that soy protein has a very high digestibility rating (96) (Schaafsma et al. 2000). Although insulin is orexigenic, our ELISA results suggest that the basal group would have the greatest consumption if this was the salient mechanism

(Crespo et al. 2014). A complex mechanism resulting in an orexigenic effect from wheat gluten is possible, but further research would need to be done in order to determine if such a mechanism exists.

More research needs to be done in order to further elucidate the relationship between protein source and satiety. An analysis of plasma amino acids after consumption of the the two protein sources would be particularly beneficial in examining their relationship to insulin secretion. Furthermore, analysis of amino acids in the brain might illuminate neural mechanisms underlying our results. Understanding these mechanisms might allow us to design diets which are more effective at inducing long-term satiety.

In summary, both protein level and source affect acute satiety after a breakfast-like meal in rats. At high-protein levels, egg white protein increases satiety while wheat gluten decreases it. Protein source did not affect satiety when animals were given a breakfast containing a normal level of protein. While the mechanisms of action of these effects are unclear, the results provide new support for further research examining the effects of protein source and level.

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