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THE PRE MEAL PRIMING OF GASTROINTESTINAL SATIETY FACTORS

by

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THESIS DEFENCE

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ABSTRACT

Background: Although experimental studies on meal frequency are few, results show that increasing the number of daily meals leads to decreased energy intake (EI). No studies have yet looked at the type of meal patterning that would maximize this reduction.

Objective: The purpose of this study was to determine if structured preloads affects EI, appetite, and Peptide YY (PYY) and Glucagon like peptide-1 (GLP-1) concentrations in normal weight men.

Design: Eight normal weight men (25.3 ± 2.6 kg/m^2) participated in a randomized crossover trial. In condition 1 the subjects were able to self select their snacks *ad libitum.* In condition 2 the subjects consumed a standardized preload (300 Kcal: 40 % protein, 40% carbohydrate and 20 % fat) 15 minutes prior to lunch and dinner. During condition 3, participants consumed the preload, at times that maximized their pre meal fullness levels. During each condition, a standardized breakfast was served, while lunch and dinner were self-selected from a 5 item menu, and eaten *ad libitum.* Daily EI, fasting and postprandial appetite, PYY, and GLP-1 were sampled every 30 minutes for 9 hours for all 3 conditions.

Results: No difference in daily EI, AUC GLP-1, and PYY was noted between conditions. Desire, hunger, and PFC were found to be lowest during the control session ($P<0.05$). Finally, GLP-1 concentrations were correlated to fullness levels with significance during conditions 1 ($R=0.81$, $P<0.05$) and 3 ($R=0.89$, $P<0.01$).

Conclusion: Structured snacking does not affect EI over the short term.

Key words: Energy intake, appetite, PYY, GLP-1, preload, meal frequency

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CHAPTER 1: INTRODUCTION

OBESITY PANDEMIC

Obesity has become an increasing concern over the past several years due mostly to its related comorbidities. Statistics Canada released a report in 2005 suggesting that 23.1% (5.5 million) of the adult population in Canada (over 18 yrs) was obese (BMI≥30 kg/m^2) and another 36.1% (8.6 million) was overweight (BMI≥27 kg/m^2). Similarly, rates have increased worldwide. In 2006, the World Health Organization reported 1.6 billion overweight and another 400 million obese adults (over 15 yrs) worldwide. The rising numbers of affected individuals has lead to increased need for understanding in the area of obesity and weight regulation.

ENERGY BALANCE

Energy balance is achieved when Energy Intake (EI) (ie. macronutrient intake) equals energy expenditure (EE) and nutrient partitioning (Flatt 1988). Thus, in order to maintain weight one must be in a state of energy balance. The notion of energy balance is complex as one of side on the equation, EI is episodic, occurring only when one ingests food; however, on the other side of the equation, EE is continuous with the majority of the expenditure (70%) occurring at rest and labelled the resting metabolic rate. For this reason, the body is constantly fluctuating between positive and negative energy balances, and it is the sum of these fluctuations that will determine energy balance during weight stability. When the body is in a state of increased EE or energy restriction, the sum of the energy fluctuations leads to a state of negative energy balance and

ultimately weight loss. On the other hand, it is only when we eat that energy balance becomes positive, so weight increase is achieved when the sum of the fluctuations leads to a continuous state of positive energy balance. Therefore, when the sum of positive energy balances exceeds the sum of the negative energy balances over extended periods of time, the likelihood of becoming overweight or obese increases and is proportional to the energy imbalance. This positive energy balance can occur in several different ways, including: 1) An increase in EI, 2) a decrease in EE, and/or 3) a combination of the two. For the purpose of this literature review, EI and its impact on body weight regulation will be examined.

OBESITY TREATMENT

Current treatment options vary depending on the severity of the obesity (in terms of BMI), the health complications involved, and the willingness of the individual to change. However, it is important to note that individuals are able to receive the treatment they prefer, resources permitting, with the exception of surgery, which is available for specific at risk populations only. For example, in order to be eligible for surgery, individuals must have a BMI of over 40 kg/m2, or a BMI of over 35 kg/m2 with medical comorbidities (NIHR, 1992). The surgical method has shown to be most effective for the treatment of severe obesity (Mun, Blackburn et al. 2001; Brolin 2002; Buchwald, Avidor et al. 2004; Christou, Sampalis et al. 2004). Unfortunately, due to the expenses involved, the selection criteria needed to undergo the surgery and the possible post operation complications following the surgery, this method is not applicable to everyone.

Lifestyle changes, however, are available to everyone, and when looking at these weight loss techniques, it has been estimated that with over 30% of the US

population reporting recent weight loss attempts, it would seem that current lifestyle treatment programs are not effective over the long term (Williamson, Serdula et al. 1992; Serdula, Williamson et al. 1994). In fact, research has shown that a common regain in successful losers occurs after 5 years (Andersen, Backer et al. 1984; Brownell, Steen et al. 1987).

The most accessible treatment includes these aforementioned lifestyle changes (ie. through a decrease in EI and/or increase in EE). Many commercial diets have been created such as Weight Watchers, the Atkins Diet, Jenny Craig etc... Diets can be formed using two types of energy restriction: non macronutrient specific reduction and macronutrient specific energy restriction. Non macronutrient specific reductions include very low calorie diets (less than 800 Kcal per day) where individuals have been found to lose up to 20 kg in 12-16 weeks, and low calorie diets (between 800 and 1200 Kcal/day) causing a weight loss of approximately 8.5 kg in 20 weeks (Wadden 1993) where as macronutrient specific diets include manipulating energy substrates from ingested foods. An example of a macronutrient specific energy restriction is the low fat diet that has been commonly attempted in the past, with the rationale being that fat is the most concentrated source of energy and is preferentially stored in adipose tissue (Jequier and Bray 2002). Further, randomized control trials have supported the notion that limiting fat intake leads to weight loss (Yu-Poth, Zhao et al. 1999). However, it has also been found that low fat diets yielded similar results to low calorie diets when looking at weight loss (Pirozzo, Summerbell et al. 2002). For this reason, further emphasis has been placed on playing with other macronutrients such as decreasing carbohydrate (CHO) intake (Westman 2002; Westman, Yancy et al. 2002; Westman, Mavropoulos et al. 2003) or increasing protein intake (Nair, Halliday et al. 1983; Skov, Toubro et al. 1999).

As supported by a number of recent studies, it would seem that the success rate of most of these conventional diets is short-lived. In fact, one study showed that individuals had a weight relapse of 71-77% (depending on the weight loss technique used) of their original weight loss after 4-5 years (Anderson, Konz et al. 2001). This relapse to initial body weight may be attributed to the concept of body weight set point (Leibel, Rosenbaum et al. 1995), i.e. that body weight is carefully regulated within a specific range. More specifically, individuals become predisposed to post weight loss adaptations as a mechanism of maintaining this body weight set point. Some of these adaptations include, adaptive thermogenesis (Leibel, Rosenbaum et al. 1995), decreased lipid oxidation (Larson, Ferraro et al. 1995), decreased sympathetic nervous system activity (Arone, Mackintosh et al. 1995), increased appetite (Doucet, Imbeault et al. 2000), and decreased leptin concentration (Doucet, Imbeault et al. 2000). These adaptations are in turn able to antagonize further weight loss and possibly even cause weight re-gain. With the realization that the post weight loss adaptations arise when in a state of negative energy balance, research needs to focus on the "vulnerable state" post weight loss, and ultimately on methods that could minimize these susceptibilities to weight re-gain.

BODY WEIGHT REGULATION

One study that examined the effects of energy deprivation on the body was the Minnesota experiment. This experiment looked at the effects of food deprivation on the autoregulatory phenomenon, which attempts to restore body weight (Dulloo, Jacquet et al. 1997). More specifically, the study consisted of 4 phases in which the first included several weeks of baseline data collection (control), the second phase consisted of several weeks of semi-starvation where the EI was 6.61 MJ including 25% Protein, 17% Fat and 58% CHO, followed by

the third phase of restricted refeeding and finally the final stage of ad libitum refeeding (approximate average of 20 MJ/day where 14% came from protein, 35% from fat and 51% from CHOs). The results from this study showed that hyperphagia (45-60% of control EI) in the refeeding phases were mainly explained by the degree of fat and fat free mass (FFM) depletion. In other words, with a greater depletion in fat and fat free mass stores, there is a simultaneous increase in EI. Further, figure 1 shows that fat recovery occurs at a faster rate than FFM recovery, so EI is initially increased to restore fat mass and continues to remain elevated once fat stores are replenished as a method of restoring the fat free mass stores.

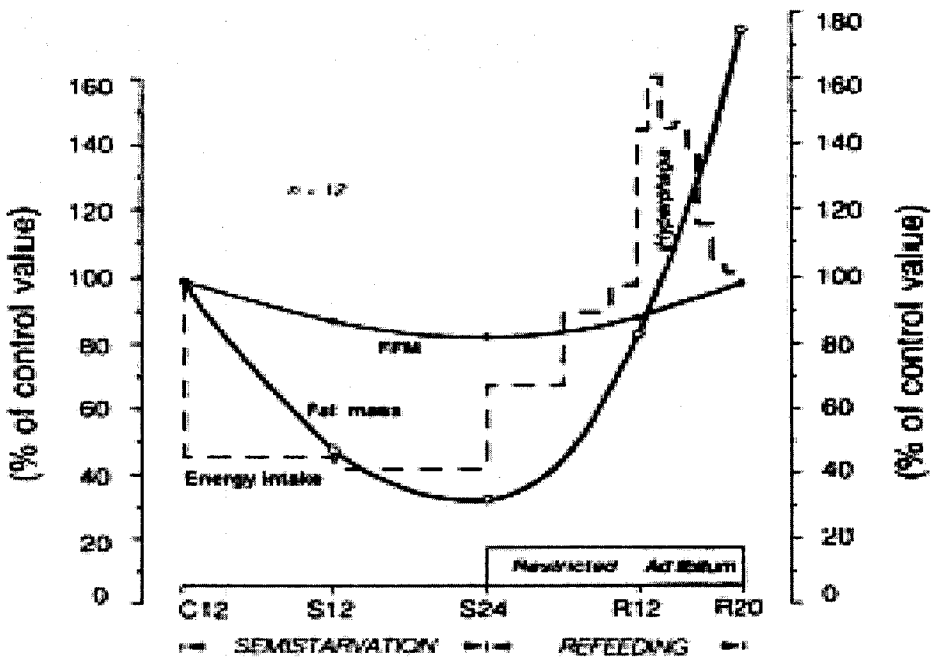

FIGURE 1. Pattern of changes in energy intake, body fat, and fat-free mass (FFM) during semistarvation and refeeding. All values are expressed as a percentage of corresponding values during the control (prestarvation) period.

(Dulloo, Jacquet et al. 1997)

This idea of increased EI post semi-starvation can be attributed to one of the two tiers of the feeding model. Briefly, the feeding model includes the bottom-up control mechanism and the top down control mechanism(Cameron and Doucet 2007). In the case of weight loss, increased EI as a result of starvation could be argued as following the theory of bottom-up control. Further, bottom-up control refers to the "drive theory", whereby an individual will be driven to eat based on signaling from the periphery, i.e. appetite regulation hormones, chemo and mechanoreceptors, and gastrointestinal peptides. The periphery will then signal the appetite centers in the brain, leading to appetite changes. Conversely, the top down theory of eating suggests that an individual will eat for emotional or social reasons. This model will further be developed in the review of literature.

Seeing as the Minnesota experiment explores the effects of semistarvation on EI, I would argue that the main cause of feeding in this situation would be a result of the periphery sending signals to the central nervous system concerning the depleted FM and FFM, hence bottom up control. Further, possible mechanisms of autoregulation could be witnessed in the changes in nutrient partitioning, thermogenesis, and/or appetite, which were all previously shown to be affected following weight loss. This review will attempt to focus on the third of these control system, namely the one involving hunger and appetite, by concentrating on two of the novel anorectic gastrointestinal peptides including Peptide YY PYY) (Adrian, Ferri et al. 1985)and Glucagon like peptide-1 (GLP-1) (Holst 1994), which are released in from the distal small intestine in response to EI. Finally, although the effects of weight loss on appetite justify our rationale,

this study will focus on the short term effects of manipulated meal frequency on peptide profiles, meal time and total daily EI during energy balance.

THE PROBLEM

A common weight loss method is through a decrease in EI. Although results are often successful in the short term (Andersen, Backer et al. 1984; Brownell, Steen et al. 1987), leading to weight loss, patients tend to regain the weight they lost, possibly due to the weight set point theory (Leibel, Rosenbaum et al. 1995). It has been shown that EI can be significantly altered following a period of weight loss or food deprivation (Dulloo, Jacquet et al. 1997), which in turn could cause a person to regain the weight they once lost on an energy restriction diet. This may be due to a loss of appetite control, which in turn could be explained by changes in gut peptides.

We aim to develop a strategy that may help control weight by first attempting to control appetite through dietary manipulations of some of the novel peptides.

PURPOSE

The main purpose of the study was to determine whether structured preloads in the form of snacks could lead to a decrease in EI. More specifically this study investigated the effects of a high protein/high CHO preload offered twice a day (before lunch and dinner) at times that would maximize meal time fullness which would be expected to be at peak PYY and GLP-1 levels. The main outcome was then to investigate the effects of this elevated fullness on subsequent meal time EI. A secondary purpose was also to investigate daily profiles of GLP-1 and PYY.

HYPOTHESES

The first hypothesis proposed that the timing of the preloads, so that fullness was peaked prior to meal initiation, would cause the participant to eat less during that meal, which would in turn cause a decrease in total daily caloric intake during the experimental session.

The second hypothesis assumed that daily total PYY and GLP-1 levels would be higher during the timed preload condition.

DEFINITIONS

In the present document, ad libitum food intake can be defined as the amount of food consumed during free feeding. Gastrointestinal peptides (or Gut or GI peptides) can be defined as peptides that are secreted into the small intestine and that work in conjunction with the central nervous system to affect appetite and food intake. And a preload can be defined as a milkshake of 300 Kcal (40% protein, 40% CHO, 20% fat). Finally a timed preload, is one that is given at times that will further induce meal time (lunch and dinner) fullness.

LIMITATIONS, DELIMITATIONS, AND ASSUMPTIONS

During the course of this study it was assumed that the participants would follow the protocols that were asked of them, including the 12 hour overnight fast prior to the experimental sessions. It was also assumed that the individuals would answer truthfully to the questionnaires given during both screening and experimental sessions, including the visual analogue scales.

The study was also delimited to a sample of normal weight (20<BMI<30 kg/m2) males that were healthy, non-smokers, and between the ages of 20 and

55 years. Further, these men had to be regular eaters (eat at least 3 meals a day for a minimum of 5 days per week).

Also, there were some limitations to the study that were uncontrollable. One of these included the influence that the top down control theory of feeding had on appetite, such as eating for emotional or motivational purposes. In fact, this study focused only on the bottom up theory of feeding, and more specifically the influence of fullness and the short-term appetite regulatory peptides on subsequent EI, which may have limited generalizability to outside of the laboratory. Another factor that may have limited generalizability was the small sample size, which was chosen due to time and monetary constraints related mainly to multiple peptides assays.

Finally, it is important to note that we were not able to compare peptide concentrations and appetite measurements at specific time-points because the statistical power was too low. Although a larger sample size would have allowed us to explore this option, we decided to go ahead with multiple sampling to obtain daily profiles of these gut hormones.

RELEVANCE OF THE STUDY

As opposed to the long-term appetite regulators, few studies have looked at the effect that some of the short-term GI peptides have on EI. In this study, we examined the influence that fullness, total PYY and GLP-1 had on appetite and EI in lean men. These two peptides were chosen because they have been found to have additive, inhibitory effects of EI (Neary, Small et al. 2005). No study had

yet examined the effects of a preload, given at times that further maximized fullness and the effects of the peptides at meal times, on meal time EI and daily peptide profiles. Seeing as eating is a main component of energy balance and weight loss, and that weight loss in turn affects the control of appetite, an increased understanding in the area of short-term appetite regulators is merited.

CHAPTER 2: LITERATURE REVIEW

BODY WEIGHT REGULATION

One of the problems with body weight regulation is the increase in EI post weight loss (Blundell and King 1998). In fact there is a tendency for the body to return to basal levels of weight and adiposity once ad libitum feeding is resumed(Havel 2001) . As mentioned previously, the weight returns to basal levels after a weight loss attempt due to decreased thermogenesis (Leibel, Rosenbaum et al. 1995), decreased leptin concentration (Doucet, Imbeault et al. 2000), and increased appetite (Doucet, Imbeault et al. 2000). Seeing as EI is central to the regulation of energy balance, and thus weight stability, and that appetite affects EI, the increased appetite that is witnessed post weight loss is an essential area in the study of weight maintenance. Further, it is important to recognize the factors that initiate changes in appetite.

The changes in appetite post weight loss could in part be explained by changes in some of the gut peptides. In order to understand these changes we

need to reexamine the two-tiered feeding model developed by(Cameron and Doucet 2007). Briefly, this model is demonstrated in figure two below.

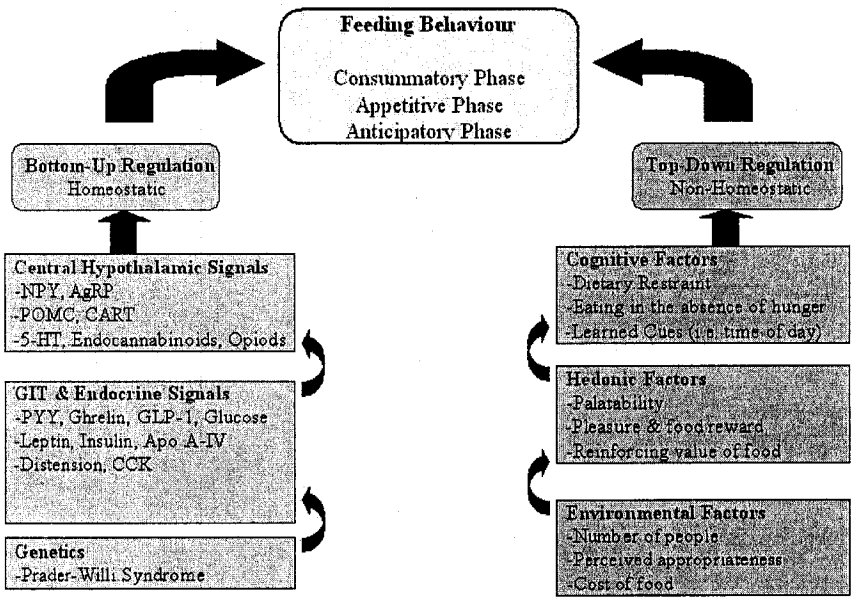

Figure 2: Demonstrating the two-tier regulation of feeding. (Cameron and Doucet 2007)

Although the top- down regulation does play a large role in feeding, this review will focus on the bottom-up regulation in order to alleviate possible changes in appetite that could arise from food deprivation, in other words, to minimize the cues/feeding signals that the periphery relays to the CNS. Finally, the following discussion of bottom up regulation will be broken into two sections including: Long term body weight regulation and short-term EI regulation.

LONG TERM BODY WEIGHT REGULATION: APPETITE REGULATION

When looking at the body as a whole, there are large, daily variations in CHO and protein stores in adults, so it is proposed then that body weight

regulation is achieved as a function of the adipose tissue stores (Kennedy 1953). Further, many studies have indicated that adipose tissue stores in human and other mammals are tightly regulated (Adolph 1947; Hervey 1969; Leibel, Rosenbaum et al. 1995). Recently, even more emphasis has been placed on the study of adipose tissue as it secretes a number of endocrine and paracrine mediators including leptin, which has been shown to influence appetite (Heini, Lara-Castro et al. 1998). This idea is further supported in the Minnesota experiment as hyperphagia is increased during the ad libitum phase in individuals that had lost weight (ie. Fat mass) due to energy restriction. This increased hyperphagia in response to depleted fat and fat free mass stores would seem to have resulted from the signaling of energy reserves between the periphery and the brain.

These long-term peripheral signals are hormones that are released in response to changes in adipose tissue stores as well as through the amount of energy consumed over a long period of time. These hormones act to regulate body weight through 3 steps: 1) peripheral sensors monitor the energy stores, 2) the signals are transmitted to the hypothalamic centers, which receive and integrate the intensity of the signals through the hormone receptors in the brain and 3) the effector systems are activated which in turn influence the 2 components of the energy balance equation (EI and EE) (Jequier and Tappy 1999). Insulin and leptin are both examples of hormones that have effects on body weight regulation, and their mechanisms will be further described below.

Also, seeing as both of these hormones act on the central nervous system, an explanation of the appetite centers in the brain will be given.

Central Nervous System:

In the bottom up control, or homeostatic theory of feeding, changes in appetite can be explained by the communication between signals in the periphery and the appetite centers in the brain. The main area in the brain that receives the hormonal signals is the arcuate nucleus (ARC), which is located in the mediobasal hypothalamus, alongside the third ventricle (Broberger and Hokfelt 2001). Located in the ARC are two neurochemically different sets of first order neurons, including: 1) Neuropeptide Y (NPY) and agouti-gene-related protein (AgRP) and 2) proopiomelanocortin (POMC). Activation of NPY and AgRP causes decreases in EE and increases in EI while activation of POMC leads to increases in EE and decreases in EI. These neurons are considered first order because they have direct contact with the peripheral hunger and satiety factors. This is due to the absence of the blood brain barrier in the median eminence resulting in axon terminals that are in direct contact with the bloodstream. Once peripheral signals (ie. Insulin and/ or leptin) come into contact with the first order neurons, a series of events results in the activation of second order neurons. For example, POMC is a large precursor protein which gives rise to several bioactive peptides including alpha and gama melanocyte-stimulating hormone (MSH). Both of these secondary proteins have been shown to exert anorexigenic effects when administered (Poggioli, Vergoni et al. 1986) (Fan, Boston et al. 1997). Similarly, activation of POMC can increase the release

of another anorexigenic peptide known as cocaine- and amphetamine-regulated transcript (CART).

When looking at the combined effects of NPY and POMC, it is found that NPY neurons are able to control POMC cells through NPY's co-expression of AgRP, which is an endogenous melanorcortin antagonist (Ollmann, Wilson et al. 1997; Broberger, Johansen et al. 1998; Hahn, Breininger et al. 1998). In other words, the release of AgRP will cause the blockade of melanocortin action, which is turn will cause an increase in food intake. Secondly, it seems that there is an asymmetrical interaction in the ARC, which favours the orexigenic NPY/AgRP over the anorexigenic melanocortin signaling. Although their brain centers seem to favor the orexigenic effect, most of the appetite-regulating hormones have been shown to be anorexigenic. A few of these hormones are discussed below.

Insulin:

Insulin is a hormone that is released from the beta cells in the pancreas in proportion to visceral fat content (Wajchenberg 2000) and in response to increased glucose concentration in the blood. Visceral fat content has the highest lypolitic rate in comparison to other fat depots (Martin ML 1991), therefore increases in visceral fat lead to increased lypolysis and in turn increased levels of plasma free fatty acids. The higher levels fatty acids in the hepatic artery block insulin binding and degradation in the liver leading to inhibited glucose suppression (Svedberg J 1990). Seeing as the glucose levels in the periphery remain at high levels, insulin is continually released, as it is essential for glucose uptake and metabolism in peripheral tissues (Ferreannini E

1983; Bevilacqua S 1987). Although little electrophysiology has been done on the mechanism of insulin (Jobst, Enriori et al. 2004), both physiological and anatomical evidence have shown that the activation of central insulin receptors decreases food intake and body weight (Woods, Porte et al. 1985) through the activation of the anorexigenic POMC neurons and the inhibition of the orexigenic NPY/AgRP neurons. In fact, knocking out the insulin receptor in POMC neurons results in hyperphagia and obesity in mice (Bruning, Gautam et al. 2000).

Peripherally, however, insulin is anabolic where it is released in response to sugar and acts to increase lipid synthesis and storage. In healthy individuals, insulin will peak in response to glucose ingestion and then return to basal levels once glucose uptake has occurred, however this trend is not seen in individuals with hyperinsulinemia. This condition will lead to increased lipid synthesis or inhibited lipolysis (Cahill 1971), which in turn could result in increased fat stores and weight gain. These anabolic effects can further be seen where the exogenous administration of the hormone causes increases in body fat in individuals with type 2 diabetes(Groop, Widen et al. 1989; Makimattila, Nikkila et al. 1999) (Rigalleau, Delafaye et al. 1999) (Sinha, Formica et al. 1996; Bagg, Plank et al. 2001).

Leptin:

Leptin is a product of the obese (ob) gene that is present in mouse and human plasma (Zhang, Proenca et al. 1994). Therefore, in the ob/ob mouse, which either has 1) a mutation in the ob gene leading to a premature stop codon or 2) the total absence of mRNA, there is no leptin production, and a tendency

towards obesity (Coleman 1978). This tendency towards obesity may result from the absence of leptin, as this hormone has been found to reduce EI and increase EE (Halaas, Gajiwala et al. 1995), which can further be seen with leptin injection. Further, injections of leptin into the ob/ob mouse and normal animals results in decreased body weight and decreased EI (Halaas, Gajiwala et al. 1995; Maffei, Halaas et al. 1995).

Interestingly, another type of mouse was studied, the db/db mouse, which was shown to be phenotypically identical to the ob/ob mouse, yet had high circulating levels of leptin. Thus, the tendency towards obesity occurred as a result of the db mutation, which encodes the cytoplasmic domain of the leptin receptor causing resistance to the hormone through a mutated receptor (Lee, Proenca et al. 1996). Furthermore, injecting leptin into these mice had no appetite reducing effects nor did it have any effects of weight reduction.

A similar trend was seen in obese participants, where leptin was released in proportion to adipose tissue stores, indicating that these individuals were insensitive to endogenous leptin, and for this reason, became obese (Considine, Sinha et al. 1996). Also important to note is that there is a decrease in leptin post weight loss (Considine, Sinha et al. 1996; Doucet, Imbeault et al. 2000) (Sinha, Ohannesian et al. 1996), which is associated with increased appetite ratings, suggesting leptin's role in long term energy regulation. Finally, the postprandial rise in insulin is not associated with increased leptin levels in humans (Considine, Sinha et al. 1996). In other words, meal size does not affect leptin production. This further suggests that leptin's main role is in the regulation of long term

weight regulation and that other factors must be involved in the daily regulation of appetite.

SHORT-TERM APPETITE REGULATORS

Understanding the short-term appetite regulators requires an understanding of the feeding cycle. As mentioned previously, eating occurs in episodes, and it is hunger, or the drive to eat, that usually results in meal initiation. During the next phase, satiation is said to occur once eating has commenced and usually suppresses hunger resulting in meal termination. Further, satiation depends on the size of each meal as well as the length for which the meal is ingested. Satiety is reached following the meal, once the feelings of hunger are completely suppressed (Jequier and Tappy 1999). Understanding the feeding cycle allows us to study the causes of hunger, satiation and satiety, or in other words to identify the signals caused by the absence and/ or presence of food. Furthermore, the following sections will describe the regulation of feeding through bottom up control, only, but it is important to note that there are other factors involved in the feeding process (ie. top down control) that we will not cover here.

Some of the peripheral mechanisms that regulate short-term energy stores include chemo and mechanoreceptors, which send information to the brain through the vagus nerve following nutrient ingestion (Mei 1986). This regulation occurs during the feeding process itself and occurs in response to the content and volume of food ingested. All short-term appetite regulators act in response to the size and or absence of a meal as well as to the frequency of a

meal over a short period of time. Gastrointestinal peptides work along with the mechano and chemoreceptors, as factors that affect short-term EI. These peptides are also released in response to a meal.

Gastrointestinal peptides work via several different mechanisms. Some are considered endocrine, that is, they circulate in the blood in order to reach their target cell while others are considered paracrine as they are released into the interstitial fluid in order to affect nearby cells. Other cells are categorized as autocrine whereby they inhibit or activate the function of their cell origin and finally there are neurocrine cells which are neurotransmitters that act within neurons (Greenspan 1997). The following sections will focus on some of the gastrointestinal peptides that are released as a result of the absence or presence of feeding, and the resulting effects that these peptides have on appetite.

CCK:

Cholecystokinin (CCK) was discovered in 1928 by Ivy and Goldberg, and has been accepted as being a hormonal regulator of gallbladder contractions(Ivy AC 1928). It is a hormone produced by the endocrine cells in the upper small intestine, is released following a meal, and remains elevated for 5 hours (Liddle, Goldfine et al. 1985). CCK acts through two receptors known as CCK1 and CCK2, and it is CCK1 that has the most influence on appetite regulation (Asin, Gore et al. 1992). Further, it has been shown to stimulate gallbladder contractions, pancreatic enzyme secretion and to inhibit gastric emptying (Moran and McHugh 1982; Liddle, Goldfine et al. 1985) and food intake in humans and rodents (Ballinger, McLoughlin et al. 1995; Kissileff, Carretta et al. 2003).

Unfortunately, its half life is short so its ability to be helpful in decreasing hunger enough to be considered an adequate obesity treatment is questionable. In fact, with a half life of 1-2 min, it has not been found to reduce meal size when administered more than 15 minutes prior to a meal (Gibbs, Young et al. 1973).

Ghrelin:

Ghrelin is a growth hormone secretagogue (Kojima, Hosoda et al. 1999) (Bowers 2001), was purified and identified in the rat stomach, and was found to contain 28 amino acids in which the serine 3 residue is n-octanoylated. In this form, the acylated peptide specifically releases Growth Hormone (GH), and its administration (and not that of desacyl ghrelin) results in increased appetite. In fact, desacyl ghrelin does not seem to have a role in appetite stimulation or GH release, however recent evidence suggests that it may be involved in the stimulation of adipogenesis (Thompson, Gill et al. 2004). Moreover, in recent studies, it has been found that ghrelin also contributes to appetite stimulation and increased adiposity in rodents (Wren, Small et al. 2001) (Tschop, Smiley et al. 2000). The increase in adiposity is shown when the rodents are injected with ghrelin, which causes an increase in their respiratory quotient leading to more CHO oxidation and less fatty acid oxidation (Tschop, Smiley et al. 2000). Finally, this gastric peptide has also been found to stimulate gastric motility in humans and increase gastric acid secretion (Masuda, Tanaka et al. 2000), which may also play a role in its correlation with increased EI.

The hunger stimulation effects of ghrelin was studied in 9 lean individuals, and when comparing their EI after saline injection vs. those following ghrelin

injections, it was found that ghrelin caused a 28% increase in food intake which can be demonstrated in the figure A below (Wren, Seal et al. 2001). Also important to note from this study is the increase in hunger prior to meal time after ghrelin infusion compared to saline infusion. The participants self reported hunger ratings from the visual analogue scale are shown in figure C below (Wren, Seal et al. 2001).

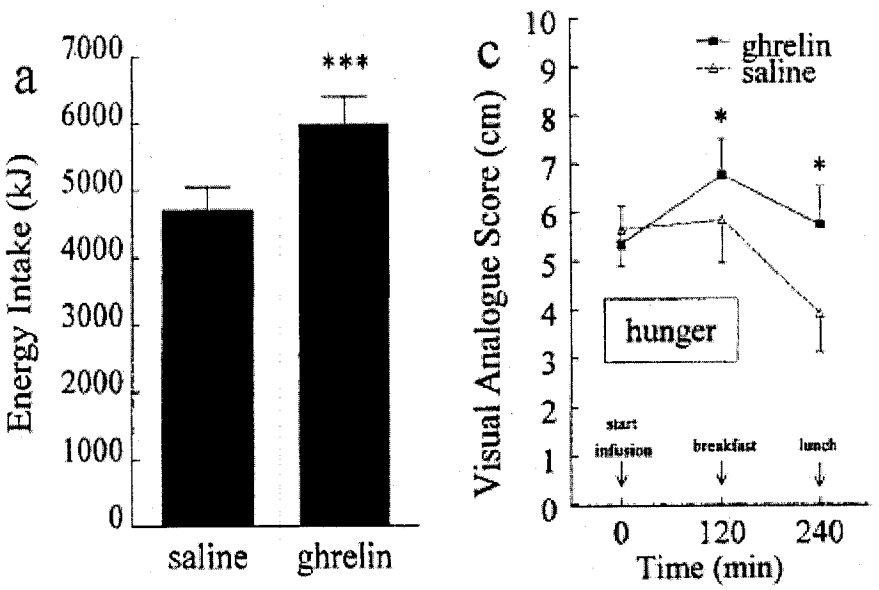

Figure 3. Mean EI from free choice buffet and c) mean visual analogue score for hunger during saline and ghrelin infusion. *p<0.05 and ***p<0.001.(Wren, Seal et al. 2001)

Finally, it is important to look at the daily profiles of ghrelin, and how the peptide levels are affected by the ingestion of nutrients or the lack of. It has been noted that ghrelin levels follow a regulated daily pattern, with peaks occurring prior to (Horvath, Diano et al. 2001) and troughs occurring 1 to 2 hours following meals (Ariyasu, Takaya et al. 2001). This suppression of ghrelin is dependent on caloric load and macronutrient content, with CHOs yielding the highest

suppression along with higher caloric loads (Callahan, Cummings et al. 2004). So far, ghrelin is the only known orexigenic peptide and its levels have been shown to be suppressed by increased levels of PYY (Batterham, Cohen et al. 2003). Its orexigenic character comes from its ability to stimulate electrical activity in most ARC neurons that are inhibited by leptin (Cowley, Cone et al. 2003) by directly activating NPY/AgRP neurons (Cowley, Cone et al. 2003). Finally, ghrelin has been shown to have inhibitory effects on POMC neurons.

MECHANISM OF PYY AND GLP-1

PYY 3-36 is released in response to a meal and is thought to act through the Y2 receptor in the hypothalamic arcuate nucleus (ARC) to regulate EI. The activation of the Y2 receptor is said to inhibit neuropeptide Y (NPY) and the agouti-related peptide (AgRP) and thereby disinhibiting pro-opiomelanocortin (POMC). NPY and AgRP are orexigenic or appetite-stimulating peptides where as POMC and cocaine- and amphetamine-regulated transcript (CART) express anorexigenic or appetite suppressing peptides (Chen, Li et al. 1999). All of these neurons reside in the ARC, and once activated, innervate second order hypothalamic targets that express melanocortin-4 (MC4) and NPY receptors (Liu, Kishi et al. 2003).

Similar to PYY, GLP-1's feeding regulation acts primarily through the ARC. The mechanisms of PYY and GLP-1 prove to be very similar seeing as they result in the same effects on EI. Although not many studies have looked at the mechanism of GLP-1, recent evidence shows that it directly activates the anorexigenic POMC neurons (Ma, Bruning et al. 2007)

GLP-1:

Glucagon-like Peptide 1 (GLP-1) is a 30 amino acid peptide hormone secreted by the L-cells of the ileum in response to nutrient intake (Holst 1994; Naslund, Barkeling et al. 1999), with its main effect being its ability to stimulate insulin release (Kreymann, Williams et al. 1987). It has a blood half-disappearance time of 5 minutes and a metabolic clearance rate of 13 ml/kgmin (Holst 1994). GLP-1 has also been shown to affect appetite ratings and food intake. Its peripheral administration inhibits food intake in both rats and humans compared with saline infusions (Gutzwiller, Drewe et al. 1999; Toft-Nielsen, Madsbad et al. 1999) and with nutrient stimulated GLP-1 secretion, a reduction in appetite and food intake is also observed, although this reduction is smaller (Flint, Raben et al. 2001; Verdich, Toubro et al. 2001). Similarly, lower hunger ratings and decreased EI were found during an ad libitum meal in obese patients (Fukase, Igarashi et al. 1993).

Postprandial GLP-1 levels have been reported as attenuated in obese subjects compared to lean subjects (Ranganath, Beety et al. 1996). The lower GLP-1 levels in the obese have been suggested as a result of the increased concentrations of non-esterified fatty acids, which are associated with obesity (Ranganath, Norris et al. 1999). Also, some studies show that weight loss causes an additional decrease in plasma GLP-1 concentration in obese and overweight participants (Adam, Lejeune et al. 2006) as well as abolishes the secretion of GLP-1 caused by nutrient ingestion (Adam, Jocken et al. 2005). While other studies report decreased concentrations of appetite related peptides

post weight loss(Cigaina and Hirschberg 2003). Lowered levels of this peptide could be a factor involved in the increased appetite found in individuals following a weight loss. However these peptides do not remain at low levels as, after a period of weight maintenance, GLP-1 concentrations returned to basal levels suggesting that the degree of energy balance could play a role(Adam, Lejeune et al. 2006).

Increases in GLP-1 caused by meal intake are often small (about 2.5 pmol/L) (Adam, Lejeune et al. 2006), where as through infusions are often much larger (about 50 pmol/L depending on the infusion rate) (Flint, Raben et al. 1998).This dramatic difference between the two GLP-1 concentrations may be a reason for the increased feelings of fullness caused by infusion vs. food intake seeing as the effect of GLP-1 on the reduction of EI is dose dependent (Verdich, Flint et al. 2001). For this reason then it would be harder to induce lower total caloric intake using meal patterning to affect GLP-1 alone. Still, research has shown that the greatest release of GLP-1 occurs when CHOs are ingested (Elliott, Morgan et al. 1993). Although its role in appetite control is still unclear, GLP-1 has been shown to decrease gastric emptying which may in turn affect food intake (Naslund, Gutniak et al. 1998) by acting as an ileal brake .

PYY:

PYY, a 36 amino acid peptide with a tyrosine at the C and N terminals, is released from the L cells in the GI tract with its release being highest in the rectum followed by the ileum and finally with the lowest concentration released from the colon (Adrian, Ferri et al. 1985; Ekblad and Sundler 2002). Once PYY

is released into the blood, it has a blood half-disappearance time of 11.7 ± 2.1 minutes and a metabolic clearance rate of 13.8 ± 1.6 ml/kgmin (Pappas TN 1985). This peptide is released in proportion to caloric consumption and is also influenced by the macronutrient composition of the meal, where it was initially thought that fats triggered the highest release, followed by proteins and finally CHOs (Lin and Chey 2003). Results from our lab have shown the opposite, where a high protein, high CHO snack caused the highest release of PYY when compared to the other possible combinations. Our findings can be supported by a recent study conducted by Batterham et al., which confirmed the fact that protein elicited the highest secretion of PYY (Batterham, Heffron et al. 2006).

PYY is present in the circulation in two forms, PYY 1-36, which is found in its highest concentration in the fasting state and PYY 3-36 (a 34 amino acid peptide with cleaved N-terminal Tyr-Pro residues), which is more abundant after a meal (63%) vs during fasting (36%)(Grandt, Schimiczek et al. 1994). PYY 3-36 is released into the circulation after food intake where its concentration is elevated within 15 minutes, peaked at 90 min followed by a plateau for 1-2 hours (Adrian, Ferri et al. 1985). During this time, PYY inhibits fluid and electrolyte secretion in the small bowel and delays the meals transport through the intestine, acting as an ileal brake, so that nutrients can continually be absorbed by the small intestine (Taylor 1993). This inhibition of gastric emptying may also play a role in PYY's ability to decrease feelings of hunger when peaked.

Seeing as PYY is affected by food intake there are interesting findings from studies in the obese population. A study in morbidly obese patients resulted

in the discovery that fasting and postprandial PYY levels were suppressed in these individuals (Batterham, Cohen et al. 2003). Another study showed that obese patients treated with surgery, had increases in PYY levels following the operation (Naslund, Melin et al. 1997; Alvarez Bartolome, Borque et al. 2002). These increased PYY levels could be a possible explanation for the decreased hunger that these patients feel post surgery. The suppressed PYY levels in the obese and the increase in PYY levels in the post obese state lead to the question of whether obesity causes low levels of PYY or whether it is the low levels of PYY that would in turn cause obesity. A study in rats found that plasma PYY levels were lower in diet induced obese mice, suggesting that it is obesity that leads to the lower plasma PYY levels. This same study compared 20 lean and 20 obese humans and found that the obese group needed a greater meal calorie content to increase plasma PYY levels to those similar to the lean group (le Roux et.al, 2004). This study then led to the speculation of whether obese individuals had low PYY synthesis or whether they had impaired PYY release. More research is needed in this area to differentiate between tissue and plasma PYY levels in both lean and obese individuals. Nonetheless, these studies reinforce the idea that PYY 3-36 reduces food intake and could in turn decrease body weight in animals and humans (Batterham, Cowley et al. 2002; Challis, Pinnock et al. 2003; Halatchev, Ellacott et al. 2004).

The anorectic effects of PYY have lead to inquiry into the possibility of it acting as a pharmacological aid in the treatment of obesity. For this reason, studies have looked at the effects of infused PYY into the body, and have found

that the peptide decreased food intake by over 30% approximately 2 hours after the infusion in both lean and obese individuals (Batterham, Cowley et al. 2002; Batterham, Cohen et al. 2003). Also, in these same individuals, caloric intake remained decreased for 24 hours following the infusion in both groups. However, PYY levels in obese patients were lower than lean participants. These results suggest that PYY may be beneficial as a treatment method in the obese population as it does not show resistant effects as did leptin in this same population (Hukshorn, Saris et al. 2000; Torekov, Larsen et al. 2005).

Finally, another benefit of PYY infusion is its ability to decrease plasma ghrelin levels (Batterham, Cowley et al. 2002; Batterham, Cohen et al. 2003) and work additively with GLP-1 (Neary, Small et al. 2005). Considering the appetite stimulating effects of ghrelin, its reduction prior to meal time along with increased levels of PYY and GLP-1 would cause a trio effect by which appetite would be inhibited even further. It is however important to note, that although infused PYY resulted in decreased plasma ghrelin and decreased food intake, the inhibition was found to be significant only at pharmacologic plasma concentrations (Degen, Oesch et al. 2005). This would suggest that meal induced maximal PYY levels may not be sufficient at causing the appetite inhibition that was seen with the infusions, however no studies have yet confirmed this idea. As mentioned previously, patients after a gastric surgery have been found to elicit high PYY (Naslund, Melin et al. 1997) and GLP-1 levels (Strader, Vahl et al. 2005; le Roux, Aylwin et al. 2006; Morinigo, Moize et al. 2006), suggesting that these increased levels may play a role in the reduced appetite found in these patients. At the

same time, ghrelin levels were reduced in these patients post demonstrating that these low levels may be responsible for the reduced appetite associated with the surgery (Cummings, Weigle et al. 2002). These post surgery and infusion observations reinforce the interplay that PYY and ghrelin have on reducing appetite.

Finally, although it is clear that PYY injections cause a decrease in feeding, it would also be important to note how to increase PYY levels through feeding alone, such as through nutrient manipulation. Research in this domain has shown contradictory results, with an earlier study suggesting that fat initiated the highest PYY secretion (Onaga, Zabielski et al. 2002; Zabielski, Morisset et al. 2002), and a more recent study showing that protein elicited the highest release (Batterham, Heffron et al. 2006). Knowing the nutrient specific release for each of the peptides will allow us to manipulate snacks and meals in order to maximize the peptide responses.

All of the peptides signals described above are summarized in table 1 below.

Table 1. Long and short term signals that regulate food intake.

Signals	Secretion	FI	Central Mechanism	Exogenous Administration		References
				Peripheral	Central	
Long Term						
Insulin	-beta cells in the pancreas in response to increased glucose concentration in the blood	↓	-activate POMC and inhibit NPY/AgRP	↓	↓	Wajchenberg 2000; Woods et al. 1985; Bruning et al. 2000
Leptin	-adipocytes in proportion to fat mass	↓	-activate POMC and inhibit NPY/AgRP	↓	↓	Halaas et al. 1995; Considine et al. 1996; Doucet et al. 2000; Sinha et al. 1996
Short Term						
CCK	-Endocrine I cells in upper SI in response to FI	↓	-stimulates CCK1	↓	↓	Liddle et al. 1985; Ballinger et al. 1995; Kissileff et al. 2003; Asin et al. 1992
Ghrelin	-Oxyntic cells of the stomach in response to the absence of FI	↑	-activates NPY/AgRP and inhibits POMC	↑	↑	Kojima et al. 1999; Wren et al. 2001; Tschop et al. 2000; Horvath et al. 2001; Cowley et al. 2003
GLP-1	-Endocrine L cells of the distal SI in response to FI and CHO	↓	-activates POMC	↓	↓	Ma et al. 2007; Holst et al. 1994; Barkeling et al. 1999; Kreymann et al. 1987; Naslund et al. 1998; Gutzwiller et al. 1999; Toft Nielsen et al. 1999; Ranganath et al. 1996; Adam et al. 2006; Flint et al. 1998; and Elliot et al. 1993
PYY	-Endocrine L cell of the distal SI in response to FI and Protein	↓	-inhibits NPY (Y2)	↓	↓	Adrian et al. 1985; Eckblad et al. 2002; Al-Saffar et al. 1985; Batterham et al. 2002; Batterham et al. 2003; Batterham et al. 2006; Lin et al. 2003; and Onaga et al. 2002

Note. The table is to be read with the understand that each parameter in responding to increases in the above signals. CCK, cholecystokinin; GLP-1, glycagon-like peptide 1; PYY, peptide tyrosine tyrosine, POMC, proopiomelanocortin, NPY, neuropeptide Y, AgRP, agouti-related protein; FI, food intake; SI, small intestine; ↓, decrease in food intake; ↑, increase in food intake.

MANIPULATING PERIPHERAL FEEDING SIGNALS

Although the studies above have examined the effects of infused PYY and GLP-1 on appetite, it would also be important to study how PYY and GLP-1 can be maximized without having it infused into the body as most people are not open to this type of solution. The next step would then be to examine the type of meal patterning and macronutrient intake that would cause the highest PYY and GLP-1 response.

The studies looking at a meal's effect on peptide responses involve increased meal frequency and snacking protocols as they seem to elicit a decrease in total daily EI (Drummond, Crombie et al. 1998; Speechly, Rogers et al. 1999; Westerterp-Plantenga, Kovacs et al. 2002; Farshchi, Taylor et al. 2005) With this understanding then, it would be interesting to examine the causes of this decreased EI, and now with the increased knowledge of gastrointestinal satiety factors, it is possible to manipulate meal patterning, caloric consumption, and macronutrient content. This manipulation would then allow us to attempt to maximize the release of anorectic peptides. One study looking at the effects of a snack of 320 Kcal (50% CHO, 35% protein and 15% lipid) prior to meal time found that the snack elicited a 100 % increase in PYY from fasting to peak levels (Korner, Bessler et al. 2005). Similarly, a study in our lab has shown that a 300 Kcal preload (40% CHO, 40% Protein and 20% Fats) resulted in an 85% increase in PYY response and an 11% increase in GLP-1 response. This pilot

study included 3 normal weight participants that participated in a crossover study involving 5 sessions. The first three sessions included the ingestion of a 300 Kcal preload (1- 40%CHO, 40%protein, 20% fat; 2- 40%CHO, 40%fat, 20%CHO; 3- 40%protein, 40%fat, 20% CHO) in the form of a shake followed by blood sampling every 20 min for 2 hours in order to determine PYY and GLP-1 profiles. From this experiment, it was determined that the high protein, high CHO shake produced the highest increases in PYY and GLP-1 concentrations. Next, the participants came back into the lab on 2 different occasions and we examined the effects of a 225 Kcal and 150 Kcal shake that was high in protein and CHO on PYY and GLP-1. The results of this study suggest that the 300Kcal shake that was high in protein and CHO produced the largest changes in PYY and GLP-1.

As reviewed previously, PYY and GLP-1 secretion are maximized with protein (Batterham, Heffron et al. 2006) and CHO (Ranganath, Schaper et al. 1999) intake respectively. Further, a study in our lab confirmed that protein and CHO elicited the highest PYY and GLP-1 secretion. For the purpose of this study then, we will investigate the effects that the above preload, (40% CHO, 40% Protein and 20% Fat), in the form of a milkshake, given at times to maximize fullness levels, will have on meal time EI and total daily EI. We did, however, decide to keep the energy and macronutrient content the same for each participant even though there were differences in body weight. The reason we did this was two fold. First, deciding to base the caloric content of the snack on body weight would yield very minimal differences between each individuals

snack content seeing as they were all normal weight individuals. Second, PYY and GLP-1 secretion are affected by caloric content, so having different snack amounts would make it difficult to compare PYY and GLP-1 across participants. Due to the short half life of CCK and because of this its inability to affect appetite and EI, the main focus of this study will be on the postprandial changes in two other anorectic peptides, PYY and GLP-1, and there resulting effects on meal time and total daily EI. A detailed explanation of the study will be given below in the methodology section.

CHAPTER 3 AND 4: METHODOLOGY AND RESULTS

To avoid redundancy, the methodology and results sections are presented in the form of a paper. The paper is entitled: 'The pre meal priming of gastrointestinal satiety factors'. The paper will be submitted for publication.

THE PRE MEAL PRIMING OF GASTROINTESTINAL SATIETY FACTORS

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ABSTRACT

Background: Although experimental studies on meal frequency are few, results show that increasing the number of daily meals leads to decreased energy intake (EI). No studies have yet looked at the type of meal patterning that would maximize this reduction.

Objective: The purpose of this study was to determine if structured snacking affects EI (EI), appetite, and PYY and GLP-1 concentrations in normal weight men.

Design: Eight normal weight men (79.9 ± 11.1 kg) participated in a randomized crossover trial. In condition 1, the subjects were able to self select their snacks *ad libitum*. In condition 2, the subjects consumed a standardized preload (300 Kcal: 40 % protein, 40% CHO and 20 % fat) 15 minutes prior to lunch and dinner. During condition 3, participants consumed the preload at times that maximized their pre meal fullness levels. During each condition, a standardized breakfast was served, while lunch and dinner were self-selected from a 5 item menu, and eaten *ad libitum*. Daily EI, fasting and postprandial appetite, PYY, and GLP-1 sampled every 30 minutes for 9 hours for all 3 conditions.

Results: No difference in daily EI, AUC GLP-1, and PYY was noted between conditions. Pre meal peptide levels tended to be higher during condition 3. Desire to eat, hunger, and prospective food consumption were found to be lowest during the control session ($P<0.05$). Finally, GLP-1

concentrations were correlated to fullness levels with significance during conditions 1 ($R=0.81$, $P<0.05$) and 3 ($R=0.89$, $P<0.01$).

Conclusion: Structured snacking does not affect EI over the short term.

Key words: Energy intake, appetite, PYY, GLP-1, preload, meal frequency

INTRODUCTION

Appetite is in part regulated by gastrointestinal satiety signals (Korner, Bessler et al. 2005). Among the signals contributing to the inhibition of appetite are Peptide YY (PYY)(Batterham and Bloom 2003; Challis, Pinnock et al. 2003) and Glucagon Like Peptide 1 (GLP-1) (Gutzwiller, Drewe et al. 1999; Toft-Nielsen, Madsbad et al. 1999; Flint, Raben et al. 2001). GLP-1 is a 30 amino acid peptide hormone secreted by the L-cells of the ileum in response to nutrient intake (Holst 1994; Naslund, Barkeling et al. 1999). Its peripheral administration inhibits food intake in both rats and humans compared with saline infusions (Gutzwiller, Drewe et al. 1999; Toft-Nielsen, Madsbad et al. 1999). Nutrient stimulated increases in GLP-1 are also associated with a reduction in appetite, although this reduction is smaller (Flint, Raben et al. 2001; Verdich, Toubro et al. 2001).

PYY is a 36 amino acid peptide with a tyrosine at the C and N terminals and similar to GLP-1 is also released from the L cells of the GI tract (Adrian, Ferri et al. 1985; Ekblad and Sundler 2002). Its secretion is proportional to caloric intake and is also affected by macronutrient composition (Onaga, Zabielski et al. 2002; Zabielski, Morisset et al. 2002; Lin and Chey 2003; Batterham, Heffron et al. 2006). PYY 3-36, a truncated form of PYY 1-36, is the most abundant form in circulation postprandially (Grandt, Schimiczek et al. 1994). When administered intravenously, PYY 3-36 has been shown to reduce food intake and appetite (Batterham, Cowley et al. 2002; Batterham, Cohen et al. 2003; Challis, Pinnock

et al. 2003; Halatchev, Ellacott et al. 2004; Degen, Oesch et al. 2005; le Roux, Batterham et al. 2006; Sloth, Holst et al. 2007) . PYY has also been shown to inhibit gastric motility by triggering the ileal break, which may also have some influence of increasing satiety (Allen, Fitzpatrick et al. 1984; Savage, Adrian et al. 1987)

As mentioned previously, both PYY and GLP-1 increase in proportion to caloric intake. In addition, there also seems to be macronutrient specific effects on the secretion of these peptides. Studies on PYY in animals showed that fat caused the highest release (Onaga, Zabielski et al. 2002; Lin and Chey 2003) however more recent findings suggest that it is protein (Batterham, Heffron et al. 2006). When looking at GLP-1 studies, secretion is greatest after CHOs are ingested (Elliott, Morgan et al. 1993). These findings suggest that composing a preload with high protein and high CHO could possibly maximize PYY and GLP-1 levels respectively.

Few experimental studies (Drummond, Crombie et al. 1998; Speechly, Rogers et al. 1999; Westerterp-Plantenga, Kovacs et al. 2002; Farshchi, Taylor et al. 2005) have looked at the effect of meal frequency on subsequent EI, and seeing as PYY and GLP-1, both peptides that exhibit anorectic properties, seem to be increased following a meal, a question that remains to be answered is whether these peptides, amongst others, could modulate the effects of meal frequency on EI. The few studies that have been conducted so far have shown that lower EI was associated with increased meal frequency (Drummond,

Crombie et al. 1998; Speechly, Rogers et al. 1999; Westerterp-Plantenga, Kovacs et al. 2002; Farshchi, Taylor et al. 2005).

To our knowledge, no study has yet examined if meal patterning modifies daily PYY and GLP-1 profiles. Seeing as both of these peptides have been shown to be associated to fullness and decreased EI, increasing their levels prior to meals could increase meal time fullness. The objectives of this study were then twofold. The first objective was to determine whether ingesting preloads, at times that would optimize pre meal fullness, would lead to a decrease in total EI. The second objective was to determine whether this approach would lead to increases in pre meal and daily PYY and GLP-1 levels.

METHODS

Subjects

Eight normal weight men (based on BMI) were recruited from the University of Ottawa. The inclusion criteria were the following: (1) adult men (18-55); (2) weight stable (+/ 2 kg during the previous 6 months); (3) non-smoker; (4) normal weight (BMI between 20 to 29 kg/m^2). Men were excluded if: 1) they had a history of eating disorders; 2) had conditions such as thyroid, chronic liver and renal disorders; 3) been on medication that may influence their EI or energy expenditure (EE). Subjects' characteristics are presented in **Table 1**. Informed consent was received from each participant before beginning the study, and both the University of Ottawa and Montfort Hospital research ethics boards approved the study.

Experimental sessions and measurements

All tests were performed at the Behavioural and Metabolic Research Unit (BMRU), at the Montfort Hospital in Ottawa between May 2007 and September 2007. Subjects came to the laboratory on 4 different occasions. During the first of these visits, all anthropometric measures including body composition, height, body weight and waist circumference were measured. The participants were given a standardized breakfast at 8h00 followed by questionnaires pertaining to their medical history. Finally, the session was concluded with the determination of the time needed to reach peak fullness following the ingestion of the preload. The participants were asked to ingest a preload in the form of a shake containing

300 Kcal with 40% CHO, 40% protein and 20 % fat (**Appendix C**) at 10:15 am (2 hours post breakfast consumption). Fasting and postprandial appetite measurements were taken every 20 minutes after breakfast until two hours after the ingestion of the preload. The fullness scores from the visual analogue scales where then plotted for each participant to determine the time post shake to reach maximum fullness. The mean time to fullness was 53.1±18.5 min (range: 25-80min).

The experimental sessions were divided into 3 randomly assigned conditions each consisting of one testing day (9h). Condition 1 involved no imposed preloads. Condition 2 involved asking the participants to ingest the preload 15 minutes before lunch and dinner and finally condition 3 consisted of asking the participants to ingest the preload at a specific time (as determined for each individual during the screening visit) prior to lunch and dinner. This time was individualized as our pilot studies' results showed that it took different times for each individual to reach maximum levels of PYY and GLP-1. Further, no study has examined the individualized profiles of PYY and GLP-1. A diagram detailing experimental sessions is shown in **Appendix B**.

For all 3 experimental sessions, participants were asked to come to the laboratory for 7h45 after an overnight fast at which point body weight was measured. Although at least 1 week had elapsed between each experimental session, body weight remained stable across conditions (1 = 79.5±11.6 kg; 2 = 80.2±10.9 kg; 3 = 79.6±11.8 kg; NS). Shortly after, participants were asked to consume a standardized breakfast consisting of two slices of whole wheat bread

(80g), 2 small containers of peanut butter (20g), 2 small containers of jam (20g), a piece of cheddar cheese (20g) and 250 ml of orange juice. The energy content of this breakfast was 462 Kcal and the food quotient was 0.89. Appetite ratings were measured using the visual analogue scale before and after breakfast and every 30 minutes for a period of 9 hours, during each condition. Also, blood was sampled fasting and every 30 minutes post breakfast (18 samples) during each condition. The samples were then assayed to determine daily hormone profiles.

After breakfast, a list of snacks (**Appendix C**) was given to each participant during condition 1, and they were given the option to self-select as many of the snacks as they wanted. During condition 2, the preload was given to each participant exactly 15 minutes prior to lunch and dinner (11h45) and each person was instructed to ingest the preload within 5 minutes. Finally, during condition 3, the preload was given to each participant 53.1±18.5 min (range: 25-80min) prior to lunch and dinner (depending on the time to reach peak fullness, as was determined during the screening session). Lunch and dinner were consumed at 12h00 and 17h00, respectively. Subjects chose their foods from a list of 5 meal items at the BMRU. Participants were able to self-select from a variety of foods (**Appendix C**).

Measurements

Anthropometric Measurements: Height and body weight were measured (HR-100 Height Rod and BWB-800AS Digital Scale from Tanita Corporation of America, Inc. Arlington Heights, Illinois, USA). Body composition was measured using

dual energy x-ray absorptiometry (DEXA) (Lunar Prodigy, GE Medical Systems). Coefficient of variation and correlation for the DEXA was 1.8% (R=0.99) as determined in 12 healthy subjects.

Preloads: Preloads were composed of 40% CHO, 40% protein, and 20% fat (see **appendix C**) and a caloric content of 300 kcal/serving. This composition was determined from a study in our lab, which confirmed that protein and CHO elicited the highest PYY and GLP-1 secretion respectively. The preload consisted of 15g of 35 % m.f. cream; 318 g of fruit yogurt; 85 g of 2 % m.f. lactose-free milk and 15 g of protein powder. All ingredients were measured on a scale to the nearest 0.1 g, then mixed in a blender and chilled at 4 degrees Celsius prior to serving.

Experimental Food Intake: The participants were able to self-select their meals (lunch and dinner) from a menu of 5 meal items and the conditions were designed so that they always selected from the same menu during each of the 3 experimental conditions. The meals consisted of a variety of foods differing in macronutrient content. The meals included a Chinese stir-fry, pizza (1- spinach and cheese, 2- ham, mushrooms, and pepperoni, 3- vegetarian), beef lasagna, beef stew, and BBQ chicken and potatoes. These foods were offered in large amounts and subjects were instructed to eat until satiated. More food was made available to participants in the event that they consumed all that was laid out for them at the beginning of the measurement. Energy and macronutrient content of

snacks, preloads and meals were assessed using Food Processor SQL from ESHA Research, Inc.

Appetite: Participants were asked to rate their appetite on a 150 mm visual analogue scale adapted from Hill and Blundell (Hill 1986). The scales assessed the individual's appetite, hunger, desire to eat, fullness and prospective food consumption (PFC). Each component was assessed by asking the participant to rank their current state by marking a vertical line across the horizontal continuum of 150 mm. Questions were asked as follow: 1) "How strong is your desire to eat?" (Very weak-Very strong); 2) "How hungry do you feel" (Not hungry at all-As hungry as I ever felt); 3) "How full do you feel" (Not full at all-Very full), and 4) "How much food do you think you could eat?"(Nothing at all-A large amount). The measurements were always performed in the same environment, where VAS measures have been shown to produce reliable results (Flint, Raben et al. 2000).

Blood Sampling and Peptide Assays: Blood samples were also taken during each experimental condition for 8.5 hours (fasting and every half hour post breakfast). This blood was collected in tubes containing EDTA from a catheter, which a qualified nurse had inserted upon the participants arrival to the laboratory. After collection, aprotinin (anti-protease) was added to one tube for PYY treatment and DPP-4 was added to another tube for GLP-1 treatment, then both samples were centrifuged at 3000 rpm for 10 min at 4 degrees Celsius.

After centrifugation, all samples were pipetted into 2 ml tubes and frozen down at –80 degrees Celsius until the assays were performed (maximum 3 months post collection). PYY and GLP-1 were assayed with commercially available ELISA (PYY ELISA Kit (CV= 4.36%) and GLP-1 ELISA kit (CV= 4.16%), LINCO Research, St-Louis, MO., USA). Although PYY 3-36 is the major circulating form postprandially and is the most potent anorectic form; Batterham et al. found that the temporal plasma profiles and the intergrated levels of PYY 3-36 mirrored those of total PYY(Batterham, Heffron et al. 2006). Further, the total PYY kit from Linco is very stable producing an intrakit CV of 4.36%, so for these reasons we decided to do our analyses using total PYY. Finally, it is also important to note is that each participant's samples were done with the same kit.

Area under the curve (AUC): The trapezoid method was used to calculate AUC for appetite scores, total PYY, and GLP-1. Fasting measurements and those obtained at times 30 to 510 min post breakfast (at 30 min intervals). The trapezoid method is a procedure used to approximate the AUC. This is done by inscribing or circumscribing the number of trapezoids under a curve, in which the areas are then summed to obtain the total area under the given curve.

Statistics: The primary outcome of this experiment was to measure total daily EI. A secondary outcome of this project was to determine the peptide and appetite profiles across the 3 different conditions. An ANOVA for repeated measures was used to detect the differences in total and meal time EI. Furthermore, the effects

of the different experimental conditions on the AUC for the appetite measurements, hormonal profiles, and pre meal (t=240 and 510) peptide levels were also assessed with an ANOVA for repeated measures with condition as the within subject factor. Correlation analyses were performed between AUC of PYY and GLP-1 and 1) total daily EI, lunch EI, and dinner EI and 2) total daily macronutrient content and 3) daily appetite profiles. Finally, one last set of correlations was done to determine the relationship between pre meal (lunch and dinner) peptide levels and EI during lunch and dinner. Effects were considered significant at $p \leq 0.05$ and data are presented as mean ± SD unless otherwise specified.

RESULTS

Subjects' characteristics are presented in **Table 1**. The subjects (25.1±1.6 yrs) had a normal body weight (79.9 ± 11.1 kg) and were non obese (25.3 ± 2.6 kg/m^2). Of note is the fact that body weight was stable across conditions (1 = 79.5±11.6 kg; 2 = 80.2±10.9 kg; 3 = 79.6±11.8 kg; NS), which is a gross index of the maintenance of energy balance in between experimental conditions.

Total daily EI was 2823 ± 798 Kcal, 2759 ± 286 Kcal, and 2900 ± 420 Kcal for conditions 1, 2 and 3 respectively. Total and meal time energy content are presented in **Table 2**. No significant differences were noted for total, lunch and dinner energy content across the three conditions.

Daily profiles and AUC's are shown for PYY and GLP-1 in **Figure 1** and **2,** respectively. The AUC for both PYY (1= 53467±21910; 2= 51158±18744; 3=

58363±24604; NS) and GLP-1 (1= 2287±1623; 2= 2500±1618; 3= 2923±1328; NS) tended to be higher during condition 3 however no significance was found. Further, when analyzing the pre meal values (t=240 and 510), both PYY (Lunch: 1- 97±40; 2- 87±25; 3-115±4 pg/ml, $p < 0.05$ and Dinner: 1- 112±45; 2- 130±68; 3- 132±58 pg/ml, NS) and GLP-1 (Lunch: 1- 4.2±3.5; 2- 5.7±3.9; 3-4.7±4.4 pmol, NS and Dinner: 1-4.0±3.4; 2- 5.9±4.0; 3-7.1±4.6 pmol, NS) showed either a trend of being higher or were significantly higher during condition 3 for both lunch and dinner. Finally, post hoc comparisons showed a significantly higher PYY concentration during condition 3 ($p<0.05$) pre-lunch in comparison to condition 2, but significance was not found when comparing to condition 1.

Daily profiles of appetite scores are shown in **Figure 3**.The AUC appetite scores for desire to eat (1-31423±12065; 2-39298±15227; 3-39964±8481; $p<0.05$), hunger (1-28982±13806; 2-37787±14369; 3-38188±8286; $p<0.01$), and PFC (1-36311±9025; 2-42461±9487; 3-42053±6466; $p<0.05$) were significantly lower during condition 1 than the conditions 2 and 3. The results for fullness showed no significant differences, although there was a tendency for the AUC to be higher during the control session (1=43474±7660; 2=40763±11120; 3=41303±7765, NS). No trends were noted between the pre meal appetite measures across conditions.

Correlation analyses were done to examine the potential association between AUC of the peptides and total EI, and between the pre meal peptide concentrations and the EI of the subsequent meal. No significant correlations were noted for the AUC of the peptides and total daily EI. Similarly, no

relationships were found between the pre meal peptide levels and the EI at lunch and dinner.

No significant relationships were noted between the AUC for PYY and GLP-1 across all three conditions. However, when examining the correlation between the appetite scores and the peptide AUC's, it was found that the AUC for GLP-1 was associated with increased fullness scores during condition 1 ($r=0.8$, $p < 0.01$) and condition 3 ($r=0.9$, $p <0..01$).

DISCUSSION

Recent studies reported that increased meal frequency or snacking seemed to elicit a decrease in total daily EI (Speechly, Rogers et al. 1999; Farshchi, Taylor et al. 2005). Further, food intake induces the secretion of GLP-1 (Holst 1994; Naslund, Barkeling et al. 1999) and PYY (Adrian, Ferri et al. 1985) into circulation, which has been associated with fullness levels(Flint, Raben et al. 1998; Naslund, Gutniak et al. 1998; Gutzwiller, Drewe et al. 1999; Naslund, Barkeling et al. 1999; Challis, Pinnock et al. 2003; Halatchev, Ellacott et al. 2004; Degen, Oesch et al. 2005). The present study was performed to investigate whether structured snacking, in the form of preloads, at times specified to maximize pre meal fullness, would in turn cause a decrease in EI at meal time as well as daily EI and an increase in meal time and total PYY, GLP-1 and fullness. Experimental sessions were rigorously respected as shown by the 12 hr fast and by a stable weight across conditions. The findings of this study were two fold. First, there were no differences for EI across conditions. Second, there was no

difference between AUC PYY and GLP-1 but there was a trend that these levels were highest during condition 3, with a similar trend occurring for the pre meal concentrations of these peptides. Post hoc analysis revealed that pre-lunch [PYY] was significantly higher during condition 3 than condition 2.

Increased meal frequency has often been associated with decreased EI (Drummond, Crombie et al. 1998; Speechly, Rogers et al. 1999; Westerterp-Plantenga, Kovacs et al. 2002; Farshchi, Taylor et al. 2005), but to our knowledge no study has looked at both snack composition and timing as having an effect. This led us to time the snack intake for each participant so that fullness scores were maximized at meal times. This was done on an individual basis to take into account different responses that each participant may have to a 300 Kcal preload. We did, however, decide to keep the energy and macronutrient content the same for each participant even though there were differences in body weight. The reason we did this was two fold. First, all of our participants were of normal weight so basing the caloric content of the snack on body weight would yield minimal differences between each individuals' snack content. Second, PYY and GLP-1 secretion are affected by caloric content, so having different snack amounts would make it difficult to compare PYY and GLP-1 across participants.

Energy Intake:

Even with our attempt at maximizing pre meal fullness with our structured preloads, we found no differences in EI between conditions. Important to note

here is the large individual variation in EI found in **table 2**. Still with re-analysis of individual cases, no trend in mealtime and total EI was found across conditions. A reason for this could be that although we tried to time the snacks so that maximum fullness was reached pre meal, as determined during the screening session, we found no differences in pre meal fullness levels across conditions. This supports the fact that EI was also similar across conditions and the findings are in line with one study which found that fullness was the best indicator of EI (Drapeau, King et al. 2007).

We also analyzed pre meal peptide levels and the results showed a trend indicating higher GLP-1 and PYY concentrations during condition 3. Post hoc analysis revealed no strong differences, however PYY concentrations pre-lunch were significantly higher during condition 3 than condition 2 ($p<0.05$), but no such differences were noted for condition 1. But again, EI remained similar across conditions. It is possible, that with a greater number of participants, the post hoc analyses would have shown stronger differences across conditions, but these differences may still not have been enough to elicit further differences in EI. It is difficult to explain why this may have occurred, seeing as very few experimental studies have been done on meal frequency, but it is possible that the duration of the conditions may not have been long enough for the participants to become accustomed to the type of structured eating. Further, it is possible that this type of manipulated meal frequency may not work for normal weight men. In fact, no matter what condition they were in, they tended to eat similar amounts, suggesting that they were able to adjust their caloric intake no matter

what manipulations they were placed under. This pattern was also seen in a study that looked at alternating temporal patterns of EI with isoenergetically dense foods where they found that normal weight men had no significant differences in total EI across a condition where mandatory snacks were given vs. a condition were no snacks were given (Johnstone, Shannon et al. 2000). These results may lead to the question as to whether normal weight men are simply able to adjust their food consumption in any situation (whether placed under manipulated feeding frequencies, or allowed to eat freely in a habitual environment).

Peptides:

Although PYY and GLP-1 tended to be higher before meals, this did not impact on meal time EI. These observations are intriguing, seeing as GLP-1 ((Blundell and Naslund 1999) and PYY (Batterham, Cohen et al. 2003) are both anorectic peptides. Important to note here is that the studies that showed decreased appetite and EI where those where PYY (Degen, Oesch et al. 2005) and GLP-1 (Flint, Raben et al. 1998; Naslund, Gutniak et al. 1998; Gutzwiller, Drewe et al. 1999; Naslund, Barkeling et al. 1999) were infused. In fact, the lowest infusion rate that elicited these changes in appetite and EI produced GLP-1 concentrations of 50pmol/L (Flint, Raben et al. 1998) and % changes in PYY of approximately 424% (Batterham, Cowley et al. 2002). However, our highest pre meal [GLP-1] was 7.1 pmol/L (139% change from baseline) and our highest pre meal [PYY] was 132pg/ml (48% change from baseline). Interestingly, both of

these peak values occurred pre-dinner during condition 3, however post hoc analyses revealed no significant differences. Still, there is a large discrepancy between the values we found, and the values previously found to show impacts on satiety and EI. Further, no studies had yet looked at the influence of snacking on these peptide levels and whether PYY and GLP-1 would be increased to a high enough level to elicit changes in total daily EI. It is possible that the snack composition and timing during condition 3 was not sufficient to trigger responses in PYY and GLP-1 that were high enough to elicit subsequent changes in appetite and EI in comparison to the other conditions.

Two studies have looked at the effects of meal induced increases in GLP-1 and found that an increase of 2.5 pmol/l (Adam, Lejeune et al. 2006) and one of 6 pmol/l (Verdich, Toubro et al. 2001) were not enough to have a significant impact on satiety. However, as mentioned previously, our study found that increased GLP-1 levels was associated with fullness during condition 1 ($\triangle$4.1 pmol/L) and condition 3 ($\triangle$9.16 pmol/l), however no association was noted for condition 2 ($\triangle$7.8pmol/l). Still, these GLP-1 changes from baseline were not enough to show associations with all of the appetite measures.

To our knowledge, no studies have yet looked at the effects of nutrient stimulated PYY secretion on EI. Also important to note, is that we measured total PYY and not PYY 3-36. Perhaps having measured PYY 3-36 only would have been beneficial when looking at changes from baseline. Baseline measures usually have higher levels of PYY 1-36 and postprandial levels are mostly composed of PYY 3-36 (Grandt, Schimiczek et al. 1994), so not having

isolated these may be a reason why our % changes in PYY are so low. However, it has been suggested that changes in PYY 3-36 can be mostly explained by changes it total PYY, and seeing as the total PYY ELISA kit is very stable, we decided to use this option. Our results have thus confirmed that perhaps the peptide levels needed to elicit changes in appetite may be too high then those observed under normal physiological conditions.

In summary, our two hypotheses were rejected. Contrary to what we had initially hypothesized, timing the preload so that maximum fullness was achieved prior to meals did not yield decreases in meal time or total EI. Similarly, the timing of the preload had no affects on overall GLP-1 and PYY concentrations.

Table 1. Subjects' Characteristics. n=8

	Mean	Range
Weight (kg)	79.9±11.1	64.2-94.4
Height (cm)	177.8±8.9	164.4-189.6
BMI (kg/m2)	25.3±2.6	21.9-28.9
Waist Circumference (cm)	82.8±6.6	75.8-91.3
Fat mass (kg)	19.9±8.6	10.4-29.7
Fat free mass (kg)	59.0±7.4	50.1-70.7
Percentage Body Fat (%)	24.4±8.2	13.9-35.0

Table 2. EI for the control (1), the pre snack (2) and the timed snack (3) condition.

	CONDITION 1		CONDITION 2		CONDITION 3	
	Mean	**Range**	**Mean**	**Range**	**Mean**	**Range**
Lunch (Kcal)	944 ± 253	591-1349	889 ± 221	546-1266	978 ± 365	565-1643
Dinner (Kcal)	784 ± 385	258-1560	808 ± 261	547-1338	860 ± 336	366.7-1185
AM Snack (Kcal)	377 ± 235	156-800	300 ± 0	300	300 ± 0	300
PM Snack (Kcal)	255 ± 156	0-434	300 ± 0	300	300 ± 0	300
Total EI (Kcal)	2823 ± 798	1743-4271	2760 ± 286	2182-3143	2900 ± 420	2306-3785

Table 3. Correlation analyses between AUC GLP-1 and PYY scores and AUC VAS, and EI throughout the day.

	AUC GLP-1			AUC PYY		
CONDITION	**1**	**2**	**3**	**1**	**2**	**3**
Total EI	-0.30	-0.35	0.15	-0.22	-0.33	-0.37
AUC Desire	0.32	0.24	0.09	-0.00	-0.10	-0.01
AUC Hunger	0.27	0.25	0.02	-0.05	0.02	-0.07
AUC Fullness	0.81**	0.20	0.89†	0.25	-0.05	0.28
AUC PFC	0.42	0.27	-0.34	0.12	0.06	-0.26

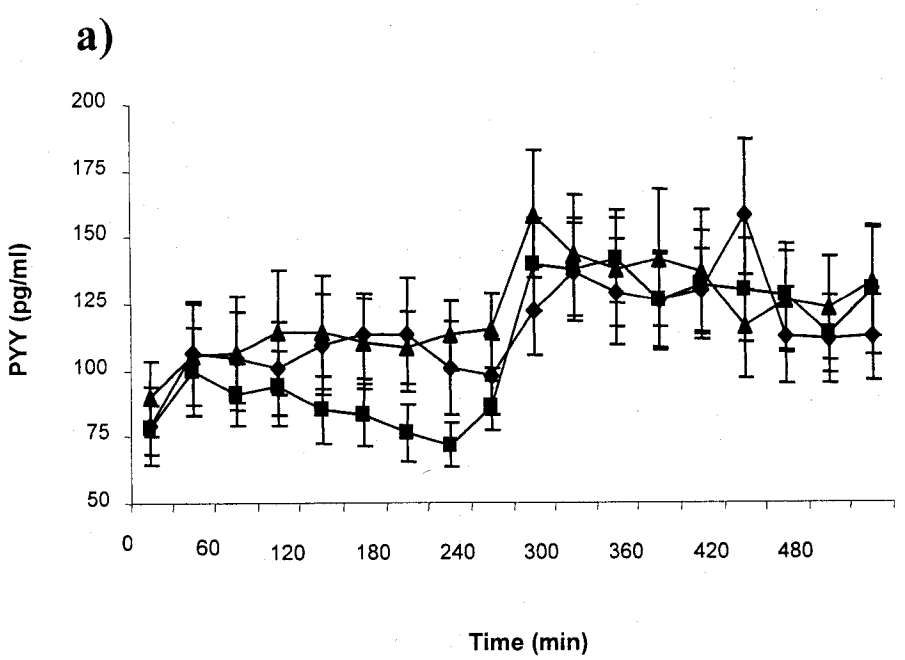
1, 2, and 3= control, pre snack, and timed snack conditions respectively. *, ** and †; p value ≤ 0.1, 0.05 and 0.01, respectively. N = 8.

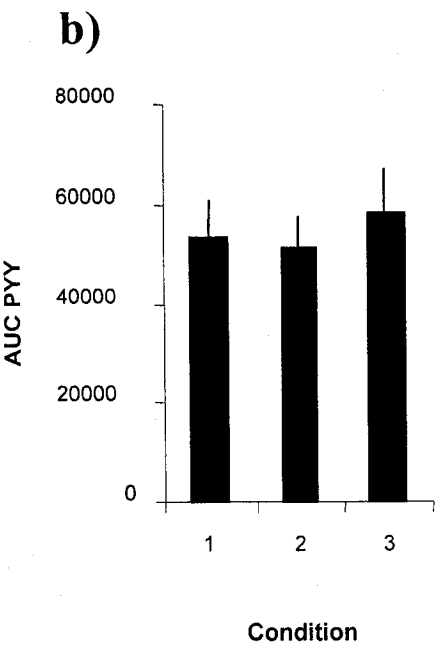
Figure Legends:

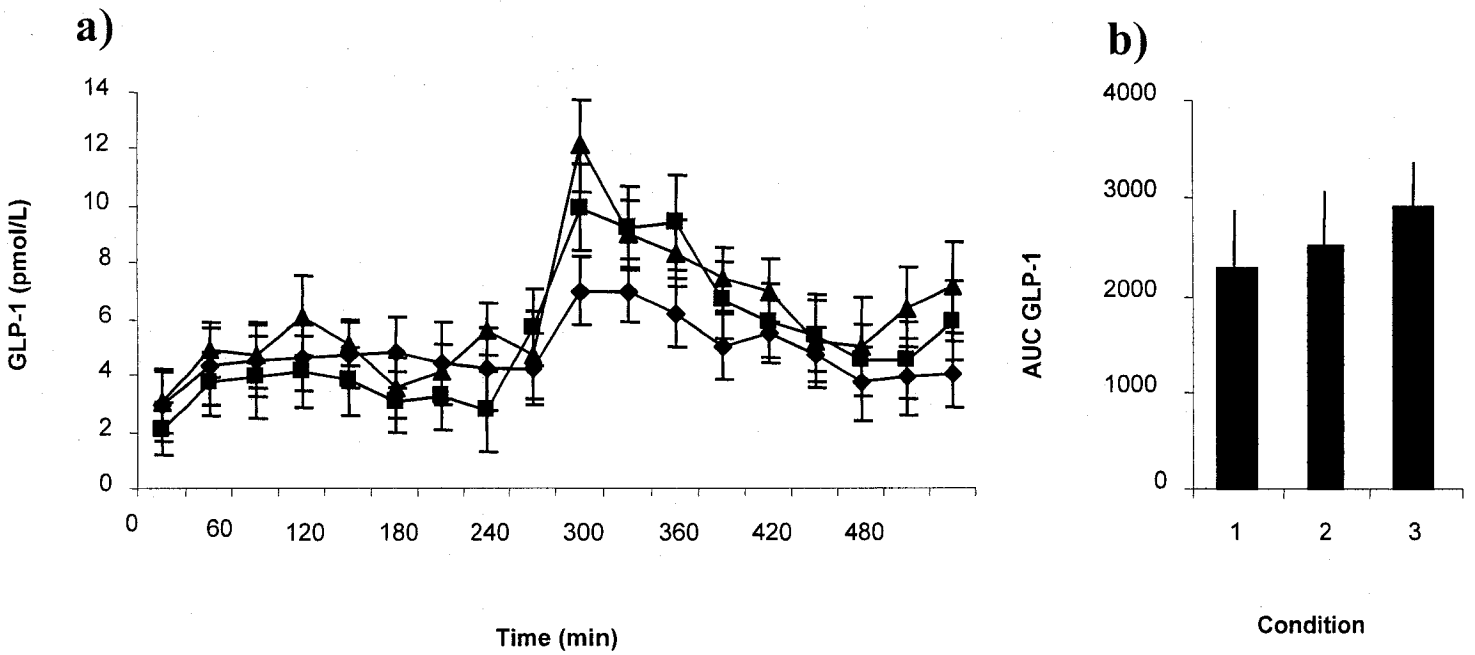
Figure 1. Daily profile (A) and AUC (B) for PYY across condition 1 ♦, condition 2 ■, and condition 3 ▲ conditions. No differences in AUC PYY across conditions. N=8.

Figure 2. Daily profile (A) and AUC (B) for GLP-1 across condition 1 ♦, condition 2 ■, and condition 3 ▲. No differences in AUC GLP-1 across conditions. N=8.

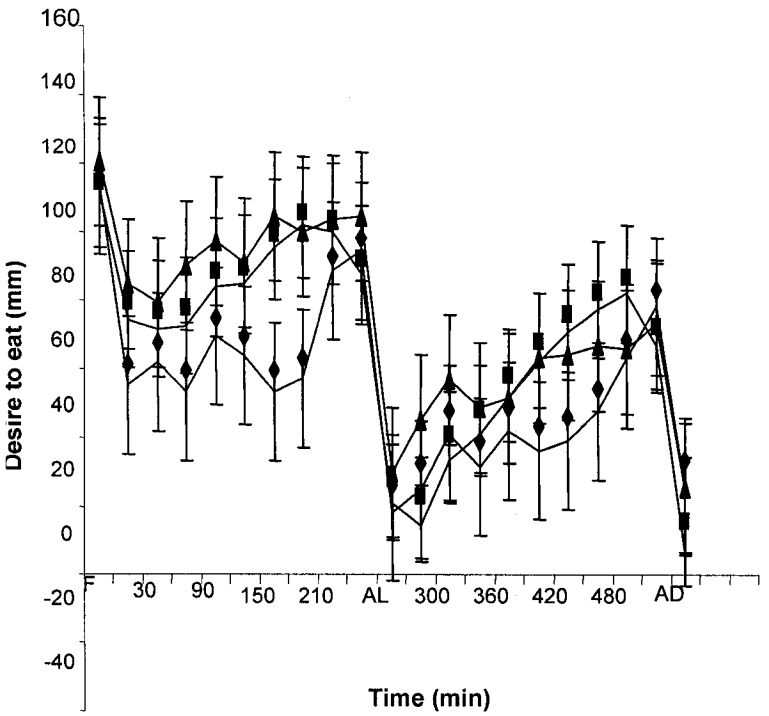
Figure 3. Daily profile of desire to eat (A), appetite (B), fullness (C) and Prospective Food Consumption (D) across condition 1 ♦, condition 2 ■, and condition 3 ▲. No differences in AUC VAS scores across conditions. N=8.


a)
200
175
150
125
100
75
50
PYY (pg/ml)
0
60
120
180
240
300
360
420
480
Time (min)

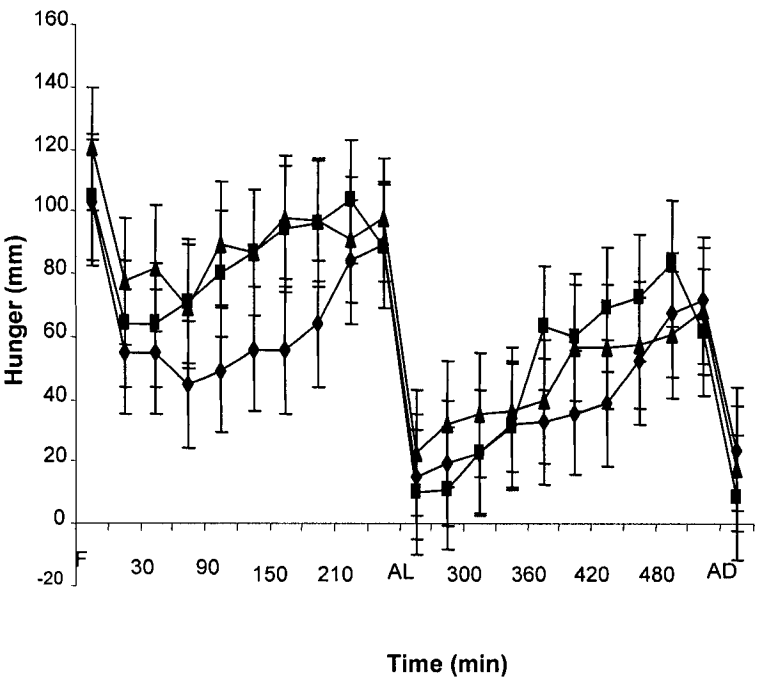

b)
80000
60000
40000
20000
0
AUC PYY
1
2
3
Condition


a)
GLP-1 (pmol/L)
14
12
10
8
6
4
2
0
0
60
120
180
240
300
360
420
480
Time (min)
b)
AUC GLP-1
4000
3000
2000
1000
0
1
2
3
Condition

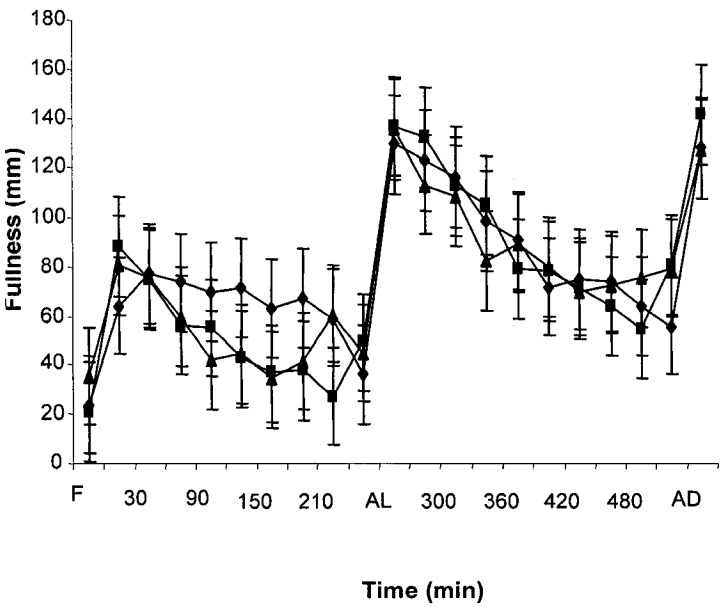
a)


Desire to eat (mm)
160
140
120
100
80
60
40
20
0
-20
-40
F
30
90
150
210
AL
300
360
420
480
AD
Time (min)

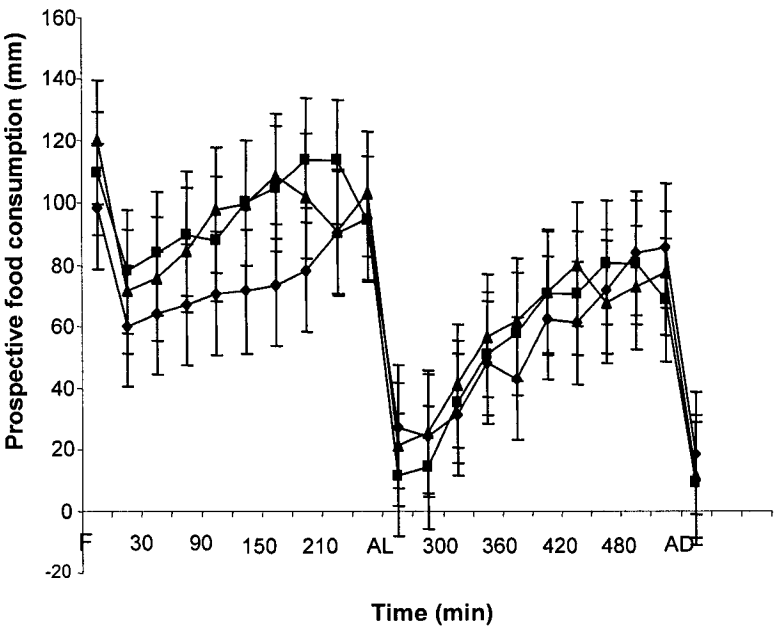
b)


Hunger (mm)
160
140
120
100
80
60
40
20
0
-20
F
30
90
150
210
AL
300
360
420
480
AD
Time (min)

c)


Fullness (mm)
180
160
140
120
100
80
60
40
20
0
F
30
90
150
210
AL
300
360
420
480
AD
Time (min)

d)


Prospective food consumption (mm)
160
140
120
100
80
60
40
20
0
-20
F
30
90
150
210
AL
300
360
420
480
AD
Time (min)

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CHAPTER 5: GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

FUTURE PERSPECTIVES

When looking back on the study design, there are definitely some recommendations to be made. One is that allotting one day per condition was simply too little time for the participant to become accustomed to the eating pattern. They were in a new environment and offered new types of food, so it is quite likely that the top down mechanism was at work here. As mentioned previously, this study focused only on the bottom up control of feeding, and not on the cognitive, environmental, and hedonic reasons for eating (ie. Top down control). It would be interesting to see the results if there participants were asked to come into the laboratory for 5 or 7 days, so that they are given a chance to become used to the meals and scheduling of the meals, however this would have required more funds and been more time consuming for a masters project.

Also, it would have been interesting to see if the participants had consumed any snacks once they went home at 5pm. Our data for daily intake included foods eaten in between 8 am and 5 pm, so it is quite possible that they consumed more foods after they went home, which could have altered our findings.

Important to note here is that we conducted the study in young men who were weight stable (ie. +/- 2 kg over the past six months). During this time, both the top down and bottom up regulation of feeding are at play. Further, our rationale was developed with the idea that appetite increases post weight loss, more specifically after a period of energy restriction (Dulloo, Jacquet et al. 1997; Doucet, Imbeault et al. 2000; Doucet and Cameron 2007). During such a trial, it is possible that individuals rely more of physiological cues (ie. Bottom up regulation) to initiate and terminate feeding. An interesting idea would then be to conduct a similar study in individuals following a weight loss program. In other words, we would want to use our preloads as a type of meal frequency manipulation in order to minimize this increased appetite that often occurs after a weight loss trial. The rationale here would be that PYY and GLP-1 are often down regulated during a weight loss trial (Adam, Jocken et al. 2005), so it is possible that increasing meal frequency would elicit higher peptide levels throughout the day. Further, seeing as energy balance is often compromised in the reduced obese state, increasing meal frequency may help antagonize the increase in appetite. A similar trend is seen in patients following a gastric bypass surgery, where prior to the surgery, they have low levels of PYY and GLP-1, and following the surgery, these levels rise to levels seen in normal weight individuals (Alvarez Bartolome, Borque et al. 2002; Korner, Bessler et al. 2005; Strader, Vahl et al. 2005; Chan, Mun et al. 2006; le Roux, Aylwin et al. 2006; Morinigo, Moize et al. 2006).

In the end, it is possible that nutrient stimulated peptide secretion, under normal physiological conditions, does not yield the pre meal peak values necessary to elicit changes in EI and appetite. Further, seeing as these men are lean, they are accustomed to monitoring their EI as a method of maintaining energy balance and weight maintenance, so it is likely that they are able to regulate their feeding patterns and intake as a method of maintaining this stable energy balance.

CONCLUSION

In conclusion, structured snacking resulted in no difference in EI in comparison to *ad libitum* snacking, in normal weight men maintained in energy balance. In other words, the pre meal priming of fullness via maximization of PYY and GLP-1 at meal times was not enough to cause changes in EI and appetite. These results are contrary to what was originally hypothesized. Our hypothesis was based on the rationale that priming these anorectic peptides would cause decreases in EI and increases in satiety. It is possible that the increases in PYY and GLP-1 caused by meal patterning are not enough to elicit appetite changes, however this idea should be revisited with longer experimental conditions, more subjects, and a trial with individuals during and following and energy restricted diet.

Finally, although we were able to somewhat induce higher PYY and GLP-1 conditions during condition 3, the increases were not robust enough to elicit changes in satiety and total EI over the short term.

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APPENDIX A- Poster, forms, and Questionnaires

Université d'Ottawa · University of Ottawa

Faculté des sciences de la santé — Faculty of Health Sciences
École des sciences de l'activité physique — School of Human Kinetics

Looking for volunteers

For a study on food intake

Selection criteria:

- Men aged 20 to 55 years;
- Moderately active: (< than 3 hours exercise/week);
- No major health problems (specifically thyroid, liver and kidney);
- Non-smokers;
- Body mass index (20<BMI<30)
- Must be weight stable (past 6 months)
- Have no history of eating disorders
- Be on no medication that may affect energy intake or expenditure.

** Free evaluation of body composition**

For more information, contact Stephanie Willbond or Gabrielle Mifsud:

Stephanie Willbond or Gabrielle Mifsud	Stephanie Willbond or Gabrielle Mifsud	Stephanie Willbond or Gabrielle Mifsud	Stephanie Willbond or Gabrielle Mifsud	Stephanie Willbond or Gabrielle Mifsud	Stephanie Willbond or Gabrielle Mifsud	Stephanie Willbond or Gabrielle Mifsud	Stephanie Willbond or Gabrielle Mifsud	Stephanie Willbond or Gabrielle Mifsud	Stephanie Willbond or Gabrielle Mifsud	Stephanie Willbond or Gabrielle Mifsud	Stephanie Willbond or Gabrielle Mifsud	Stephanie Willbond or Gabrielle Mifsud	Stephanie Willbond or Gabrielle Mifsud

RECRUITMENT SHEET

Inclusion criteria questionnaire for the GISS study:

1) How old are you? _____

2) What is your weight? _____

3) What is your height? _____

4) Are you a smoker? Yes ☐ No ☐

5) Have you had a stable (± 2 kg) body weight for at least the last 6 months? Yes ☐ No ☐

6) Do you have plans to change your dietary habits in the coming 7 months? Yes ☐ No ☐

7) Are you sedentary? Yes ☐ No ☐

If not, how many minutes of physical activity do you do each week?

_____

8) Do you take medication? Yes ☐ No ☐

If yes, which ones?

_____

9) Are you diabetic? Yes ☐ No ☐

10) Do you suffer from heart disease? Yes ☐ No ☐

11) Do you suffer from hypertension? Yes ☐ No ☐

12) Do you suffer from asthma or other respiratory problems? Yes ☐ No ☐

13) Has your doctor ever said that you suffered from thyroid gland disorder? Yes ☐ No ☐

14) Have you ever, or do you currently suffer from an eating disorder? Yes ☐ No ☐

If yes, which one:

15) Do you suffer from chronic liver disease or renal disorders? Yes ☐ No ☐

T :

Visual Analogue Scale (150 mm)

Procedure	**Please quantify your sensation for the feeling mentioned below. Consider the line as two extremes of this sensation. Draw a vertical line that best represents this sensation at this moment in time.**

1.How strong is your desire to eat?

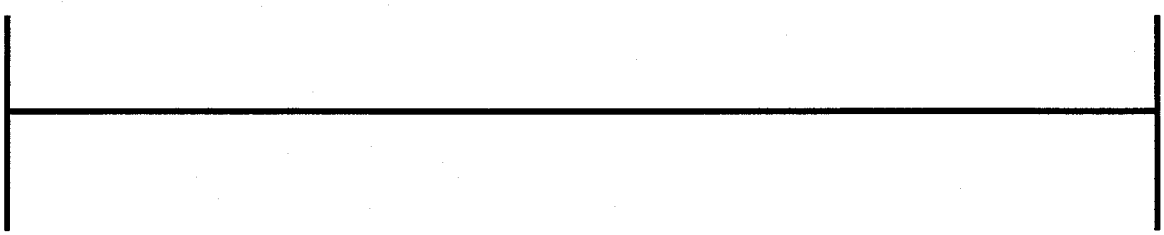
Very weak Very strong

T :

Visual Analogue Scale (150 mm)

Procedure	**Please quantify your sensation for the feeling mentioned below. Consider the line as two extremes of this sensation. Draw a vertical line that best represents this sensation at this moment in time.**

2.How hungry do you feel?



Not hungry at all felt

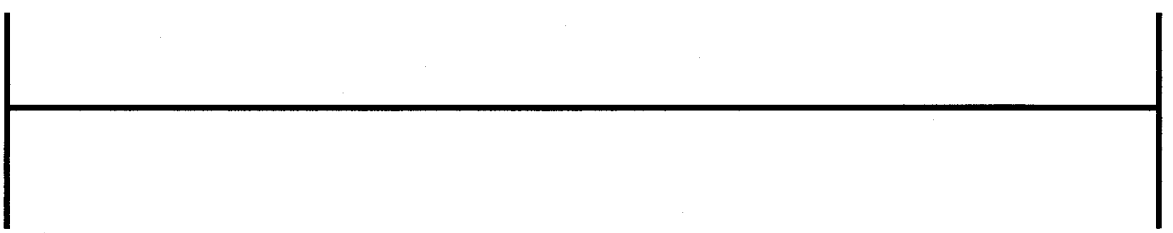
As hungry as I have ever

T :

Visual Analogue Scale (150 mm)

Procedure	Please quantify your sensation for the feeling mentioned below. Consider the line as two extremes of this sensation. Draw a vertical line that best represents this sensation at this moment in time.

3.How full do you feel?



Not full at all

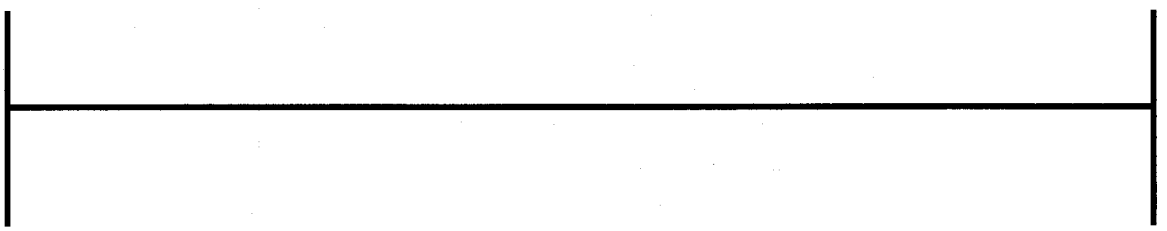
Very full

T :

Visual Analogue Scale (150 mm)

Procedure	**Please quantify your sensation for the feeling mentioned below. Consider the line as two extremes of this sensation. Draw a vertical line that best represents this sensation at this moment in time.**

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



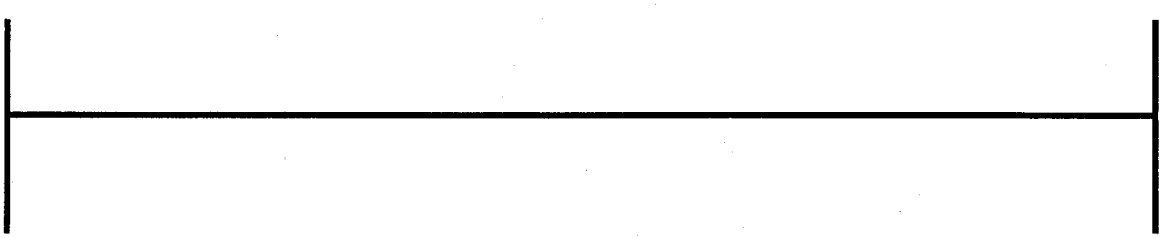
Nothing at all A large amount

T :

Visual Analogue Scale (150 mm)

Procedure	Please quantify your sensation for the feeling mentioned below. Consider the line as two extremes of this sensation. Draw a vertical line that best represents this sensation at this moment in time.

5.How did you appreciate this meal?



Not at all **Very much**

DEXA

Anthropometric measurment :

Weight : _____.____ kg BMI : _____.____ kg/m^2

Height : _____.____ cm

Waist circumference: _____.____ cm

Questions to ask prior to the DEXA :

- Did you eat 2 hours before? Yes ☐ No ☐
- Are you pregnant? Yes ☐ No ☐
- Did you get any magnetic resonance test in the previous 7 days? Yes ☐ No ☐
- Did you empty your bladder? Yes ☐ No ☐

* Remove all the metal.

Fat mass : _____.____ kg Percentage body fat : _____.____ %

Fat-free mass : _____.____ cm

MEDICAL AND DIETARY HISTORY

PARTICIPANT HISTORY:	YES	NO
Have you ever participated in a research study before?	☐	☐
Where? _____		
Are you in a study now other than the present study?	☐	☐

FAMILY HISTORY	YES	NO	If « yes » : specify Code : 1-Father 2- Mother 3- Siblings 4- Grandparents
Diabetes	☐	☐	_____
Heart disease	☐	☐	_____
Other	☐	☐	_____

SIGNIFICANT MEDICAL/SURGICAL HISTORY (and not active): NONE ☐

(If condition is ongoing, list under history currently active)

CONDITION	YEAR
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

MEDICAL AND DIETARY HISTORY continued...

MEDICATION :

List all medications the participant has taken in the last month:

DRUG NAME (GENERIC ONLY)	**TOTAL DAILY DOSE** (MG/DAY)	**DATE STARTED** (DAY/MTH/YR)	**DATE ENDED*** (DAY/MTH/YR)
		___/___/___	___/___/___
		___/___/___	___/___/___
		___/___/___	___/___/___
		___/___/___	___/___/___
		___/___/___	___/___/___
		___/___/___	___/___/___
		___/___/___	___/___/___
		___/___/___	___/___/___
		___/___/___	___/___/___
		___/___/___	___/___/___
		___/___/___	___/___/___

* If medication is continuing, check box and leave date ended blank.

MEDICAL AND DIETARY HISTORY continued...

SMOKING HISTORY:

		CURRENT/SMOKERS	QUIT SMOKING	NEVER SMOKED
A.	Cigarettes	☐	☐	☐
B.	Cigars	☐	☐	☐
C.	Pipes	☐	☐	☐

Age when you first started? ____years Duration? ____years

A) How many of the above per day (last year)? _____/day

B) How old were you when you stopped smoking? _____ years

C) If you quit smoking, how long ago did you stop smoking? ____ years

D) Number of trial? _____

EXERCISE PROGRAM:

Do you exercise 30 minutes (continous moderate physical activity « higher than 60 % of VO_2max ») or more per week? **YES** ☐ **NO** ☐

If **YES**, what type of exercise? _____

If **YES**, how many times per week? _____ per week

DIETARY PATTERN (SELECT ONLY ONE ITEM):

Unrestricted	☐	Low fat/cholesterol	☐	Low sodium	☐
Watching what you eat	☐				
Low calorie	☐	Diabetic	☐	Vegetarian	☐

WEIGHT CYCLING HISTORY BEFORE TRIAL MEDICATION:

Have you ever been on a diet (weight loss ≥ 4 kg or 10 lbs)? **YES** ☐ **NO** ☐

If «YES», weight before the first diet: _____ kg

Your age at that period: _____ years

If «YES», specify how many times did you follow a diet: _____

MEDICAL AND DIETARY HISTORY continued...

☐ None

SPECIFICATION OF THE DIET

Name of the diet	Age (yr.)	Period on diet (days /weeks/months)	Weight lost (kg/lbs)	Weight regain after the diet (kg/lbs)	Time until weight regain (days/weeks/months)
☐ Weight Watcher	____ yr.	_____	_____.__	_____.__	_____
☐ Scarsdel	____ yr.	_____	_____.__	_____.__	_____
☐ Nutri-bars	____ yr.	_____	_____.__	_____.__	_____
☐ Diuretics	____ yr.	_____	_____.__	_____.__	_____
☐ Laxative	____ yr.	_____	_____.__	_____.__	_____
☐ Pills	____ yr.	_____	_____.__	_____.__	_____
☐ Protein diet	____ yr.	_____	_____.__	_____.__	_____
☐ Chirurgical intervention	____ yr.	_____	_____.__	_____.__	_____
☐ Montignac	____ yr.	_____	_____.__	_____.__	_____
☐ Other, specify	____ yr.	_____	_____.__	_____.__	_____
_____	____ yr.	_____	_____.__	_____.__	_____
_____	____ yr.	_____	_____.__	_____.__	_____
_____	____ yr.	_____	_____.__	_____.__	_____
_____	____ yr.	_____	_____.__	_____.__	_____
_____	____ yr.	_____	_____.__	_____.__	_____
_____	____ yr.	_____	_____.__	_____.__	_____
_____	____ yr.	_____	_____.__	_____.__	_____

HEIGHT and BODY WEIGHT

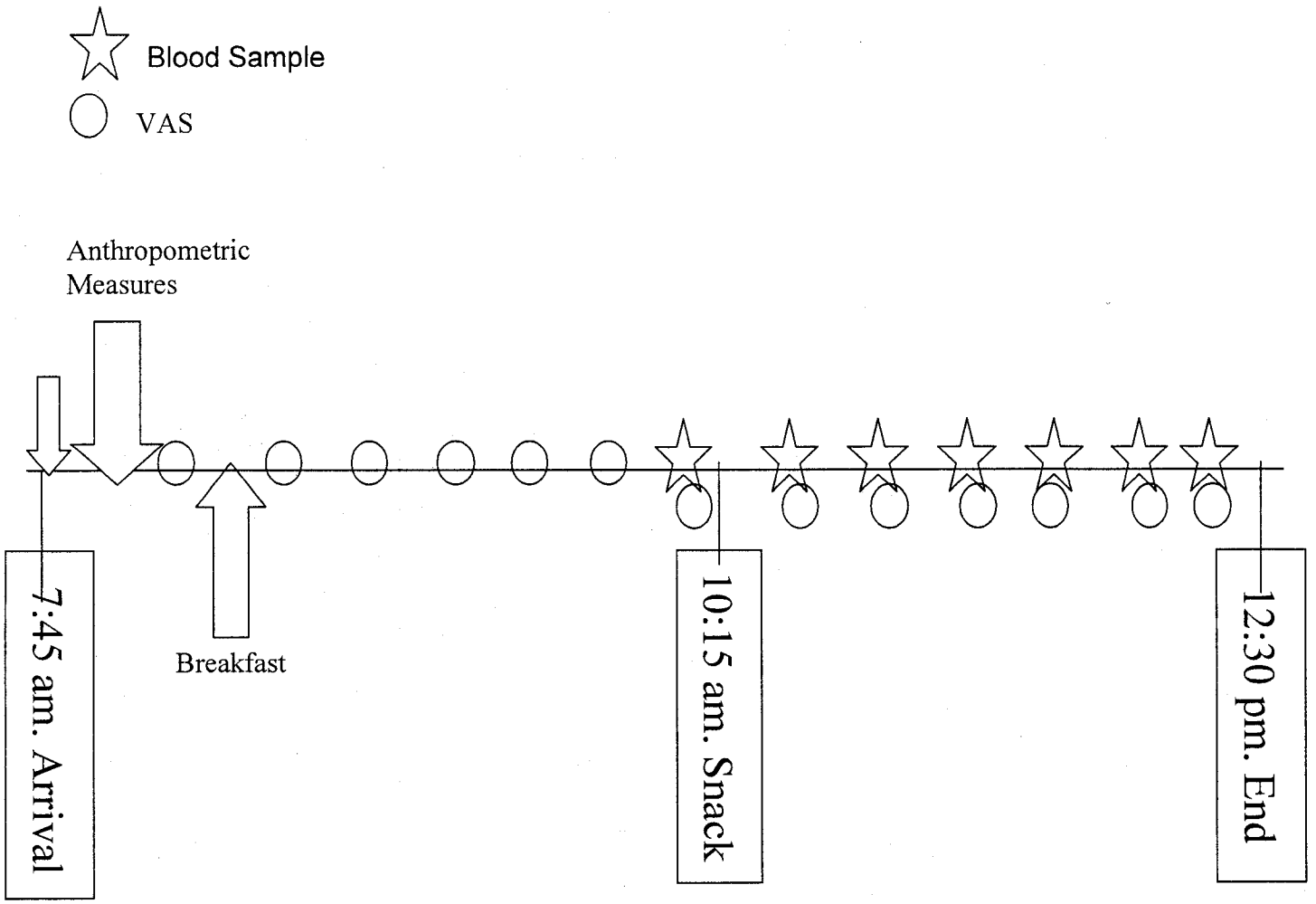
HEIGHT AND BODY WEIGHT

Height _____.___ cm

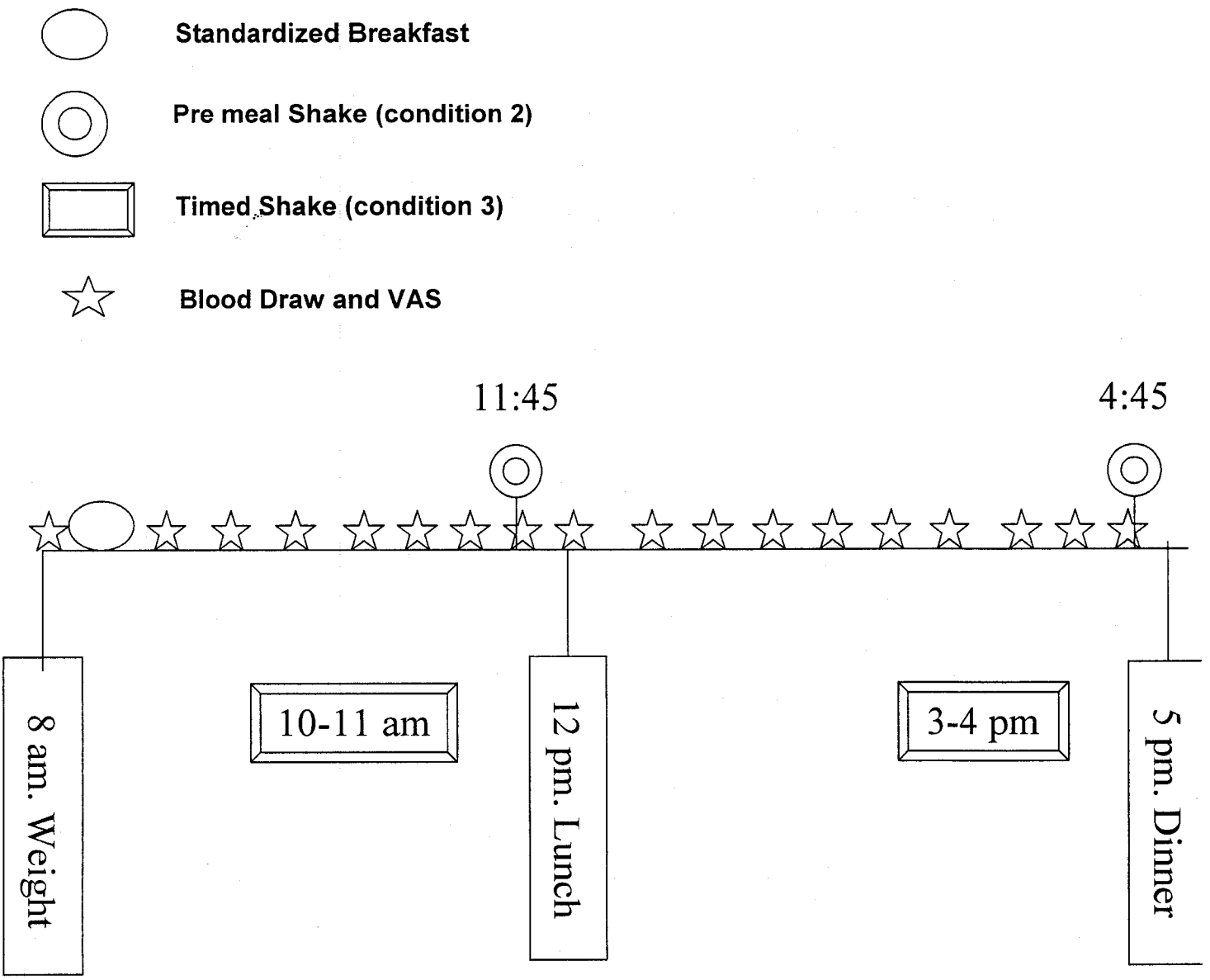
Weight _____.___ kg

BMI _____.___ kg/m^2

APPENDIXB-Design



SCREENING SESSION



EXPERIMENTAL SESSION

APPENDIX C- Snack and food composition

GISS EXPERIMENTAL SESSION

MEAL BREAKDOWN

Food Type	Energy (Kcal)/serving	Protein (g)	Carbohydrate (g)	Fats (g)
Special Pizza (mushrooms, ham, peperonni)	200 Kcal/83g	9	19	10
Spinach Pizza	220 Kcal/98g	7	21	12
Vegetarian Pizza (tomatoes, bell peppers, onions)	190 Kcal/96g	7	21	9
Chinese Stir Fry (with steamed rice)	410 Kcal/365g	22	59	10
Barbecue Chicken with Potatoes	330 Kcal/250g	13	49	9
Beef Pot Roast (Beef, green beans, roasted potatoes, rich gravy)	320 Kcal/446g	17	44	8
Beef Lasagna	340 Kcal/340g	20	43	10

Drink Type	Energy (Kcal/Serving)	Protein (g)	Carbohydrate (g)	Fats (g)
Sprite	160 Kcal/355ml	0	43	0
Coke	160 Kcal/355ml	0	42	0
Milk 1%	80 Kcal/250ml	9	12	0
Milk 2%	130 Kcal/250ml	9	12	5
Orange Juice	110Kcal/250ml	1	26	0
Water	0	0	0	0

Snack List Composition (Condition 1)

Snack Type	Energy (Kcal/serving)	Protein (g)	Carbohydrate (g)	Fats (g)
Yogurt	150 Kcal/175g	6	27	2.5
Orange Juice	110 Kcal/250ml	1	26	0
Milk 1%	80 Kcal/250ml	9	12	0
Milk 2%	130 Kcal/250ml	9	12	5
Cheddar Cheese	80 Kcal/21 g	5	0	7
Coke	160 Kcal/355ml	0	42	0
Sprite	160 Kcal/355ml	0	43	0
Lays Regular Chips	240 Kcal/43g	3	22	15
Chocolate Chip Cookies	141Kcal/30 g	1.5	22	4.6

Snack Composition (Condition 2 and 3)

Snack Type	Energy (Kcal)	Protein (g)	Carbohydrate (g)	Fats (g)
Yogurt	152.4	10.9	27.18	0
Crème	47.4	0.3	0.3	5
Milk	41.4	3.74	2.72	1.7
Protein	61.2	15.26	0	0
Total	**302.4**	**30.2**	**30.2**	**6.7**

APPENDIX D-Consent Form

CONSENT FORM

Pre meal priming of gastrointestinal satiety factors through structured snacking

Principal Investigator: Eric Doucet
Faculty of Health Sciences, University of Ottawa
School of Human Kinetics

I, _____, agree to participate in the research Eric Doucet of the School of Human Kinetics at the University of Ottawa.

PURPOSE OF THE RESEARCH

In this study sponsored by the Canadian Institutes of Health Research (CIHR), we will investigate whether structured snacking can lead to a reduction in Energy Intake. In fact, we will investigate the effects of a high-carbohydrate/high-protein snack offered twice a day (mid-morning and mid-afternoon) at times that will maximize pre-lunch and pre-dinner fullness levels. The specific times will have been determined for each subject during a session where they will have been exposed to the snack prior to the beginning of the experimental session.

DESCRIPTION OF THE PROPOSAL

My participation will consist essentially of attending a screening visit (4.5 hrs) and three experimental conditions, each consisting of 1 testing day (approximately 9 hours per day) during which many measurements will be made. The experimental sessions will be performed between 7h45 and 5h00 pm. A detailed description of the visits is given in the following sections.

Screening

A first visit will be necessary to insure that I meet the selection criteria of this study. I will be asked to come into the laboratory in the morning after an overnight fast. During this first visit, I will read and sign the consent form for the study and I will also be asked to fill-out a questionnaire on my medical and dietary histories.
Also, anthropometric data (height, body weight, body composition (Dual Energy X-ray Absorptiometry), and waist circumference) will be collected as well as information on my current eating habits. Finally the session will conclude with the determination of the timed snack.

The intervention

If my characteristics correspond to all the inclusion criteria and I accept to participate in this study, I will be subjected to the three experimental conditions of which the order will be randomized. During these days, I will be asked to eat foods in the BMRU experimental kitchen from a 5-item menu that offers a wide selection of foods. For the duration of my participation in this study, I will be able to take part in my regular daily activities.

DESCRIPTION OF MEASURES

I will also be subjected to a series of measurements that will be performed during three experimental conditions, each consisting of 1 testing day. A detailed description of these sessions is given below.

Screening Session (7h30-12h00)

A. Arrival at the laboratory 7h30.

B. Consent form (7h30-7h45)

C. Body composition (7h45-8h00) - Body weight, height and waist circumference will be measured. A method called dual-photon x-ray absorptiometry (DEXA) will be used to measure bone density, percent body fat and percent lean body mass. I will have to lie on an examination table, fully clothed, while a low intensity x-ray will scan the entire body. The measurement takes 20 minutes. The only risk is a minimal x-ray exposure of less than 0.5 millirem. This exposure is less than the natural background from 1 day of exposure to sunlight.

D. Standardized breakfast test meal and appetite ratings (8h00-8h15) - After an overnight fast, I will be served a variety of foods at 8h00. I will be asked to eat a standardized breakfast test meal, which will consist of two slices of whole wheat bread, peanut butter, jam and 250 ml (1 cup) of orange juice, and 30 g of cheddar cheese. The meal will be designed to have an energy content of 462 kcal. Appetite ratings (ie. How hungry I feel?) will be measured before and after breakfast and every 20 minutes for a period of 4.5 hours on the visual analogue scale. From this, my peak fullness levels will be determined.

E. Determination of Timed Snack (10h15-12h15)- I will be asked to ingest a shake containing 300 kcal with 40 % carbohydrates , 40 % protein and 20 % fat. After this, an needle catheter will be inserted into my vein of the non-dominant arm. Fasting and postprandial (after meal) blood will be drawn every 20 minute for 2 hours (7 samples).

Experimental Sessions

Condition 1: No imposed snacking (consisting of 1 testing days).
Condition 2: Snack intake immediately before lunch and dinner (consisting of 1 testing days).

Condition 3: Timed snack intake as determined during the Screening visit (consisting of 1 testing days).

For each experimental condition, I will have to come to the laboratory and subject myself to the sequence of measurements described below.

A. Arrival at the laboratory 7h45.

B. Body composition (7h45-7h50) - Body weight will be taken.

C. Standardized breakfast test meal and appetite ratings (8h00-8h15) - After an overnight fast, I will be served a variety of foods at 8h00. I will be asked to eat a standardized breakfast test meal, which will consist of two slices of whole wheat bread, peanut butter, jam and 250 ml (1 cup) of orange juice, and 30 g of cheddar cheese. The meal will be designed to have an energy content of 462 kcal. Appetite ratings (ie. How hungry I feel?) will be measured before and after breakfast and every 30 minutes for a period of 9 hours on the visual analogue scale. From this, my peak fullness levels will be determined.

D. Blood samples (8h00-17h00). I will be inserted with a cathether – I will rest comfortably in a reclining bed. An intraveneous cathether will be placed in a vein in my arm. Fasting, and every hour after breakfast for 8.5 hours blood will be sampled. The samples will be used to measure the circulating peptide concentrations in my blood. My blood samples will be stored in a freezer at –70° C for the duration of the study (1 year).

E. AM Snack (10h00-11h45) – The snacks will either be consumed ad libitum from a list of different snacks *(Experimental condition 1)*, in the form of a shake immediately before lunch *(Experimental condition 2)*, or in the form of a shake at the time determined during the Screening visit *(Experimental condition 3)*.

F. Lunch (12h00-12h30) - A lunch will be served to me consisting of a variety of foods from a 5-item menu in the experimental kitchen of the BMRU.

G. PM Snack - The snacks will either be consumed ad libitum from a list of different snacks *(Experimental condition 1)*, in the form of a shake immediately before dinner *(Experimental condition 2)*, or in the form of a shake at the time determined during the Screening visit *(Experimental condition 3)*.

H. Dinner (18h00-18h30) - A dinner will be served to me consisting of a variety of foods from a 5-item menu in the experimental kitchen of the BMRU.

H. END OF SESSION 17h15.

FORESEEABLE RISKS

The risks associated with this study are low. The proposed measurements, such as anthropometrical measurements, blood samples, and body composition pose very little risk to me.

BENEFITS

My participation in this study will allow me to gather information on my body composition as well as on other health indicators (e.g. peptide concentrations...).

MONETARY COMPENSATION

Parking at the research center is free for participants, as are all scientific tests. I will receive a compensation of $100.00 which will be paid in increments of 25$ at the beginning of the screening visits, as well as the beginning of each condition. I will not be compensated for a session for which I did not show up.

CONFIDENTIALITY AND ANONYMITY

In order to guarantee the confidentiality and anonymity of participants, all precautions and necessary measures will be taken to ensure that results and personal information of participants is kept under the strictest of confidentiality.

☐ My name will not appear on any reports. A number code will be used to identify me on all research documents.

☐ All material and information which can be linked to myself will not be made public and will be kept under the strictest confidentiality.

☐ The data collected will be kept in a locked file cabinet with limited access at the behavioural and metabolic unit in the montfort hospital. In addition, the computer files will be protected by a password. The data will be destroyed five years following their publication.

VOLUNTARY PARTICIPATION

☐ My participation in this study is entirely voluntary.

☐ I will be made aware of new findings that might influence my decision to take part in the present study.

RIGHTS OF THE PARTICIPANTS

The researcher guaranties that:

- I can withdraw from the project at any time.

- The confidentiality of the information gathered as well as the anonymity of all participants will be rigorously protected as indicated above.

Any information about my rights as a research participant may be addressed to the Protocol Officer for Ethics in Research, 550 Cumberland Street, Room 159, Ottawa, ON K1N 6N5.
(613) 562-5387 or ethics@uottawa.ca .

There are two copies of the consent form, one of which I may keep.

If I have any questions about the conduct of the research project, I may contact the researcher (Eric Doucet, 613-562-5800 ext. 4271, 353 Montpetit Hall, edoucet@uottawa.ca).

Researcher's signature: _____ Date: _____

Research Subject's signature: _____ Date: _____

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