EFFECT OF YELLOW PEA PROTEIN AND FIBRE ON SHORT-TERM FOOD INTAKE, SUBJECTIVE APPETITE AND GLYCAEMIC RESPONSE IN HEALTHY YOUNG MEN

By

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ABSTRACT

In order to elucidate the component(s) of yellow peas responsible for their health benefits, the effects of 10 or 20 g of isolated yellow pea protein (P10 and P20) or fibre (F10 and F20) on food intake (FI) at an \textit{ad libitum} pizza meal served at 30 min (experiment 1) or 120 min (experiment 2), blood glucose (BG) and appetite in young healthy males (20-30 y) were investigated. In experiment 1, P20 suppressed FI compared to all other treatments and lowered cumulative FI (pizza meal kcal + treatment kcal) compared to F10. Protein treatments suppressed pre-meal (0-30 min) BG compared to control, whereas only P20 suppressed post-meal (50-120 min) BG. In experiment 2, there was no effect of treatment on any outcome measures. Thus, protein is the component responsible for the short-term effects of yellow peas on glycaemia and FI, but its second-meal effects diminish by 2 hours post-consumption.
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<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>BG</td>
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<td>Resistant Starch</td>
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<td>SEM</td>
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<td>SDS</td>
<td>Slowly Digestible Starch</td>
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1 INTRODUCTION

The emergence of the current obesity pandemic has been accompanied by a concomitant increase in related morbidities, especially metabolic syndrome, type 2 diabetes mellitus, cardiovascular disease and some forms of cancer (Lawrence and Kopelman 2004; WHO 2006; Kopelman 2007; Danaei et al. 2011). In 2005, the Canadian Health Measures Survey, which relies on direct anthropometric measurement, found that 24.1% of Canadian adults were obese (BMI > 30 kg/m²) with another 39.7% being overweight (BMI of 25.0 – 29.9 kg/m²); a sharp increase over the last few decades (Health 2003; 2010; Shields et al. 2011). Many other developed and even developing countries are reporting equally alarming rates of obesity, especially the United States of America and the United Kingdom (House 2004; Finucane et al. 2011; Shields et al. 2011). In addition to the high morbidity and mortality that accompanies this increased prevalence of obesity are the economic costs. The direct costs associated with the treatment of overweight and obesity in Canada were estimated at $6 billion in 2006 (Anis et al. 2010).

The rapid rise in overweight and obesity has made it increasingly important to discover and develop foods, or functional food ingredients, that suppress food intake and improve metabolic regulation. Obesity is caused by a chronic energy imbalance characterized by energy intakes that are consistently higher than energy expenditures. Foods, or food ingredients, that suppress appetite and food intake have the potential to correct this imbalance, thus offering an inexpensive and safe alternative to other obesity treatments such as pharmaceuticals or surgery.

Pulses are the edible seeds of pod-bearing leguminous plants including chickpeas, lentils, beans and yellow peas. They have a low glycemic index and are very high in protein and dietary fibre that, on a kilocalorie per kilocalorie basis, are more satiating than other
macronutrients (Anderson and Li 1987; Rolls et al. 1988; Anderson and Moore 2004; Gerstein et al. 2004; Anderson et al. 2006). Epidemiological studies have consistently shown an inverse association between consumption of pulses and risk of obesity, diabetes mellitus and components of metabolic syndrome (Sichieri 2002; Papanikolaou and Fulgoni 2008; Cunha et al. 2010). Although this relationship may be, at least in part, attributable to the link between pulse (especially bean) consumption and a healthy lifestyle, it is seen consistently in cross-sectional population studies despite rigorous statistical adjustment for possible confounders such as exercise, smoking, socioeconomic status and other markers of a healthy lifestyle.

There is a general consensus that pulses are a ‘healthy’ food and many national nutrition guidelines, such as Canada’s Food Guide and the USDA’s ‘My Plate’, have special recommendations for their consumption (Health Canada 2007; USDA 2011). However, it is not yet clear which component, or components, of pulses are responsible for their benefits. Thus, the current study sought to determine whether the short-term suppression of appetite and glycemia following consumption of whole pulses, specifically yellow peas (Pisum sativum), is due, at least in part, to their fibre or protein content. Furthermore, this research provides evidence to determine whether fractionated yellow pea protein or fibre would be efficacious as value-added ingredients in functional foods aimed at suppressing food intake and controlling glycemia. In addition to their high protein and fibre content, fractionated yellow peas were chosen for several reasons: Canada is the largest producer and exporter of yellow peas in the world (Faye 2007; FAOSTAT 2011); Canadians only consume 1% of domestic production (Faye 2007; Pulse Canada 2007); and inexpensive, food-grade isolated yellow pea fibre and protein products are commercially available in large quantities.
2 LITERATURE REVIEW

2.1 INTRODUCTION

The following is a review of the current state of knowledge regarding the interrelationship among pulses (particularly yellow peas), obesity, food intake and other metabolic disorders starting with an overview of the mechanisms of short-term food intake regulation in humans. It then continues with a review of \textit{in vivo} studies in humans and animals to illustrate that the epidemiological associations between pulse consumption and reduced risk of obesity and other metabolic disorders are causal. Finally, the review will cover the possible mechanisms that explain the benefits of pulse consumption including the role of different macronutrient classes and other components.

2.2 MECHANISMS OF FOOD INTAKE REGULATION

The physiological and neurological mechanisms that govern when, what and how much we eat are extremely complex. They involve the integration of a plethora of signals originating from the gastrointestinal tract, the peripheral and central nervous systems, adipose and muscle tissues, environmental cues and hormonal action. The following section will give a brief overview of the current understanding of the mechanisms behind short-term food intake regulation in humans.

2.2.1 HUNGER, SATIATION AND SATIETY

The collective term ‘appetite’ is used to describe three distinct aspects of food intake regulation: hunger, satiation and satiety. Hunger is the physiological drive to eat that triggers the initiation of feeding. Satiation is the opposite; during a meal, hunger subsides until a state of ‘fullness’ is reached. This state of fullness that triggers the termination of feeding is dubbed satiation (Tolkamp \textit{et al.} 2011). The postprandial feeling of continued fullness and suppression of hunger is called satiety. In other words, satiety is the lasting
suppression of hunger between meals that helps determine when the next meal is initiated. The study of how hunger, satiation and satiety are affected by different foods or food components may help elucidate the cause of the current obesity epidemic and may help to quell it.

2.2.2 Short-term Regulation of Food Intake

Short-term food intake is essentially a function of how often we eat (determined by satiety) and how much we eat at each meal (determined by satiation). Blundell (1991) describes the short-term physiological control of food intake as a ‘satiety cascade’ (Blundell 1991). This is somewhat of a misnomer since the cascade describes the stages of satiation and satiety. His satiety cascade has four main stages, which are, in order: sensory, cognitive, pre-absorptive and post-absorptive.

The sensory and cognitive stages include the mental and physical preparation in anticipation of feeding (the cephalic phase) as well as the psychological responses arising in response to environmental and hedonistic cues during a meal. At the onset of a meal, the act of chewing, tasting, smelling and swallowing food has a positive effect on hunger (Bellisle 2003).

2.2.2.1 Gastric Distention

Pre-absorptive satiety signaling begins when food enters the stomach, causing an increase in gastric volume, which triggers appetite-suppressing signals directly to the brain. Independent of nutrient content, stretch receptors in the gastric wall signal gastric distention, mostly through the afferent fibres of the vagus nerve (Gonzalez et al. 1986), to the brain, which has a negative feedback on hunger (Wang et al. 2008a). Using functional magnetic resonance imaging (fMRI), Wang et al. (2008) measured brain activation in 18 adult healthy humans (3 females, 15 male) in response to gastric balloon distention (Wang
et al. 2008a). They found distinct patterns of neuronal activation in regions of the brain thought to be associated with food intake (amygdala, insula, etc.) during the high volume (500 mL) and low volume (0 mL) conditions. Furthermore, the high and low gastric distention-induced brain activation patterns were accompanied by higher and lower ratings of fullness, respectively, as measured in real-time on a four-point fullness scale. Two studies by Geliebter (1988) in healthy-weight and obese adults found that a gastric balloon with volume ≥ 400 mL was sufficient to significantly suppress food intake in healthy-weight and obese adults (Geliebter 1988). The results of these studies show that increasing gastric distention during a meal suppresses food intake via almost instantaneous stimulation of particular regions of the brain regardless of the content of the ingested food. It is thus one of the earliest mechanisms to modulate appetite during (satiation) and after (satiety) the ingestion of food.

Gastric distention also contributes to the suppression of food intake by interfering with the peristaltic motion of the distal stomach and proximal small intestine (Dalton et al. 1992). Dalton et al. (1992) placed pliable inflatable bags into the stomachs of dogs to measure the effect of 0 to 25 ml/kg of gastric volume on peristalsis in the stomach and small intestine (Dalton et al. 1992). They found that at gastric volumes > 12.5 ml/kg, peristalsis was completely abolished in the antrum and duodenum, and inhibited by 62% and 25% in the proximal and distal jejunum, respectively. After transthoracic vagotomy, the artificial gastric distention failed to inhibit peristalsis. These findings show that regardless of nutrient content, the ingestion of food stretches the stomach walls, leading to the slowing of peristalsis mediated through the vagus nerve, thus slowing the passage of chyme through the upper gastrointestinal tract and suppressing appetite. This depression of peristalsis also works to prolong the duration that the stomach is distended postprandially, thus extending feelings of fullness between meals (Wang et al. 2008a).
2.2.2.2 Gastric Emptying

The rate at which chyme passes through the pyloric sphincter into the duodenum (the gastric emptying rate) is also a major pre-absorptive determinant of satiety. Gastric emptying rate contributes to short-term satiation and satiety in two ways: reducing or prolonging the time that the stomach is distended; and by controlling the rate at which the proximal small intestine is exposed to nutrients (Janssen et al. 2011).

Many factors alter the gastric emptying rate and thus influence satiety and satiation. Due to the stomach’s lack of chemoreceptors, the gastric emptying rate during a meal is largely independent of the macronutrient content of a meal. However, in a randomized control trial in healthy middle-aged adults, gastric emptying rate was slowest following a high fat milkshake (90 g of 50% cream) versus isovolumetric low-fat milkshakes (8 g of 50% cream) and glucose control drinks (Hadi et al. 2002). This could be due to the difference in energy content (596, 238 and 180 kcal, respectively) between the drinks and not the macronutrient content itself. Similarly, middle-aged men suffering from type-2 diabetes, but otherwise healthy, have greatly suppressed gastric emptying in response to a 30 mL preload of olive oil compared to 30 mL of water (Gentilcore et al. 2006). The lack of an isocaloric carbohydrate or protein treatment, both of which also suppress gastric emptying, for comparison in these two studies leaves the question of the role of macronutrients in determining gastric emptying rate unanswered (Burn-Murdoch et al. 1978; Haberer et al. 2011).

Energy density modulates gastric emptying rate and is likely responsible for the suppression of gastric emptying following the consumption of all macronutrient types (Marciani et al. 2001; Faas et al. 2002). Faas et al. (2002) showed that four equicaloric and isovolumetric meals varying in their carbohydrate to protein ratio as well as consistency (homogeneous versus heterogeneous) had similar gastric emptying rates as measured by
magnetic resonance imaging in healthy adult males (Faas et al. 2002). Furthermore, Calbet and MacLean (1997) fed six healthy adults four isovolumetric drinks containing glucose, pea protein, whey protein or milk protein (Calbet and MacLean 1997). Although the drinks were not isocaloric, they found that the caloric density of the drinks was highly correlated with both gastric emptying rate ($r = 0.96, p < 0.05$) and the rate of energy delivery to the duodenum ($r = 0.99, p < 0.001$) (Calbet and MacLean 1997). Taken together, these results suggest that although gastric emptying rate appears to differ in response to different macronutrients, the effect is driven by caloric density (4 kcal/g for protein and carbohydrates versus 9 kcal/g for fat).

The diminished ability to suppress gastric emptying in response to higher density foods may contribute to suppressed satiety and satiation leading to greater energy intake. A systematic review by Xing and Chen (2004) concluded that the gastric emptying rates of obese individuals are not reduced in response to the ingestion of solid, energy-dense foods to the same extent as their lean counterparts (Xing and Chen 2004). Another review specifically investigated the relationship between gastric emptying and food intake and found that slowed gastric emptying was associated with lower food intake (Hunt 1980). Furthermore, several short-term randomized controlled trials have found a strong inverse relationship between gastric emptying rate and satiety, as measured by food intake or visual analog scales (Di Lorenzo et al. 1988; Wisen and Hellstrom 1995).

Although it is clear that gastric emptying rate itself is directly related to food intake and satiety, it is largely controlled by feedback loops from the small intestine in response to nutrient-sensing by enterocytes. Thus, perhaps the most important role of gastric emptying, besides controlling gastric distention, is modulating the rate at which chyme reaches the small intestine where the digestion and absorption of nutrients
initiates a cascade of gastrointestinal peptide release that characterizes the next period of satiation and satiety.

2.2.2.3 Gastrointestinal Peptides

After a lag phase where little chyme reaches the duodenum, chemoreceptive intestinal mucosa cells in the duodenum begin responding to the influx of nutrients by secreting a cornucopia of peptides that act both locally (autocrine or paracrine signaling) and globally (endocrine signaling) to prepare the body for the incoming torrent of nutrients (Blundell 1991; Badman and Flier 2005). The best characterized of these peptides include ghrelin, the only confirmed orexigenic gastrointestinal peptide, and cholecystokinin (CCK), peptide YY (PYY) and glucagon-like-peptide-1 (GLP-1), which are generally considered anorexigenic.

In between meals, as satiety wanes and once again gives rise to hunger, ghrelin is secreted into peripheral circulation by the parietal cells of the gastric pits (Kojima et al. 1999). This rise in plasma ghrelin stimulates appetite by binding to receptors in the appetite-regulating regions of the arcuate nucleus of the hypothalamus; an area that is not protected by the blood brain barrier and therefore able to respond to peripheral ghrelin levels (Moran and Dailey 2011). The specific role of ghrelin in stimulating appetite is supported by studies that found an increase in food intake following ghrelin injection. This increase in food intake seems to be primarily driven by an increase in meal frequency rather than energy intake at any given meal (Tschop et al. 2000; Faulconbridge et al. 2003). Following a meal, plasma ghrelin levels quickly drop, giving way to appetite suppressing hormones (Moran and Dailey 2011).

Gastrointestinal CCK is secreted from ‘I’ cells which are mostly located in the mucosa of the proximal duodenum (Wright et al. 2011). These enteroendocrine cells respond quickly, within 10-20 minutes of meal initiation, most potently in response to the
presence of luminal protein and fat, but also carbohydrate (Liddle et al. 1985; McLaughlin et al. 1999; Bellissimo and Anderson 2003). CCK then enters peripheral blood circulation. Two CCK receptors, CCK-A and CCK-B, have been identified in peripheral and CNS tissues, with CCK-A being more highly concentrated in the brain (Davidowa et al. 1997; Mercer and Beart 1997). Activation of the CCK-A receptor by CCK or other agonists induce satiety and reductions in food intake (Corwin et al. 1991; Crawley and Corwin 1994; Miyasaka et al. 1994), whereas CCK-B receptor agonists induce feelings of anxiety and nausea (Fink et al. 1998).

The appetite-suppressing effects of CCK can be explained by several mechanisms acting in concert. By activating CCK-A receptors on vagal neurons and on the smooth muscle cells of the muscularis externa of the stomach and pyloric sphincter, CCK inhibits gastric emptying (Liddle et al. 1985; Liddle et al. 1986; Crawley and Corwin 1994). This delays the onset of the post-absorptive stages of satiety as well as increases gastric distention, the net result of which is increased satiety and satiation (Lateef et al. 2011). CCK enters the CNS via specific and non-specific transport mechanisms (Banks and Kastin 1996), subsequently activating neurons in appetite-regulating regions of the brain, such as the hypothalamus, amygdala and hippocampus, suppressing hunger and short-term food intake (Gibbs et al. 1973a; Gibbs et al. 1973b; Crawley and Corwin 1994; Zhu et al. 2011).

PYY and GLP-1 are anorexigenic peptides secreted by the enteroendocrine L cells along the entire small intestine in response to direct contact to luminal nutrients and, in the case of PYY, by the paracrine action of CCK (Brennan et al. 2007; Degen et al. 2007; Moran and Dailey 2011). Endogenous or exogenous excursions of PYY and/or GLP-1 have been shown to induce satiety and suppress short-term food intake in rats and humans (Scott and Moran 2007; Moran and Dailey 2011).
Once in circulation, the N-terminus of the 36 residue PYY peptide is cleaved to leave the active PYY(3-36) form (Mentlein et al. 1993). PYY(3-36) then acts on Y2 receptors in the arcuate nucleus and vagal neurons to suppress appetite via appetite-center signaling and suppression of gastric emptying (Batterham et al. 2002; Moran et al. 2005).

GLP-1 is most potently secreted in response to fat or protein ingestion, but a slight rise can be seen after the ingestion of readily-digestible carbohydrate (Elliott et al. 1993). GLP-1 is rapidly degraded in the plasma (two min half-life in rats) by dipeptidyl peptidase-4 (DPP IV) and thus is likely to mediate its anorexigenic effects by activation of vagal afferents proximate to their site of secretion and subsequent activations of food intake regulatory regions of the hindbrain (Kieffer et al. 1995). GLP-1 infusions have also been shown to suppress gastric emptying rate (Meier et al. 2003). In addition to its direct effects on food intake, GLP-1 suppresses appetite via several indirect mechanisms. It enhances the survival of pancreatic β-cells, increases insulin secretion and improves insulin sensitivity of peripheral tissues in rats and humans (Drucker and Nauck 2006; Wu et al. 2011).

After a meal, CCK, PYY, GLP-1 and other hormones act synergistically to quickly and effectively suppress postprandial appetite during and after a meal (De Silva et al. 2011). Once the nutrients in a meal begin to be fully digested and absorbed in large quantities in the small intestine, the post-absorptive stage of postprandial satiety begins.

2.2.2.4 Post-absorptive Control of Appetite

Once chyme reaches the small intestine, pancreatic digestive enzymes begin breaking down carbohydrates (excluding fibre and resistant starches), proteins and triglycerides into their component parts; namely mono- and disaccharides, oligopeptides and amino acids, and free fatty acids and monoglycerides, respectively. As these end
products of macronutrient digestion are absorbed and their plasma levels rise, they induce satiety via direct and indirect mechanisms. Two of the most potent determinants of post-absorptive satiety are insulin and glucose, the physiological mechanisms of which will be the focus of this section.

The glucostatic theory of appetite control was first suggested by Dr. Jean Mayer in 1952 (Mayer 1991). He proposed the logical hypothesis that food intake was regulated by the body in such a way as to minimize perturbations in blood glucose concentrations and that this was mainly driven by glucose-sensing neurons in the hypothalamus (Mayer 1991). Although we now know there are many other factors contributing to food intake regulation, blood glucose levels are generally found to be inversely proportional to food intake (Anderson and Woodend 2003b; Anderson and Woodend 2003a). Furthermore, peripheral glucose infusions acutely suppress food intake in rats and humans (Langhans et al. 2001; Cha et al. 2008). This suggests that meals that cause the largest postprandial blood glucose rise will suppress appetite the most. A study by Anderson et al. (2002) found food intake to be inversely correlated with blood glucose at an ad libitum test meal served one hour after carbohydrate drinks of varying digestibility (Anderson et al. 2002). This is likely mediated by several factors including direct sensing of blood glucose levels by the hypothalamus, with lower levels leading to higher desire for food (Page et al. 2011), delayed gastric emptying (Vella et al. 2004) and interactions with gut hormones (Nauck et al. 2003) and insulin (Anderson et al. 2006).

Although some postprandial appetite suppression can be attributed to blood glucose levels alone, insulin levels also rise in concert with glucose and exert several satiating effects. Produced from the pancreatic β-cells of the islets of Langerhans, insulin is secreted in a pulsatile fashion in response to nutrient absorption (Steinert and Beglinger 2011). The main effects of insulin are to increase glucose uptake in peripheral tissues and
fatty acid synthesis, promote glycogen storage and generally shift the body from the fasting catabolic state to the fed anabolic state (Plum et al. 2006).

The most potent predictor of insulin secretion is plasma glucose and it affects short-term food intake and satiety in several ways (Drazen and Woods 2003). Insulin acts to increase the satiating properties of blood glucose, and vice versa. Insulin-sensitive GLUT4 and GLUT8 have been detected in appetite-regulating areas of the brain, including the hypothalamus (Alquier et al. 2006). These glucose transporters are translocated to the plasma membrane of glucose-sensing hypothalamic neurons, increasing their sensitivity to blood glucose levels and therefore suppressing appetite (Alquier et al. 2006). At the same time, glucose metabolism in pancreatic β-cells enhances secretion of insulin (Steinert and Beglinger 2011). The products of glycolysis trigger membrane depolarization in β-cells and subsequent release of insulin (Steinert and Beglinger 2011). This positive feedback loop leads to elevated glucose and insulin levels following a meal and consequently enhances postprandial satiety.

Insulin concentrations are inversely proportional to appetite. Rats given exogenous insulin eat less and rapidly lose body weight (Langhans et al. 2001; Woods and Seeley 2001; Woods et al. 2006). Conversely, rats with antibody-inactivated insulin have increased food intake (Brown et al. 2006). The same relationships have been confirmed in humans (Hallschmid et al. 2004a; Hallschmid et al. 2004b).

2.2.2.5 Environment, Behaviour and Organoleptic Properties

Despite all of these physiological negative feedback systems controlling short-term food intake, obesity rates continue to rise. This is because, at least in humans, freedom of choice means that preferences for certain types of foods and environmental factors such as food availability, social settings, serving size, the presence of distractions, boredom and convenience can override physiological appetite signals, causing more frequent
consumption of large energy-dense meals (Bellisle 2003; Wansink et al. 2006; Bellissimo et al. 2007). It has been shown that during a meal, sensory characteristics including the sight, taste, smell and texture (which together comprise palatability) of foods influence the time to meal termination and thus food intake (Rogers and Schutz 1992; De Graaf et al. 1999; Sorensen et al. 2003).

All of these mechanisms of short-term food intake control act before, during and following a meal; however, they do so while overlapping a background of long-term regulation. This regulation involves many complex physiological processes. For example, leptin secretion from adipose tissue acts as a measure of fat storage and suppresses appetite (Brown et al. 2006; do Carmo et al. 2011; Dubinion et al. 2011). No one mechanism, short- or long-term, can fully account for food intake at any given meal or satiety in between meals. Rather, it is the complex interaction, whether synergistic or antagonistic, between all of these signals that determines food intake and ultimately energy balance.

2.3 MEASUREMENT OF APPETITE, FOOD INTAKE AND BLOOD GLUCOSE

This section discusses the materials and methods used in the literature to measure subjective appetite, food intake and blood glucose. Furthermore, where available, it presents data on the accuracy and precision of said measurement techniques.

2.3.1 Measurement of Subjective Appetite

Subjective feelings of appetite (and thus satiety) are measured via visual analogue scales (VAS). A VAS consists of a 100 mm printed horizontal line with opposing statements at either end. Associated with each line is a question and the opposing statements correspond to answers at opposing extremes. Study participants are asked to place an “X”
on the line, in between the two opposing statements, that corresponds to their feelings regarding the question. For example, the question "how hungry do you feel?" is followed by a VAS with the statement “not hungry at all” on the left, and the opposing statement “as hungry as I have ever felt” on the right. The “X” placed on the line indicates their level of hunger. These qualitative measures of subjective feelings are then quantified by recording (by way of a ruler) the distance, in millimeters, between the left limit of the VAS and the intersection of the subject’s “X.” VAS are used to measure subjective feelings of hunger, desire to eat, fullness, prospective food consumption and thirst.

Because variables measured with VAS are so subjective, they tend to have high between-subject variation. Therefore VAS are best used for within-subject repeated measures designs where each subject’s VAS response is only compared to their own on different occasions. Indeed, the use of VAS for the measurement of subjective appetite has been validated in adults and in adolescents (Stratton et al. 1998; Flint et al. 2000; Bellissimo et al. 2008). In addition, a review by Stubbs et al. (2000) on the use of VAS to measure appetite concluded VAS to be a useful and reliable tool for the measurement of appetite, especially for within-subject experimental designs (Stubbs et al. 2000). Furthermore, the authors found VAS appetite measures to be highly reproducible and sensitive to experimental manipulations (Stubbs et al. 2000). The plethora of recent literature utilizing VAS for the measurement of appetite in adults also attests to its utility (Akhavan and Anderson 2007; Samra and Anderson 2007; Hamedani et al. 2009; Akhavan et al. 2010).

2.3.2 Measurement of Short-term Food Intake

There are various protocols for assessing short-term food intake, but most involve providing study participants with a test meal and measuring the amount of food consumed. In our laboratory, we serve participants an ad libitum pizza meal in purpose-
made isolation cubicles. The pizzas are of the frozen factory-made variety with no crusts. This ensures uniformity of macronutrient distribution and caloric density. The pizzas are weighed after cooking and then served to the subjects until they refuse more. The leftover pizza is weighed and the difference is used to calculate energy intake. This method has been used in many within-subject repeated measures experiments with great success (Akhavan and Anderson 2007; Samra and Anderson 2007; Wong et al. 2009; Anderson et al. 2010) and inter-session reliability of food intake responses measured in this manner is high (Nair et al. 2009).

2.3.3 Measurement of Blood Glucose

Determination of blood glucose concentrations is performed by chemical or enzymatic means. The latter is more common and usually involves glucose oxidase enzyme combined with a colorimetric analysis (King and Garner 1947; Raja and Sankaranarayanan 2006). After enzymatic oxidation of glucose, a coloured substance (e.g. copper(1) oxide) is formed. The intensity of the resulting colour is directly proportional to the concentration of glucose.

This technology has been miniaturized for use in hand-held self-monitoring devices. Although the coefficient of variation of these hand-held devices is larger than for laboratory-grade equipment, their reproducibility and inter-test variation is within acceptable limits (Thomas et al. 2008). Furthermore, within-subject correlation of blood glucose measurements using some of the most common hand-held glucometers, including the one used in the current research, on separate days is also high (r > 0.7) (Nair et al. 2009).
2.4 WHOLE PULSES, OBESITY AND METABOLIC REGULATION

Pulses are the seeds of pod-bearing leguminous plants and include, but are not limited to, beans, lentils, chickpeas and yellow peas. Canada is one of the world’s largest producers and exporters of pulses, especially yellow peas, chickpeas and lentils, but domestic human consumption is very low (Faye 2007; Pulse Canada 2007). This is a lost opportunity to take advantage of the proven and potential health benefits of these healthy, inexpensive and widely available foods.

It is well known that pulses are a good source of protein, fibre and many vitamins and minerals, but their ability to aid in short- and long-term food intake regulation and glycemic management is less so and has been the focus of much investigation. This section will review the current literature regarding whole pulses, obesity and their role in food intake and glycemic regulation starting with observational studies and then moving on to short- and long-term randomized controlled trials.

2.4.1 WHOLE PULSES AND FOOD INTAKE CONTROL

There has been a lot of interest on the effect of pulses on food intake over the past few decades. The following sections review relevant observational and experimental studies regarding pulses and food intake control.

2.4.1.1 Observational Studies

There are several epidemiological studies that show an inverse relationship between the consumption of pulses and various indicators of obesity and related metabolic disorders. Brazil’s Annual National Survey of Households in 1996 used an 80-item semi-quantitative food frequency questionnaire (FFQ) to assess nutrient and food intakes of 2040 households (Sichieri 2002; Sichieri et al. 2003). Although this cross-sectional study did not attempt to correlate specific foods with obesity, it found a
significant relationship between what they called a ‘traditional’ diet and obesity protection. Furthermore, the most striking difference between the obesity-protective traditional diet and the Western diet (associated with obesity) was higher bean consumption in the former. This led the author to conclude that beans are the main diet component responsible for reducing the risk of obesity in those following a traditional diet (Sichieri 2002). The same group has recently reaffirmed the seemingly protective effect of bean consumption against obesity and a high waist circumference using up-to-date data in a low-income Brazilian neighbourhood (Cunha et al. 2010).

An association between pulse consumption and reduced risk of obesity and related disorders in the North American population has also been established. Using cross-sectional data from over 21000 non-institutionalized Americans as part of the National Health and Examination Survey (NHANES) 1999-2002, Papanikolaou and Fulgoni (2008) showed that adult (20 years of age or older) bean consumers had a 23% lower risk of having a high waist circumference and 22% lower risk of being obese than non-bean consumers (Papanikolaou and Fulgoni 2008). Bean consumption was also associated with lower systolic blood pressure. Using the same dataset, Fulgoni et al. (2006) reported a similar relationship between bean consumption and reduced risk of obesity and high waist circumference in American children (Fulgoni et al. 2006). These observational studies focus on beans because they are the most commonly consumed pulses among their respective populations. Unfortunately, the food frequency questionnaires used did not survey the consumption of other common pulses, such as chickpeas, lentils and yellow peas. As such, it is not known whether this association would extend beyond beans. However, given the very similar nutritional profiles of various pulses, it is likely that consumption of non-bean pulses is also associated with reduced risk of being obese.
It is possible that these associations are the result of pulses acting as a marker of a healthy lifestyle since people who eat beans or other pulses tend to have other healthy dietary patterns (Sichieri 2002). However, there is a growing body of evidence from randomized controlled trials that suggest at least part of the reduction in obesity risk associated with pulse consumption is due to the pulses themselves. Specifically, these benefits may be the result of the suppression of appetite after consumption of pulses leading to lower energy intakes and the correction of the chronic energy imbalance that leads to obesity.

2.4.1.2 Clinical Studies

In an attempt to determine if the observational relationship between pulse consumption and obesity is a causal one, several short-term randomized controlled trials have been conducted with varying results.

There have been several studies that show the consumption of pulses suppresses short-term subjective appetite and postprandial glycemia. Leathwood and Pollet (1988) investigated the effects of equicaloric mixed-macronutrient ‘Shepherd's pie’ containing either potato or bean puree (each made from processed and dried flakes) on blood glucose and subjective appetite over 180 minutes in 6 men and women of varying age and weights (Leathwood and Pollet 1988). Using a 24-question ten-point scale to assess hunger, fullness, prospective food consumption and gourmandise (the desire to eat tasty foods), they found that subjects were more full, less hungry and had lower gourmandise ratings after consumption of the bean-containing meals compared to the potato meals by the end of the 180 minute postprandial period (Leathwood and Pollet 1988). Although peak blood glucose response was significantly lower immediately following the bean meal compared to the potato meal (approx. 30% reduction), the bean meal had a more sustained excursion, leading to slightly higher blood glucose by the end of the study period.
Unfortunately, this study suffers from shortcomings such as low number of subjects and poor subject homogeneity, but nonetheless provides evidence that pulses are more satiating than other carbohydrate sources, even when highly processed.

In a similar experiment conducted by Pai et al. (2005), 40 healthy females aged 19-24 years were given one of five equicaloric test meals, three wheat-based varying in fibre content, one rice-based and finally a rice-pulse mixture (using slightly fermented mung beans) (Pai et al. 2005). Subjective hunger was measured using a 7-point hedonic scale and a 100 mm visual analogue scale was used for the prospective food consumption. Using the ‘satiety index’ first developed by Holt et al. (1995), which compares the satiety area under the curve (AUC) following a particular treatment to that after a white bread control, they found the rice-pulse treatment to be the most satiating of all the treatments (206.2% versus 112.7% for the comparable rice meal) (Holt et al. 1995; Pai et al. 2005). The treatments were not isovolumetric, however, and thus differed greatly in energy density (range of 1.1 to 3.0 kcal/g) and weight, both of which are likely to confound satiety scores (Rolls et al. 1998). Indeed, they found a significant inverse association between satiety score and energy density of the treatments ($r = -0.477$, $p < 0.01$) and a positive association between satiety score and fibre content of the treatments ($0.467$, $p < 0.01$), indicating these factors play a role in predicting the satiety score of a meal (Pai et al. 2005). However, since the rice-pulse treatment had the highest satiety score, but intermediate fibre and energy density, there must be some other factor, or factors, contributing to the pulses’ satiating effects. Also, Pai et al. (2005) did not measure food intake and thus it is not known if the pulse treatment would lead to a suppression of food intake.

The preceding experimental studies did not measure ad libitum food intake after consumption of pulses and thus it is not known if the observed reduction of perceived satiety would actually lead to reduced energy intakes. To fill this gap in the literature,
Wong *et al.* (2009) conducted 3 experiments to determine if the satiating effects of pulses lead to lower food intake (Wong *et al.* 2009). The authors also sought to determine whether effects on subjective appetite, blood glucose and food intake would differ depending on the processing, recipe and variety of pulses. Wong *et al.* (2009) tested the effects of lentils, chickpeas, navy beans and yellow peas on food intake at an *ad libitum* pizza meal served 120 minutes later, and on subjective appetite and glycemic response before (pre-meal) and after (post-meal) the test meal in 15 young healthy men (Wong *et al.* 2009). Except for chickpeas, all pulse treatments led to lower food intake at the test meal compared to a water control, but not compared to an equicaloric white bread treatment. However, none of the pulse treatments suppressed cumulative food intake (the sum of energy intake from the treatments and test meal) compared to the water control or white bread. This suggests that the putative long-term benefits of pulse consumption are not related to short-term suppression of food intake (Wong *et al.* 2009). However, the consumption of glucose or other high glycemic index (GI) carbohydrates (such as white bread) is very well compensated for at a subsequent meal (Anderson and Woodend 2003b; Anderson and Woodend 2003a; Anderson *et al.* 2006). Therefore the failure of the pulse treatments, which contain a mix of carbohydrate, protein and fat, to suppress short-term food intake may have been due to the comparison to white bread, which is almost entirely carbohydrate. All pulse treatments led to lower blood glucose than the white bread treatments, which was expected given their low GI on the glucose and white bread scales (Wong *et al.* 2009).

### 2.4.2 Pulses and Glycemic Control

Adherents to the glucostatic theory of food intake control have suggested the satiating properties of pulses are largely due to their effects on glycemia (Sievenpiper *et al.* 2009). This is supported by the fact that all pulses have a very low glycemic index
between 15-40 on the glucose scale (Jenkins et al. 1980; Jenkins et al. 1981; Jenkins et al. 1988; Jenkins et al. 1989; Foster-Powell et al. 2002; Araya et al. 2003). This means that the carbohydrates in pulses take very long to digest and therefore postprandial gut hormones and insulin, and their associated satiating effects, are also elevated long after meal completion (Sievenpiper et al. 2009).

Beyond the benefits of improved glycemic control on appetite, the glycemic properties of pulses have other inherent benefits beyond increased satiety which are very well characterized (Sievenpiper et al. 2009). In individuals suffering from diabetes, three weeks of a high legume diet decreases fasting BG and urinary glucose excretion compared to a similar diet without legumes (Karlstrom et al. 1987). This also could have been due to the difference in fibre content of the diets (24 versus 37 g/day), but nevertheless provides evidence that a diet rich in pulses improves glycemic control in individuals with diabetes (Karlstrom et al. 1987). Improved glycemic control may help reduce the risk of morbidities associated with hyperglycemia such as microvascular degeneration.

2.5 FRACTIONATED YELLOW PEAS (PISUM SATIVUM), FOOD INTAKE AND GLYCEMIC REGULATION

Despite a growing body of evidence that whole pulses, alone or as part of a meal, contribute to increased satiation, satiety and improved blood glucose control in the short- and long-term, there has been surprisingly little research conducted to investigate the specific component, or components, responsible for these benefits.

It is generally accepted that on a per kilocalorie basis, protein and fibre are more satiating than digestible carbohydrate, which in turn, is more satiating than fat (Westerterp-Plantenga 2003; Anderson et al. 2006). However, the short-term satiating effects of each macronutrient depend largely on the digestion kinetics, source and processing (Beaton et al. 1992; Anderson et al. 2006; Diepvens et al. 2008; Aziz et al. 2009;
Bodinham et al. 2010; Jahan-mihan et al. 2011b; Jahan-Mihan et al. 2011a). Because pulses are very high in non-digestible carbohydrates and protein, both of which are thought to have a large effect on food intake and metabolic regulatory systems, these components have received the most investigation. However, food grade isolated pulse fractions have only become available in large quantities relatively recently and fractionation methods vary greatly from processor to processor, making rigorous and reproducible investigation difficult.

The following section summarizes what little research has been done on the food intake and metabolic regulatory effects of pulse fractions, with a particular focus on yellow pea protein and fibre. As mentioned previously, yellow peas (Pisum sativum) were chosen because of their Canadian economic importance, domestic underuse and commercial availability.

2.5.1 **Yellow Pea Fibres**

Dietary fibre is associated with slowed gastric emptying and increased satiety compared to readily digestible carbohydrates (Anderson et al. 2006; Slavin and Green 2007; Hamedani et al. 2009). This may be due to the fact that fibre is resistant to digestion and therefore offers fewer available kilocalories per gram compared to other carbohydrates; leading to fullness when relatively little energy has been consumed (Rolls et al. 1998). The reduction of gastric emptying, combined with increased physical bulk of high fibre foods, may then sustain higher levels of satiety.

Fibre may help regulate food intake indirectly by lowering the blood glucose response to foods. In order for carbohydrate to be absorbed, it must diffuse from the center of intestinal digesta and come in contact with the intestinal epithelium where digestion and absorption occurs. Some dietary fibres, when hydrated, have a higher viscosity than other macronutrients, thus potentially impeding carbohydrate absorption.
and decreasing glycemic response, leading to increase satiety (Dikeman et al. 2006; Vuksan et al. 2009). Vuksan et al. (2009) found an inverse association between the viscosity of fibre pre-loads and subsequent food intake (Vuksan et al. 2009). This delay of blood glucose absorption and subsequent rise in blood glucose may also contribute to the satiating effects of fibre by modulating the concentrations of appetite-regulating hormones such as insulin and glucagon (Anderson et al. 2006).

Whole yellow peas contain 14-26% fibre dry weight, depending on the cultivar and method of analysis, of which approximately 10-15% and 2-9% is insoluble and soluble fibre, respectively (Tosh and Yada 2010). It has been suggested that this high fibre content may help explain their satiating effects (Tosh and Yada 2010). The hull of yellow peas contain up to 90% insoluble fibre, whereas the inner cotyledon contains only ~5% (Tosh and Yada 2010). The very high insoluble fibre content of the hulls makes it easy to isolate and process. Thus, most commercially available yellow pea fibre fractions are made from pea hulls. Because of this, there has been more research on the satiating effects of pea hull fibre compared to cotyledon fibre.

The short-term effects of yellow pea hull fibre are partly mediated by its bulking and gelling properties. Canibe and Knudsen (2002) measured the viscosity and other physicochemical characteristics of ileal, caecal, and faecal digesta in pigs after feeding with diets composed of dehulled barley, barley hulls, yellow pea cotyledon or yellow pea hulls (Canibe and Knudsen 2002). They found that in the ileum, the main site of carbohydrate absorption, the viscosity of the digesta was significantly greater after consumption of the pea hull diet (Canibe and Knudsen 2002). In contrast, a study in dogs showed no difference in food intake behavior following 2% dietary supplementation with pea fibre (Butterwick et al. 1994). It is possible that the lack of effect in this study was due to the relatively low level of pea fibre added. In humans, an enteral formula with pea fibre (10
g/l) and fructo-oligosaccharides (5 g/l) led to greater fullness and low appetite as measured via visual analogue scales (VAS), compared to an identical formula with no added fibre (Whelan et al. 2006).

2.5.2 **YELLOW PEA PROTEIN**

Protein is also thought to contribute to pulses' beneficial effects. Yellow peas, like all pulses, are high in protein. Depending on the cultivar, their protein content ranges from 19 to 34% dry weight (Boye et al. 2010). Protein may increase satiety and improve glycemic control by directly modulating blood concentrations of appetite-regulating hormones such as insulin, glucagon, ghrelin, CCK, GLP-1 and PYY (Anderson and Li 1987; Aziz and Anderson 2003; Anderson and Moore 2004; Aziz et al. 2005).

The rate at which proteins are digested and amino acids appear in the plasma is related to their degree of satiation after their ingestion. In general, proteins that are readily digestible, 'fast' proteins, result in peak plasma amino acid concentrations between 20 – 40 min after consumption, whereas 'slow' proteins take an hour or longer (Calbet and MacLean 2002). Whey protein, a characteristic fast protein, suppresses appetite by stimulating the secretion of satiety hormones (Luhovyy et al. 2007). As little as 20 g of whey protein suppressed *ad libitum* food intake 30 min later in young adults (Akhavan et al. 2010). Few studies have investigated the short-term satiety effects of isolated yellow pea protein. However, the digestion kinetics, and therefore the subsequent pattern of gut peptide release, of yellow pea protein are very similar to whey and therefore may have similar effects (Calbet and MacLean 2002). Furthermore, pea protein, like whey, is rich in the branched-chain amino acids leucine, isoleucine and valine, which have been shown to stimulate the release of insulin, with subsequent reductions in blood glucose and suppression of food intake (Holt and Miller 1995; Drazen and Woods 2003).
2.5.3 **Yellow Pea Starch**

The starch fraction accounts for approximately 50% of the dry weight of yellow peas (Boye *et al.* 2010). Due to high levels of amylose, the resistant starch (RS) content of yellow peas is considerable and has been proposed to contribute to their beneficial effects on glycemic and appetite control (Araya *et al.* 2003; Chibbar *et al.* 2010; Hoover *et al.* 2010). RS is any starch or starch degradation components that evade complete digestion in the human small intestine, to be partially or completely fermented by resident flora in the proximal colon (Englyst *et al.* 1992). This differs from rapidly digestible (RDS) and slowly digestible (SDS) starches, which are digested by pancreatic amylases in the upper and lower small intestine, respectively.

As predicted by the glucostatic theory of food intake regulation, patterns of postprandial satiety are related to the rate of carbohydrate digestion. A recent study in our laboratory showed that 50 g of maltodextrin (a rapidly digestible carbohydrate) suppressed appetite and food intake, with a concomitant increase in blood glucose, compared to a water control, at an *ad libitum* test meal served 30 minutes post-treatment (Anderson *et al.* 2010). Three other starches with increasing amounts of slowly-digesting carbohydrate failed to suppressed food intake at 30 minutes. However, all three high-SDS and RS starches suppressed food intake at an *ad libitum* meal served at 120 minutes compared to the maltodextrin (Anderson *et al.* 2010). This suggests that the starch fraction may be responsible for some of the short-term benefits of whole yellow peas.

Although the starch fraction of yellow peas is the largest, it is also the most sensitive to processing. It has consistently been shown that the RS content of pulses, including yellow peas, varies greatly depending on the degree of processing and cooking; from 35.2% RS in raw peas to 1.5% RS in pressure-cooked split peas (Eyaru *et al.* 2009). Thus processing of pea starch may alter its satiating and glycemic properties, making
rigorous study of the short-term effects of yellow pea starch difficult, which may explain the dearth of pea starch research compared to the protein and fibre fractions.

2.5.4 *Yellow Pea Fat*

Although intestinal fat stimulates the release of CCK and some other gut peptides, it is the least satiating of the macronutrients (Anderson *et al.* 2006). This could be due to the high caloric density of fat, approximately 9 kcal/g compared to 4 kcal/g for protein and carbohydrates. Meals that have a high energy density are less satiating than those with a low energy density in adults and children (Rolls *et al.* 1988; Rolls *et al.* 1998; Overduin *et al.* 2005; McCrory *et al.* 2006; Leahy *et al.* 2008a; Leahy *et al.* 2008b). Because of the poor satiating properties of fats, and the low content of fat in yellow peas (< 3 % dry weight), it is unlikely the short-term benefits of yellow pea are due to the fat component (Frias *et al.* 2011).
3 HYPOTHESIS, OBJECTIVES AND DESIGN

3.1 HYPOTHESIS

It is hypothesized that yellow pea protein, but not fibre, will suppress food intake at the pizza meal served 30 min after consumption, as well as pre-meal and post-meal subjective appetite and BG. Furthermore, it is hypothesized that yellow pea fibre, but not protein, will suppress food intake at the pizza meal served at 120 min post consumption, as well as pre- and post-meal subjective appetite and BG.

3.2 OBJECTIVES AND DESIGN

3.2.1 MAIN OBJECTIVE

The main objective of this study was to determine the effect of different doses of isolated yellow pea protein and fibre, given alone, on food intake at an *ad libitum* meal served 30 (experiment 1) or 120 min (experiment 2) after consumption.

3.2.2 SECONDARY OBJECTIVES

Secondary objectives of this study were to determine the effects of different doses of isolated yellow pea protein and fibre, given individually, on subjective appetite and glycemic response after consumption as well as after a subsequent *ad libitum* meal served at 30 or 120 min.

3.3 EXPERIMENTAL DESIGN

Two experiments were designed to test the above hypothesis and meet the study's objectives. Experiment 1 investigated the effects of different doses of isolated yellow pea protein and fibre on food intake at an *ad libitum* test meal served at 30 mins after consumption of the fractions as well as pre- and post-meal subjective appetite and BG. Experiment 2 investigated the effects of different doses of isolated yellow pea protein and
fibre on food intake at an *ad libitum* test meal served at 120 mins after consumption of the fractions as well as pre- and post-meal subjective appetite and BG.
CHAPTER 4

THE EFFECT OF YELLOW PEA PROTEIN AND FIBRE ON SHORT-TERM FOOD INTAKE, SUBJECTIVE APPETITE AND GLYCEMIC RESPONSE IN HEALTHY YOUNG MEN

The following chapter is a reproduction of a manuscript that has been submitted and accepted to the British Journal of Nutrition
THE EFFECT OF YELLOW PEA PROTEIN AND FIBRE ON SHORT-TERM FOOD INTAKE, SUBJECTIVE APPETITE AND GLYCEMIC RESPONSE IN HEALTHY YOUNG MEN

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Running Head: Yellow pea fractions, food intake and glycemia

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4.1 ABSTRACT

Pulses are low-glycemic foods rich in protein (20-25%), resistant starch and fibre that suppress appetite and glycemia. The objective of this study was to elucidate the component(s) of yellow peas responsible for these benefits and assess their efficacy as value-added food ingredients. We investigated the effects of 10 or 20 g of isolated yellow pea protein (P10 and P20) or fibre (F10 and F20) on food intake (FI) at an *ad libitum* pizza meal served at 30 min (experiment 1, n = 19) or 120 min (experiment 2, n = 20) and blood glucose (BG) and appetite in young healthy males (20-30 y). In experiment 1, P20 led to lower FI than control (1180 ± 120 vs. 1346 ± 111 kcal) and all other treatments (p< 0.01) and lower cumulative FI (pizza meal kcal + treatment kcal) compared to F10 (1305 ± 119 vs. 1454 ± 108 kcal) (p=0.033). Both protein treatments suppressed pre-meal (0 – 30 min) BG compared to control (p<0.05), whereas only P20 suppressed post-meal (50 – 120 min) BG (p<0.01). There was no effect of treatment on pre-meal or post-meal appetite. In experiment 2, there was no effect of treatment on FI, CFI, or pre- or post-meal BG or appetite. In conclusion, protein is the component responsible for the short-term effects of yellow peas on the regulation of glycemia and FI, but its second-meal effects diminish by 2 hours post-consumption.
4.2 INTRODUCTION

The emergence of the current obesity pandemic has been accompanied by a concomitant increase in related morbidities, especially metabolic syndrome and diabetes mellitus (WHO 2006). This has made it increasingly important to discover and develop foods, or functional food ingredients, that suppress food intake and improve metabolic regulation. Foods, or food ingredients, that suppress appetite and food intake have the potential to correct the chronic energy imbalance that leads to obesity, thus offering an inexpensive and safe alternative to other obesity treatments such as pharmaceuticals or surgery.

Pulses, the edible seeds of pod-bearing leguminous plants including chickpeas, lentils, beans and yellow peas, have a low glycemic index and are very high in protein and dietary fibre which are more satiating than other macronutrients (Anderson and Li 1987; Gerstein et al. 2004; Anderson et al. 2006). Epidemiological studies have consistently shown an association between consumption of pulses and reduced risk of obesity, diabetes mellitus and components of metabolic syndrome (Sichieri 2002; Papanikolaou and Fulgoni 2008; Cunha et al. 2010).

Pulses are also reported to have significant short-term physiological benefits. When consumed alone or as part of a mixed-macronutrient meal, pulses suppress appetite and blood glucose not only to the meal, but also following a subsequent meal served up to four hours later (Wong et al. 2009), (Mollard RC et al., unpublished results). Although the health benefits associated with pulse consumption are well documented, the component, or components, responsible for their effects has not been investigated. This led us to investigate the isolated fibre and protein fractions of yellow peas to determine if these components are responsible for the observed beneficial effects on glycemic response and weight management of whole pulses. Yellow peas (Pisum sativum) were chosen because,
despite being among the least expensive and most abundant non-oilseed pulses, their consumption in the countries with the highest rates of overweight and obesity (mostly North American and European markets) is extremely low (Schneider 2002). Yellow peas are also safe, inexpensive and high-quality food-grade fibre and protein fractions are commercially available and approved for human consumption.

The hypothesis of the current study was that the suppression of short-term food intake, appetite and blood glucose after consumption of whole yellow peas is due, at least in part, to their protein and fibre fractions. Therefore, the objective of the present study was to investigate the effects of isolated yellow pea protein and fibre, given individually, on short-term food intake 30 and 120 minutes later as well as subjective appetite, and glycemic response in young healthy men. Furthermore, this study provides insight into the efficacy of yellow pea fractions as value-added ingredients aimed at suppressing food intake and controlling blood glucose response.

4.3 EXPERIMENTAL METHODS

4.3.1 SUBJECTS

Healthy male participants 20-30 years of age with a body mass index (BMI) of 20.0 – 24.9 kg/m² were recruited via advertisements placed around the University of Toronto St. George campus (Appendix 8.2), local newspaper classifieds and on student websites. Females, smokers, breakfast skippers and those on medication, with metabolic disorders or scoring greater than or equal to eleven on an Eating Habits Questionnaire (Velangi et al. 2005) (Appendix 8.3.1) were excluded from the study. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the University of Toronto Health Sciences Research Ethics Board. Written informed consent was obtained from all subjects (Appendix 8.3.2).
4.3.2 Experimental Design

Two experiments were conducted in which food intake (FI) was measured at 30 min (experiment 1) and at 120 min (experiment 2) after consumption of the treatments. Both experiments were single blind, randomized, repeated measures designs in which subjects received one treatment per week one week apart.

4.3.3 Treatments

The same five treatments were used in both experiment 1 and experiment 2 and were as follows: tomato soup (control) with either 10 g (F10) or 20 g (F20) yellow pea fibre or 10 g (P10) or 20 g (P20) yellow pea protein added. Commercially available yellow pea protein and fibre, by the trade names Propulse (82% protein dry weight) and Centara5 (94% fibre dry weight from yellow pea hulls), respectively, were provided by Nutri‐Pea Ltd. (Portage la Prairie, Manitoba). The protein fraction is isolated from the cotyledon, whereas the fibre is made from yellow pea hulls and contains approximately 85% insoluble and 15% soluble fibre (Nutri‐Pea Limited, Portage la Prairie, Manitoba, personal communication). The F10 and F20 treatments contained 10.6 and 21.3 g of the pea fibre isolate, respectively, to yield 10 and 20 g of net fibre. Similarly, the P10 and P20 treatments contained 12.2 and 24.4 g of pea protein isolate, respectively, to yield 10 or 20 g of pea protein. The control was a tomato soup with no added fractions. All treatments were isovolumetric (300 mL) and contained the following ingredients: tomato paste (55 g), pepper (0.6 mL, Verona, Montreal, QC), garlic herbs (2.5 mL, McCormick Garlic and Herb Seasoning, London, ON), worcestershire sauce (2.5 mL, Lea & Perrins, North York, Ontario), dried basil (0.6 mL, McCormick Canada Inc., London, ON), Tabasco sauce (0.25 mL, McIlhenny Company, Avery Island, LA), and lemon juice (1.2 mL, Canada Dry Mott’s, Mississauga, ON). The sodium content of a meal has been linked to altered postprandial satiety and glycemia (Thorburn et al. 1986; Driver 1988). Sodium content, determined
using a modified version of AOAC method 985.01, was highest in the P20 treatment (294 mg) and thus 570.8, 454.9, 338.0 and 285.4 mg of salt (Sifto table salt, Mississauga, ON) was added to the control, F10, F20 and P10 treatments, respectively, to equalize their sodium content and eliminate any confounded effects of sodium. The treatments were prepared 20 minutes before the scheduled arrival of the subjects and 85°C water was added upon their arrival to make up a total treatment volume of 300 mL. All treatments were served with 100 mL of bottled water as palate cleansers. The order of treatments was determined via random number generator for each subject. The nutritional composition of the five treatments is given in Table 4.1.

4.3.4 Protocol

As described previously (Anderson et al. 2002; Anderson et al. 2004), subjects were asked to pick a time between 10:00 and 13:00 and a day of the week they wished to begin each of their sessions. Before each session, subjects fasted for 10-12 hours overnight, after which they consumed a standardized breakfast consisting of 26 g of Honey Nut Cheerios cereal (General Mills, Mississauga, Ontario), 250 mL of Beatrice 2% milk (Parmalat Canada, Toronto, Ontario), and 250 mL Tropicana orange juice (Tropicana Products Inc., Bradenton, Florida). A 500 mL bottle of water was also provided to be consumed between breakfast and 2 hours prior to the start of their session, after which no food or drink was to be consumed. Subjects were asked to abstain from caffeine and alcohol the night before their sessions and to maintain their normal routine of food intake and physical activity.

Upon arrival in the lab for each session, subjects completed a Sleep Habits and Stress Factors questionnaire and a Food Intake and Activity Questionnaire in order to assess their compliance with fasting and physical activity instructions (Appendix 8.4.3). Sessions were rescheduled if any irregularities were found that might have altered the
subject's appetite or metabolism. Subjects then completed the baseline (0 min) Visual Analogue Scales (VAS) to measure motivation to eat (to quantify subjective appetite), thirst, physical comfort, and energy and fatigue (Appendix 8.4.4). VAS consist of a 100 mm printed line anchored to opposing statements on either side. Subjects were informed to place an 'X' on the line at a location that reflects their level of agreement with the two statements. The VAS were scored by measuring the distance, in millimeters, from the leftmost statement to the intersection of the marked 'X'. This allowed for the accurate measurement of variables on a continuum instead of discrete categories to which the subjects' feelings may not correspond. The utility of VAS for measuring subjective appetite has previously been validated in children as well as adults (Flint et al. 2000; Bellissimo et al. 2008).

A baseline blood glucose measurement was then taken via finger prick using a Monojector lancet (Sherwood Medical, St. Louis, Missouri) and capillary blood glucose measured and converted to plasma glucose equivalent using an Accu-check Compact-Plus glucose monitor (Roche Diagnostics Canada, Laval, Quebec). A baseline measurement of greater than 5.5 mmol/L suggested non-compliance with the fasting instructions and subjects were rescheduled accordingly. As per the manufacturer's instructions, the first drop of blood was wiped away and the second drop placed on the testing strip. Subjects were assigned specific glucose monitors and testing strip batch codes for the duration of the study. Quality control measurements using two concentrations (6.3 and 10.0 mmol/L) of Assayed Human Multi-Sera (Randox Laboratories Ltd., Antrim, United Kingdom) were performed before each session to ensure glucose monitors and test strips were within an acceptable range of accuracy.

Following the completion of the baseline measurements, subjects were given 5 min to consume their treatment, along with 100 mL of bottled water to cleanse their
palate. VAS measuring treatment palatability were then filled out to ensure appetite and
FI were not affected by the subject’s dislike of a treatment. The palatability VAS consisted
of three questions assessing the pleasantness, taste and texture of the treatments. In
experiment 1, blood glucose (via finger prick), subjective appetite, physical comfort and
energy fatigue (via VAS) were measured at 15 and 30 (pre-meal) as well as at 50, 65, 80,
95, 110, 140 and 170 (post-meal) min after consumption of the treatment. In experiment
2, the same outcomes were measured at 15, 30, 45, 60, 90 and 120 min (pre-meal) as well
as 140, 155, 170, 185 and 200 (post-meal) min after consumption of the treatments.

FI was measured at 30 (experiment 1) or 120 (experiment 2) min after
consumption of the treatments. Subjects were instructed to eat until ”comfortably full”
and given 20 min during which they were served an *ad libitum* pizza meal consisting of
McCain Deep ‘N Delicious (McCain Foods Canada, Florenceville, New Brunswick) pizzas,
prepared, as suggested by the manufacturer, by baking for 8 minutes in a 221°C oven,
along with *ad libitum* bottled water. Nutritional content of the pizzas can be found in
Appendix 8.1. Pepperoni, Three cheese, and Deluxe pizzas were served, four at a time, with
two pizzas of their first choice as determined during screening, and one each of the others.
A fresh batch of four pizzas was served every 7 minutes and water was given as needed.
Food and water intake (WI) was measured by weighing the cooked pizza and bottled
water before and after the meal without the subjects’ knowledge. The manufacturers’
nutritional information was used to calculate energy intake in kJ. The three varieties of
pizza averaged 10.0 g of protein, 7.6 g of fat, 26.6 g of carbohydrate and 945.6 kJ per 100 g.
Following the pizza meal, subjects filled out a VAS measuring the palatability of the pizza
meal.
4.3.5 **DATA ANALYSIS**

Average subjective appetite was calculated as the average of the four questions on the Motivation to Eat VAS as follows: Average appetite score = [desire to eat + hunger + (100 – fullness) + prospective consumption]/4. Average treatment palatability was calculated as the average of the three questions on the treatment palatability VAS as follows: (pleasantness + taste + texture)/3.

All statistical analyses were conducted using the SAS version 9.2 (Statistical Analysis Systems, SAS Institute Inc., Cary, North Carolina) software suite. Two-way repeated measures analysis of variance (ANOVA) tests were used to test for treatment, time and treatment by time interaction effects on glycemic response, subjective appetite, physical comfort and energy and fatigue. For variables with a significant treatment and/or interaction effect (p<0.05), one-way repeated-measures ANOVA and Tukey-Kramer’s post-hoc test was used to determine between-treatment differences at individual time points. Treatment effects on the following variables were tested via one-way repeated measured ANOVA: food and water intake; and treatment and pizza palatability.

Correlation analyses among treatments and outcome measures were performed using the Pearson’s Correlation Coefficient. All results are presented as mean ± standard error of the mean (SEM). Statistical significance was concluded with the P-value less than 0.05.
4.4 RESULTS

4.4.1 Subject Characteristics

In experiment 1, nineteen subjects were recruited with a mean age of 23.2 ± 0.5 years and BMI of 22.5 ± 0.3 kg/m². In experiment 2, twenty subjects were recruited with a mean age of 22.3 ± 0.5 years and BMI of 21.8 ± 0.3 kg/m².

4.4.2 Food and Water Intake

In experiment 1, there was a significant effect of treatment on FI at 30 min (P = 0.0008). FI following P20 was lower compared to all other treatments (P < 0.05). No other differences between the treatments were found (Table 4.2). Cumulative food intake, the sum of energy consumed at the treatment and at the pizza meal (CFI), was also significantly affected by treatment (P = 0.03). CFI was suppressed by P20 compared to F10 (P = 0.03); all other treatments led to intermediate CFI. WI was not affected by treatment (P = 0.92).

In experiment 2, there was no effect of treatment on FI (P = 0.45), CFI (P = 0.41) or WI (P = 0.40) at 120 min (Table 4.2).

4.4.3 Blood Glucose

In experiment 1, pre-meal mean BG (0, 15 and 30 min) was significantly affected by time (P < 0.0001) and treatment (P = 0.02), but not by their interaction (P = 0.28). Over the entire pre-meal period, BG was lower following both protein treatments compared to control (P < 0.05) (Table 4.3). BG was lowest upon arrival at the lab and gradually increased following the treatment until 30 min. BG at 15 min was lower following P10 compared to control (P = 0.03) (Figure 4.1A). Post-meal (50, 65, 80, 95, 110, 140 and 170 min) BG was significantly affected by time (P < 0.0001), treatment (P = 0.001) and their interaction (P < 0.0001); the latter of which is explained by variable treatment effects over time. Overall post-meal BG was suppressed following P20 compared to control and F10 (P
BG was highest at 65 min (15 min following completion of the meal) and gradually declined until the end of the study period. BG immediately following the pizza meal (50 min) was lower following both doses of protein compared to after the control and F10 ($P < 0.0001$) (**Figure 4.1A**). BG at 50 min following P20 was also lower than after F20 ($P < 0.0001$). At 65 min, BG was lower following P20 compared to after the control and both fibre treatments ($P < 0.0001$), while P10 resulted in intermediate BG.

In experiment 2, mean pre-meal BG over time (0, 15, 30, 45, 60, 90 and 120 min) significantly affected by time ($P < 0.0001$), but not treatment ($P = 0.67$) or treatment by time interaction ($P = 0.16$) (**Table 4.3**). Pre-meal BG was lowest at baseline, increased slightly to 30 min and then returned to baseline before 120 min. There was an effect of time ($P < 0.0001$) and treatment ($P = 0.03$) but no effect of treatment by time interaction ($P = 0.17$) on post-meal (140, 155, 170, 185 and 200 min) BG (**Figure 4.1**). BG peaked at 155 min, regardless of treatment, and declined steadily until 200 min, without reaching baseline. Although there was a significant treatment effect, Tukey-Kramer’s post-hoc test did not declare any differences between treatments at any time point (**Figure 4.1B**).

4.4.4 **SUBJECTIVE APPETITE**

In experiment 1, the average of pre-meal appetites was significantly affected by time ($P < 0.0001$), but not treatment ($P = 0.42$) or treatment by time interaction ($P = 0.58$) (**Table 4.4**). Pre-meal appetite was highest at baseline and dropped slightly after the treatment (**Figure 4.2A**). Post-meal appetite was significantly affected by time ($p < 0.0001$), but not treatment ($P = 0.17$) or their interaction ($P = 0.54$). Post-meal appetite dropped immediately post-meal and slowly rose until the end of the study period. There were no differences between any of the treatments at any time point pre- or post-meal (**Figure 4.2A**).
In experiment 2, there was a significant effect of time (P < 0.0001), but not of treatment (P = 0.37) or treatment by time interaction (P = 0.81) on pre-meal appetite (Table 4.4). Pre-meal appetite was high at baseline, dropped slightly after the treatment, followed by a slow raise to above baseline at 120 min (Figure 4.2B). Post-meal subjective appetite was also affected by time (P < 0.0001), but not by treatment (P = 0.58) or time by treatment interaction (P = 0.21). Post-meal subjective appetite was lowest immediate following the meal and gradually rose until 200 min. There were no differences between any of the treatments at any time point pre- or post-meal (Figure 4.2B).

4.4.5 Palatability

Average palatability [(pleasantness + taste + texture)/3] of treatments varied in both experiments, with the ratings for the F20 treatment being significantly lower compared to control in both experiments (p < 0.05). The average palatability of the F10 and P20 treatments were also lower than the control in experiment 2 (P < 0.05), but not experiment 1. Average palatability ratings (mean ± SE) for the C, F10, F20, P10 and P20 treatments, respectively, were 60.0 ± 5.0, 53.7 ± 5.7, 43.3 ± 5.8, 61.1 ± 4.9 and 51.8 ± 5.8 mm in experiment 1, and 68.4 ± 4.2, 53.6 ± 4.4, 50.4 ± 4.8, 59.8 ± 5.4 and 49.2 ± 4.9 mm in experiment 2. However, Pearson correlation analysis showed no relationship between treatment palatability and FI at the test meal (experiment 1: r = 0.16, P = 0.13; experiment 2: r = -0.13, P = 0.26). Average palatability of the pizza meal was high (74.2 ± 1.6 mm in experiment 1; 80.9 ± 1.4 mm in experiment 2) in both experiments and was not affected by the treatments (data not shown).
4.5 DISCUSSION

The results of these studies support a role for yellow pea protein in regulating short-term FI and satiety and glycemic responses to a second meal. Although the protein preloads did not affect subjective appetite, they led to suppression of FI at 30 min, and reduced the glycemic response to the treatment as well as the response to the second meal at 30 min, but not at 120 minutes. Furthermore, yellow pea protein’s effects on pre-meal glycemic response were independent of dose; whereas its effects on glycemic response to a second meal and FI at the meal were dose-dependent. These results support the hypothesis that the protein and not the fibre fraction of whole yellow peas is responsible for the physiological effects up to 30 min after their consumption. However, the effects of the protein fractions are transient and thus the benefits of whole yellow peas beyond 30 min cannot be explained by their fibre or protein content alone.

The effect of pea protein at 30 min but not at 120 min may be explained by the observation that it is both fast digesting (defined as peaking plasma amino acid concentrations approximately 30 minutes after consumption) and high in branched-chain amino acids (BCAA), thus eliciting similar short-term physiological benefits on BG and FI as known for whey (Calbet and MacLean 2002; Nilsson et al. 2007). The suppression of FI by yellow pea protein at 30 min corresponds with the peak in plasma amino acids following consumption of fast-digesting protein. Whey, a characteristic fast-digesting protein, peaks plasma amino acid concentrations as well as levels of the anorexigenic hormones insulin and GLP-1, between 20 and 40 min after consumption (Nilsson et al. 2007). Calbet and MacLean (2002) found yellow pea protein to affect plasma amino acid and hormone levels in a similar manner as whey (Calbet and MacLean 2002). Plasma amino acid and anorexigenic hormone levels return to baseline well before 120 min after
ingestion of a fast digesting protein (including pea) (Calbet and MacLean 2002; Nilsson et al. 2007), which may explain the transient nature of pea protein’s appetite suppression effects. However, it has been suggested that fast proteins have synergistic effects on plasma insulin levels when consumed with available carbohydrates (Calbet and MacLean 2002). Thus it is possible that the effects of the pea protein may persist past 30 min if consumed with carbohydrate. Indeed, Manders et al. (2005) found that the insulinemic response to a glucose drink was 3-fold higher when consumed with a protein hydrolysate mixture (Manders et al. 2005). In the current study, pea protein was served with very low amounts of available carbohydrates and unfortunately, plasma insulin levels were not measured. Thus these hypotheses cannot be assessed, but warrant further investigation.

It is surprising that no effects were detected following the fibre treatments. There are several possible explanations for this. First, the fibre used in the current study was isolated from the hulls of yellow peas. Legume hulls are a byproduct of most processing techniques and contain very high amounts of fibre (Arrigoni et al. 1986); therefore, the majority of pulse-derived fibre fractions for commercial use are from the hull. However, not only are the hulls much higher in total fibre, but they have very different soluble:insoluble fibre ratios and functional properties compared to fibre from the cotyledon (Arrigoni et al. 1986; Wang et al. 2008b). Dehulling peas reduces the insoluble fibre content of whole peas by 45.8% and the soluble fibre content by just 21.0% (Wang et al. 2008b). Yellow peas are most commonly consumed dehulled and thus the fibre treatments in the current study may not be representative of the effects of consuming fibre from whole yellow peas. Similar studies are needed to investigate possible benefits of pea fibre derived from the cotyledon.

Secondly, our studies to only 120 min may not be of sufficient duration to see the benefits associated with fibre consumption. Many fibres are fermented by bacteria in the
large intestine, especially in the caecum and proximal portions of the colon, with beneficial outcomes likely mediated by the production of short-chain fatty acids (Wong et al. 2006; Nilsson et al. 2008). Indeed, there have been several studies showing short-term metabolic benefits of fermentable fibres. An evening meal including fermentable fibres led to suppressed appetite, FL and BG response to a standardized breakfast the following morning (10.5 hours after the evening meal) compared to similar low-fibre meals (Nilsson et al. 2008). Furthermore, breath hydrogen, a marker of fermentation, was inversely related to BG response to the morning meal, as well as to GLP-1, indicating that the effects of fibre may have been mediated through changes in gut microflora and fermentation that require longer than 2 hours to occur.

Lastly, it may be that the amount of yellow pea fibre was not sufficient to exert short-term effects. A study by Samra et al. (2007) showed that 33 g of insoluble fibre served in a breakfast cereal reduced glycemic response to a meal 75 minutes later (Samra and Anderson 2007). This discrepancy with the current study could be due to the different doses of fibre used (20 g versus 33 g), and because those results were in comparison to high glycemic index cereal and white bread treatments. A maximum fibre dose of 20 g was chosen because this is the maximum amount of fibre an individual would normally consume if they ate approximately two servings of whole pulses. Despite the null results of fibre in the current study, further research into the effects of pea fibre is warranted before it can be ruled out as at least partially responsible for the physiological benefits of consumption of whole yellow peas. Studies investigating the cotyledon fibre, larger amounts of fibre and/or a fixed energy test-meal in order to reduce confounding effects of food intake and isolate the effect of fibre on satiety and glycemic response are needed.
A weakness of the current study was the absence of a treatment containing whole yellow peas. Thus it is unclear how much of the benefits of whole peas are due to the protein alone. Although protein improved BG and suppressed BG in experiment 1, neither of yellow pea fractions alone in this study replicated the effects of whole yellow peas on short-term FI, appetite or BG (Marinangeli et al. 2009a; Marinangeli et al. 2009b; Marinangeli and Jones 2010). This means that not enough of the fractions were used, or perhaps the short-term benefits of whole yellow peas are due to synergistic effects of these and other components. Yellow peas are also high in resistant starch, which has been associated with improved glycemic response and suppression of FI (Mikulíková et al. 2008; Zhou et al. 2008; Aziz et al. 2009; Bodinham et al. 2010). Further studies investigating the effects of the starch fraction, as well as with different pea fractions in combination, are required to further understand the mechanism of action of whole pulses.

Nevertheless, this research provides evidence for the efficacy of yellow pea protein as a value-added ingredient in functional foods aimed at suppressing short-term energy intake and blood glucose. More research on the functional properties of yellow pea fractions, as well as on fibre and other fractions, is warranted.

4.6 CONCLUSION

In conclusion, protein and not fibre, is the primary component of yellow peas responsible for the suppression of FI and glycemic response at a meal served at 30 min after the consumption of whole yellow peas. However, the effects of the protein fraction are transient and thus the benefits of whole yellow peas beyond 30 min cannot be explained by their fibre or protein content alone.
**TABLE 4-1 Nutritional composition of tomato soup treatments.**

<table>
<thead>
<tr>
<th></th>
<th>Treatment 1, 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>177.8</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0.2</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>2.1</td>
</tr>
<tr>
<td>Available carbohydrate (g)</td>
<td>9.5</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>2.0</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>294.2</td>
</tr>
</tbody>
</table>

1 Energy content and composition of common ingredients provided by the manufacturer. Composition of yellow pea fractions determined using the following methods: Fat, AOAC 983.23; Protein, AOAC 992.15; Fibre, AOAC 43-A14; Sodium, AOAC 985.01. Amounts given are per 300 mL serving.

2 C, control; F10, 10 g fibre; F20, 20 g fibre; P10, 10 g protein; P20, 20 g protein.

3 Calculated as [Total carbohydrate – fibre].
### TABLE 4-2 Energy intake and cumulative energy intake at the *ad libitum* pizza meal

<table>
<thead>
<tr>
<th>Treatment 5</th>
<th>Energy intake 2 (kJ)</th>
<th>Cumulative energy intake 3 (kJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>5633.2 a</td>
<td>463.9</td>
</tr>
<tr>
<td>F10</td>
<td>5894.9 a</td>
<td>454.3</td>
</tr>
<tr>
<td>F20</td>
<td>5832.8 a</td>
<td>471.0</td>
</tr>
<tr>
<td>P10</td>
<td>5677.9 a</td>
<td>398.3</td>
</tr>
<tr>
<td>P20</td>
<td>4938.0 b</td>
<td>500.7</td>
</tr>
<tr>
<td>P</td>
<td>0.0008</td>
<td></td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>6090.5</td>
<td>365.2</td>
</tr>
<tr>
<td>F10</td>
<td>6127.4</td>
<td>360.1</td>
</tr>
<tr>
<td>F20</td>
<td>6403.9</td>
<td>443.7</td>
</tr>
<tr>
<td>P10</td>
<td>5819.9</td>
<td>384.8</td>
</tr>
<tr>
<td>P20</td>
<td>6015.6</td>
<td>374.4</td>
</tr>
<tr>
<td>P</td>
<td>0.4521</td>
<td></td>
</tr>
</tbody>
</table>

1 All values are mean ± SEM (n = 19 in experiment 1, n = 20 in experiment 2).

2 Energy consumption in a test meal was measured at 30 min in experiment 1 and 120 min in experiment 2 following treatments.

3 Energy from treatment + energy from test meal.

4 Means in the same column with different superscript letters are significantly different from each other (one-way ANOVA, Tukey-Kramer post hoc test, P < 0.05).

5 C, control; F10, 10 g fibre; F20, 20 g fibre; P10, 10 g protein; P20, 20 g protein.
<table>
<thead>
<tr>
<th>Treatment(^5)</th>
<th>Pre-meal (^2) (mmol/L)</th>
<th>Post-meal (^3) (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td><strong>Experiment 1(^4)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>5.31(^a)</td>
<td>0.11</td>
</tr>
<tr>
<td>F10</td>
<td>5.12(^{ab})</td>
<td>0.07</td>
</tr>
<tr>
<td>F20</td>
<td>5.06(^{ab})</td>
<td>0.06</td>
</tr>
<tr>
<td>P10</td>
<td>4.95(^b)</td>
<td>0.07</td>
</tr>
<tr>
<td>P20</td>
<td>5.05(^b)</td>
<td>0.06</td>
</tr>
<tr>
<td>P</td>
<td>0.0253</td>
<td></td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>5.03</td>
<td>0.04</td>
</tr>
<tr>
<td>F10</td>
<td>4.97</td>
<td>0.04</td>
</tr>
<tr>
<td>F20</td>
<td>5.07</td>
<td>0.04</td>
</tr>
<tr>
<td>P10</td>
<td>5.08</td>
<td>0.04</td>
</tr>
<tr>
<td>P20</td>
<td>5.01</td>
<td>0.04</td>
</tr>
<tr>
<td>P</td>
<td>0.6691</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) All values are mean ± SEM (n = 19 in experiment 1, n = 20 in experiment 2).
\(^2\) Pre-meal values are means of all observations before the test meal: 0, 15 and 30 min in experiment 1 and 0, 15, 30, 45, 60, 90 and 120 min in experiment 2.
\(^3\) Post-meal values are means of all observations after the test meal: 50, 65, 80, 95, 110, 140 and 170 min in experiment 1 and 140, 155, 170, 185 and 200 min in experiment 2.
\(^4\) Means in the same column and within each experiment with different superscript letters are significantly different from each other (two-way ANOVA, Tukey-Kramer post hoc test, P < 0.05).
\(^5\) C, control; F10, 10 g fibre; F20, 20 g fibre; P10, 10 g protein; P20, 20 g protein.
<table>
<thead>
<tr>
<th>Treatment 5</th>
<th>Pre-meal  2 (mmol/L)</th>
<th>Post-meal 3 (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Experiment 1 4</td>
<td>C</td>
<td>65.74</td>
</tr>
<tr>
<td></td>
<td>F10</td>
<td>68.90</td>
</tr>
<tr>
<td></td>
<td>F20</td>
<td>64.90</td>
</tr>
<tr>
<td></td>
<td>P10</td>
<td>65.80</td>
</tr>
<tr>
<td></td>
<td>P20</td>
<td>62.44</td>
</tr>
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<td></td>
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<td></td>
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<td>66.92</td>
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<td></td>
<td>P</td>
<td>0.3664</td>
</tr>
</tbody>
</table>

1 All values are mean ± SEM (n = 19 in experiment 1, n = 20 in experiment 2).
2 Pre-meal values are means of all observations before the test meal: 0, 15 and 30 min in experiment 1 and 0, 15, 30, 45, 60, 90 and 120 min in experiment 2.
3 Post-meal values are means of all observations after the test meal: 50, 65, 80, 95, 110, 140 and 170 min in experiment 1 and 140, 155, 170, 185 and 200 min in experiment 2.
4 Means in the same column and within each experiment with different superscript letters are significantly different from each other (two-way ANOVA, Tukey-Kramer post hoc test, P < 0.05).
5 C, control; F10, 10 g fibre; F20, 20 g fibre; P10, 10 g protein; P20, 20 g protein.
FIGURE 4-1 Effect of treatments on blood glucose concentrations over time

A) Experiment 1. B) Experiment 2. Treatments were served in a tomato soup with 10 (F10) or 20 (F20) g of yellow pea fibre, 10 (P10) or 20 (P20) g of yellow pea protein, or a control with no added fractions. Means with different superscripts are significantly different at each measured time (one-way ANOVA, Tukey-Kramer post hoc test, P < 0.05). All values are mean ± SEM (n = 19 in experiment 1; n = 20 in experiment 2).
FIGURE 4-2 Effect of treatments on average appetite over time

A) Experiment 1. B) Experiment 2. Treatments were served in a tomato soup with 10 (F10) or 20 (F20) g of yellow pea fibre, 10 (P10) or 20 (P20) g of yellow pea protein, or a control with no added fractions. Means with different superscripts are significantly different at each measured time (one-way ANOVA, Tukey-Kramer post hoc test, P < 0.05). All values are mean ± SEM (n = 19 in experiment 1; n = 20 in experiment 2).
5 FUTURE DIRECTIONS

The results from this study support the hypothesis that the short-term suppression of food intake following yellow pea consumption is due to the protein content; however, many questions regarding physiological effects of consumption of yellow pea fractions remain to be investigated. The following section further discusses some of these questions as well as the limitations of this research and how they may be addressed in the future.

The proportion of food intake and glycemic suppression attributable to the protein fraction of whole yellow peas is still unknown. Because the effects of only the protein and fibre fractions were compared in these studies, it is not clear whether protein is responsible for all of the satiating and glycemic properties of whole yellow peas, or only a portion of it. A comparison of the protein fractions to an equivalent dose of whole peas as a control is required. If, as expected, the magnitude of appetite and glycemic suppression of the protein fraction alone does not match that of whole yellow peas, then combining realistic proportions of protein, fibre, starch and fat fractions in all possible permutations would help elucidate the combination of macronutrients responsible.

Due to the strict inclusion criteria in the current studies, the study population is not representative of the population as a whole. Age, BMI, sex and the presence of metabolic disorders can affect the interaction of dietary component and satiety. The blood glucose modulating effects of pea protein are particularly encouraging for those suffering from type-2 diabetes mellitus or metabolic syndrome. These disorders are often associated with obesity and therefore the combined effects of suppressed appetite and glycemia would be especially beneficial. Further studies are needed to determine if the benefits of yellow pea protein will extend to these populations.
Bioactive peptides could be responsible for some of the effect of yellow pea protein in experiment 1. Several bioactive peptides with physiological significance have been identified in yellow pea protein (Agboola et al. 2010; Roy et al. 2010). A recent study by Geraedts et al. (2011) found that direct intraduodenal infusions of intact pea protein in 20 lean and obese adults suppressed food intake compared to a placebo (Geraedts et al. 2011b). Unfortunately, the authors did not compare the effect of the intraduodenal infusion to the oral ingestion of pea protein. Thus it is unclear whether the effect was due to pea-specific bioactive peptides or pea protein itself. Intact pea protein, but not yellow pea hydrolysates, increased the secretion of appetite-suppressing hormones from duodenal enterocytes, suggesting a role of bioactives in appetite and glycemic control (Geraedts et al. 2010; Geraedts et al. 2011a). Similarly, a peptide isolated from yellow peas, dubbed PA1b, induced membrane depolarization, and thus insulin release, in rat pancreatic β-cells in vitro (ZhiTao et al. 2007). The authors do not speculate as to whether this bioactive peptide could reach the pancreas intact and increase insulin secretion in vivo, but it warrants further research nonetheless.

Despite the lack of an effect of yellow pea fibre in the current study, there is a growing body of evidence suggesting its consumption may have significant long-term benefits on energy balance and metabolic outcomes. Sparti et al. (2000) compared the effects of a diet high or low in fibre (from pulses) in young healthy adults (Sparti et al. 2000). Those fed the high pulse-fibre diet had significantly lower hunger scores than the low-fibre group. Furthermore, this was associated with high breath hydrogen and thus possibly was mediated by fermentation of pulse fibres (Sparti et al. 2000). This supports the hypothesis that while yellow pea fibre had little effect in the short-term in this study, it could lead to greater satiety and perhaps lower food intake in longer studies once fermentation takes place.
Although this research is encouraging, the mechanisms explaining the short-term glycemic and satiating benefits of pulses have not been fully elucidated. Additional *in vitro* and *in vivo* studies, especially in animal models where all environmental conditions can be strictly controlled and more invasive techniques can be employed, are required. Once these mechanisms are understood, they will add to the understanding of the physiological effects of pulses and their fractions on satiety, food intake and glycemic control and provide evidence required for the development of satiety- and blood glucose-related health claims for foods containing pulses or their fractions.
6 CONCLUSION

This research is the first to show that yellow pea protein, and not fibre, contributes to the short-term benefits of whole yellow peas up to 30 minutes after consumption.

The results of this research suggest that isolated yellow pea protein could be used in the development of functional foods aimed at reducing blood glucose and suppressing food intake and appetite. Research has already begun on the functional, physicochemical and organoleptic properties of novel foods containing yellow pea fractions. These include meat products, baked goods, snack foods, pastas and breads and the results are very promising (Kaack and Pedersen 2005a; Kaack and Pedersen 2005b; Zhao et al. 2005; Marinangeli et al. 2009a; Marinangeli and Jones 2011). Once on the market, not only may these foods help curb the rising rates of obesity and related diseases, but they could also help increase domestic consumption of an economically important Canadian crop. This means that the results of this and future studies on pulse fractions have the potential to strengthen the economy of Canada while simultaneously improving the health of its citizens.
7 REFERENCES


Eyaru, R., A. Shrestha and J. Arcot (2009). Effect of various processing techniques on digestibility of starch in Red kidney bean (Phaseolus vulgaris) and two varieties of peas (Pisum sativum). *Food Research International* **42**: 7.


Papanikolaou, Y. and V. L. Fulgoni, 3rd (2008). Bean consumption is associated with greater nutrient intake, reduced systolic blood pressure, lower body weight, and a smaller waist circumference in adults: results from the National Health and


Stratton, R. J., R. J. Stubbs, D. Hughes, N. King, J. E. Blundell and M. Elia (1998). Comparison of the traditional paper visual analogue scale questionnaire with an


## 8 APPENDICES

### 8.1 Nutritional Composition of the Pizza Served at Test Meals

<table>
<thead>
<tr>
<th>Per 1 pizza</th>
<th>Pepperoni (87 g)</th>
<th>Three Cheese (96 g)</th>
<th>Deluxe (92 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories</td>
<td>180</td>
<td>180</td>
<td>180</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>2.5</td>
<td>2.5</td>
<td>2</td>
</tr>
<tr>
<td>Trans Fat (g)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>400</td>
<td>360</td>
<td>380</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>23</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>9</td>
<td>9</td>
<td>8</td>
</tr>
</tbody>
</table>
Study in the Department of Nutrition

Flexible start dates

Healthy Males Needed!

Requirements: age 20-30, non-smoking

Involves: Five 3-hour sessions

$ Compensation, Breakfast and Lunch are provided $
8.3 Screening Questionnaires

8.3.1 Recruitment Screening Questionnaire
Recruitment Screening Questionnaire
(Note: After you are recruited for the study, you will be assigned an ID # which will be used on your forms and data throughout the study)

NAME: __________________________ AGE: _____
ADDRESS: ____________________________________
Postal Code: __________________________________

PHONE # : (____)____________________ email:__________

HEIGHT: _________ WEIGHT: _________ BMI: ______

PARTICIPATION IN ATHLETICS/ EXERCISE:
ACTIVITY HOW OFTEN? HOW LONG? (HOURS)
________________________________________________________________________

Do you usually eat breakfast? YES _____ NO _____
If YES, what do you usually eat for breakfast? __________________________
________________________________________________________________________

Health Status:
Do you have diabetes? YES _____ NO _____
Do you have any other major disease? YES _____ NO _____
If YES, please specify ________________________________
Are you taking any medications? YES _____ NO _____
Do you have reactions to any foods? YES _____ NO _____
If YES, please specify ________________________________
Are you on a special diet? YES _____ NO _____
If YES, please specify ________________________________
Have you recently lost or gained weight? YES _____ NO _____
If YES, please specify ________________________________
Do you smoke? YES ______ NO ______

How many Alcoholic Beverages do you consume per day? _____ per week? _____

**Sleep Habits**

1. What time do you normally wake up in the morning?
   - during the week: ______
   - weekends/ days off: ______

2. What time do you normally get out of bed? (if different from the above)
   - during the week: ______
   - weekends/ days off: ______

3. What is the earliest you would get up in a normal week?
   - during the week: ______
   - weekends/ days off: ______

4. What is the latest you would get up in a normal week?
   - during the week: ______
   - weekends/ days off: ______

5. How long do you wait to eat after rising
   - during the week: ______
   - weekends/ days off: ______
**Eating Habit Questionnaire**

Choose the appropriate answer to best describe your personal situation.

1. How often are you dieting?
   
   Never ____ rarely ____ sometimes ____ often ____ always ____

2. What is the maximum amount of weight (in pounds) that you have ever lost within one month?
   
   1-4 ____ 5-9 ____ 10-14 ____ 15-19 ____ 20+ ____

3. What is your maximum weight gain within one week?
   
   0-1 ____ 1.1-2 ____ 2.1-3 ____ 3.1-5 ____ 5.1+ ____

4. In a typical week, how much does your weight fluctuate?
   
   0-1 ____ 1.1-2 ____ 2.1-3 ____ 3.1-5 ____ 5.1+ ____

5. Would a weight fluctuation of 5 lbs affect the way you live your life?
   
   Not At all ____ slightly ____ moderately ____ very much ____

6. Do you eat sensibly in front of others and splurge alone?
   
   Never ____ rarely ____ often ____ always ____

7. Do you give too much time and thought to food?
   
   Never ____ rarely ____ often ____ always ____

8. Do you have feelings of guilt after overeating?
   
   Never ____ rarely ____ often ____ always ____

9. How conscious are you of what you are eating?
   
   Not at all ____ slightly ____ moderately ____ extremely ____

10. How many pounds over your desirable weight were you at your maximum weight?
    
    0-1 ____ 2-5 ____ 6-10 ____ 11-20 ____ 21+ ____
**Food Acceptability**

Please indicate with a rating between 1 and 10 how much you enjoy the following foods (1= not at all, 10 = very much) and how often you eat them (never, daily, weekly, monthly)

<table>
<thead>
<tr>
<th>Enjoyment?</th>
<th>How often?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Pasta</td>
<td></td>
</tr>
<tr>
<td>2 Rice</td>
<td></td>
</tr>
<tr>
<td>3 Potatoes (Mashed, roasted)</td>
<td></td>
</tr>
<tr>
<td>4 French fries</td>
<td></td>
</tr>
<tr>
<td>5 Pizza</td>
<td></td>
</tr>
<tr>
<td>6 Bread, Bagels, Dinner rolls</td>
<td></td>
</tr>
<tr>
<td>7 Sandwiches, Subs</td>
<td></td>
</tr>
<tr>
<td>8 Cereal</td>
<td></td>
</tr>
<tr>
<td>9 Cake, Donuts, Cookies</td>
<td></td>
</tr>
<tr>
<td>10 Tomato Soup</td>
<td></td>
</tr>
</tbody>
</table>

Will you be able to eat tomato soup?

YES ____ NO _____

At the end of each of the sessions, you will be provided with pizza. In order to provide you with a meal that you will enjoy, we ask that you rank the following pizzas according to your personal preferences (i.e. 1, 2, 3) in the space provided. If you do NOT like a particular type of pizza, then do not rank it, but place an "X" in the space provided.

**Pepperoni (Cheese, pepperoni)**

**Deluxe (cheese, pepperoni, peppers, mushrooms)**

**Three Cheese (mozzarella, cheddar, parmesan)**
8.3.2 Study Information and Informed Consent Forms
The Effect of Different Doses of Pea Protein and Fibre on Glycemic Response and Subjective Appetite in Young Men

Information Sheet and Consent Form

**Investigators:** Dr. G. Harvey Anderson  
Department of Nutritional Sciences, University of Toronto  
Phone: (416) 978-1832  
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Email: rebecca.mollard@utoronto.ca

Chris Smith  
Department of Nutritional Sciences, University of Toronto  
Phone: (416) 978-4153  
Email: chris.smith@utoronto.ca

**Funding Source:**

Funding for this project is provided by Pulse Canada.

**Background and Purpose of Research:**

In 2004, almost 60% of adult Canadians were overweight or obese. This is a serious health problem because obesity and being overweight are related to many risk factors of disease, including increased blood sugar. Overweight and obesity can be treated by changing what we eat. It is important to find food-based ways to prevent and treat overweight and obesity.

Protein and fibre are two types of food components that can used to decrease hunger and control blood glucose. This study will investigate whether protein and fibre from yellow peas can be used for these purposes. The information obtained from this study will be used to better understand the effects of different types of protein and fibre on the health of young men and may lead to future studies in other groups, including women.

The purpose of this research project is to determine the effect of eating yellow pea fibre and protein on blood sugar and appetite in young men.

This study will have 16 participants.
Invitation to Participate:

You are being invited to take part in this study. If you chose to take part, you will be asked to eat a tomato soup treatment five times (five sessions) one week apart. Four of the treatments will be soup containing yellow pea protein or fibre and one will be soup without added protein and fibre. Your appetite and blood sugar will be measured after eating the treatment and a pizza lunch. Each session will take up to 3 ½ hours of your time.

Eligibility:

To participate in this study you must be healthy male and between the ages of 20-30. You must be a nonsmoker and you cannot be taking any medications. The study will take place in the Department of Nutritional Sciences, Rm 305, 334, 331 and 331A, FitzGerald Building, 150 College Street, Toronto, ON.

Procedure:

To find out if you can take part in this study, you will be asked to fill out questionnaires, which ask questions about your age, if you smoke, exercise, your health, if you are on any medications and your eating habits. You height and weight will be measured.

If you can take part, you will be asked to fill out questionnaires about the foods you like. You will be scheduled to meet with us for five sessions over five weeks.

You will be asked to eat a standard breakfast on the day of the session following a 10 hour fast (no eating for 10 hours before eating breakfast). We will give you the standard breakfast (cereal, milk and orange juice) the day before the session.

You will be asked to arrive at the FitzGerald Building between 9:45 a.m. and 12:45 p.m., 3 ¼ hours after eating breakfast. Please do not eat between breakfast and meeting with us. You will be asked to stick to your normal routine, including exercise and to eat a similar meal the night before each session. You can drink water up to one hour before meeting with us.

At each session you will be asked to eat a tomato soup treatment, give blood samples and to complete questionnaires at the times outlined in the table below. Ten times during each session, for a total of 50 times over the whole study, you will be asked to provide a small drop of blood by finger prick. Blood will be sampled at before eating the treatment and 15, 30, 50, 65, 80, 95, 110, 140 and 170 minutes after eating the treatment. You will be asked to fill out visual analog scale (VAS) questionnaires measuring your appetite, physical comfort and energy/fatigue as well as the palatability (pleasantness) of the treatment and pizza throughout the study sessions. You will be served a pizza meal 30 minutes after you eat the treatment. Each session will last up to 3.5 hours.

Time and Activity Schedule for Each Session

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:00</td>
<td>Consumption of breakfast</td>
</tr>
<tr>
<td>Time</td>
<td>Activity</td>
</tr>
<tr>
<td>------------</td>
<td>-------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>10:45</td>
<td>Arrive at the laboratory</td>
</tr>
<tr>
<td>10:50</td>
<td>Fill in Sleep, Stress, and VAS questionnaires and take first blood sample</td>
</tr>
<tr>
<td>11:00 – 11:05</td>
<td>Eat the treatment (-5 minutes)</td>
</tr>
<tr>
<td>11:05-11:35</td>
<td>Blood sampling and VAS questionnaires at 15 and 30 minutes</td>
</tr>
<tr>
<td>11:35-11:55</td>
<td>Pizza served and eaten (30 minutes)</td>
</tr>
<tr>
<td>11:55-1:55</td>
<td>Blood sampling and VAS questionnaires at 50, 65, 80, 95, 110, 140 and 170 minutes</td>
</tr>
</tbody>
</table>

VAS: Visual analogue scale

**Voluntary Participation and Early Withdrawal:**

It is hoped that you will finish all five sessions. However, you may choose to stop being in the study at anytime without any problems.

**Early Termination:**

Not applicable

**Risks:**

All of the foods and beverages (water) that you will be asked to consume are prepared hygienically in the kitchen and present minimal risk.

The risks and discomfort will come from the blood sampling procedure. Great care will be taken when taking your finger prick blood samples. The investigator will help you. To make sure that you are not exposed to another person's needle, we will ask you to sit away from other study participants. We will put a lancet into the finger prick gun before taking each blood sample and then put it into the safety container. We will swab your finger with alcohol before and after each finger prick and will use a new sterile needle each time.

Some discomfort will be felt as a result of a sharp momentary pain caused as the needle enters the skin. However, because the lancet needle is very small the pain felt is usually less than you might feel from skin puncture during vaccination or if a blood sample is taken by a needle inserted in a vein.

A total of 10 finger pricks will be conducted per session and may result in some discomfort.

There is very little risk of infection. Before the finger is pricked the area is cleaned with an alcohol swab. There might be slight bruising under the skin, but this will be minimized by applying pressure after the finger is pricked and blood sugar is measured.

You may experience flatulence (passing gas) and feelings of gastrointestinal discomfort (bloating) from the treatments if they are high in fibre. This hardly ever happens and there is no health risk linked with these effects.

**Benefits:**
You will not benefit directly from taking part in this study. You will be shown your blood sugar results and if they are not normal you will be told and advised to talk to your doctor. The foods and drinks (water) will be provided for free.

**Confidentiality and Privacy:**

Confidentiality will be respected and no information that shows your identity will be released or published without your permission unless required by law. Your name, personal information and signed consent form will be kept in a locked filing cabinet in the investigator's office. Your results will not be kept in the same place as your name. Your results will be recorded on data sheets and in computer records that have an ID number for identification, but will not include your name. Your results, identified only by an ID number, will be made available to the study sponsor if requested. Only study investigators will have access to your individual results.

**Publication of Results:**

The results of the study may be presented at scientific meetings and published in a scientific journal. If the results are published, only average and not individual values will be reported.

**Possible Commercialization of Findings:**

This study is preliminary. Once these products are tested more widely in future studies, results may lead to commercialization of a product, new product formulation, changes in the labeling of a product and/or changes in the marketing of a product; you will not share in any way from the possible gains or money made by commercial application of findings.

**Alternative Treatment/ Therapy:**

Not applicable.

**New Findings:**

If anything is found during the course of this research which may change your decision to continue, you will be told about it.

**Compensation:**

You will be paid $30 per session. You will also be given $6 per session for travel (bus, subway). If you withdraw from the study before finishing or asked to withdraw, you will be paid for the sessions you have already finished.

**Injury Statement:**

Not applicable

**Rights of Subjects:**

Not applicable
Before agreeing to take part in this research study, it is important that you read and understand your role as described here in this study information sheet and consent form. You waive no legal rights by taking part in this study. If you have any questions or concerns about your rights as a participant you can contact the Ethics Review Office at ethics.review@utoronto.ca or call 416-946-3273.

If you have any questions after you read through this information please do not hesitate to ask the investigators for further clarification.

**Dissemination of findings:**

A summary of results will be made available to you to pick up after the study is done

**Copy of informed consent for participant:**

You are given a copy of this informed consent to keep for your own records.

**Consent:**

I acknowledge that the research study described above has been explained to me and that any questions that I have asked have been answered to my satisfaction. I have been informed of the alternatives to participation in this study, including the right not to participate and the right to withdraw. As well, the potential risks, harms and discomforts have been explained to me. I understand that I will receive compensation for my time spent participating in the study.

As part of my participation in this study, I understand that I may come in contact with certain confidential information. I agree to keep the confidentiality of such, if any, information unless it is necessary to disclose it to my health care provider(s), or to my legal representative(s).

I hereby agree and give my authorized consent to participate in the study and to treat confidential information in a restrictive manner as described above. I have been given a copy of the consent form to keep for my own records.

<table>
<thead>
<tr>
<th>Participant Name</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Witness Name</td>
<td>Signature</td>
<td>Date</td>
</tr>
<tr>
<td>Investigator Name</td>
<td>Signature</td>
<td>Date</td>
</tr>
</tbody>
</table>
The Effect of Different Doses of Pea Protein and Fibre on Glycemic Response, Subjective Appetite and Food Intake over 200 minutes in Young Men

Information Sheet and Consent Form

**Investigators:** Dr. G. Harvey Anderson, Principle Investigator  
Department of Nutritional Sciences, University of Toronto  
Phone: (416) 978-1832  
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Dr. Bohdan Luhovyy, Research Associate  
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Chris Smith, Undergraduate Student  
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Email: chris.smith@utoronto.ca

**Funding Source:**  
Funding for this project is provided by Purenet.

**Background and Purpose of Research:**

In 2004, almost 60% of adult Canadians were overweight or obese. This is a serious health problem because obesity and being overweight are related to many risk factors of disease, including increased blood sugar. Overweight and obesity can be treated by changing what we eat. It is important to find food-based ways to prevent and treat overweight and obesity.

Eating foods which maintain a moderate (not high) level of blood sugar for a longer period of time may prevent many diseases like diabetes which are related to obesity. We wish to measure your blood sugar 12 times over the session for two reasons. First, it is known that eaten foods change your blood sugar. Second, it is believed that food that help maintain moderate levels of blood sugar for longer times are more healthy than those that cause large fluctuations (swings) in blood sugar. Our goal is to identify foods and food components that contribute to stable blood sugar levels.

The information obtained from this study may help us understand the potential of proteins and fibres derived from peas in the control of blood sugar and in the prevention and management of obesity and related diseases.
This study will have 16 participants.

**Invitation to Participate:**
You are being invited to take part in this study. If you chose to take part, you will be asked to eat a tomato soup treatment five times (five sessions) one week apart. Four of the treatments will be soup containing yellow pea protein or fibre and one will be soup without added protein and fibre. Your appetite and blood sugar will be measured after eating the treatment and a pizza lunch. Each session will take up to 3 ½ hours of your time.

**Eligibility:**
To participate in this study you must be a healthy male and between the ages of 20-30. You must be a nonsmoker and you cannot be taking any medications. The study will take place in the Department of Nutritional Sciences, Rm 305, 334, 331 and 331A, FitzGerald Building, 150 College Street, Toronto, ON.

**Procedure:**
To find out if you can take part in this study, you will be asked to fill out questionnaires, which ask questions about your age, if you smoke, exercise, your health, if you are on any medications and your eating habits. Your height and weight will be measured.

If you can take part, you will be asked to fill out questionnaires about the foods you like. You will be scheduled to meet with us for five sessions over five weeks.

You will be asked to eat a standard breakfast on the day of the session following a 10 hour fast (no eating for 10 hours before eating breakfast). We will give you the standard breakfast (cereal, milk and orange juice) the day before the session.

You will be asked to arrive at the FitzGerald Building between 9:45 a.m. and 12:45 p.m., 3 ¾ hours after eating breakfast. Please do not eat between breakfast and meeting with us. You will be asked to stick to your normal routine, including exercise and to eat a similar meal the night before each session. You can drink water up to one hour before meeting with us.

At each session you will be asked to eat a tomato soup treatment (300 ml), give blood samples and to complete questionnaires at the times outlined in the table below. Twelve times during each session, for a total of 60 times over the whole study, you will be asked to provide a small drop of blood by finger prick. Blood will be sampled at before eating the treatment and 15, 30, 45, 60, 90, 120, 140, 155, 170, 185 and 200 minutes after eating the treatment. You will be asked to fill out visual analog scale (VAS) questionnaires measuring your appetite and physical comfort as well as the palatability (pleasantness) of the treatment and pizza throughout the study sessions. You will be served a pizza meal 120 minutes after you eat the treatment. Each session will last up to 4 hours.

**Time and Activity Schedule for Each Session**

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:00</td>
<td>Consumption of breakfast</td>
</tr>
<tr>
<td>10:45</td>
<td>Arrive at the laboratory</td>
</tr>
<tr>
<td>Time</td>
<td>Activity</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>10:50</td>
<td>Fill in Sleep, Stress, and VAS questionnaires and take first blood sample</td>
</tr>
<tr>
<td>11:00-11:05</td>
<td>Eat the treatment (-5 minutes)</td>
</tr>
<tr>
<td>11:05-1:05</td>
<td>Blood sampling and VAS questionnaires at 15, 30, 45, 60, 90 and 120 minutes</td>
</tr>
<tr>
<td>1:05-1:25</td>
<td>Pizza served and eaten at 120 minutes</td>
</tr>
<tr>
<td>1:25-2:25</td>
<td>Blood sampling and VAS questionnaires at 140, 155, 170, 185 and 200 minutes</td>
</tr>
</tbody>
</table>

VAS: Visual analogue scale

**Voluntary Participation and Early Withdrawal:**
It is hoped that you will finish all five sessions. **However, you may choose to stop being in the study at anytime without any consequences to you.**

**Early Termination:**
Not applicable

**Risks:**
All of the foods and beverages (water) that you will be asked to consume are prepared hygienically in the kitchen and present minimal risk.

After the overnight fast you may feel faint or dizzy, however the risk of this is minimal.

The risks and discomfort will come from the blood sampling procedure. Great care will be taken when taking your finger prick blood samples. The investigator will help you. To make sure that you are not exposed to another person’s needle, we will ask you to sit away from other study participants. We will put a needle into the finger prick gun before taking each blood sample and then put it into the safety container. We will swab your finger with alcohol before and after each finger prick and will use a new sterile needle each time.

Some discomfort will be felt as a result of a sharp momentary pain caused as the needle enters the skin. However, because the lancet needle is very small the pain felt is usually less than you might feel from skin puncture during vaccination or if a blood sample is taken by a needle inserted in a vein. You will be provided with your own finger prick gun for the entire study.

A total of 12 finger pricks will be conducted per session and may result in some discomfort.

There is very little risk of infection. Before the finger is pricked the area is cleaned with an alcohol swab. There might be slight bruising under the skin, but this will be minimized by applying pressure after the finger is pricked and blood sugar is measured.

You may experience flatulence (passing gas) and feelings of gastrointestinal discomfort (bloating) from the treatments if they are high in fibre. This hardly ever happens and there is no health risk linked with these effects.

**There is always a possibility that you will become ill following consumption of food, but that is very unlikely in this study. All treatments as well as pizza are freshly...**
prepared at the time of your session. The pizzas are stored frozen and cooked accordingly to the manufacturer's instructions immediately before you are served.

Benefits:
You will not benefit directly from taking part in this study. You will be shown your blood sugar results and if they are not normal you will be told and advised to talk to your doctor. The foods and drinks (water) will be provided for free.

Confidentiality and Privacy:
Confidentiality will be respected and no information that shows your identity will be released or published without your permission unless required by law. Your name, personal information and signed consent form will be kept in a locked filing cabinet in the investigator's office. Your results will not be kept in the same place as your name. Your results will be recorded on data sheets and in computer records that have an ID number for identification, but will not include your name. Your results, identified only by an ID number, will be made available to the study sponsor if requested. Only study investigators will have access to your individual results.

Publication of Results:
The results of the study may be presented at scientific meetings and published in a scientific journal. If the results are published, only average and not individual values will be reported.

Possible Commercialization of Findings:
This study is preliminary. Once these products are tested more widely in future studies, results may lead to commercialization of a product, new product formulation, changes in the labeling of a product and/or changes in the marketing of a product; you will not share in any way from the possible gains or money made by commercial application of findings.

Alternative Treatment/Therapy:
Not applicable.

New Findings:
If anything is found during the course of this research which may change your decision to continue, you will be told about it.

Compensation:
You will be paid $40 per session. You will also be given $6 per session for travel (bus, subway). If you withdraw from the study before finishing or asked to withdraw, you will be paid for the sessions you have already finished.

Injury Statement:
If you begin to feel sick following participation in the study, please seek medical advice as soon as possible. We will provide your medical specialist with information about the food you have consumed during the session, so take our phone number with you.
**Rights of Subjects:**

Before agreeing to take part in this research study, it is important that you read and understand your role as described here in this study information sheet and consent form. You waive no legal rights by taking part in this study. If you have any questions or concerns about your rights as a participant you can contact the Ethics Review Office at ethics.review@utoronto.ca or call 416-946-3273.

If you have any questions after you read through this information please do not hesitate to ask the investigators for further clarification.

**Dissemination of findings:**

A summary of results will be made available to you to pick up after the study is done.

**Copy of informed consent for participant:**

You are given a copy of this informed consent to keep for your own records.

**Consent:**

I acknowledge that the research study described above has been explained to me and that any questions that I have asked have been answered to my satisfaction. I have been informed of the alternatives to participation in this study, including the right not to participate and the right to withdraw. As well, the potential risks, harms and discomforts have been explained to me. I understand that I will receive compensation for my time spent participating in the study.

As part of my participation in this study, I understand that I may come in contact with other study participants because our session times overlap. I agree to keep anything I learn about other participants confidential and know that other participants have agreed to do the same for me.

I hereby agree and give my authorized consent to participate in the study and to treat confidential information in a restrictive manner as described above. I have been given a copy of the consent form to keep for my own records.

<table>
<thead>
<tr>
<th>Participant Name</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Witness Name</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Investigator Name</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
8.3.3 Food Acceptability List
8.4 STUDY SESSION FORMS

8.4.1 FOOD INTAKE SHEET
<table>
<thead>
<tr>
<th>ID: ____ Date: ____________________</th>
<th>ID: ____ Date: ____________________</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Session: ____ Treatment: ________</strong></td>
<td><strong>Session: ____ Treatment: ________</strong></td>
</tr>
<tr>
<td><strong>Tray 1</strong></td>
<td><strong>Tray 1</strong></td>
</tr>
<tr>
<td>Pepperoni (g)</td>
<td>Pepperoni (g)</td>
</tr>
<tr>
<td>Deluxe (g)</td>
<td>Deluxe (g)</td>
</tr>
<tr>
<td>3-cheese (g)</td>
<td>3-cheese (g)</td>
</tr>
<tr>
<td><strong>Tray 2</strong></td>
<td><strong>Tray 2</strong></td>
</tr>
<tr>
<td>Pepperoni (g)</td>
<td>Pepperoni (g)</td>
</tr>
<tr>
<td>Deluxe (g)</td>
<td>Deluxe (g)</td>
</tr>
<tr>
<td>3-cheese (g)</td>
<td>3-cheese (g)</td>
</tr>
<tr>
<td><strong>Tray 3</strong></td>
<td><strong>Tray 3</strong></td>
</tr>
<tr>
<td>Pepperoni (g)</td>
<td>Pepperoni (g)</td>
</tr>
<tr>
<td>Deluxe (g)</td>
<td>Deluxe (g)</td>
</tr>
<tr>
<td>3-cheese (g)</td>
<td>3-cheese (g)</td>
</tr>
<tr>
<td>Water 1 (g)</td>
<td>Water 1 (g)</td>
</tr>
<tr>
<td>Water 2 (g)</td>
<td>Water 2 (g)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ID: ____ Date: ____________________</th>
<th>ID: ____ Date: ____________________</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Session: ____ Treatment: ________</strong></td>
<td><strong>Session: ____ Treatment: ________</strong></td>
</tr>
<tr>
<td><strong>Tray 1</strong></td>
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<tr>
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<td>3-cheese (g)</td>
</tr>
<tr>
<td><strong>Tray 2</strong></td>
<td><strong>Tray 2</strong></td>
</tr>
<tr>
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<td>Deluxe (g)</td>
</tr>
<tr>
<td>3-cheese (g)</td>
<td>3-cheese (g)</td>
</tr>
<tr>
<td>Water 1 (g)</td>
<td>Water 1 (g)</td>
</tr>
<tr>
<td>Water 2 (g)</td>
<td>Water 2 (g)</td>
</tr>
</tbody>
</table>
8.4.2 *Blood Glucose Sheets*
Subject ID: ___  Treatment: _________
Date/Time: _________________
Session #: ________________
Monitor: _________________
Standards: high _____  low _____

**Blood Glucose Measurement**

<table>
<thead>
<tr>
<th>TIME</th>
<th>READING (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (0 min)</td>
<td></td>
</tr>
<tr>
<td>15 min</td>
<td></td>
</tr>
<tr>
<td>Pre – meal (30 min)</td>
<td></td>
</tr>
<tr>
<td>Post – meal (50 min)</td>
<td></td>
</tr>
<tr>
<td>65 min</td>
<td></td>
</tr>
<tr>
<td>80 min</td>
<td></td>
</tr>
<tr>
<td>95 min</td>
<td></td>
</tr>
<tr>
<td>110 min</td>
<td></td>
</tr>
<tr>
<td>140 min</td>
<td></td>
</tr>
<tr>
<td>170 min</td>
<td></td>
</tr>
</tbody>
</table>
Subject ID: ___  Treatment: __________

Date/Time: ______________

Session #: ______________

Monitor: ______________

Standards:  high _____  low _____

**Blood Glucose Measurement**

<table>
<thead>
<tr>
<th>TIME</th>
<th>READING (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (0 min)</td>
<td></td>
</tr>
<tr>
<td>15 min</td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td></td>
</tr>
<tr>
<td>45 min</td>
<td></td>
</tr>
<tr>
<td>60 min</td>
<td></td>
</tr>
<tr>
<td>90 min</td>
<td></td>
</tr>
<tr>
<td>Pre – meal (120 min)</td>
<td></td>
</tr>
<tr>
<td>Post – meal (140 min)</td>
<td></td>
</tr>
<tr>
<td>155 min</td>
<td></td>
</tr>
<tr>
<td>170 min</td>
<td></td>
</tr>
<tr>
<td>185 min</td>
<td></td>
</tr>
<tr>
<td>200 min</td>
<td></td>
</tr>
</tbody>
</table>
8.4.3 Sleep Habits and Stress Factors Questionnaire
Subject ID: ___

Date/Time: ___________

Session #: ___________

ID: ____________

1. Did you have a normal night’s sleep last night?
   ☐ YES ☐ NO

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

3. At what time did you go to bed?
   _______________________

4. At what time did you wake up this morning?
   _______________________

5. Recount your activity since waking up:
   TIME ACTIVITY
   _______ ________________________
   _______ ________________________
   _______ ________________________
   _______ ________________________
   _______ ________________________

6. Are you experiencing any feelings of illness or discomfort other than those from hunger?
   Today: ☐ YES ☐ NO
   Past 24 hrs: ☐ YES ☐ NO

7. Are you under any unusual stress? (i.e. exams, reports, work deadlines, personal)
   Today: ☐ YES ☐ NO
   Past 24 hrs: ☐ YES ☐ NO

8. Have you been involved in any physical activity, unusual to your normal routine within the past 24 hours?

9. Have you had anything to eat or drink other than water for the past 12 hours?
   ☐ YES ☐ NO

If yes, please describe briefly:
   _______________________
   _______________________

If yes, please describe briefly:
   _______________________
   _______________________
   _______________________

If yes, please describe briefly:
   _______________________

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RECENT FOOD INTAKE AND ACTIVITY QUESTIONNAIRE

What time did you have dinner? ________

Please describe your dinner last night (list all food and drink and give an estimate of the portion size):

______________________________________________________________________
______________________________________________________________________
______________________________________________________________________

The following three questions relate to your food intake, activity and stress over the last 24 hours. Please rate yourself by placing a small “x” across the horizontal line at the point which best reflects your present feelings.

How would you describe your food intake over the past 24 hours?

Much LESS ____________________________ Much MORE than usual

How would you describe your level of activity over the last 24 hours?

Much LESS ____________________________ Much MORE than usual

How would you describe your level of stress over the last 24 hours?

Much LESS ____________________________ Much MORE than usual
8.4.4 VAS

8.4.4.1 Motivation to Eat
Visual Analogue Scales
Motivation to Eat: BASELINE (0 min)

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

1. How strong is your desire to eat?

   VERY ____________________________ VERY
   weak                                            strong

2. How hungry do you feel?

   NOT__________________________________________ As hungry
   hungry                                          as I have
   at all                                           ever felt

3. How full do you feel?

   NOT__________________________________________ VERY
   full                                             full
   at all                                           at all

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

   NOTHING________________________________________ A LARGE
   at all                                           amount

5. How thirsty do you feel?

   NOT__________________________________________ As thirsty
   thirsty                                          as I have
   at all                                           ever felt
8.4.4.3 Physical Comfort
Visual Analogue Scales
Physical Comfort: BASELINE (0 min)

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

1. Do you feel nauseous?
   NOT ___________________________ VERY much
   at all

2. Does your stomach hurt?
   NOT ___________________________ VERY much
   at all

3. How well do you feel?
   NOT ___________________________ VERY well
   at all

4. Do you feel like you have gas?
   NOT ___________________________ VERY much
   at all

5. Do you feel like you have diarrhea?
   NOT ___________________________ VERY much
   at all
8.4.4.4 Energy and Fatigue
Visual Analogue Scales
Energy and Fatigue: BASELINE (0 min)

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

1. How energetic do you feel right now?

NOT
at all.................................................................................................................VERY
energetic

2. How tired do you feel right now?

NOT
at all.................................................................................................................VERY
tired
8.4.4.5 Palatability of the Treatments
Visual Analogue Scales 
Palatability: Treatment 5 min

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

1. How pleasant have you found the beverage/food?

NOT at all _________________________ very pleasant
pleasant

2. How tasty have you found the treatment?

NOT at all _________________________ very tasty
tasty

3. How did you like the texture of the treatment?

NOT at all _________________________ very much
at all
8.4.4.6 Palatability of the Pizza Meal
Visual Analogue Scales
Palatability: Pizza

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

How pleasant have you found the beverage/food?

NOT at all pleasant

---------------------------------------------

VERY pleasant