

The Acute Effects of Faba Bean-Containing Pasta on Glycaemia, Satiety and Metabolic Control in Healthy Young Adult Males

by

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Abstract

The hypothesis that addition of faba bean (FB) flours and fractions into pasta reduces postprandial glycaemia and increases satiety has been tested in young adult males. Experiment 1 investigated the effects in young adult males. They consumed a serving of pasta made from (1) durum wheat semolina or substituted with 25% of flour from faba bean (2) split flour, (3) high starch fraction, (4) protein concentrate, or (5) protein isolate. Measurements included postprandial blood glucose, insulin, C-peptide, GLP-1 and PYY, and appetite and second meal food intake. Experiments 2A and 2B measured second-meal effects after an *ad libitum* or fixed size meal (12 kcal/kg). Addition of high protein faba bean flours reduced postprandial glycaemia, and second meal appetite, and increased PYY and C-peptide but did not affect insulin or GLP-1. Consumption of pastas with added faba bean protein can have value-added nutritional benefits compared to conventional pasta.

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Abbreviations

- i. ANOVA, analysis of variance
- ii. BG, blood glucose
- iii. BMI, body mass index
- iv. CAD \$, Canadian \$
- v. dm, dry matter
- vi. DWS, durum wheat semolina
- vii. FBF, faba bean flour
- viii. FBPC, faba bean protein concentrate
- ix. FBPI, faba bean protein isolate
- x. FBS, faba bean protein starch
- xi. FI, food intake
- xii. G6DP, glucose-6-phosphate dehydrogenase
- xiii. GI, glycaemic index
- xiv. GLP-1, glucagon-like peptide 1
- xv. HC, Health Canada
- xvi. iAUC, incremental area under the curve
- xvii. IV, intravenous
- xviii. kcal, kilocalories
- xix. kg/m², kilograms per meter squared
- xx. min, minutes
- xxi. mIU/L, milli-International Units per litre
- xxii. mIU*min/L, milli-International Units*minutes per litre

- xxiii. mmol/L, millimoles per litre
- xxiv. non-IV, non-intravenous
- xxv. P, P-value
- xxvi. pg/mL, picograms per millilitre
- xxvii. pg*min/mL, picograms*minutes per milliliter
- xxviii. PPG, postprandial glycaemia
- xxix. PYY, peptide tyrosine tyrosine
- xxx. r, R-value
- xxxi. RCT, randomized controlled trial
- xxxii. RDS, rapidly digestible starch
- xxxiii. RS, resistant starch
- xxxiv. SEM, standard error of means
- xxxv. SDS, slowly digestible starch
- xxxvi. tAUC, total area under the curve
- xxxvii. VAS, visual analog scale
- xxxviii. y, year

Chapter 1

1 Introduction

Obesity and type 2 diabetes (T2D) require attention and effective action for their prevention and management. A potential approach to counteract weight gain and its associated conditions is to identify and promote satiating and low energy-dense foods. For this purpose, pulses and their components are a potential food source in diets due to their composition of high protein and fibre, known to lower glycaemic response and increase meal satiety [1-4]. Pulses have also become central to the prairie economy in Canada, one of the leading global producers and exporters of pulses [5].

Despite the health benefits and large national production of pulse, Canadians are low consumers. To stimulate their utilization in foods, the industry is encouraging processing of pulses to flours and the extraction of value-added components. However, the health benefits attributed to whole pulses for prevention and management of obesity and T2D may be lost by processing to easily digested flours [6]. The objective of this research was to describe the functionality of pulse flours for utilization in processed foods, using pasta as the example. Faba beans flours were utilized as they are one of the highest in protein among pulses, as well as an environmentally sustainable and robust crop [7].

Pasta was selected as a food for demonstrating the food and physiological functionality of faba bean flours because pasta is easy to cook, inexpensive and a staple food, making its fortification relevant to consumers and the food industry. Through fortification of pasta with faba beans flours, the nutritional quality of pastas was improved by addition of protein containing flours and by decreasing easily digested carbohydrate.

Chapter 2

2 Literature Review

2.1 Introduction

To provide background for the research rationale, the following topics are addressed: (1) current Canadian health and nutrition status (2) Health Canada policies and regulation, (3) the health benefits of pulses (4) health benefits of faba beans, (5) pulse flours as value-added benefits in foods, and (6) health benefits of the faba bean-containing pasta.

2.2 Canadian health and nutrition status

2.2.1 Prevalence of obesity and type 2 diabetes mellitus

There is an urgency to address worldwide obesity and the rise in its related diseases. The consequences of obesity are well-known, and they greatly reduce an individual's quality of life. Obesity is co-morbid to the metabolic syndrome and life-threatening chronic diseases, such as T2D mellitus, cardiovascular disease and certain types of cancers [8]. Obesity affects 1 in 5 adult Canadians and the same proportion is affected by the metabolic syndrome [9, 10]. By 2030, obesity is projected to grow by 33% [11]. Moreover, the 2015 Canadian Community Health Survey reported that 1 in 3 adult Canadians had pre-diabetes or diabetes, and the numbers are projected to grow 41% by 2026 [12]. The annual economic burden of obesity in Canada was estimated to be CAD \$1 billion in health care costs and indirect costs of an individual's loss of productivity; and a further \$3.4 billion for cost of diabetes to the Canadian health care system [12, 13].

2.2.2 Health Canada's new healthy eating strategy

Consumer education is important to help drive healthy food choices aimed at preventing growing nutrition-related epidemics, like obesity and T2D. Despite the efforts of Health Canada in the last decade to reduce fat and sodium levels in foods by working with the food industry, consumption remains high [14]. Canada is undergoing an urgent shift of focus

towards plant-based diets, lower consumption of sugar, sodium, and saturated fats to help manage weight gain and its associated conditions [15]. To help inform the public, Health Canada is currently implementing new healthy eating strategies, to be completed by 2019, with goals to “*improve healthy eating information, improve nutrition quality of foods, protect vulnerable populations, and support increased access to availability of nutritious foods*” [16]. Some major goals of Health Canada for the upcoming year includes informing Canadians to limit foods and beverages high in calories, sugar and/or salt and banning all trans-fats in foods by September, 2018 [14-16]. Moreover, new regulations are being established for mandatory front-of-package labelling symbols on food products to indicate products high in saturated fat, sugars and/or sodium [14]. Revisions to the Canada’s Food Guide are to be completed by 2019 with focus plant-based meat alternatives rather than meat products to promote consumption of cooked legumes, tofu, peanut/nut butters and shelled nuts [15]. Health Canada is updating the new food guide based on the latest scientific evidence and striving to make it useful to a wider range of audiences by providing recommendation for healthy meals and snacks using both pictorial and written information. With new goals in place by 2019, it is anticipated that food and nutrition in Canada will shift towards healthier foods and food choices in the market, as well as help buyers make informed decisions.

2.3 Food label claims in Canada

In conjunction with the new healthy eating strategies, health claims on food labels can help consumers make informed purchase decisions. For health claims to be approved, there must be sufficient scientific evidence to receive approval by Health Canada for use. With greater awareness about lower caloric consumption, “filling” foods, and the importance of glycaemic control, Health Canada released draft guidance documents for satiety and postprandial glycaemic (PPG) control health claims in 2012 and 2013, respectively. These claims fall under ‘Function Claims’ [17].

2.3.1 Satiety claims

Satiation refers to the process that leads to inhibition of further eating during a meal through a satiety cascade, where sensory, cognitive, post-ingestive and post-absorptive aspects

collectively determine meal size and satiety [18]. Satiety is the feeling of fullness that persists after eating, possibly suppressing desire-to-eat until hunger returns [19]. Some measures to support satiety claims include self-reported measures of subjective appetite, *ad libitum* food intake (FI) and physiological biomarkers.

2.3.1.1 Test food, reference food and *ad libitum* meal

The HC draft guidance for satiety states that the amount of food tested and the reference food should match the serving size in the Canadian Nutrition Facts table [20]. The test food should not have higher energy content compared to the reference food. An *ad libitum* test meal should follow the treatment pre-load to measure FI to determine satiety-related effects of the treatment pre-load. The time of day when the food is given to participants should be appropriate and follow when this type of food is normally consumed. Moreover, the time interval between test meal and *ad libitum* FI meal should represent a realistic gap between meal times [20].

2.3.1.2 Subjective appetite

Subjective appetite questionnaires provided in specified time intervals determine change in appetite over time [20]. Appetite is a combination of sensations that lead to seeking, choosing, and ingesting food. Quantifying appetite and satiety for nutrition research is a challenge, due to difficulties in capturing all the aspects that determine satiety beyond eating for energy requirements, such as environmental stimuli and sensory aspects of food [18]. The visual analog scale is a tool to quantify subjective appetite by rating levels of hunger, desire-to-eat, fullness and prospective food consumption on a line scale [18].

2.3.1.3 Physiological biomarkers of metabolic control

Some physiological biomarkers used to support a satiety claim include (but are not limited to), ghrelin, cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1) and peptide tyrosine tyrosine (PYY). Their mechanisms of action are summarized in Table 2.1. Orexigenic hormones promote hunger and in contrast, anorexigenic hormones increase satiety, reduce appetite and FI. They are described in Table 2.1.

2.3.2 Postprandial glycaemia claims

2.3.2.1 Reference and test food

According to HC draft guidelines, if the test food contains a substitution of ingredients, the reference food should be the same food without the ingredient substitution [21]. Moreover, the serving size of the test and reference food should match and the claimed effect should be achieved by 1 serving of the food prepared conventionally [21].

2.3.2.2 Blood glucose measure

HC Draft guidelines for postprandial glycaemic response claims require measures of BG in acute human intervention studies [22]. To support a claim, statistically significant difference of a minimum of 20% decrease in the incremental area under the curve compared to the reference food is required [22]. BG measures should be done for at least 2 hours after eating the test meal, in 15 minute increments up to the first hour and in 30 minute increments, thereafter [22].

2.3.2.3 Insulin measures

In response to rise in BG after the meal, insulin is released from the pancreas to drive tissue uptake of glucose [23]. In healthy individuals, insulin increase should correspond to a proportional decrease of glucose in the blood stream. For postprandial glycaemia claims, insulin measures should accompany data for BG to show that the decrease in BG concentrations does not correspond to disproportionate insulin increase. Insulin measures are especially important when there are major differences in protein between the test and reference food [22]. C-peptide is a bioactive peptide responsible for the correct folding of pro-insulin and released in proportional amounts to insulin. It is often measured in conjunction with insulin to confirm proper function of the pancreas. Expression of glycaemic and insulinaemic responses are required to be shown as incremental area under the curve above baseline using the trapezoidal rule [21, 24]. A summary of the primary hormones and peptides used as biomarkers of glycaemic and appetite controls are shown in Table 2.1.

Table 2.1: Appetite regulatory hormones and peptides and their site of production, effect on appetite and mechanisms of action

Hormones and peptides	Site of production	Effect on appetite	Mechanism	Key notes
Ghrelin	Stomach	↑ appetite	Via ghrelin receptors in brain	-Gut hormone -Long-term effect on energy balance
Cholecystokinin (CCK)	Duodenum and jejunum	↓ appetite	Via vagus nerve	-Gut hormone -Delays gastric emptying -Stimulates pancreatic enzyme secretion -Stimulates gall bladder contraction -Acts as neurotransmitter
Peptide tyrosine tyrosine (PYY)	Ileum, colon and rectum	↓ appetite	Via Y2 receptors in brain	-Gut hormone -Slow gastric emptying and intestinal transport
Glucagon-like peptide-1 (GLP-1)	Intestine and brain	↓ appetite	Via GLP-IR in brain	-Gut hormone -Incretin (stimulates insulin production)
Insulin	Pancreas	↓ appetite (indirect)	Via glucose uptake from bloodstream	-Long term adiposity signal -Production stimulated by GLP-
C-peptide	Pancreas	-	-	-Proinsulin splits apart and forms one molecule of insulin and one molecule of C-peptide -Serum concentration proportional to serum insulin

Adapted from [19, 25]

PPG and satiety health claims may aid healthier consumer decisions aimed at preventing obesity and T2D. But in conjunction with consumer awareness, effective intervention on weight management requires healthier, yet tasty, food choices in the market that are affordable and appeal to the public. Incorporating plant-based ingredients (i.e. legume or vegetable powders) into commonly consumed foods may help to promote their consumption, which is in line with HC’s new focus on plant-based diets.

2.4 Pulses

Pulse crops are important for their health benefits and agricultural sustainability; however, they are underexploited, with low global average consumption. Moreover, Canada is the third largest producer of pulse crops globally, making them important for the national economy [26]. Pulses are the edible seeds of plants in the legume family (*Fabaceae* or *Leguminosae*), excluding oil-seed legumes [27]. Pulses have been a major protein source in the diet around the world, including the Mediterranean, Middle-East and Asian countries, but now competes with the rise in meat consumption [28]. Moreover, the Food and Agriculture Organization (FAO) of the United Nations declared 2016 as the “International Year of the Pulses” to recognize and promote the utilization and consumption of pulse crops [29].

There is extensive knowledge on the health benefits of pulses and reduction in biomarkers and incidence of diet-related chronic diseases. Between 1983 and 2017, a large body of evidence has been published showing that pulses are low glycaemic and satiating foods. Randomized controlled trials (RCTs) and observational studies report both acute and long-term benefits, while several systematic reviews and meta-analyses have also summarized the knowledge.

2.4.1 Relationship between pulses and glycaemic regulation

The relationship between whole pulses and the regulation of PPG has been well-recognized including an approved health claim for postprandial glycaemic reduction. Available evidence from 1980 to 2012 was reviewed, and included 11 reports on whole lentils, 7 reports on whole beans, 7 reports on whole peas and 4 reports on whole chickpeas [30]. The report concluded that 1 cup (250 ml) of cooked whole pulse in place of low fibre starchy foods resulted in reduced PPG response after a meal in healthy and diabetic consumers. In the same year, a review by Ramdath et al. evaluated the role of pulses in the dietary management of diabetes in 3 epidemiological studies (8.5-15 years), 9 short-term RCTs (3-11 meals, approximately 1 week apart), and 6 long-term RCTs (8-22 weeks) [31]. They concluded that from short-term human trials, postprandial BG was significantly lower after a single serving of pulses between $\frac{3}{4}$ to 1 cup compared to a non-pulse starchy equivalent. Moreover, long-term pulse consumption of 5 cups/week consistently improved glycaemic control [31].

2.4.1.1 Systematic reviews and meta-analyses

Several systematic reviews and meta-analyses have also summarized similar data from RCTs and epidemiological studies. In 2009, Sievenpiper et al. conducted a systematic review and meta-analysis of 41 randomized control trials (RCT) on pulses and diabetes management. Data was collected between 1981 to 2007 consisting of 1,674 diabetic and non-diabetic participants. Studies were assigned into a group: (1) pulse alone (11 trials), (2) pulses as components of a low-GI diet (19 trials) and (3) pulses as components of a high-fibre diet (11 trials). They concluded that all three categories showed improved markers of longer term glycaemic control in diabetic and non-diabetic participants [32]. Pulses alone attenuated

fasting BG and insulin [32]. The study also reported that chickpeas exhibited more prominent effects compared to other pulses.

In 2014, a meta-analysis of 38 acute clinical trials (n=714) reported that pulses of many types, including chickpeas, beans, lentils and split peas significantly reduced PPG by 50% compared to a control with equal carbohydrate content [33].

2.4.1.2 Randomized controlled trials

Anderson and colleagues have conducted several acute randomized controlled trials and these results are summarized in Table 2 [1-4]. These have been extensively cited looking at the effects of whole pulses on glycaemic control and were included in the aforementioned reviews by various authors and used in the HC briefing document for PPG claims [31-34]. In a recent study, acute randomized controlled trials investigated the effects of chickpeas, lentils, yellow peas and navy beans on PPG immediately after the test meal and after a second pizza meal 2-5 hours later. All pulses resulted in cumulative BG reduction in at least two of the four studies and most consistently by chickpeas and lentils [2-4]. Lentils led to a cumulative reduction of PPG and post-meal PPG suppression in two studies [1, 2]. Meanwhile, chickpeas suppressed cumulative PPG in all studies and post-meal suppression in one study [1].

Recently, Mollard et al. reported the effects of consuming 5 cups/week of mixed pulses for 8 weeks on the management of risk factors for the metabolic syndrome (MetS) (n=40; age=47.3±5.9y energy-restricted group, age=43.5±6.7y pulse group; BMI=32.8kg/m²) [35]. An *ad libitum* diet including 5 cups/week of pulses reduced the risk factors for MetS similar to an energy-restricted diet achieved through counselling. These included lower waist circumference, energy intake, systolic blood pressure, HbA1c, and improved BG control and insulin sensitivity [35]. Thus, it can be hypothesized that that management of obesity can be achieved by spontaneous reductions in energy intake by regular consumption of satiating foods such as pulses in an *ad libitum* diet.

Pulse consumption, especially lentils and chickpeas, not only reduces PPG immediately after eating but also in response to a meal consumed several hours later [1-3]. Sievenpiper et al.'s meta-analysis also demonstrated that chickpeas showed more prominent effects on the

management of biomarkers of diabetes [32]. Thus, it is clear that metabolic effects are dependent on pulse type [1]. As such, not all pulses have the same magnitude of effect on postprandial glycaemia. This has been well-described for the effect of whole pulse consumption on acute glycaemic control, specifically for the more popular pulse types, such as lentils, chickpeas, common beans and peas. However, less common pulse types, such as faba beans, black-eyed peas and runner beans, have received little investigation.

Table 2.2: Acute randomized controlled trials from our lab investigating relationship between whole pulses, appetite, and glycaemic regulation

Author, year	Subject Characteristics	Treatments	Session duration	Appetite			Glycaemic response			Second meal ad libitum food intake
				Cumulative	Pre-meal	Post-meal	Cumulative	Pre-meal	Post-meal	
Wong et al., 2009	n=14M (Exp 1) n=14M (Exp 2) n=15M (Exp 3) age=18-35y; BMI=20-25kg/m ²	C: White bread T: Pulse with Sauce -Chickpeas -Lentils -Navy beans -Yellow Peas	120 min	↔	↔	N/A	↓ chickpeas ↓ lentil ↓ yellow peas	↓ chickpeas ↓ lentil ↓ yellow peas	N/A	↔ (120 min)
Mollard et al., 2011	n=25M; age=21.5±0.5y; BMI=21.6±0.3 kg/m ²	C: Macaroni + cheese T: Pulse + macaroni + sauce -Chickpeas -Lentils -Yellow peas	340 min	↔	↔	↔	↓ chickpeas	↔	↓ chickpeas ↓ lentil	↓ lentil ↓ yellow peas
Mollard et al., 2012	n=24M; age=24.3±3.6y; BMI=22.8±1.4 kg/m ²	C: Macaroni + sauce T: Pulse + macaroni + sauce -Chickpeas -Lentils -Navy beans -Yellow Peas	340 min	↔	↔	↔	↓ chickpeas ↓ lentil ↓ navy beans ↓ yellow peas	↓ chickpeas ↓ lentil ↓ navy beans	↔	↓ lentil (260-280 min)
Mollard et al., 2014	n=15M; age=22.5±0.8y; BMI=22.9±0.4 kg/m ²	C: White bread T: Pulse + macaroni + sauce -Chickpeas -Lentils -Navy beans -Yellow Peas	210 min	↓ chickpeas ↓ lentil ↓ navy beans	↓ lentil	↔	↓ chickpeas ↓ lentil ↓ navy beans ↓ yellow peas	↓ chickpeas ↓ lentil ↓ navy beans	↓ lentil	↔ (135-150 min)

2.4.2 Relationship between pulses, appetite and food intake regulation

2.4.2.1 Systematic reviews and meta-analyses

A systematic review reported the effect of pulses on acute satiety and second meal FI from 9 randomized controlled trials (n=126 with a ratio male:female=6:5; n=98 for FI; median age=31.6y; median BMI=24.7kg/m²) [34]. All studies were conducted in less than a day with a single isocaloric test meal. Satiety incremental area under the curve (iAUC) was increased by a remarkable 31% compared to control; however, second meal FI was not significantly different. Authors proposed that measures of second meal effects should reflect realistic meal time intervals (3-6 h after the first test meal), which allows satiety effects of low-GI macronutrients, like protein, to take place. Only two trials scheduled the second meal ≥ 3 hours from the test meal and these demonstrated the greatest FI reduction [34]. Moreover, another systematic review and meta-analysis reported the effects of dietary pulse consumption on body weight from 21 trials (n=940) on overweight or obese middle-aged men and women over median duration of 6 weeks [36]. They concluded that in this population, 1 serving of pulses per day may be beneficial for weight loss in both usual *ad libitum* (neutral energy-balance) and calorically restricted (negative energy-balance) diets [36].

2.4.2.2 Observational studies

A decade ago, a the National Health and Nutrition Examination Survey (NHANES) of data from 1999-2002 reported that bean consumers had 23% lower body weight and 22% lower risk of obesity [37]. This well-cited report led to a greater popularity of pulses for appetite regulation and satiety research.

2.4.2.3 Randomized controlled trials

Several aforementioned randomized controlled trials in our lab investigated the effects of whole chickpeas, lentils, navy beans and chickpeas on satiety and FI of healthy, young men with healthy BMI [1-4]. These studies have also been considered in the previously discussed systematic reviews and meta-analyses for appetite regulation and satiety control. Treatments were compared to a starchy, non-pulse carbohydrate, such as white bread or pasta with sauce

by measuring subjective appetite and second meal FI and summarized in Table 2.2. A second *ad libitum* pizza meal was served several hours later to assess FI. Measures for appetite and satiety were taken pre-pizza meal and post-pizza meal. Lentils demonstrated most prominent effects on appetite regulation and FI. In one study, lentils suppressed cumulative and pre-meal appetite compared to white bread; but despite appetite reduction, FI was not different (Mollard et al., 2014). In one study, chickpeas and navy beans also reduced cumulative appetite compared to white bread but no specific effects premeal or postmeal [2]. Interestingly, in two studies where appetite regulation of pulses showed no effect, lentils suppressed second meal FI [1, 3]. Although the RCT evidence to support acute appetite regulation and FI control is inconsistent, long term evidence still suggests pulses are considered in the diet for weight-loss strategy and reduced risk of obesity, along with many other benefits for chronic disease risk reduction.

As discussed here, many studies have investigated the health benefits of some popular pulses such as lentils, chickpeas and peas. However, faba beans have received little attention, despite their valuable nutritional content and important role on environmental sustainability.

2.5 Faba beans

Faba beans (*Vicia faba* L.), also known as the broad bean, horse bean and field bean, is a pulse crop which has received less attention in research and development.

2.5.1 Faba beans and agricultural sustainability

Faba beans are robust crops with potential to thrive under global warming and climate changing conditions currently faced in agriculture. It is highly adaptable in a wide range of soil conditions with minimal input, has high tolerance to biotic and abiotic stress and able to tolerate frost [7].

For the past half century, use of synthetic fertilizers grew by almost tenfold for nitrogen (N) between 1961 and 2013 [38]. One of the unique ability of pulses in cropping systems is to fix atmospheric nitrogen (N) into the soil. Emissions of greenhouse gas, N₂O, is higher from

fertilized crops than from legume-based systems with almost 3 times higher net global warming potential index (GWP) [39]. Amongst the pulses, faba beans are the highest N fixer where about 77% of N in the crop is derived from N fixation, possibly explaining their high protein content [39].

2.5.2 Nutritional composition of faba beans

Faba bean nutrient content is approximately: 52.2-60.5% carbohydrates, 25.0-30.14% protein, 3.27-4.97% ash, 1.65-2.24% fat and 7.09-7.59% moisture [40, 41].

2.5.2.1 Protein

Overall, pulses are recognized as a protein source by Health Canada and is a well-known meat alternative. Protein content of beans are comprised of albumins and globulins. Albumins are water soluble and are enzymatic proteins, protease inhibitors, amylase inhibitors and lectins [42]. Meanwhile, globulins are salt-soluble storage proteins and comprised primarily of legumin (11S) and vicilin (7S). Percentage of these proteins vary greatly in faba beans and depends on climate, region and time of growing season but are present at approximately 45% 11S and 33% 7S proteins. Legumin is the plant equivalent to casein from animal milk, with similar physicochemical properties and has a long digestion time upon consumption [43]. Cooking tends to increase protein content in certain pulses, including faba beans, and are considered highly digestible of over 80% [44]. Moreover, cooking possibly releases carbohydrates, increasing protein percentage and inactivates antinutritive compounds, like trypsin and chymotrypsin inhibitors which interferes with protein availability [44].

Pulses and cereal grains are often viewed as complementary amino acid profiles which improves protein quality when they are consumed together [45]. Of the essential amino acids, pulses are high in lysine and leucine, but low in sulfur-containing essential amino acids like tryptophan and methionine [42, 45]. Lysine content was reported for 12 faba bean varieties to be an average of 4.09% (g/100g protein, dry matter) compared to 2.70% in whole wheat, and when milled into semolina is only 1.95% [46]. Investigation of protein concentrates made from faba beans showed 39% greater protein concentration compared to other pulse protein concentrates [47]. Thus, there can be a high protein yield by fractionating faba bean flour into

protein concentrates and isolates with strong potential in nutrient enrichment applications for the food industry.

2.5.2.2 Carbohydrates

Starch is the main reserve component of pulses seeds and makes up 37%-44% of faba beans [41, 48]. Based on in-vitro starch digestion, faba bean starch content is comprised of two thirds slowly digestible starch (SDS=20-120 min) and resistant starch (RS), with one third rapidly digestible starch (RDS=first 20 min) [48, 49]. This is an indicator that faba beans are a good source of SDS and RS. Greater contents of SDS and RS generally leads to slower release of glucose into the blood stream, which may be associated with improved glycaemic control [50]. However, there is a wide range of reported values for starch contents in pulses, ranging from a few percent to 80% RS of total starch because processing parameters can hugely influence starch ratios [48, 51].

Faba beans are high in dietary fibre amounting to 20-26% of the dry matter (dm) with about 3-4% dm being soluble fraction while the rest is insoluble [48]. However, much of the fibre, especially insoluble fibre is contained in the seed coat at about 90% dm and this is removed during processing. [48].

2.5.2.3 Fats and oils

Faba beans and pulses in general are low in fat and considered non-oil seed grains, unlike other legume grains that are pressed for oil (i.e. soy beans and peanuts); thus, may attribute to lower energy density.

2.5.2.4 Bioactive components

Amongst the *Fabaceae* crop family, pulse seeds contain several antinutritional factors which may have adverse effects in humans, but studies have shown possible benefits in low concentrations [52]. Adverse effects may be direct interference with nutrient breakdown and absorption, or antinutritional factors may cause the creation of undesirable secondary metabolites [52]. Some examples of antinutritional factors in faba beans are primarily proteins which include: trypsin inhibitors, tannins, hemagglutinins, phytates and α -amylase inhibitors

[53]. Inhibition of α -amylase, an enzyme involved with the breakdown of carbohydrates for digestion, can significantly reduce and/or slow glucose release into the bloodstream [52, 54]. Inhibition of α -amylase is attributed to phenolic compounds associated with protein substances [53]. In low levels, amylase inhibitors may be a strategy for managing glycaemic response to starchy foods [52].

2.5.3 Favism

A barrier for encouraging faba bean consumption and hindering greater exploitation of the crop is its bioactive components that cause favism [55]. It is a rare disease affecting glucose-6-phosphate dehydrogenase (G6PD) deficient individuals causing acute hemolysis upon ingesting faba beans. A redox reaction converts glucosidic aminopyrimidine derivatives, vicine and convicine, to divicine 2,6-diamino-4,5-dihydroxypyrimidine and isouramil (6-amino-2,4,5-trihydroxypyrimidine), respectively, which are toxic compounds to G6PD deficient individuals. Upon intoxication, red blood cells are destroyed within 24-36 h after faba bean ingestion and can cause up to 80% destruction of circulating red blood cells, leading to death [56]. Fortunately, beta-glucosidase enzyme is inactivated upon seed drying, cooking, food processing, use of acid (even that of hydrochloric acid at gastric concentrations) [56]. Favism occurrence is usually from raw fresh seeds or only partially cooked seeds; although frozen fresh faba beans can carry the active enzyme as well [56]. Although young beans are commonly consumed raw due to their sweet taste and tenderness and are very high in vicine and convicine they are safe to consume because they lack the beta-glucosidase enzyme that produces the toxic compounds. Thus, less than 20% of G6PD-deficient individuals experience favism in their life, despite frequent faba bean consumption [56]. Utilization of faba bean in cooked and processed forms for food applications eliminates the risk of favism.

2.6 Mechanism of action of pulses and faba beans on glycaemic and appetite regulation

The nutritional profiles of faba beans and pulses, in general, are unique and may curb weight gain through a variety of their macronutrient characteristics. Faba beans contain slowly digestible carbohydrates, including resistant starch, high fibre (soluble and insoluble), high quality proteins and are low caloric density which may all play a role in glycaemia, satiety

and FI regulation [57, 58]. Bioactive components, such as phenolic compounds may affect metabolic regulation by modulating glucose absorption through interference with glucose transporters [59, 60].

2.6.1 Gastric distension and emptying rate

Pulse starch has a high amylose:amylopectin ratio compared to cereal and tuber starches. As well, the amylose:amylopectin ratio is particularly high in faba beans compared to other pulses [48]. Upon heating, amylose and external amylopectin branches gelatinize and upon cooling, they reassociate in a process called retrogradation [57]. Retrograded material increases gastric distension and slows emptying rates. After retrogradation, α -glucosidase enzymes responsible for starch digestion cannot reach internal branches, thus reducing rate of starch digestion by limiting the enzyme access to starch [57].

Also, viscous soluble fibre content of about 3-4% of the total dietary fibre in faba beans may increase gastric distension and help slow gastric emptying rate [48]. This occurs via a gel formation in the small intestine which slows the rate of nutrient absorption by causing the ileal brake [57]. The ileal brake is a series of mechanisms that controls the transit of food from the stomach into the ileum of the intestines [61].

2.6.2 Gastrointestinal hormones and peptides

It is well-known that protein is more satiating than the isoenergetic equivalent of carbohydrate or fats [62, 63]. A study reported that a high-protein meal compared to high-carbohydrate or high-fat meal suppressed postprandial ghrelin levels, possibly leading to longer feelings of fullness and suppression of appetite [64]. A study of various bean protein extracts found that a country bean-derived peptide stimulated the *in vitro* release of cholecystokinin (CCK) [65]. CCK is a hormone which increases satiety, reduces subsequent FI to mediate meal size [66]. Protein content is highest in faba beans compared to other pulses, which may be the primary factor leading to satiation compared to grains and other seed crops [67].

2.6.3 Postprandial glucose absorption

To aid in weight management, retrograded starch in pulses may contribute to reduced caloric intake because of the inaccessibility of glucose from retrograded starch. Moreover, postprandial glycaemic control may be mediated by the lower rate of glucose release into the bloodstream after retrogradation. Bioactive compounds may play a role in metabolic regulation by slowing digestion rates through enzyme inhibitory effects [52, 57]. For example, phenolic compounds reduce glucose absorption by inhibiting glucose transporters and carbohydrate metabolism enzyme, α -amylase, which delay postprandial glucose absorption [53, 59, 60]. Phytic acid has also been shown to reduce the rate of *in vitro* starch digestion and rate of postprandial glucose absorption [68].

2.6.4 Protein digestion

The protein quality of pulses has been investigated, and as previously mentioned, pulse proteins are highly digestible and are considered a “source of protein” under Health Canada’s standards. However, the time course of absorption of dietary amino acids of pulse proteins in humans remains unclear. Pulses contain large storage proteins, such as 60-80% globulin (legumin and vicilin) and 15-25% albumins (enzymes and active compounds, such as trypsin and chymotrypsin inhibitors) (Agarwal, 2017). Vegetable legumin physicochemical characteristics are similar to that of milk casein proteins, which are considered slowly digested [43, 69]. The concept of “fast and slow” protein originated from Boirie et al.’s study on milk proteins demonstrated that soluble whey protein peak absorption is at 2 hours and returns to baseline at ~ 4 hours while the insoluble casein protein do not peak but rather have study absorption over a 7 hour period [43]. This is a similar to the concept of carbohydrate metabolism where the physicochemical behavior of proteins to the environment during digestion, such as gastric pH and water solubility, which may affect the digestion and absorption of amino acids of the protein, leading to different postprandial metabolic responses and possibly longer feelings of satiety [43, 70]. Along with slow digestion of legumins, albumins in pulses are similar to those in eggs, which have shown to take 7 hours to fully absorb [71]. Moreover, a greater energy cost may be associated with slow protein digestion, aiding with energy balance and weight management [57]. Thus, pulses may have slowly

digested proteins which result in slower gastric emptying rate and thus, provide a longer feeling of satiety [70]. In addition, the aforementioned complementary amino acid profile of pulses and cereal grains may help to increase satiety when consumed together due to slower digestion rates of complete essential amino acid profiles [57].

2.6.5 Short chain fatty acid production

The fermentable fibre content in faba beans resist digestion in the upper intestinal tract and is fermented in the colon. As well, oligosaccharides, a prebiotic, also facilitate the growth of beneficial bacteria in the colon. Together, fermentation of these nutrients results in the product of short chain fatty acid (SCFA) by-products. SCFA is used for energy to spare protein and glucose and suppress hepatic glucose production [57].

Faba beans carry many factors that are responsible for their well-known benefits for regulation of glycaemia, satiety and metabolic control. Despite knowledge of their benefits, global consumption is low. Further processing of the seed to a flour to fortify commonly consumed products may be a route to promote its consumption and utilization in the food industry.

2.7 Pulse flours

Pulse flours can be prepared by grinding whole pulses and their macronutrients can be concentrated by solvent extraction to produce flours high in protein, fibre and slowly digested carbohydrates. These flours can be used to increase the nutritional and physiological function of high carbohydrate foods.

2.7.1 Relationship between pulse flours, glycaemia, satiety and food intake regulation

The processing of pulses to flour form, usually of small particle size, has led to criticism that the value of consuming the intact bean for control of glycaemia and appetite will be lost. This has been investigated by utilizing processed pulse flours in various high carbohydrate foods, such as baked goods and pasta. In general, there is improved nutritional value, especially for protein content quality with incorporation of pulse protein concentrates or isolates [72].

However, there is often a decrease in cooking and sensory properties of pulse-fortified foods [73-75]. Recent reports investigating the effects of pulse flours in meals on glycaemic and appetite control are summarized in Table 2.3. In all studies, glycaemic control was improved significantly with all pulse flour/fraction addition to meals compared to non-pulse containing equivalent but no studies reported effects on appetite reduction [72, 76, 77]. In contrast no self-reported appetite reduction was found, but one study reported reduction in second meal *ad libitum* FI by the yellow pea 20g protein fraction in tomato soup, compared to non-pulse tomato soup [77].

Table 2.3: Randomized controlled trials investigating the role of pulse flour glycaemia, appetite and food intake regulation

Author, year	Subject characteristics	Treatments	Duration	Glycaemic control		Appetite		Second meal ad libitum food intake
				Pre-meal	Post-meal	Pre-meal	Post-meal	
Smith et al., 2012	n=19M; age=23.2y; BMI=22.5kg/m ² (Exp 1) n=20M; age=22.3y; BMI=21.8kg/m ² (Exp 2)	C: tomato soup T: Yellow pea powder + tomato soup -Pea fibre, F10 (10g) -Pea fibre, F20 (20g) -Pea protein, P10 (10g) -Pea protein, P20 (20g)	200 min	↓ PPG (P10) ↓ PPG (P20)	↓ PPG (P20)	↔	↔	Exp 1: ↓ P20 (30 min) Exp 2: ↔ (120 min)
Anderson et al., 2014	n=17M; age=22.1y; BMI=22.9kg/m ² (Exp 1) n=12M; age=22.2y; BMI=23.2kg/m ² (Exp 2) n=12M; age=23.6y; BMI=22.3kg/m ² (Exp 3)	C: Whole-wheat flour T: Whole, puréed or powdered form + tomato sauce -Exp 1: navy bean -Exp 2: lentil -Exp 3: chickpea	200 min	↓ PPG (chickpea, all forms) ↓ PPG (lentil, all forms)	↓ PPG (navy beans, whole only)	N/A	N/A	N/A (120 min) -fixed quantity pizza
Mollard et al., 2014	n=15M; age=21.5±1.0y; BMI=22.5±0.4kg/m ²	C: Noodles + tomato sauce T: Yellow pea powder + noodles + tomato sauce -Pea protein (10g) -Pea hull fibre (7g) -Pea protein (10g) + fibre (7g) -Whole peas	215 min	↓ PPG (Pea protein + fibre) ↓ PPG (Yellow peas)	↔	↔	↔	↔ (135 min) -pizza
Marinangeli & Jones, 2011	N=10M; age=51.8±12.3y; BMI=31.7±5.3kg/m ² N=19F; age=52.3±10.0y; BMI=29.4±3.5kg/m ²	C: White wheat flour muffin (WF) T: Muffin made of: -whole pea flour (WPF) -fractionated pea flour (FPF) -flour equivalent to ½ cup yellow peas/day	Daily consumption for 28 days	Overall (on Day 28): ↓ Insulin (WPF) ↓ Insulin resistance (WPF, FPF) ↓ Android:genoid fat ratio (WPF, women only) ↔ PPG		N/A	N/A	N/A

2.7.2 Mechanisms of action of pulse flours on glycaemic and appetite regulation

The nutritional benefits from food fortification with pulse flours may be primarily attributed to quality proteins, carbohydrates and bioactive compounds working together. Some benefits of whole pulses and pulse flours may also arise from polyphenols that have inhibitory effects on α -amylase and α -glucosidase and lower PPG response [78]. Whether isolated macronutrients in flours from pulses retain the positive benefits of whole pulses remains unclear. Smith et al. showed that pea protein isolate at 10g and 20g additions to tomato soup attenuated PPG and pea protein at 20 g reduced second meal FI. Contrastingly, Anderson et al. showed that while the addition of a combination of yellow pea protein and hull fibre to tomato sauce and noodles significantly attenuated postprandial BG up to 135 min before the consumption of a second meal, with similar results to canned yellow peas, no effect was observed from pea protein and fibre separately [72]. Similarly, benefits of daily consumption of whole pea flour muffins was consistently beneficial for glycaemic regulation but such results were not as consistently observed with fractionated pea flour muffins [79].

Further research is necessary to determine whether fractionated flours have stronger benefits on physiological function than their intact seeds or their whole flours. Moreover, there is a need to assess evidence for pulse varieties other than peas.

2.7.3 Pasta as a carrier of pulse flour benefits

Pasta is a good delivery medium for the potential benefits of pulse flours due to its wide consumption, low cost and ease of cooking. Conventional pasta is made from refined durum wheat semolina (DWS) flour with a low to medium glycaemic index (GI) of 32-65 (glucose as reference), unlike other typical refined grain starchy foods, that usually have a high GI [80-82]. Enrichment of pasta dough with pulse flours may provide value added benefits to the end-product by increasing protein content and quality and slowly digested carbohydrates. Pulse flours improves final lysine content of cooked pasta, which typically loses 50% of its content after production and cooking [46, 80, 83].

The challenge in utilizing pulse flours in pasta is ensuring sufficient gluten network from DWS flour to maintain structural integrity of pasta during extrusion and prevent nutrient loss during cooking. Thus, the DWS to pulse flour ratio is an important consideration. 20 to 35%

substitution of DWS pasta dough with pulse flour is accepted without compromising pasta extrusion [73-75]. Consequently, undesirable texture changes and lower sensory properties have been reported for pulse-enriched food products, which may deter consumers from repurchasing such products [73-75]. Pulse-flour enrichment of high carbohydrates products is done extensively using lentils, chickpeas and peas, but not for faba beans. Extruded pulse products have shown variability in protein bioavailability compared to cooking and baking [84]. To promote wider consumption and to deliver the health benefits of faba beans, they should be used as a pulse flour in affordable, staple products, like pasta.

2.8 Faba bean-enriched pasta

Several studies have investigated *in vitro* nutritional quality of faba bean-enriched pasta, particularly on their carbohydrate content, but only two studies reported their effects on glycaemia and satiety in humans.

One study compared the effects of low temperature dried 100% DWS pasta to 35% faba bean flour substitution in DWS pasta dried at low temperature of 55 °C (F-LT) and very high temperature of 90 °C (F-VHT) on glycaemia and satiety in young adults (n=8M; n=7F; age=24±2.9 y; BMI=22.4±1.8 kg/m²). *In-vivo* glycaemic, insulinaemic and satiety responses were measured over a 180 min session by utilizing a randomized repeated measures design, controlled trial. *In-vitro* carbohydrate digestibility was also investigated [73]. Faba bean fortified pasta increased total protein and fibre contents, and decreased total carbohydrates [73]. Total lysine doubled but high temperature drying of the pasta reduced lysine availability suggesting that protein availability could decrease with high temperature drying [73]. From the *in-vitro* study, total available carbohydrate was significantly lower by 8% in F-LT and even lower by 13% in F-VHT compared to control. Moreover, resistant starch was significantly higher by 52% and 64%, in F-LT and F-VHT, respectively. Interestingly, F-VHT but not F-LT reduced rapidly available glucose by 20% and increased slowly available glucose by 43%. These results suggest that faba bean substitution, especially with higher drying temperatures induces lower glycaemic response *in vitro*. However, no differences were found between the three pastas for glycaemic and insulin responses perhaps because all pastas maintained low glycaemic and insulin index. However, treating faba bean-enriched pasta at very high temperature reduced appetite and increased satiation which may be a

result of harder texture and extended chewing time [73]. Despite harder texture, F-VHT reduced abdominal discomfort and “bloating” feeling compared to F-LT. Thus, faba bean enrichment of pasta coupled with high temperature drying could be a novel strategy to increase satiety and eliminates abdominal discomforts commonly associated with legumes.

In a non-randomized trial, Turco *et al.* investigated the effects of 35% faba bean flour substitution in DWS pasta compared to a DWS pasta control, where both pastas contained 30% eggs (dried or raw not specified) (n=2M; n=11F; age=31±10y; BMI=23±4kg/m²). Pasta was consumed at the beginning of the session and measures for BG was taken over 120 min period. The faba bean pasta resulted in a significantly lower GI of 40 ± 4.5 compared to 72 ± 8 in DWS control. iAUC was 41.4% lower for the faba bean pasta compared to control. α -glucosidase and α -amylase activities were reported where faba bean pasta had 51% and 36% higher inhibition, respectively, which may be a reason for the lower glycaemic response. Authors suggested that higher contents of total polyphenols, flavonoids and antioxidants of faba bean enriched pasta compared to control may contribute to inhibitory effects on enzymes that metabolize starch, possibly lowering glycaemic response [78].

Moreover, a recent study compared the effects of baking, cooking and extrusion on the protein quality of various beans [84]. Particularly for faba beans, extrusion resulted in lower protein efficiency ratio (PER) compared to baking and cooking, which may pose a challenge for receiving health benefits of faba beans through pasta. Nonetheless, this study aims to provide guidance to the food industry to see a wider utilization of pulses in novel products in various food categories, including high-carbohydrate staples, snacks and beverages. These should be investigated to promote the health benefits and consumption of pulses.

2.9 Conclusion and research rationale

There is a wealth of evidence reporting the benefits of whole pulse consumption on glycaemia, satiety and metabolic regulation. The benefits of pulses are multifactorial and attributed to their complex carbohydrates, fibre, protein and antinutrient properties. Benefits of several pulse varieties have been extensively studied but little evidence is reported for the benefits of faba beans. Canada has potential to reap the benefits of this underexploited crop which, among pulses, carries the highest average protein content and the most efficient nitrogen-fixing capabilities. However,

pulse consumption in Canada remains low and novel strategies for incorporation of pulses into the diet are necessary. Utilization of pulse flours and concentrated macro-nutrient pulse fractions in commonly consumed food products may have value-added benefits, but these effects are unclear. Thus, this project investigates the acute effects of faba bean fraction-enriched pasta on glycaemia, satiety and metabolic control in young adult males.

Chapter 3

3 Hypothesis and Objectives

3.1 Hypothesis

The hypothesis was that incorporation of flours from faba bean and its fractions in durum wheat semolina pasta lowers postprandial BG, increases satiety and stimulates regulatory hormones compared to durum wheat semolina pasta. Faba bean protein was expected to drive the response; thus, more prominent effects were expected from pastas containing high protein flours.

3.2 Objective

The objective of the study was to compare the acute effects of substituting 25% of durum wheat semolina in pasta with flours from faba bean and its fractions on satiety, FI, post-prandial glycaemia, insulin, C-Peptide, PYY and GLP-1 in healthy, young adult males.

3.2.1 Specific Objectives

Experiment 1: To compare acute effects of the FB pastas on glycaemia, metabolic control satiety and FI for 2 hours, immediately after pasta consumption (Exp. 1).

Experiment 2: To compare second meal effects of the FB pastas on glycaemia, food intake, satiety and metabolic control for 1 hour after an *ad libitum* pizza meal consumption (Exp. 2A) or after a fixed quantity pizza meal (Exp. 2B).

Chapter 4

4 Materials and Methods

4.1 Participants

Participants were young adult males ($n=62$) between 20 to 30 years old. Inclusion criteria were males who had regular BMI ($18.5-24.9 \text{ kg/m}^2$), healthy, non-smokers, who participated in little to moderate physical activity, regular breakfast consumers, and with no dietary restrictions other than vegetarianism. Recruitment was done through social media, Toronto Transit Commission subway ad and University of Toronto paper ads (Appendix 2, Appendix 3). Upon showing interest, participants received an email containing details about the study (Appendix 4). If participants continued to respond with interest to the email, an in-person screening interview was conducted where they were required to fill out questionnaires about their health status, dietary habits, possible favism and food preferences (Appendix 5). Favism was screened for but the risk of favism occurrence in this study was unlikely due to rigorous processing of the bean prior to consumption. Participants were financially compensated for the study.

4.1.1 Sample size

Recruitment requirement was $n=30$ for each experiment (Exp. 2A and 2B) and based on the power analysis for within-subject design from previous studies [1, 4, 72]. This sample size is sufficient to detect a treatment effect on FI of 150 kcal, and a difference of 10% in subjective appetite, with a power of 0.80, $\alpha < 0.05$ and accounting for 20% dropout rate. 15 participants were used to detect differences in hormone levels. Considering the variability of responses in physiological measures, our previously published experiments (Akhavan et al. 2011; Akhavan et al. 2012, Panahi et al. 2013) show that 12 participants are required for the examination of physiologic mechanisms. A CONSORT flow diagram is in Appendix 1. Eight participants withdrew from the study due to scheduling conflicts and/or limited nurse availability (Appendix 1). One participant switched from the IV to the non-IV protocol due to claimed discomfort of the IV insertion. Participants were permitted to participate in both Exp. 2A and Exp. 2B with a 4-week washout period (Figure 4.1). Data from Exp. 1 that was duplicated from repeated participants was removed from statistical analysis for Exp. 1 ($n=12$, Figure 4.1).

4.2 Study design

This study was designed in accordance to Health Canada's Draft Guidelines for satiety (Appendix 8) and postprandial glycaemia food health claims (Appendix 9) and the protocol was approved by Ethics Review Committee of the University of Toronto. Three experiments were carried out using a single-blinded, within-subject, randomized, repeated-measures controlled design (Figure 4.1). In Exp. 1, participants received 1 of 5 treatments weekly in randomized order, determined by a random sequence generator on Statistical Analysis Software version 9.4 (SAS v9.4). Effects of pasta treatments were measured in all participants over 120 min at which time they were divided and offered an *ad libitum* (Exp. 2A) or fixed quantity (12 kcal/kg) (Exp. 2B) pizza meal. Thereafter, post-second meal effects of the pastas were measured for another 60 min (140-200 min, Figure 4.1). Exp. 2B participants consumed a fixed quantity pizza meal to eliminating the effects of changes in pizza intake quantity anticipated due to treatment effects on *ad libitum* FI. Thus, this allowed a clearer evaluation of the second meal effect of FB flours. All participants arrived for the study to consume the singled serving of pasta for lunch and the pizza meal within the window of 10:00 am to 1:00 pm; thus, completing the session at a time between 1:30 pm to 4:30 pm, respectively.

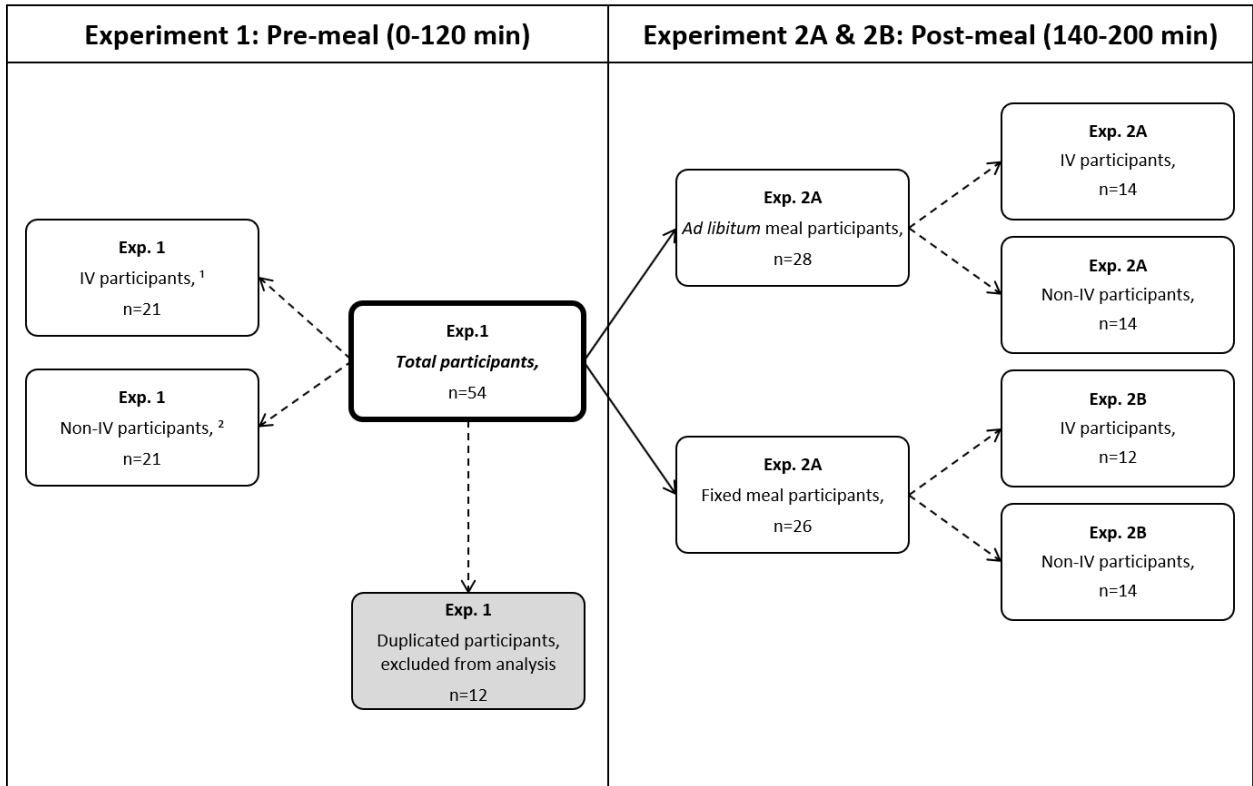


Figure 4.1: Study participation for Exp. 1, 2A and 2B in IV and non-IV groups

¹ IV, intravenous; ² Non-IV, non-intravenous

4.3 Protocol

Protocol was similar to previous reports and shown in Figure 4.2 [1-4, 76, 77, 85]. Each session was 200 min from the time of initial treatment consumption. Before attending the sessions, all participants underwent a mandatory overnight fast of 10-12 h prior to a standard breakfast consumption that was provided, consisting of 250 mL of Tropicana orange juice (Tropicana Products Inc., Bradenton, FL, US.), 237 mL of 2% milk (Sealtest, Agropur Cooperative, Saint-Hubert, QC, CA) and 60 g of Honey Nut Cheerios (General Mills' Canada Corp., Mississauga, ON, CA). Breakfast was consumed 4 h prior to the scheduled study start time. Participants were asked not to participate in rigorous physical activity nor consume alcohol 24 h prior to start of session. After the standard breakfast, participants were asked not to go back to sleep and to use a mode of transportation to the session that did not require rigorous physical activity (i.e. no running or biking). After baseline information collection (Appendix 6), participants had 10 minutes to consume all the pasta treatment and water provided. The only difference among Exp. 2A

and AB was the quantity of pizza provided during the second meal at 120 min, consisting of Dr. Oetker's Giuseppe Pizzeria: Mini Pizzas, 3 Cheese pizza (Dr. Oetker Canada, Mississauga, ON, CA.) served with 500 g water.

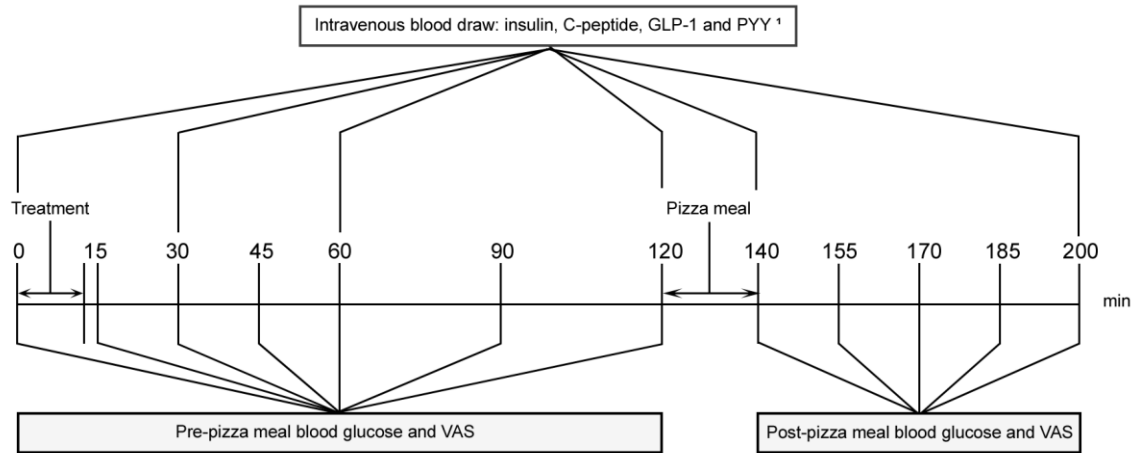


Figure 4.2: Protocol of a single session, 200 min duration

- Treatment is iso-caloric.
- Finger prick blood glucose and visual analog scale scores (VAS) collected at 0, 15, 30, 45, 60, 90, 120, 140, 155, 170, 185 and 200 min.
- Intravenous blood collected at 0, 30, 60, 120, 140 and 200 min measured for GLP-1, insulin, PYY, C-peptide.
- In Experiment 2A, participants consume *ad libitum* pizza meal at 120 min.
- In Experiment 2B, participants consume fixed quantity pizza meal at 120 min.

4.4 Treatments

Pasta samples were formulated with and produced by AGT Food and Ingredients (Regina, SK, CA). At each session, participants received 85 g of a macaroni pasta (Health Canada's recommended serving size) in randomized order: (1) control (100% DWS), and pastas containing 25% faba bean flours and fractions from (2) whole bean flour (FBF), (3) high starch fraction at 55% starch (FBS), (4) protein concentrate fraction at 60% protein (FBPC), and (5) protein isolate fraction at 85% protein (FBPI) (Table 4.1) [86].

Pastas were prepared and consumed in isocaloric amounts with 125 g Primo tomato sauce: Thick and Zesty, Original Recipe (Toronto, ON, CA). Treatment meals were served with 300 g of water that was mandatory to finish. All foods were prepared in an experimental kitchen. Pastas were weighed to 85 g dry weight and cooked in 1 L of boiling water for 8 minutes, as per supplier recommendation for control. After draining, the pasta was added to 125 g tomato sauce which was

microwaved for 30 seconds prior. All contents were stirred well for even sauce distribution. Pasta servings were approximately isocaloric at ~365 kcal (Table 4.1). Palatability questionnaires were collected in the visual analog scale format to determine overall liking of the pasta (Appendix 7). Participants were asked to consume all of the pasta and sauce.

4.5 Food intake

4.5.1 Pizza

In Exp. 2A, an *ad libitum* pizza meal was served at 120 min to measure FI. Pizzas were defrosted for 1 hour and cooked as per manufacturer's instructions (Dr. Oetker's Giuseppe Pizzeria: Mini Pizzas, 3-Cheese pizza, Mississauga, ON. CA.) at 430 °F for 8 minutes. Pizza nutrient composition is shown in Table 4.1. Pizzas were cut into quarters and rearranged in nonuniform order on serving tray. Participants were asked to eat until they were "comfortably full" over the 20 min period (120-140 min). They received 3 trays of 4 mini pizzas (total=12 mini pizzas received) at ~ 6 min 30 s intervals. Weights of the pizza trays were weighed before and after to calculate the weight of the pizza consumed. If participants finished all 3 trays of pizza, an additional tray was provided.

In Experiment 2B, the fixed quantity pizza meal was served based on a 12 kcal per kg of the participant's weight. Pizzas were prepared as described above. Participants were allowed 20 min to consume all pizza provided. Electronic devices or reading material were not allowed in the dining space. At the end of the meal period, participants were asked to fill out a palatability questionnaire in the visual analog scale format (Appendix 7).

Table 4.1: Nutritional composition of dry pasta, pasta sauce and pizza

	Weight (g)	Calories (kcal)	Protein (g)	Fat (g)	Total Carbohydrates (g)	Fibre (g)
DWS, 100% durum wheat semolina	85.0 (dry)	304**	13.09*	0.6*	63.8**	3.91*
FBF, 25% Split faba bean flour + 75% DWS	85.0 (dry)	308**	16.56*	0.5*	59.7**	5.19*
FBS, 25% high faba bean starch flour + 75% DWS	85.0 (dry)	305**	13.26*	0.4*	62.2**	4.34*
FBPC, 25% faba bean protein concentrate + 75% DWS	85.0 (dry)	307**	22.7*	0.7*	53.6**	5.70*
FBPI, 25% faba bean protein isolate + 75% DWS	85.0 (dry)	309**	25.25*	0.8*	47.8**	4.76*
Tomato sauce: Primo, Thick and Zesty	125.0	60	2.0	1.0	12.0	2.0
Dr. Oekter Giuseppe Pizzeria: Mini Pizzas, 3 Cheese	82 g	190	9.0	6.0	24.0	1.0

*Calculated from University of Saskatchewan chemical analysis report for the control and all faba bean pastas
**Calculated from AGT specification sheets for flour compositions based on 25% DWS flour substitution

4.5.2 Water

In both Exp. 2A and Exp. 2B, participants consumed an *ad libitum* quantity of water. Water was served in 500 g increments. Refill for water was provided if participants requested, were running low, and/or responded “yes” for water refill 10 min into the pizza session. Water cups were weighed before and after, and recorded to determine water intake.

4.6 Outcome measurements

Baseline information was collected at the beginning of each session. A questionnaire was provided to determine behavior of the last 24 hours for sleep, stress, physical activity, alcohol, and water consumption, as shown in Appendix 6. If reported significant deviations from usual patterns or against protocol compliance, then session was rescheduled. Measures were taken for BG concentration, visual analog scale (VAS) scores and hormone concentration (only for intravenous participants).

4.6.1 Visual analog scale calculations

Measures for VAS scores for motivation-to-eat, energy levels and physical comfort were taken at 0, 15, 30, 45, 60, 90, 120, 140, 155, 170, 185 and 200 min. Samples of the VAS questionnaires are shown in Appendix 7.

Average VAS scores were calculated for each of subjective appetite, energy level, physical comfort and palatability by taking the sum of each score divided by the number of questions

present: (i) subjective appetite = [desire to eat + hunger + (100 – fullness) + prospective consumption]/4, (ii) energy level = [energy + (100 – tiredness)]/2, (iii) Physical comfort = [(100 – nausea) + (100 – stomach pain) + wellness + (100 – gas) + (100 – diarrhea)]/5 and (iv) palatability = (pleasantness + taste + texture)/3.

4.6.2 Blood glucose

Measures for BG were taken at 0, 15, 30, 45, 60, 90, 120, 140, 155, 170, 185 and 200 min. Finger prick BG concentration was measured with a hand-held monitor Accu-Chek Compact and Compact-Plus (Roche Diagnostics Canada, Laval, QE, Canada), as previously described [1, 3, 62, 72]. The first drop of blood was wiped due to possible contamination with alcohol and interstitial fluid. The second drop was placed on the glucose strip for measurement. A baseline BG concentration >5.5 mmol/L suggested that the participant had not complied with the protocol, and the session was rescheduled.

4.6.3 Intravenous blood samples

A total of 26 participants out of 54 participants completed the IV component of the experiments where 14 participants completed Exp. 2A and 12 participants completed Exp. 2B (Figure 4.1). IV blood samples were collected to measure ghrelin, CCK, insulin, C-peptide, GLP-1 and PYY.

A registered nurse collected IV blood samples from IV participants by insertion of an indwelling catheter in the antecubital vein after baseline questionnaires. Approximately 8.5 mL of blood was collected at 0, 30, 60, 120, 140 and 200 min (Figure 4.2). Samples were collected in BD Vacutainer® spray-coated with dipotassium ethylenediaminetetraacetic acid (K₂EDTA) 10 mL tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, US). EDTA salt is an anticoagulant for hematological procedures and specimen collection [87]. Thereafter, blood samples collected were inverted 5-10 times and immediately processed. Processing included adding 1250 µL aliquot of the blood sample to a tube of prepared enzyme inhibitors containing 12 µL dipeptidyl peptidase IV (DPP-IV) and 12 µL 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride. Samples were centrifuged at 1500 rpm for 15 min. The serum was extracted and placed into a separate tube, immediately frozen, and then stored at -85 °C. All samples were sent to University of Manitoba, Department of Food and Human Nutritional Sciences to be analyzed for hormone concentration.

Upon analysis, samples were defrosted and analyzed using MESO™ SECTOR S 600 and MESO QuickPlex® SQ 120 multiplex ELISA (Meso Scale Discovery, Rockville, MD, US).

4.7 Data analysis

Statistical analyses were conducted using Statistical Analysis Software (SAS) version 9.4. All data was tested for normality of residuals. Data for mean BG response, subjective appetite, energy and fatigue, physical comfort and hormone concentrations were analyzed via the MIXED procedure using two-way repeated measures analysis of covariance (ANCOVA) for the post-treatment period (15-120 min) using baseline (t=0 min) as a covariate, followed by Tukey-Kramer's Post-Hoc test to determine and compare treatment (DWS, FBF, FBS, FBPC and FBPI), time, treatment-by-time effects before the pizza meal for Exp. 1. Mean change from baseline was determined for all responses and analyzed via the MIXED procedure using one-way repeated measures ANOVA and Tukey-Kramers Post-Hoc test to compare treatment effects, if any.

Two-way ANOVA was carried out in a similar manner for measures taken after the pizza meal for the post-meal period (140 to 200 min) separating *ad libitum* meal participants from Exp. 2A and fixed meal participants from Exp. 2B.

Incremental area under the curve (iAUC) was calculated for BG, insulin, PYY, C-peptide and GLP-1 for post-treatment (0 to 120 min) and post-meal (120 to 200 min). Incremental AUC was calculated using the trapezoidal rule of the areas enclosed between baseline and the response curve back to baseline where only positive areas of change above baseline were summed [24]. Total area under the curve (tAUC) post-treatment and post-meal were determined for subjective appetite. Total AUC does not take baseline into consideration and estimates the total value of the parameter over the course of the period observed. For tAUC, all area under the curve calculated using the trapezoid rule are summed [24].

Data for FI, water intake, treatment palatability and each of the AUC's were analyzed via the MIXED procedure using one-way ANOVA and Tukey-Kramer's Post-Hoc test to determine and compare treatment effects.

Correlation analyses among treatments and outcome measures were performed via the CORR procedure using Pearson's Correlation Coefficient (normal data) or Spearman's Rank

Correlation Coefficient (not normal data). All results are presented as mean \pm standard error of the mean (SEM). Statistical significance was concluded with the p-value (P) <0.05 .

Chapter 5

5 Results

5.1 Subject characteristics

A total of fifty-four healthy young adult male participants completed the study. Data was pooled for the post-treatment period of the protocol for only 42 participants (age=23.38±0.38y; BMI=22.21±0.26kg/m²) (Appendix 10, Appendix 11). Considering that twelve participants completed both Exp. 2A and Exp. 2B their post-treatment data was duplicated. Therefore, to eliminate duplicated data for the post-treatment period of the session, only their first set of data was used for the analyses of Exp.1 (n=42). Out of these, 21 participants (age=22.81±0.39y; BMI=21.79±0.37kg/m²) provided intravenous IV blood samples for post-treatment gut peptide and hormone measures in Exp. 1.

5.1.1 Experiment 2A

For Exp. 2A, 28 participants (age=23.50±0.51y; BMI=22.65±0.28kg/m²) completed the experiment consisting of an *ad libitum* pizza meal served at 120 min, who were analyzed together for post-meal (140-200 min) measures (Appendix 12). Out of these, 14 participants (age=23.07±0.55y; BMI=22.13±0.41kg/m²) provided intravenous post-meal blood samples for gut peptide and hormone measures in Exp. 2A (Appendix 13).

5.1.2 Experiment 2B

For Exp. 2B, 26 participants (age=23.23±0.47y; BMI=22.07±0.35kg/m²) completed it consisting of a fixed quantity pizza meal served at 120 min, who were analyzed together for post-meal measures (Appendix 14). Out of these, 12 participants (age=22.58±0.43y; BMI=22.13±0.54kg/m²) provided intravenous blood samples for post-meal gut peptide and hormone data in Exp. 2B (Appendix 15).

5.2 Food and water intake

5.2.1 Experiment 2A: food and water intake

Mean pizza intake during the *ad libitum* pizza meal was 1219.94 ± 12.0 kcal. Mean water intake was 442.66 ± 4.73 kcal. Pizza meal FI (kcal) and water (g) intake during the *ad libitum* pizza meal were not affected by treatment ($P=0.4744$; $P=0.7614$; Table 5.1).

5.2.2 Experiment 2B: food and water intake

Mean pizza caloric consumption between all participants during the fixed pizza meal was 804.7 ± 14.2 kcal. Mean water intake was 441.78 ± 6.42 kcal. Water intake during the fixed quantity pizza meal was not affected by treatment ($P=0.7074$, Table 5.1).

Table 5.1: Food (kcal) and water (g) intake

Treatment	Exp. 2A ¹				Exp. 2B	
	Food intake ² (kcal)		Water intake (g)		Water intake (g)	
	Mean	±SEM	Mean	±SEM	Mean	±SEM
DWS ⁴	1200.98	69.087	439.96	43.130	437.96	36.417
FBF	1228.29	72.876	449.54	37.437	441.07	31.452
FBS	1234.54	84.443	440.33	39.887	436.48	33.735
FBPC	1251.33	79.187	455.64	40.510	427.54	29.697
FBPI	1184.56	70.602	427.84	39.671	465.86	35.072
Treatment P ³	0.4744		0.7614		0.7074	

¹ All values are mean \pm SEM (Exp. 2A, n=28; Exp. 2B, n=26)

² Participants had 20 minutes to consume *ad libitum* food and water between 120 to 140 min

³ Food and water intakes were not significantly different after each type of pasta. (One-way ANOVA test, $P<0.05$)

⁴ DWS, 100% durum wheat semolina pasta;

FBF, 25% faba bean flour pasta;

FBS, 25% faba bean high starch pasta;

FBPC, 25% faba bean protein concentrate pasta;

FBPI, 25% faba bean protein isolate pasta

5.3 Subjective appetite

Experiment 1: Post-treatment

In Exp. 1, subjective appetite for post-treatment period was significantly affected by treatment ($P=0.0425$) and time ($P<0.0001$), but not treatment-by-time interactions ($P=0.9011$, Table 5.2). However, after Tukey-Kramer's post hoc analysis, there was no significant difference between treatments, possibly due to Type I error which displayed a false positive [36]. Subjective appetite scores for all treatments were highest at baseline (0 min) and lowest at 15 min, then gradually rose until 120 min (Figure 5.1A).

Subjective appetite tAUC (score out of $100 \cdot \text{min}$) post-treatment was not significantly different between treatments ($P=0.5667$, Table 5.2).

Experiment 2: Post-meal

Experiment 2A

In Exp. 2A, appetite during the post-meal period was significantly affected by treatment ($P=0.0380$) and time ($P<0.0001$), but not treatment-by-time interaction ($P=0.6703$, Table 5.2). However, Tukey-Kramer's post-hoc test showed no significant treatment differences. Subjective appetite scores for all treatments were reduced after the pizza meal at 140 min, then gradually increases up to 200 min (Figure 5.1A).

Subjective appetite tAUC during the post-meal period was not affected by treatment, ($P=0.4805$), Table 5.

Experiment 2B

In Exp. 2B, subjective appetite during the post-meal period after the fixed quantity pizza meal was significantly affected by treatment ($P=0.0005$) and time ($P<0.0001$), but not treatment-by-time interaction ($P=0.6703$, Table 5.2). Appetite was lower for FBPC than DWS and FBS but similar to the rest ($P=0.0005$, Table 5.2). Appetite was lower for FBPI than DWS but similar to the rest (Figure 5.1C). Appetite scores for all treatments drop lowest after the pizza meal at 140 min, then gradually increases up to 200 min (Figure 5.1C).

There were no significant differences in subjective appetite tAUC during the post-meal period (P=0.7728, Table 5.2).

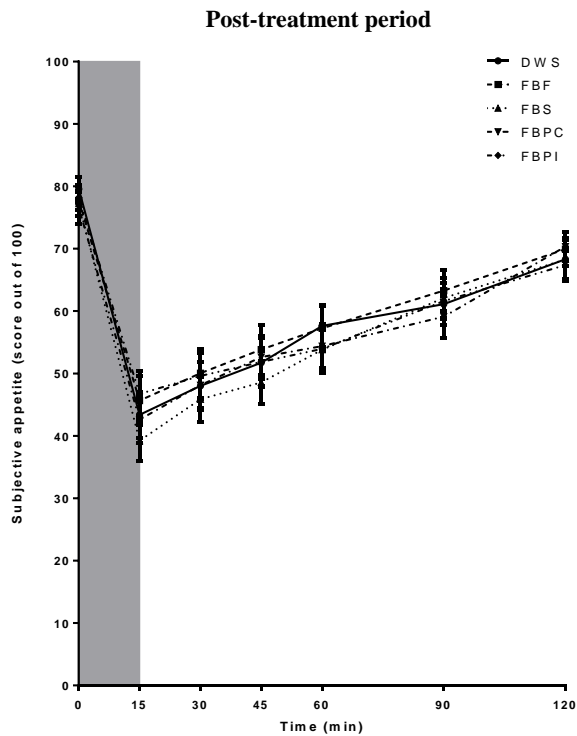


Figure 5.1A: Overall subjective appetite (score out of 100) 15 to 120 min were similar after each type of pasta. Values are means \pm SEM; n=42. (Two-way ANOVA, Pasta P=0.0425, Time P<0.0001, Pasta*time P=0.9011). Gray area not included in post-treatment analysis.

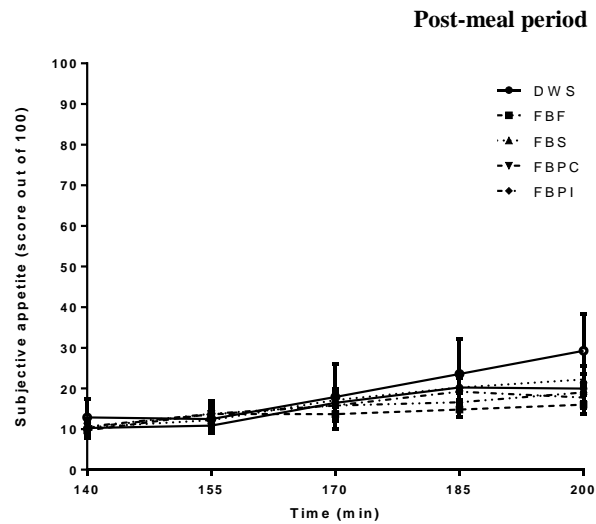


Figure 5.1B: Overall subjective appetite (score out of 100) from 140 to 200 min after an *ad libitum* pizza meal were similar for each pasta treatment. Values are means \pm SEM; n=28. (Two-way ANOVA, Pasta P=0.0380, Time P<0.0001, Pasta*time P=0.6703)

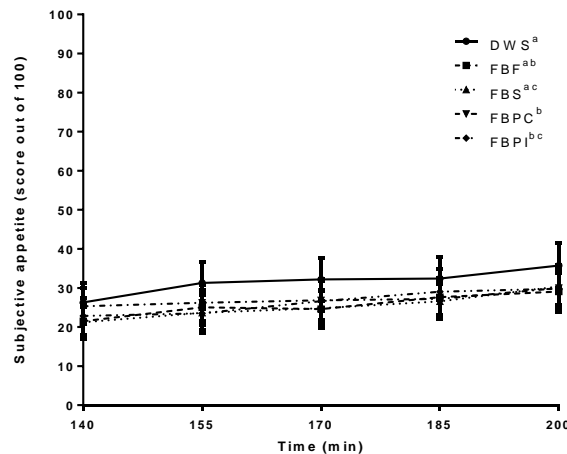


Figure 5.1C: Overall subjective appetite (score out of 100) from 140 to 200 min after a fixed quantity pizza meal were lower for FBPC compared to DWS and FBS, but similar to the rest. Overall subjective appetite scores from 140 to 200 min was lower for FBPI compared to DWS, but similar to the rest. Values are means \pm SEM; n=26. Graphs with different superscripts are significantly different. (Two-way ANOVA, Pasta P=0.0005, Time P<0.0001, Pasta*time P=0.9992)

Figure 5.1: Subjective appetite scores for the Exp. 1 (0-120 min), Exp. 2A and 2B (140-200 min) ^{1,2}

¹ 0=No appetite at all; 100=very high appetite; ² DWS, 100% durum wheat semolina pasta; FBF, 25% faba bean flour pasta; FBS, 25% faba bean high starch pasta; FBPC, 25% faba bean protein concentrate pasta; FBPI, 25% faba bean protein isolate pasta

Table 5.2. Mean subjective appetite and appetite total area under the curve (tAUC) for the post-treatment and post-meal periods¹

		Post-treatment ²		Post-meal ³			
		Pooled (Exp. 1)		<i>Ad libitum</i> (Exp. 2A)		Fixed meal (Exp. 2B)	
	Treatment	Mean	±SEM	Mean	±SEM	Mean	±SEM
App ^{4,5} (Score out of 100)	DWS ⁶	55.05	1.548	15.41	1.235	30.13 ^a	2.319
	FBF	56.68	1.549	13.81	0.980	26.85 ^{ab}	2.207
	FBS	53.00	1.521	16.90	1.221	28.70 ^{ac}	2.370
	FBPC	54.55	1.472	14.57	1.068	24.77 ^b	1.978
	FBPI	55.20	1.454	16.88	1.342	25.98 ^{bc}	2.138
	Treatment P	0.0425		0.0380		0.0005	
	Time P	<0.0001		<0.0001		<0.0001	
	Treatment*time P	0.9011		0.6703		0.9992	
App tAUC (Score out of 100*min)	DWS	6897.59	372.78	2012.44	210.34	3172.86	471.57
	FBF	7060.36	389.42	1836.27	171.22	2893.20	471.58
	FBS	6681.79	357.18	2078.17	195.13	3084.16	477.61
	FBPC	6775.49	360.43	1927.66	196.96	2755.07	388.21
	FBPI	6881.48	350.39	2111.63	228.46	2874.38	413.28
	P-value	0.5667		0.4805		0.7728	

¹ All values are mean ± SEM (Exp. 1, n=42; Exp. 2A, n=28; Exp. 2B, n=26)

² Post-treatment values are means of all observations after the treatment and before the pizza meal: 15, 30, 45, 60, 90 and 120 min

³ Post-meal values are means of all observations after the pizza meal: 120, 140, 155, 170, 185 and 200 min

⁴ Means in the same column with different superscript letters are significantly different. (Two-way ANOVA, Tukey-Kramer's post-hoc test, P<0.05)

⁵ 0=No appetite at all; 100=Very high appetite

⁶ DWS, 100% durum wheat semolina pasta;

FBF, 25% faba bean flour pasta;

FBS, 25% faba bean high starch pasta;

FBPC, 25% faba bean protein concentrate pasta;

FBPI, 25% faba bean protein isolate pasta

5.4 Blood glucose

Experiment 1: Post-treatment

Post-treatment BG was significantly affected by treatment ($P < 0.0001$) and time ($P < 0.0001$), but not treatment-by-time interaction ($P = 0.3996$, Table 5.3). Post-treatment BG concentrations were lower for FBPI compared to DWS, FBF and FBS, but was similar to FBPC. As well, BG concentrations were lower for FBPC compared to DWS and FBS, but similar to the rest (Table 5.3). BG concentrations for all treatments were lowest at baseline (0 min) and peaked at 30 min, then dropped until 120 min (Figure 5.2A).

Post-treatment BG incremental area under the curve (BG iAUC) ($\text{mmol} \cdot \text{min/L}$) was not significantly affected by treatment ($P = 0.0517$, Table 5.3).

Experiment 2: Post-meal

Experiment 2A

In Exp. 2A, BG during the post-meal period after the *ad libitum* pizza meal was significantly affected by treatment ($P = 0.0003$) and time ($P < 0.0001$), but not treatment-by-time interaction ($P = 0.8474$, Table 5.3). BG concentrations were lower for FBPC compared to DWS and FBS, but similar to FBF and FBPI. As well, post-meal BG concentrations were lower after FBPI compared to DWS, but similar to the rest (Table 5.3). From 140 min, BG concentrations for all treatments reach a slight peak at 155 min and gradually reduced until 200 min (Figure 5.2B).

There were no significant differences between treatments on post-meal BG iAUC ($P = 0.5434$, Table 5.3).

Experiment 2B

BG during the post-meal period after the fixed quantity pizza meal was significantly affected by time ($P < 0.0001$), but not by treatment ($P = 0.1134$) nor treatment-by-time interaction ($P = 0.6668$). After the pizza meal, BG peaked at 155 min for all treatments.

There were no significant differences between treatments on post-meal BG iAUC ($P = 0.2802$, Table 5.3).

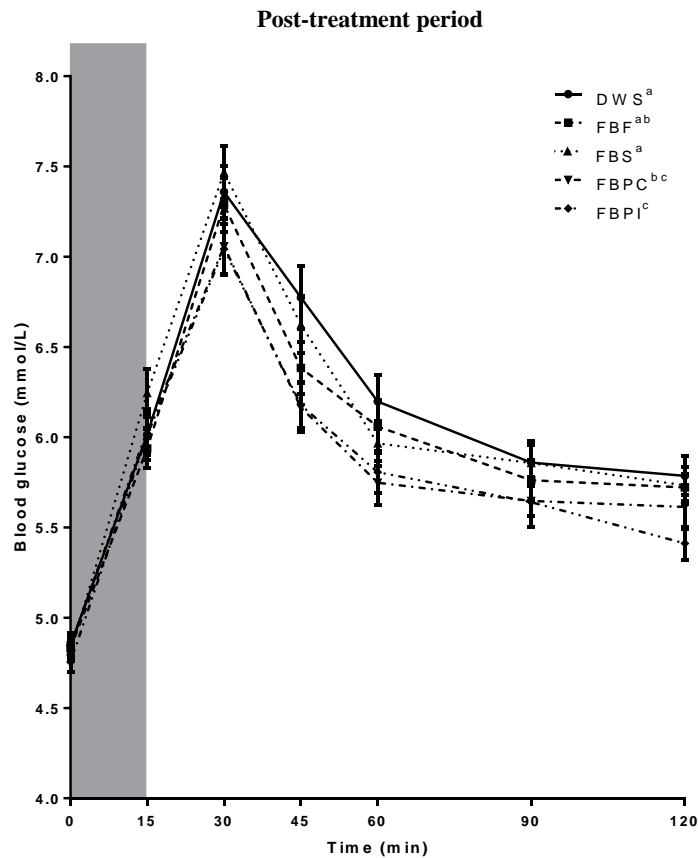


Figure 5.2A: BG concentration (mmol/L) 15 to 120 min was lower for FBPI compared to DWS, FBF and FBS, but was similar to FBPC. BG concentration up to 120 min was lower for FBPC compared to DWS and FBS. Values are means \pm SEM; n=42. Treatments with different superscripts are significantly different. (Two-way ANOVA, Pasta $P < 0.0001$, Time $P < 0.0001$, Pasta*time $P = 0.5689$). Gray area was not included in post-treatment analysis.

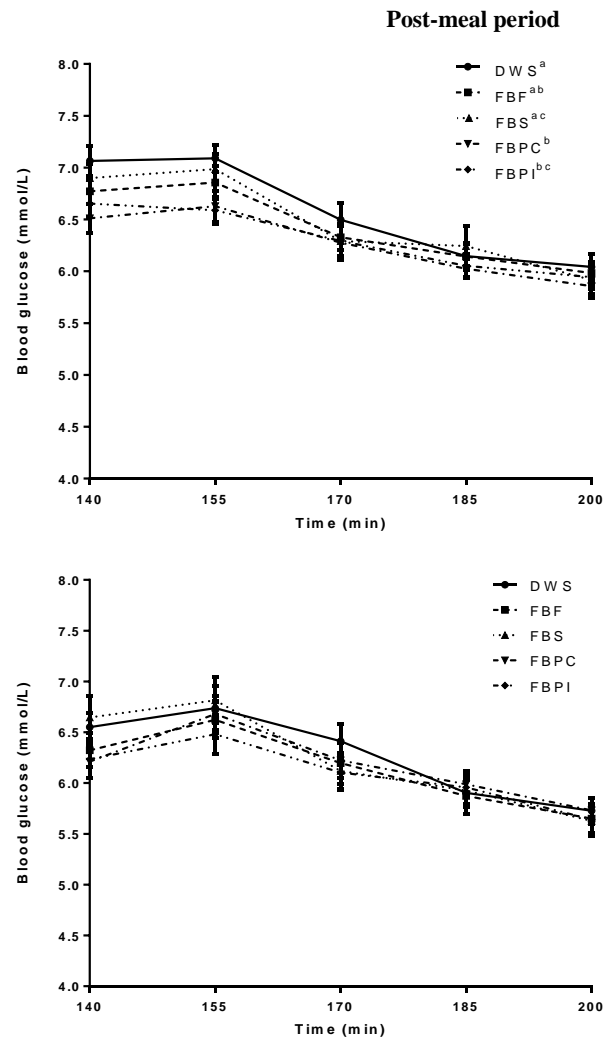


Figure 5.2B: Mean BG concentration (mmol/L) from 140 to 200 min after an *ad libitum* pizza meal were lower for FBPC compared to DWS and FBS, but similar to the rest. Me BG from 140 to 200 min was lower for FBPI compared to DWS, but similar to the rest. Values are means \pm SEM; n=28. Graphs with different superscripts are significantly different. (Two-way ANOVA, Pasta $P = 0.0003$, Time $P < 0.0001$, Pasta*time $P = 0.8474$)

Figure 5.2C: Mean BG concentrations (mmol/L) from 140 to 200 min after a fixed quantity pizza meal were similar after each type of pasta. Values are means \pm SEM; n=28. (Two-way ANOVA, Pasta $P = 0.1134$, Time $P < 0.0001$, Pasta*time $P = 0.6668$)

Figure 5.2: Serum blood glucose concentration for the Exp. 1 (0-120 min), Exp. 2A and 2B (140-200 min) ¹

¹ DWS, 100% durum wheat semolina pasta; FBF, 25% faba bean flour pasta; FBS, 25% faba bean high starch pasta; FBPC, 25% faba bean protein concentrate pasta; FBPI, 25% faba bean protein isolate pasta

Table 5.3: Mean blood glucose (BG) and BG incremental area under the curve (iAUC) for the post-treatment and post-meal periods¹

		Post-treatment ²		Post-meal ³			
		Pooled (Exp. 1)		<i>Ad libitum</i> (Exp. 2A)		Fixed meal (Exp. 2B)	
	Treatment	Mean	±SEM	Mean	±SEM	Mean	±SEM
BG ⁴ (mmol/L)	DWS ⁵	6.33 ^a	0.064	6.57 ^a	0.071	6.27	0.079
	FBF	6.19 ^{ab}	0.061	6.42 ^{ab}	0.064	6.13	0.083
	FBS	6.32 ^a	0.066	6.47 ^{ac}	0.075	6.23	0.092
	FBPC	6.04 ^{bc}	0.059	6.26 ^b	0.065	6.17	0.073
	FBPI	6.02 ^c	0.061	6.32 ^{bc}	0.061	6.08	0.080
	Treatment P	<0.0001		0.0003		0.1134	
	Time P	<0.0001		<0.0001		<0.0001	
	Treatment*time P	0.5689		0.8474		0.6668	
BG iAUC (mmol*min/L)	DWS	159.94	13.243	59.44	6.507	56.16	8.058
	FBF	147.01	11.023	60.36	7.518	49.50	9.376
	FBS	159.53	10.042	66.67	9.334	51.34	7.673
	FBPC	128.66	10.233	67.23	8.452	39.87	6.827
	FBPI	135.94	8.630	69.57	5.598	60.08	10.000
	P-value	0.0517		0.5434		0.2802	

¹ All values are mean ± SEM (Exp. 1, n=42; Exp. 2A, n=28; Exp. 2B, n=26)

² Post-treatment values are means of all observations after the treatment and before the pizza meal: 15, 30, 45, 60, 90 and 120 min

³ Post-meal values are means of all observations after the pizza meal: 120, 140, 155, 170, 185 and 200 min

⁴ Means in the same column with different superscript letters are significantly different. (Two-way ANOVA, Tukey-Kramer's post-hoc test, P<0.05)

⁵ DWS, 100% durum wheat semolina pasta;

FBF, 25% faba bean flour pasta;

FBS, 25% faba bean high starch pasta;

FBPC, 25% faba bean protein concentrate pasta;

FBPI, 25% faba bean protein isolate pasta

5.5 Gut peptides and hormones

Although ghrelin and CCK were planned to be measured, their concentrations were below detection limits using our analysis methods.

5.5.1 Insulin

Experiment 1: Post-treatment

In Exp. 1, post-treatment insulin concentration was significantly affected by time ($P < 0.0001$), but not by treatment ($P = 0.2306$) nor treatment-by-time interaction ($P = 0.7805$, Table 5.4). Insulin concentrations for all treatments were lowest at baseline (0 min) and peaked at 30 min (Figure 5.3A).

Post-treatment insulin iAUC ($\text{mIU} \cdot \text{min/L}$) was not significantly different after each pasta ($P = 0.1630$, Table 5.4).

Experiment 2: Post-meal

Experiment 2A

In Exp. 2A, mean insulin during the post-meal period after the *ad libitum* pizza meal was significantly affected by time ($P < 0.0001$), but not by treatment ($P = 0.1673$) nor treatment-by-time interaction ($P = 0.7867$, Table 5.4). Insulin concentrations for all treatments were highest at 140 min and dropped at 200 min (Figure 5.3B).

Post-meal insulin iAUC after the *ad libitum* pizza meal was not significantly affected by treatment ($P = 0.1018$, Table 5.4).

Experiment 2B

In Exp. 2B, mean insulin during the post-meal period after the fixed quantity pizza meal was significantly affected by treatment ($P = 0.0354$) and time ($P = 0.0006$), but not treatment-by-time ($P = 0.8500$, Table 5.4). Serum insulin concentration was lower for FBPC compared to FBF, but similar to the rest (Table 5.4). Insulin concentrations for all treatments were highest at 140 min and dropped at 200 min (Figure 5.3C).

Post-meal insulin iAUC after the fixed quantity pizza meal was significantly affected by treatment ($P=0.0031$). Insulin concentration was higher for FBF compared to others (Table 5.4).

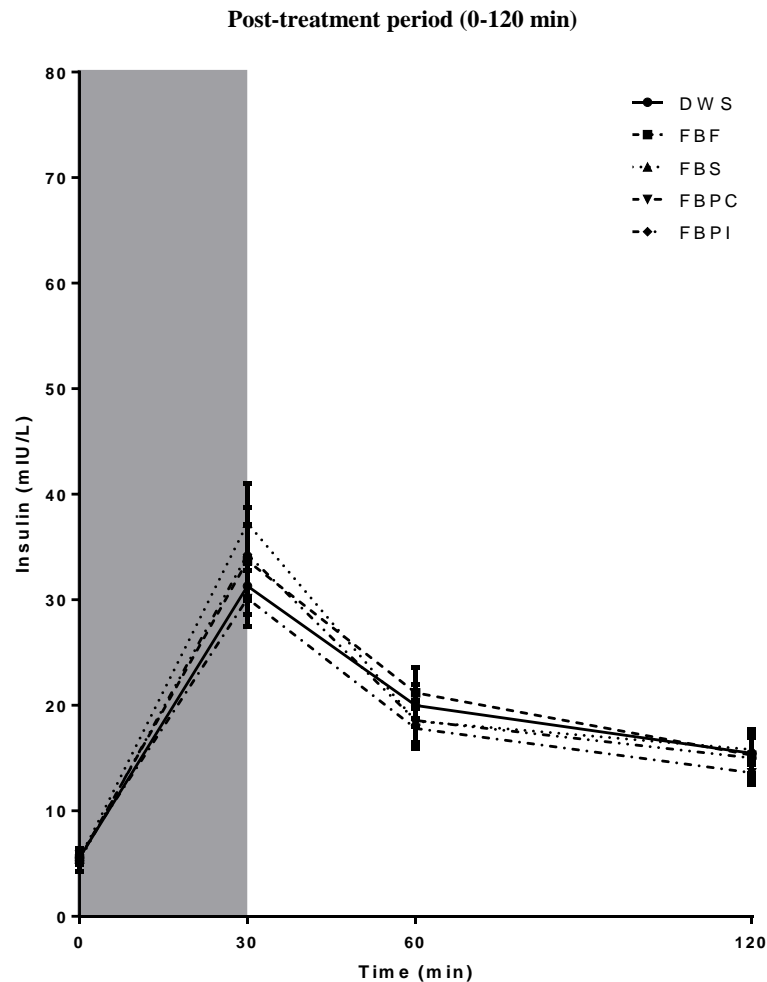


Figure 5.3A: Serum insulin concentrations (mIU/L) 30 to 120 min were similar after each type of pasta. Values are means \pm SEM; n=21. (Two-way ANOVA, Pasta P= 0.2306, Time P<0.0001, Pasta*time P= 0.7805). Gray area was not included in post-treatment analysis.

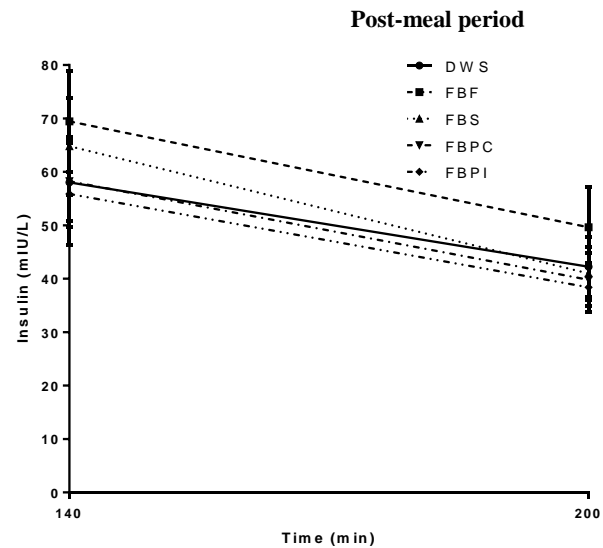


Figure 5.3B: Serum insulin concentrations (mIU/L) were similar from 140 to 200 min after an *ad libitum* pizza meal were similar for each pasta treatment. Values are means \pm SEM; n=14. (Two-way ANOVA, Pasta P= 0.1673, Time P<0.0001, Pasta*time P= 0.7867)

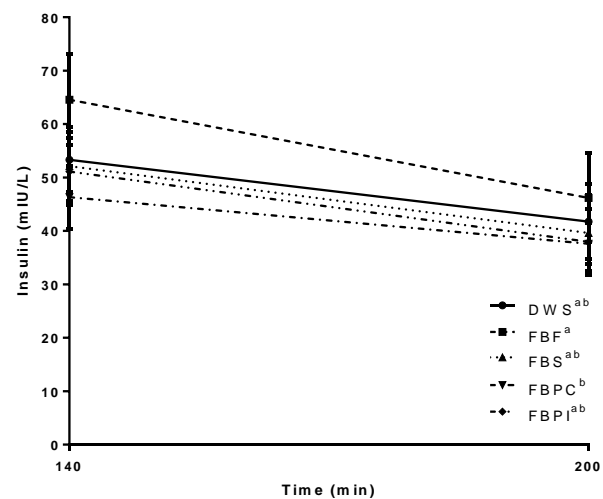


Figure 5.3C: Serum insulin concentrations (mIU/L) from 140 to 200 min after a fixed quantity pizza meal were lower for FBPC compared to FBF, but similar to the rest. Values are means \pm SEM; n=12. Treatments with different superscripts are significantly different. (Two-way ANOVA, Pasta P= 0.0354, Time P=0.0006, Pasta*time P= 0.8500)

Figure 5.3: Serum insulin concentration for the Exp. 1 (0-120 min), Exp. 2A and 2B (140-200 min) ¹

¹ DWS, 100% durum wheat semolina pasta; FBF, 25% faba bean flour pasta; FBS, 25% faba bean high starch pasta; FBPC, 25% faba bean protein concentrate pasta; FBPI, 25% faba bean protein isolate pasta

Table 5.4. Mean insulin and insulin incremental area under the curve (iAUC) for the post-treatment and post-meal periods¹

		Post-treatment ²		Post-meal ³			
		Pooled (Exp. 1)		<i>Ad libitum</i> (Exp. 2A)		Fixed meal (Exp. 2B)	
	Treatment	Mean	±SEM	Mean	±SEM	Mean	±SEM
Insulin ⁴ (mIU/L)	DWS ⁵	22.56	1.531	50.45	5.277	47.52 ^{ab}	4.701
	FBF	23.38	1.868	59.90	6.307	55.37 ^a	6.130
	FBS	23.37	1.850	52.91	5.576	45.87 ^{ab}	4.845
	FBPC	20.63	1.424	49.03	4.751	41.96 ^b	3.556
	FBPI	22.70	2.087	47.16	5.472	44.55 ^{ab}	4.499
	Treatment P	0.2306		0.1673		0.0354	
	Time P	<.0001		<0.0001		0.0006	
	Treatment*time P	0.7805		0.7867		0.8500	
Insulin iAUC (mIU*min/L)	DWS	1757.41	190.801	2473.51	340.536	2202.24 ^a	310.298
	FBF	1813.67	230.180	3158.39	434.332	2892.22 ^b	407.308
	FBS	1836.79	176.062	2837.40	421.361	2056.52 ^a	288.020
	FBPC	1513.04	122.493	2678.58	311.592	1767.44 ^a	269.164
	FBPI	1718.02	216.952	2403.16	368.143	1835.18 ^a	358.679
	P-value	0.1630		0.1018		0.0031	

¹ All values are mean ± SEM (Exp. 1, n=21; Exp. 2A, n=14; Exp. 2B, n=12)

² Post-treatment values are means of all observations after the treatment and before the pizza meal: 30, 60, and 120 min

³ Post-meal values are means of all observations after the pizza meal: 120, 140, 155, 170, 185 and 200 min

⁴ Means in the same column with different superscript letters are significantly different. (Two-way ANOVA, Tukey-Kramer's post-hoc test, P<0.05)

⁵ DWS, 100% durum wheat semolina pasta;

FBF, 25% faba bean flour pasta;

FBS, 25% faba bean high starch pasta;

FBPC, 25% faba bean protein concentrate pasta;

FBPI, 25% faba bean protein isolate pasta

5.5.2 C-peptide

Experiment 1: Post-treatment

Post-treatment serum C-peptide concentration was significantly affected by treatment ($P < 0.0001$) and time ($P < 0.0001$), but not treatment-by-time interaction ($P = 0.8649$, Table 5.5). C-peptide concentrations were lower for FBPI compared to DWS, FBF and FBS but not different from FBPC (Table 5.5). Moreover, C-peptide was lower for FBPI than FBS, but similar to the rest (Table 5.5). C-peptide concentrations for all treatments were lowest at baseline (0 min) and peaked at 30 min, except for DWS which peaked at 60 min (Figure 5.4A).

In Exp. 1, post-treatment C-peptide iAUC ($\text{pg} \cdot \text{min} / \text{mL}$) was significantly affected by treatment ($P < 0.0001$) (Table 5.5). Serum C-peptide concentration was lower for FBPC than DWS, FBF and FBS, but similar to FBPI.

Experiment 2: Post-meal

Experiment 2A

In Exp. 2A, post-meal C-peptide after the *ad libitum* pizza meal was significantly affected by treatment ($P = 0.0086$), time ($P = 0.0041$) but not treatment-by-time interaction ($P = 0.6639$, Table 5.5). C-peptide concentrations were significantly lower for FBPC compared to FBF (Table 5.5). Overall post-meal C-peptide concentrations for all treatments were highest at 140 min and dropped at 200 min (Figure 5.4B).

Post-meal C-peptide iAUC after the *ad libitum* pizza meal was not significantly affected by treatment ($P = 0.2583$, Table 5.5).

Experiment 2B

In Exp. 2B, post-meal C-peptide after the fixed quantity pizza meal were not significantly affected by treatment ($P = 0.1130$), time ($P = 0.5872$), or treatment-by-time interaction ($P = 0.6339$, Table 5.5).

Post-meal C-peptide iAUC after the fixed quantity pizza meal was significantly affected by treatment ($P=0.0180$). However, treatments were not significantly different after Tukey-Kramer's post hoc analysis (Table 5.5).

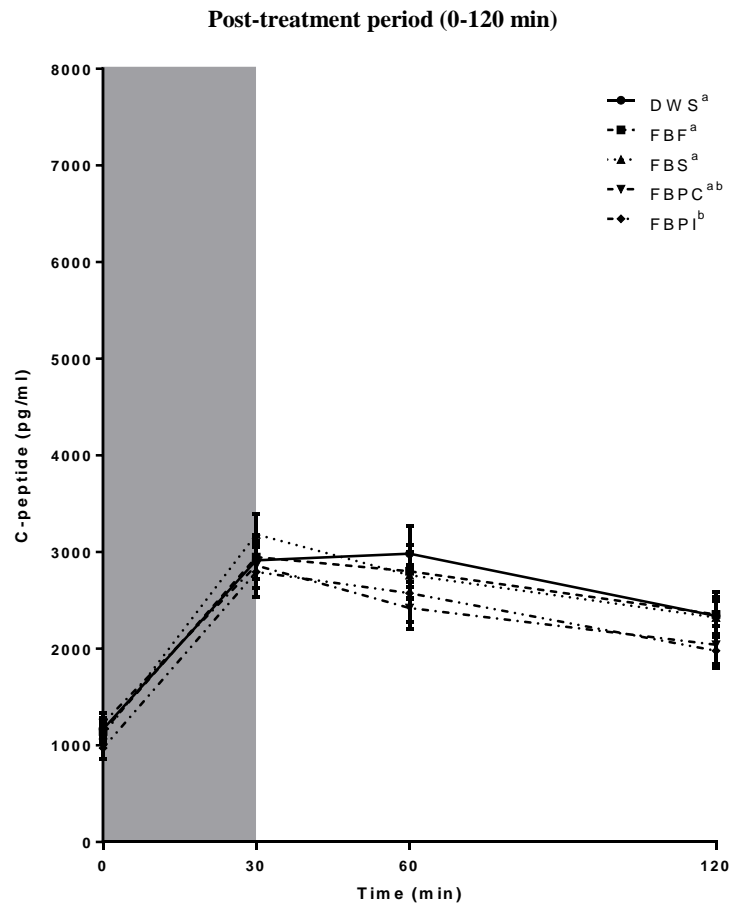


Figure 5.4A: Serum C-peptide concentration (pg/ml) 15 to 120 min was lower for FBPI compared to DWS, FBF and FBS, but was similar to FBPC. Values are means \pm SEM; n=21. Graphs with different superscripts are significantly different. (Two-way ANOVA, Pasta P<0.0001, Time P<0.0001, Pasta*time P= 0.8649). Gray area was treatment consumption period not included in post-treatment analysis.

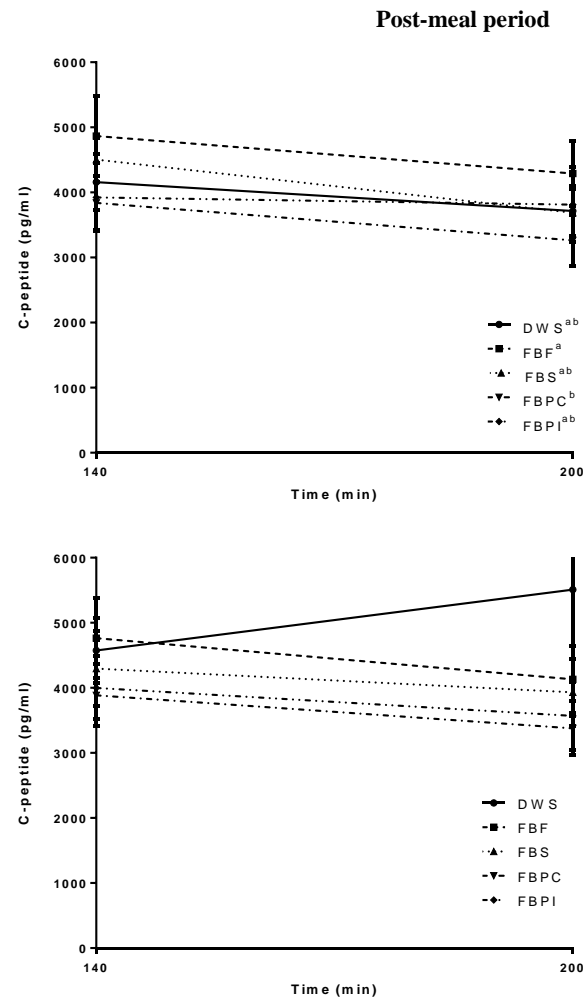


Figure 5.4B: Serum C-peptide concentration (pg/ml) from 140 to 200 min after an *ad libitum* pizza meal were lower for FBPC compared to FBF, but similar to the rest. Values are means \pm SEM; n=14. Treatments with different superscripts are significantly different. (Two-way ANOVA, Pasta P= 0.2183, Time P= 0.0776, Pasta*time P= 0.8436)

Figure 5.4C: Serum C-peptide concentration (pg/ml) from 140 to 200 min after a fixed quantity pizza meal were similar for each pasta treatment. Values are means \pm SEM; n=12. (Two-way ANOVA, Pasta P= 0.1130, Time P= 0.5872, Pasta*time P=0.6339)

Figure 5.4: Serum C-peptide concentration for the Exp. 1 (0-120 min), Exp. 2A and 2B (140-200 min) ¹

¹ DWS, 100% durum wheat semolina pasta; FBF, 25% faba bean flour pasta; FBS, 25% faba bean high starch pasta; FBPC, 25% faba bean protein concentrate pasta; FBPI, 25% faba bean protein isolate pasta

Table 5.5: Mean C-peptide and C-peptide incremental area under the curve (iAUC) for the post-treatment and post-meal periods¹

		Post-treatment ²		Post-meal ³			
Treatment		Pooled (Exp. 1)		<i>Ad libitum</i> (Exp. 2A)		Fixed meal (Exp. 2B)	
		Mean	±SEM	Mean	±SEM	Mean	±SEM
C-pep ⁴ (pg/ml)	DWS ⁵	2745.32 ^{ab}	136.113	3944.47 ^{ab}	285.517	5042.05	981.683
	FBF	2706.40 ^{ab}	143.759	4589.50 ^a	398.573	4446.54	397.830
	FBS	2755.82 ^a	127.741	4080.02 ^{ab}	260.500	4113.52	378.959
	FBPC	2441.47 ^c	129.607	3551.17 ^b	286.590	3631.40	312.287
	FBPI	2456.64 ^{bc}	149.146	3867.49 ^{ab}	382.724	3783.81	351.764
	Treatment P	<0.0001		0.0086		0.1130	
	Time P	<0.0001		0.0041		0.5872	
	Treatment*time P	0.8649		0.6639		0.6339	
C-pep iAUC (pg*min/ml)	DWS	167569.58 ^a	19357.41	128419.15	18712.67	166666.43	56308.42
	FBF	157007.43 ^a	21964.55	167297.22	28444.26	152760.79	28847.81
	FBS	169271.06 ^a	17257.06	147205.21	17711.24	114127.72	20397.06
	FBPC	124573.49 ^b	14192.99	123861.87	18498.19	88962.41	13703.52
	FBPI	146235.38 ^{ab}	19544.45	122070.46	18009.18	108838.88	23811.58
	P-value	<0.0001		0.2583		0.0180	

¹ All values are mean ± SEM (Exp. 1, n=21; Exp. 2A, n=14; Exp. 2B, n=12)

² Post-treatment values are means of all observations after the treatment and before the pizza meal: 30, 60, and 120 min

³ Post-meal values are means of all observations after the pizza meal: 120, 140, 155, 170, 185 and 200 min

⁴ Means in the same column with different superscript letters are significantly different. (Two-way ANOVA, Tukey-Kramer's post-hoc test, P<0.05)

⁵ DWS, 100% durum wheat semolina pasta;

FBF, 25% faba bean flour pasta;

FBS, 25% faba bean high starch pasta;

FBPC, 25% faba bean protein concentrate pasta;

FBPI, 25% faba bean protein isolate pasta

5.5.3 Glucagon-like peptide 1 (GLP-1)

Experiment 1: Post-treatment

In Exp. 1, post-treatment serum GLP-1 concentration (pg/mL) was not significantly affected by time ($P=0.1049$), treatment ($P=0.0571$) nor treatment-by-time interaction ($P=0.8100$, Table 5.6). GLP-1 concentrations for all treatments were lowest at baseline (0 min) and peaked at 30 min, then gradually dropped until 120 min (Figure 5.5A).

In Exp. 1, post-treatment GLP-1 iAUC (pg*min/mL) was not significantly affected by treatment ($P=0.9348$, Table 5.6).

Experiment 2: Post-meal

Experiment 2A

Post-meal GLP-1 after the *ad libitum* pizza meal was not significantly affected by treatment ($P=0.2864$), time ($P=0.6367$) nor treatment-by-time interaction ($P=0.8091$, Table 5.6, Figure 5.5B).

In Exp. 2A, post-meal GLP-1 iAUC after the *ad libitum* pizza meal were not significantly affected by treatment ($P=0.1751$, Table 5.6).

Experiment 2B

Post-meal GLP-1 after the fixed quantity pizza meal were not significantly affected by treatment ($P=0.2183$), time ($P=0.0776$), or treatment-by-time interaction ($P=0.8436$, Table 5.6, Figure 5.5C).

In Exp. 2B, post-meal GLP-1 iAUC after the fixed quantity pizza meal were not significantly affected by treatment ($P=0.8308$, Table 5.6).

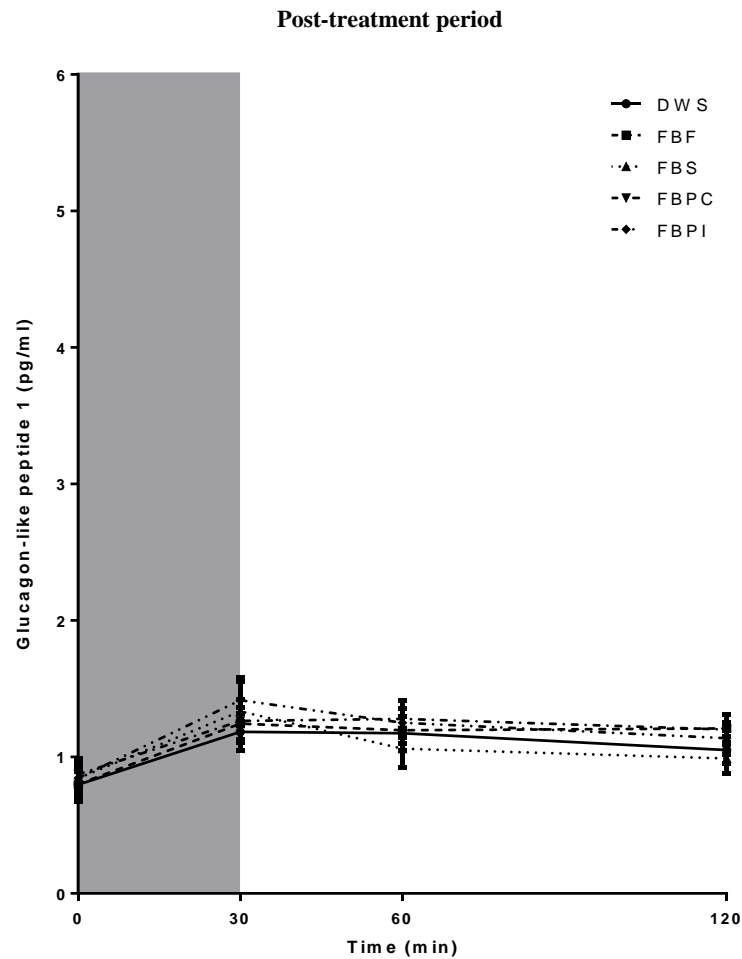


Figure 5.5A: Serum GLP-1 concentrations (pg/ml) up to 120 min were similar after each type of pasta. Values are means \pm SEM; n=21. (Two-way ANOVA, Pasta P=0.0571, Time P=0.1049, Pasta*time P=0.8100). Gray area was treatment consumption period not included in post-treatment analysis.

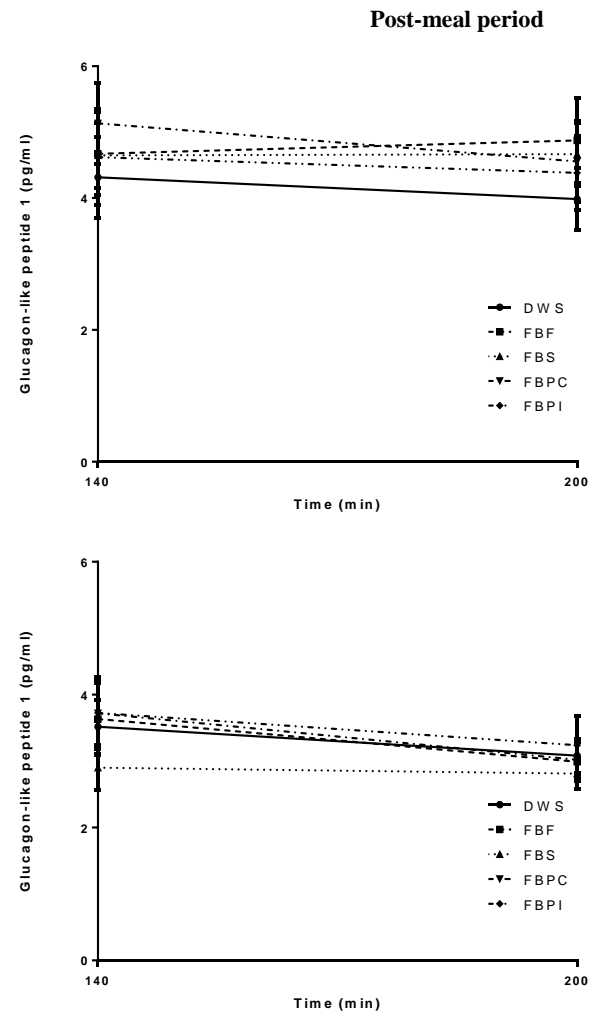


Figure 5.5B: Serum glucagon-like peptide 1 (GLP-1) concentrations (pg/ml) from 140 to 200 min after an *ad libitum* pizza meal were similar for each pasta treatment. Values are means \pm SEM; n=14. (Two-way ANOVA, Pasta P=0.2864, Time P=0.6367, Pasta*time P=0.8091)

Figure 5.5C: Serum GLP-1 concentrations (pg/ml) from 140 to 200 min after a fixed quantity pizza meal were similar for each pasta treatment. Values are means \pm SEM; n=12. (Two-way ANOVA, Pasta P=0.2183, Time P=0.0776, Pasta*time P=0.8436)

Figure 5.5: Serum glucagon-like peptide 1 (GLP-1) concentration for the Exp. 1 (0-120 min), Exp. 2A and 2B (140-200 min)¹

¹ DWS, 100% durum wheat semolina pasta; FBF, 25% faba bean flour pasta; FBS, 25% faba bean high starch pasta; FBPC, 25% faba bean protein concentrate pasta; FBPI, 25% faba bean protein isolate pasta

Table 5.6: Mean glucagon-like peptide 1 (GLP-1) and GLP-1 incremental area under the curve (iAUC, pg*min/mL) for the post-treatment and post-meal periods¹

		Post-treatment ²		Post-meal ³			
		Pooled (Exp. 1)		<i>Ad libitum</i> (Exp. 2A)		Fixed meal (Exp. 2B)	
	Treatment	Mean	±SEM	Mean	±SEM	Mean	±SEM
GLP-1 ⁴ (pg/mL)	DWS ⁵	1.13	0.069	4.17	0.384	3.30	0.240
	FBF	1.22	0.060	4.77	0.443	3.31	0.311
	FBS	1.13	0.095	4.66	0.336	2.86	0.201
	FBPC	1.25	0.076	4.84	0.431	3.37	0.308
	FBPI	1.27	0.076	4.50	0.448	3.48	0.320
	Treatment P	0.0571		0.2864		0.2183	
	Time P	0.1049		0.6367		0.0776	
	Treatment*time P	0.8100		0.8091		0.8436	
GLP-1 iAUC (pg*min/mL)	DWS	48.11	9.218	225.85	33.103	151.33	18.846
	FBF	36.56	5.781	270.44	44.530	151.65	20.693
	FBS	36.76	5.717	267.83	28.425	139.46	15.819
	FBPC	44.51	10.332	265.28	40.136	152.58	20.980
	FBPI	51.92	10.616	237.02	40.047	152.58	26.363
	P-value	0.9348		0.1751		0.8308	

¹ All values are mean ± SEM (Exp. 1, n=21; Exp. 2A, n=14; Exp. 2B, n=12)

² Post-treatment values are means of all observations after the treatment and before the pizza meal: 30, 60, and 120 min

³ Post-meal values are means of all observations after the pizza meal: 120, 140, 155, 170, 185 and 200 min

⁴ Means were not significantly different after each type of pasta (Two-way ANOVA, P>0.05)

⁵ DWS, 100% durum wheat semolina pasta;

FBF, 25% faba bean flour pasta;

FBS, 25% faba bean high starch pasta;

FBPC, 25% faba bean protein concentrate pasta;

FBPI, 25% faba bean protein isolate pasta

5.5.4 Peptide tyrosine tyrosine

Experiment 1: Post-treatment

In Exp. 1, post-treatment serum PYY concentration (pg/mL) was significantly affected by treatment ($P=0.0110$) and time ($P<0.0001$), but not treatment-by-time interaction ($P=0.9830$). PYY concentrations were significantly higher for FBPC compared to FBS and DWS, but similar to the rest (Table 5.7). PYY concentrations for all treatments peaked at 30 min, then gradually dropped below baseline by 120 min (Figure 5.6A).

In Exp. 1, post-treatment PYY iAUC (pg*min/mL) was not significantly affected by treatment ($P=0.2002$, Table 5.7).

Experiment 2: Post-meal

Experiment 2A

In Exp. 2A, post-meal PYY after the *ad libitum* pizza meal was significantly affected by treatment ($P=0.0003$) and time ($P=0.0028$), but not treatment-by-time interaction ($P=0.9294$, Table 5.7). PYY concentrations were significantly higher for FBPC compared to DWS, FBS and FBPI, but similar to FBF (Table 5.7). Overall PYY concentrations for all treatments were lower at 140 and increased at 200 min (Figure 5.6B).

In Exp. 2A, post-meal PYY iAUC after the *ad libitum* pizza meal was significantly affected by treatment ($P=0.0010$). PYY was significantly higher for FBF compared to DWS but similar to the rest (Table 5.7).

Experiment 2B

In Exp. 2B, post-meal PYY after the fixed quantity pizza meal was significantly affected by treatment ($P=0.0028$) and time ($P=0.0256$), but not treatment-by-time interaction ($P=0.4399$, Table 5.7). PYY concentrations were significantly higher for FBPC compared to FBS and higher for FBPI compared to DWS and FBS but no difference from the rest (Table 5.7, Figure 5.6C).

In Exp. 2B, post-meal PYY tAUC after the fixed quantity pizza meal was not significantly affected by treatment ($P=0.2143$, Table 5.7).

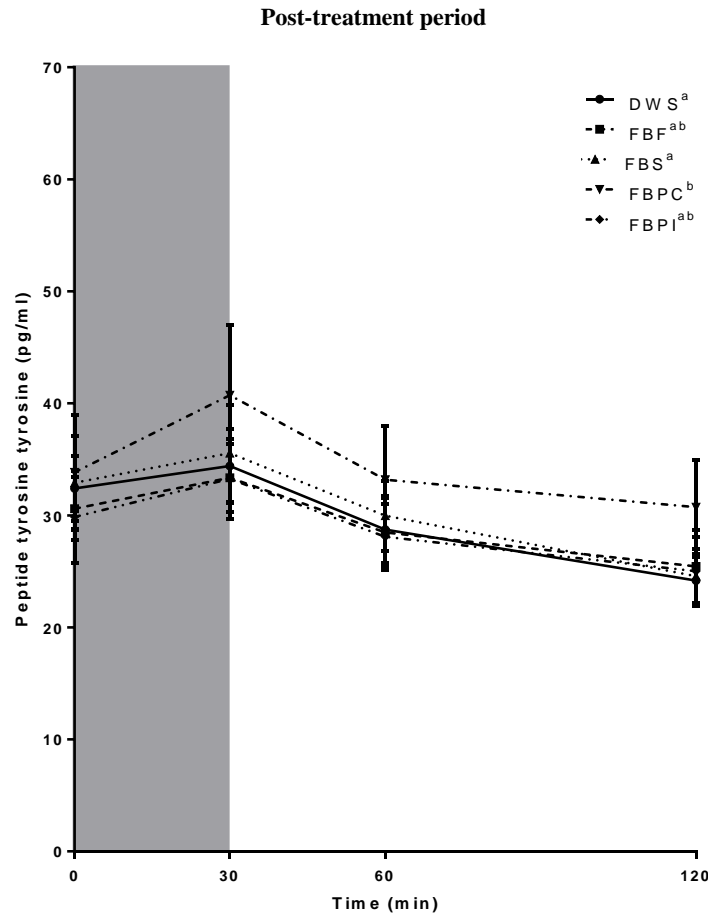


Figure 5.6A: Serum PYY concentrations (pg/ml) 15 to 120 min were similar after each type of pasta. Values are means \pm SEM; n=21. (Two-way ANOVA, Pasta P=0.0110, Time P<0.0001, Pasta*time P=0.9830). Gray area was treatment consumption period not included in post-treatment analysis.

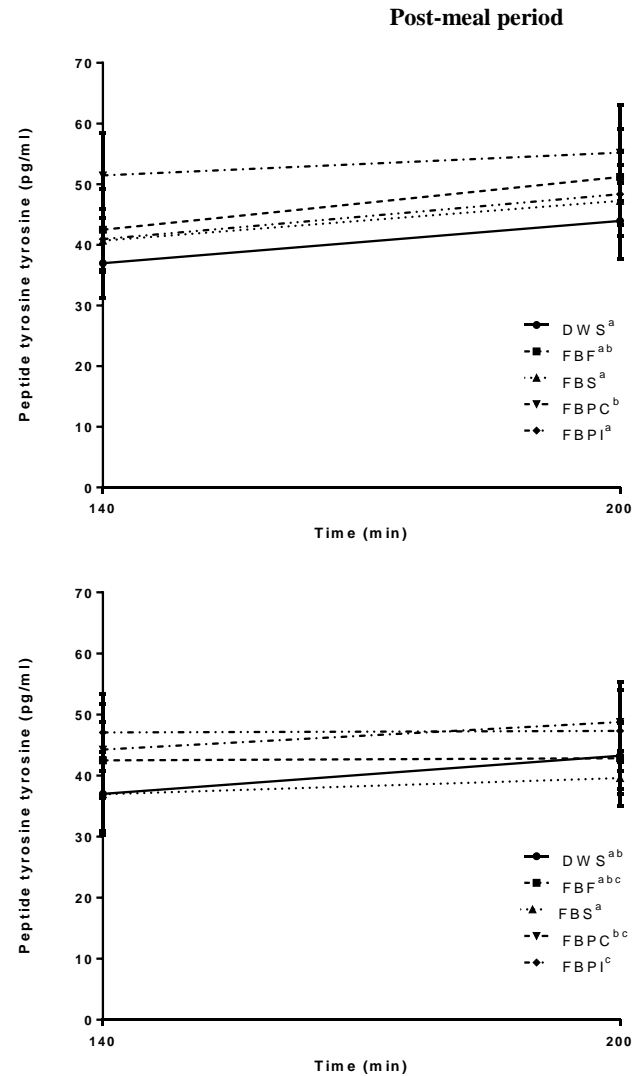


Figure 5.6B: Serum PYY concentration (pg/ml) from 140 to 200 min after an *ad libitum* pizza meal was higher for FBPC compared to DWS, FBS, FBPI but similar to FBF. Values are means \pm SEM; n=14. Treatments with different superscripts are significantly different. (Two-way ANOVA, Pasta P=0.0003, Time P=0.0028, Pasta*time P=0.0028)

Figure 5.6C: Serum PYY concentration (pg/ml) from 140 to 200 min after a fixed quantity pizza meal was higher for FBPI compared to DWS and FBS but similar to the rest, and higher for FBPC compared to FBS but similar to the rest. Values are means \pm SEM; n=12. Treatments with different superscripts are significantly different. (Two-way ANOVA, Pasta P= 0.0005, Time P<0.0001, Pasta*time P=0.9992)

Figure 5.6: Serum peptide tyrosine tyrosine (PYY) concentration for the Exp. 1 (0-120 min), Exp. 2A and 2B (140-200 min)¹

¹ DWS, 100% durum wheat semolina pasta; FBF, 25% faba bean flour pasta; FBS, 25% faba bean high starch pasta; FBPC, 25% faba bean protein concentrate pasta; FBPI, 25% faba bean protein isolate pasta

Table 5.7: Mean peptide tyrosine tyrosine (PYY) and PYY incremental area under the curve (iAUC, pg*min/mL) for the post-treatment and post-meal periods¹

		Post-treatment ²		Post-meal ³			
		Pooled (Exp. 1)		<i>Ad libitum</i> (Exp. 2A)		Fixed meal (Exp. 2B)	
	Treatment	Mean	±SEM	Mean	±SEM	Mean	±SEM
PYY ⁴ (pg/mL)	DWS ⁵	29.10 ^a	1.713	40.32 ^a	4.201	40.13 ^{ab}	4.313
	FBF	29.15 ^{ab}	1.819	46.68 ^{ab}	5.117	42.67 ^{abc}	4.159
	FBS	30.03 ^a	2.000	44.10 ^a	3.917	38.26 ^a	3.677
	FBPC	34.79 ^b	2.959	53.32 ^b	5.204	46.50 ^{bc}	4.908
	FBPI	28.84 ^{ab}	1.870	44.64 ^a	4.297	47.19 ^c	4.489
	Treatment P	0.0110		0.0003		0.0028	
	Time P	<0.0001		0.0028		0.0256	
	Treatment*time P	0.9830		0.9294		0.4399	
PYY iAUC (pg*min/mL)	DWS	147.17	67.357	1243.29 ^a	321.329	980.36	254.149
	FBF	314.07	109.798	1693.65 ^b	282.099	1112.46	250.732
	FBS	269.43	86.303	1466.77 ^{ab}	214.794	966.95	208.448
	FBPC	427.39	136.006	1617.72 ^{ab}	272.807	1128.09	223.285
	FBPI	272.72	67.437	1248.43 ^{ab}	114.357	992.08	214.530
	P-value	0.2002		0.0010		0.2143	

¹ All values are mean ± SEM (Exp. 1, n=21; Exp. 2A, n=14; Exp. 2B, n=12)

² Post-treatment values are means of all observations after the treatment and before the pizza meal: 15, 30, 45, 60, 90 and 120 min

³ Post-meal values are means of all observations after the pizza meal: 120, 140, 155, 170, 185 and 200 min

⁴ Means in the same column with different superscript letters are significantly different. (Two-way ANOVA, Tukey-Kramer's post-hoc test, P<0.05)

⁵ DWS, 100% durum wheat semolina pasta;

FBF, 25% faba bean flour pasta;

FBS, 25% faba bean high starch pasta;

FBPC, 25% faba bean protein concentrate pasta;

FBPI, 25% faba bean protein isolate pasta

5.6 Correlations

Correlations between appetite, BG, insulin, C-peptide, GLP-1 and PYY are shown in Table 5.8.

5.6.1 Post-treatment correlations

Food intake was correlated to appetite ($r=0.41271$, $P=0.0004$) and C-peptide ($r=0.24554$, $P=0.0405$). Appetite was correlated to C-peptide ($r=-0.24063$, $P=0.0134$), GLP-1 ($r=-0.36635$, $P=0.0001$) and PYY ($r=-0.23661$, $P=0.0151$). BG was correlated to C-peptide ($r=0.22723$, $P=0.0197$). Insulin was correlated to C-peptide ($r=0.71629$, $P<0.0001$), GLP-1 ($r=0.27315$, $P=0.0050$), and PYY ($r=0.55450$, $P<0.0001$). C-peptide was correlated to GLP-1 ($r=0.24397$, $P=0.0121$) and PYY ($r=0.61995$, $P<0.0001$). GLP was correlated to PYY ($r=0.49660$, $P<0.0001$). There was no correlation amongst other combination of variables assessed in Table 5.8.

5.6.2 Post-meal correlations

5.6.2.1 Experiment 2A

Food intake was correlated to BG ($r=-0.26508$, $P=0.0266$), insulin ($r=0.4502$, $P=0.0924$), C-peptide ($r=0.35980$, $P=0.0022$), GLP-1 ($r=0.48406$, $P<0.0001$), and PYY ($r=0.43702$, $P=0.0002$). Appetite was correlated to C-peptide ($r=-0.39753$, $P=0.0007$). BG was correlated to GLP-1 ($r=-0.26198$, $P=0.0285$). Insulin was correlated to C-peptide ($r=0.79949$, $P<0.0001$), GLP-1 ($r=0.27188$, $P=0.0249$) and PYY ($r=0.45616$, $P=<0.0001$). C-peptide was correlated to PYY ($r=0.58258$, $P<0.0001$). GLP-1 was correlated to PYY ($r=0.39978$, $P=0.0006$). There was no correlation amongst other combination of variables assessed in Table 5.8.

5.6.2.2 Experiment 2B

Appetite was correlated to BG ($r=-0.38325$, $P=0.0025$), GLP-1 ($r=-0.67570$, $P<0.0001$) and PYY ($r=-0.29989$, $P=0.0199$). BG was correlated to insulin ($r=0.55937$, $P<0.0001$), C-peptide ($r=0.48483$, $P<0.0001$), GLP-1 ($r=0.41768$, $P=0.0009$) and PYY ($r=0.31607$, $P=0.0139$). Insulin was correlated to C-peptide ($r=0.68302$, $P<0.0001$) and PYY ($r=0.48969$, $P<0.0001$). C-peptide was correlated to PYY ($r=0.49503$, $P<0.0001$). GLP-1 was correlated to PYY ($r=0.46552$,

P=0.0002). There was no correlation amongst other combination of variables assessed in Table 5.8.

Table 5.8: Pearson and Spearman correlations between overall means of food intake (FI) appetite, blood glucose (BG), insulin, C-peptide, glucagon-like peptide 1 (GLP-1) and peptide tyrosine tyrosine (PYY) for the pre- and post-meal periods¹

		Post-treatment ²		Post-meal ³			
		Pooled (Exp. 1)		<i>Ad libitum</i> (Exp. 2A)		Fixed meal (Exp. 2B)	
Correlated variables		R	P	r	P	r	P
FI	Appetite	0.41271	0.0004*	-0.09170	0.4502	-	-
FI	BG	-0.19024	0.1147	-0.26508	0.0266*	-	-
FI	Insulin	0.02704	0.8255	0.4502	0.0924*	-	-
FI	C-peptide	0.24554	0.0405*	0.35980	0.0022*	-	-
FI	GLP-1	0.01647	0.8923	0.48406	<.0001*	-	-
FI	PYY	0.07116	0.5583	0.43702	0.0002*	-	-
Appetite	BG	-0.16672	0.0892	0.06842	0.5736	-0.38325	0.0025*
Appetite	Insulin	-0.17226	0.0804	-0.18165	0.1382	-0.18186	0.1643
Appetite	C-peptide	-0.24063	0.0134*	-0.39753	0.0007*	0.01914	0.8846
Appetite	GLP-1	-0.36635	0.0001*	-0.04101	0.7360	-0.67570	<0.0001*
Appetite	PYY	-0.23661	0.0151*	-0.18109	0.1335	-0.29989	0.0199*
BG	Insulin	0.13014	0.1879	0.15142	0.2177	0.55937	<0.0001*
BG	C-peptide	0.22723	0.0197*	0.09000	0.4587	0.48483	<0.0001*
BG	GLP-1	-0.10559	0.2837	-0.26198	0.0285*	0.41768	0.0009*
BG	PYY	0.11685	0.2352	-0.21438	0.0747	0.31607	0.0139*
Insulin	C-peptide	0.71629	<0.0001*	0.79949	<0.0001*	0.68302	<0.0001*
Insulin	GLP-1	0.27315	0.0050*	0.27188	0.0249*	0.12014	0.3605
Insulin	PYY	0.55450	<0.0001*	0.45616	<0.0001*	0.48969	<0.0001*
C-peptide	GLP-1	0.24397	0.0121*	0.18296	0.1295	0.07358	0.5764
C-peptide	PYY	0.61995	<0.0001*	0.58258	<0.0001*	0.49503	<0.0001*
GLP-1	PYY	0.49660	<0.0001*	0.39978	0.0006*	0.46552	0.0002*

¹ Exp. 1, n=21; Exp. 2A, n=14; Exp. 2B, n=12

² Post-treatment values are means of all observations before the pizza meal: 15, 30, 45, 60, 90 and 120 min

³ Post-meal values are means of all observations after the pizza meal: 120, 140, 155, 170, 185 and 200 min

* Indicates significantly different determined using the Pearson's Correlation Coefficient (normal data) or Spearman's Rank Correlation Coefficient (not normal data).

5.7 Pasta palatability

5.7.1 Overall liking

Overall liking of the pasta was affected by treatment ($P=0.0072$, Figure 5.7). Palatability was significantly lower for FBPC compared to FBS but similar to the rest. Palatability for FBPI was significantly lower for FBPI compared to DWS and FBS but similar to the rest (Figure 5.7).

5.7.2 Pleasantness

The perceived pleasantness of the pasta was similar for each type of pasta ($P=0.6844$, Figure 5.7).

5.7.3 Tastiness

The perceived tastiness of the pasta was similar for each type of pasta ($P=0.5974$, Figure 5.7).

5.7.4 Texture

The liking for texture of the pasta was affected by treatment ($P<0.0001$, Figure 5.7). Texture-liking was lower for FBPC compared to DWS, FBF and FBS, but similar to FBPI. Texture-liking was lower for FBPI compared to DWS and FBS, but similar to the rest (Figure 5.7).

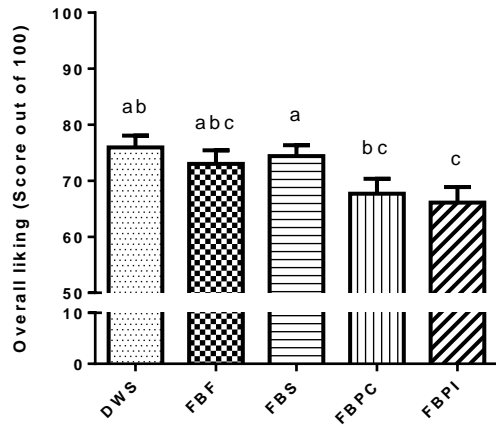


Figure 5.7A: Overall liking of pasta treatments based on average of VAS¹ scores for palatability (score out of 100). Overall liking was lower for FBPC compared to FBS but similar to the rest. Overall liking was lower for FBPI compared to DWS and FBS. Means with different superscripts are significantly different (One-way ANOVA, Tukey-Kramer's post-hoc test, P=0.0072)

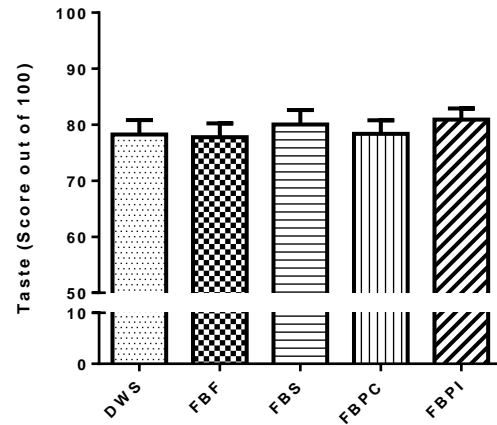


Figure 5.7C: Perceived tastiness of pasta treatments from VAS (score out of 100). Perceived tastiness was similar for each pasta (One-way ANOVA, P=0.5974)

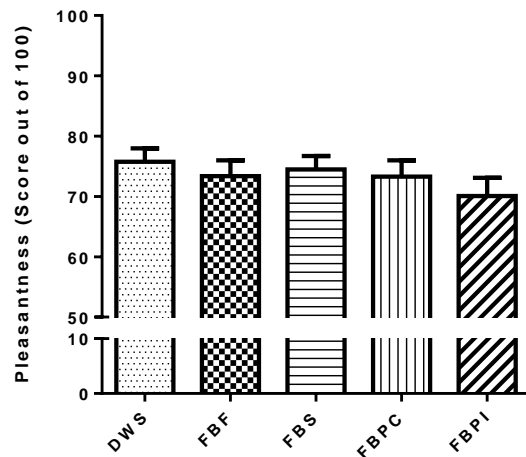


Figure 5.7B: Perceived pleasantness of pasta treatments from VAS (score out of 100). Perceived pleasantness was similar for each pasta (One-way ANOVA, P=0.6844).

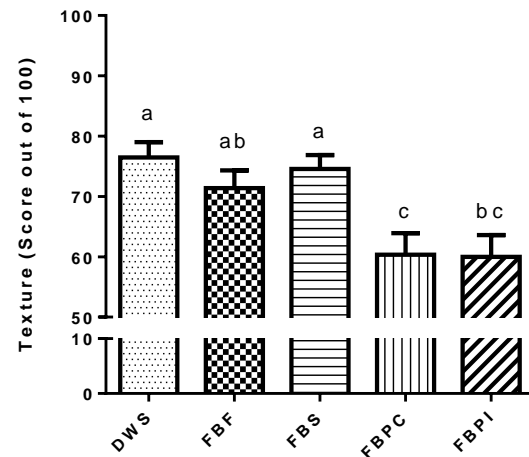


Figure 5.7D: Texture-liking of pasta treatments from VAS (score out of 100). Texture liking was lower for FBPC compared to DWS, FBF and FBS, but similar to FBPI. Texture liking was lower for FBPI compared to DWS and FBS, but similar to the rest. Means with different superscripts are significantly different (One-way ANOVA, Tukey-Kramer's post-hoc test, P<0.0001)

Figure 5.7: Palatability of pasta treatments ^{2,3,4,5,6,7}

¹ VAS, visual analog scale; ² All values are mean ± SEM (n=42); ³ 0=Not palatable at all; 100=very palatable; ⁴ 0=Not pleasant at all; 100=very pleasant; ⁵ 0=Not tasty at all; 100=very tasty; ⁶ 0=Dislike texture very much; 100=like texture very much; ⁷ DWS, 100% durum wheat semolina pasta; FBF, 25% faba bean flour pasta; FBS, 25% faba bean high starch pasta; FBPC, 25% faba bean protein concentrate pasta; FBPI, 25% faba bean protein isolate pasta

5.8 Energy level

5.8.1 Post-treatment energy level

In Exp. 1, post-treatment energy level was significantly affected by treatment ($P=0.0059$) and time ($P<0.0017$), but not treatment-by-time ($P=0.9636$) interactions (Table 5.9). Energy levels up to 120 min were lower for FBPC and FBPI compared to DWS but similar to the rest. Moreover, post-treatment energy levels for FBPC and FBPI were not different from each other (Table 5.9). Energy levels for all treatments were lowest at baseline (0 min) and peaked at 15 min and gradually dropped until 120 min (Figure 5.8A).

5.8.2 Post-meal energy level

Experiment 2A

In Exp. 2A, post-meal energy level was significantly affected by treatment ($P=0.0006$) and time ($P=0.0142$) but not treatment-by-time ($P=0.9703$) interactions. Overall post-meal energy levels after the *ad libitum* pizza meal from 140 min to 200 min were lower for FBF compared to DWS, FBS and FBPI but similar to FBPC. Energy level was lower for FBPC compared to FBS and FBPI but similar to the rest (Figure 5.8B). Post-meal energy levels after all treatments were gradually decreasing from 140 to 200 min with some fluctuations at time points in between (Figure 5.8).

Experiment 2B

In Exp. 2B, energy level was significantly affected by treatment ($P=0.2268$) but not by time ($P=0.2067$) nor treatment-by-time ($P=0.7997$) interactions (Figure 5.8). Overall post-meal energy levels after the fixed pizza meal from 140 min to 200 min were lower for FBF compared to FBPC and FBPI but similar to the rest (Figure 5.8C).

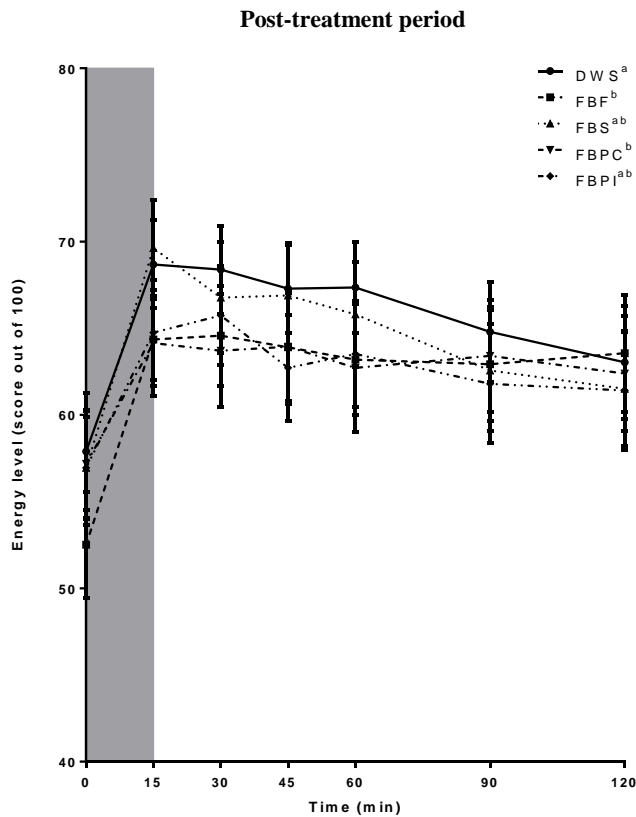


Figure 5.8A: Energy level (score out of 100) 15 to 200 min was lower for FBF and FBPC compared to DWS but similar to the rest. FBF and FBPC are similar to each other. Values are means \pm SEM; n=42. Graphs with different superscripts are significantly different (Two-way ANOVA, Pasta P=0.0059, Time P=0.0017, Pasta*time P=0.9636). Gray area was treatment consumption period not included in post-treatment analysis.

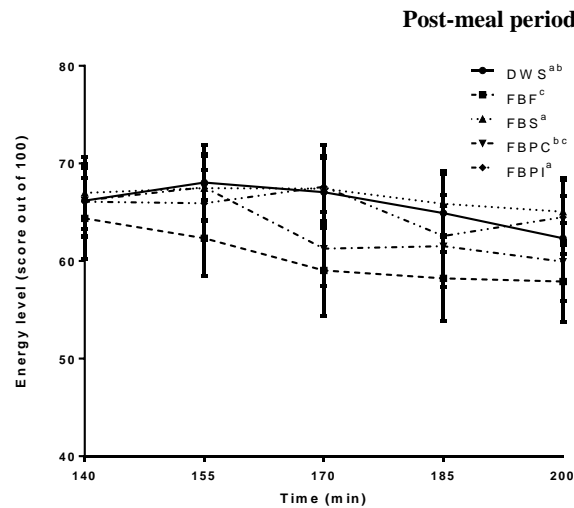


Figure 5.8B Energy level (score out of 100) from 140 to 200 min after an *ad libitum* pizza meal was lower for FBF compared to DWS, FBS and FBPI but similar to FBPC. Energy level was lower for FBPC compared to FBS and FBPI but similar to the rest. Values are means \pm SEM; n=28. Graphs with different superscripts are significantly different. (Two-way ANOVA, Pasta P=0.0006, Time P=0.0142, Pasta*time P= 0.9703)

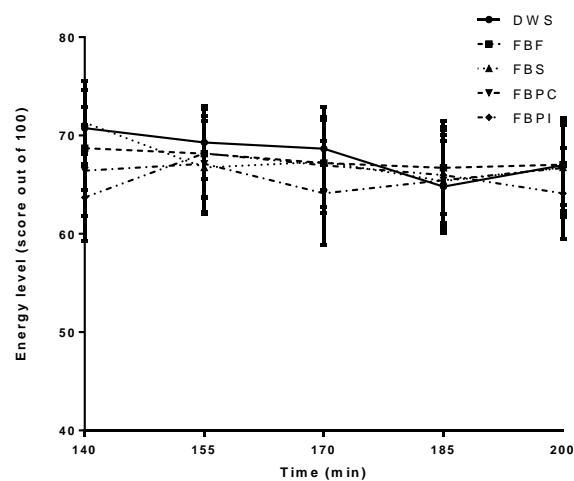


Figure 5.8C: Energy level (score out of 100) from 140 to 200 min after a fixed quantity pizza meal were similar for each pasta treatment. Values are means \pm SEM; n=26. (Two-way ANOVA, Pasta P= 0.2268, Time P=0.2067, Pasta*time P= 0.7997)

Figure 5.8: Energy levels (score out of 100) for Exp. 1 (0-120 min), Exp. 2A and 2B (140-200 min) ^{1,2}

¹0=Not energetic at all; 100=very energetic; ² DWS, 100% durum wheat semolina pasta; FBF, 25% faba bean flour pasta; FBS, 25% faba bean high starch pasta; FBPC, 25% faba bean protein concentrate pasta; FBPI, 25% faba bean protein isolate pasta

5.9 Physical comfort

5.9.1 Post-treatment physical comfort

In Exp. 1, physical comfort (score out of 100) was significantly affected by treatment ($P=0.0014$), but not time ($P=0.5665$) nor treatment-by-time ($P=0.9776$) interactions (Table 5.9). Physical comfort was lower after consuming FBPI was compared to FBS (Table 5.9). Physical comforts for all treatments were lowest at baseline (0 min) and gradually increased until 120 min with fluctuations in between (Figure 5.9A).

5.9.2 Post-meal physical comfort

Experiment 2A

In Exp. 2A, physical comfort was significantly affected by treatment ($P=0.0039$) but not time ($P=0.5901$) nor treatment-by-time ($P=0.9960$) interactions (Figure 5.9). Overall post-meal physical comforts after the *ad libitum* pizza meal from 140 min to 200 min were lower for FBS compared to DWS and FBF, but similar to the rest (Figure 5.9B).

Experiment 2B

In Exp. 2B, physical comfort was significantly affected by treatment ($P=0.0031$) but not by time ($P=0.4209$) nor treatment-by-time ($P=0.8133$) interactions (Figure 5.9). Overall post-meal physical comfort after the fixed pizza meal from 140 min to 200 min were lower for FBF compared to FBPC and FBPI but similar to the rest (Figure 5.9C).

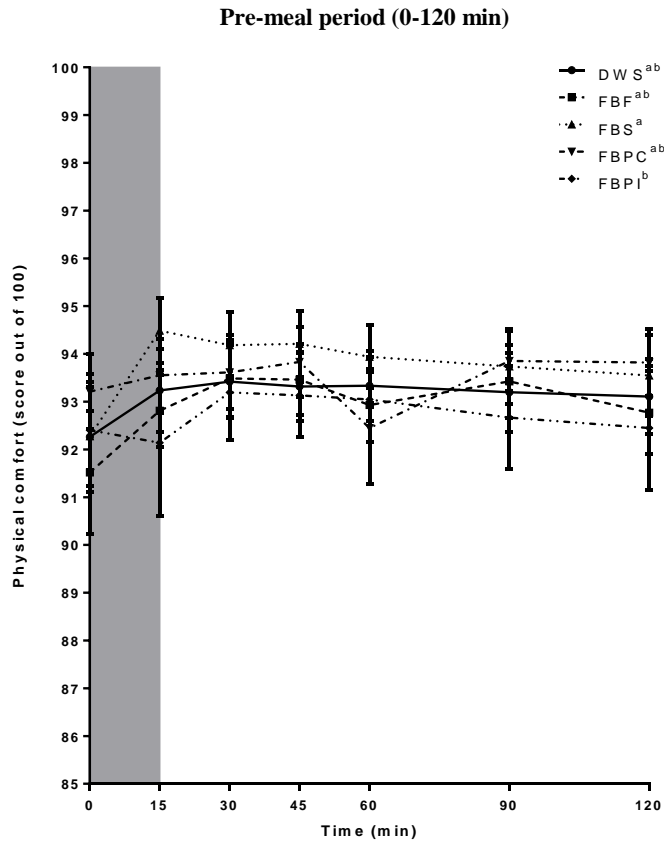


Figure 5.9A: Physical comfort (score out of 100) 15 to 200 min was higher for FBS compared to FBPI, but similar to the rest. Values are means \pm SEM; n=42. Graphs with different superscripts are significantly different (Two-way ANOVA, Pasta P=0.0114, Time P<0.0273, Pasta*time P=0.9888)

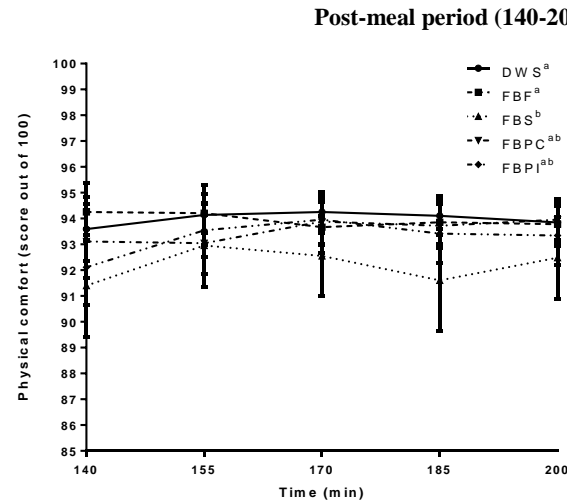


Figure 5.9B: Physical comfort (score out of 100) from 140 to 200 min after an *ad libitum* pizza meal was lower for FBS compared to DWS and FBF but similar to the rest. Values are means \pm SEM; n=28. Graphs with different superscripts are significantly different. (Two-way ANOVA, Pasta P=0.0039, Time P=0.5901, Pasta*time P=0.9960)

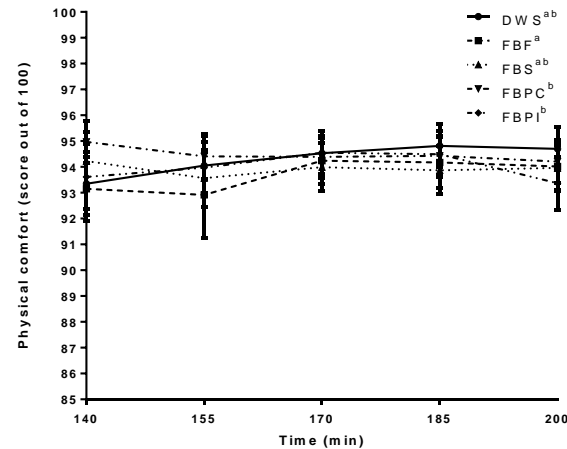


Figure 5.9C: Physical comfort (score out of 100) from 140 to 200 min after a fixed quantity pizza meal were similar for each pasta treatment. Values are means \pm SEM; n=26. Graphs with different superscripts are significantly different. (Two-way ANOVA, Pasta P=0.0031, Time P=0.4209, Pasta*time P=0.8133)

Figure 5.9: Physical comfort (score out of 100) for Exp. 1 (0-120 min), Exp. 2A and 2B (140-200 min) ^{1,2}

¹ 0=Not comfortable at all; 100=very comfortable; ² DWS, 100% durum wheat semolina pasta; FBF, 25% faba bean flour pasta; FBS, 25% faba bean high starch pasta; FBPC, 25% faba bean protein concentrate pasta; FBPI, 25% faba bean protein isolate pasta

Table 5.9: Summary of results for energy level and physical comfort for the post-treatment and post-meal periods¹

		Post-treatment ²		Post-meal ³			
		Pooled (Exp. 1)		<i>Ad libitum</i> (Exp. 2A)		Fixed meal (Exp. 2B)	
	Treatment	Mean	±SEM	Mean	±SEM	Mean	±SEM
Energy and fatigue ^{4,5} (Score out of 100)	DWS ⁷	66.59 ^a	1.115	65.72 ^{ab}	1.721	68.10	1.829
	FBF	63.75 ^{ab}	1.233	60.38 ^c	1.891	67.56	1.993
	FBS	65.53 ^{ab}	1.283	66.55 ^a	1.520	67.50	2.017
	FBPC	63.38 ^b	1.338	63.30 ^{bc}	1.698	65.96	2.198
	FBPI	63.29 ^b	1.280	65.34 ^a	1.695	65.79	2.046
	Treatment P	0.0059		0.0006		0.2268	
	Time P	0.0017		0.0142		0.2067	
	Treatment*time P	0.9636		0.9703		0.7997	
Physical Comfort ⁶ (Score out of 100)	DWS	93.27 ^{ab}	0.318	93.99 ^a	0.426	94.09 ^{ab}	0.465
	FBF	93.15 ^{ab}	0.329	93.95 ^a	0.406	93.43 ^a	0.555
	FBS	94.02 ^a	0.295	92.20 ^b	0.774	94.20 ^{ab}	0.460
	FBPC	93.52 ^{ab}	0.305	93.54 ^{ab}	0.466	94.76 ^b	0.361
	FBPI	92.77 ^b	0.428	93.54 ^{ab}	0.516	94.45 ^b	0.363
	Treatment P	0.0014		0.0039		0.0031	
	Time P	0.5665		0.5901		0.4209	
	Treatment*time P	0.9776		0.9960		0.8133	

¹ All values are mean ± SEM (Exp. 1, n=21; Exp. 2A, n=14; Exp. 2B, n=12)

² Post-treatment values are means of all observations after the treatment and before the pizza meal: 15, 30, 45, 60, 90 and 120 min

³ Post-meal values are means of all observations after the pizza meal: 120, 140, 155, 170, 185 and 200 min

⁴ Means in the same column with different superscript letters are significantly different. (Two-way ANOVA, Tukey-Kramer's post-hoc test, P<0.05)

⁵ DWS, 100% durum wheat semolina pasta;

FBF, 25% faba bean flour pasta;

FBS, 25% faba bean high starch pasta;

FBPC, 25% faba bean protein concentrate pasta;

FBPI, 25% faba bean protein isolate pasta

5.10 Summary: Post-treatment mean change from baseline for dependent measures

The post-treatment mean change from baseline was significantly affected by treatment for BG ($P=0.0002$) and C-peptide ($P<0.0001$) but not the other outcomes (Table 5.10). BG concentration was lower after FBPC compared to DWS, FBF and FBS but not FBPI ($P=0.0002$, Table 5.10). C-peptide concentration was lower after FBPC and FBPI compared to DWS and FBS, but not different from FBF; while FBPC and FBPI were similar to each other ($P<0.0001$, Table 5.10).

Table 5.10: Mean change from baseline in Exp. 1 (post-treatment period)

	DWS		FBF		FBS		FBPC		FBPI		P-value
	Mean Δ	\pm SEM	Mean Δ	\pm SEM	Mean Δ	\pm SEM	Mean Δ	\pm SEM	Mean Δ	\pm SEM	
Appetite	-24.54	2.609	-21.03	2.883	-24.24	3.177	-22.04	2.560	-23.06	2.973	0.6508
BG	1.48 ^a	0.118	1.36 ^{ab}	0.094	1.50 ^a	0.087	1.20 ^b	0.097	1.26 ^b	0.073	<0.0001
Insulin	16.97	1.875	18.30	2.249	18.17	1.719	14.79	1.185	17.77	2.168	0.1218
C-peptide	1576.60 ^a	173.495	1559.32 ^a	182.209	1647.43 ^a	148.971	1210.56 ^b	129.123	1509.82 ^{ab}	188.418	<0.0001
GLP-1	0.339	0.0771	0.419	0.0820	0.283	0.0639	0.376	0.1055	0.433	0.1232	0.6119
PYY	-3.31	1.185	-0.77	1.754	-2.89	1.895	0.57	2.050	-0.85	2.045	0.2096
Energy	8.70	2.763	11.24	2.872	8.58	2.420	6.20	2.369	6.34	2.221	0.2528
Comfort	1.01	0.716	1.63	0.878	1.74	0.878	0.31	0.469	0.37	0.482	0.1489

Chapter 6

6 Discussion

6.1 General discussion

The hypothesis that the addition of faba bean flours to DWS pasta reduces PPG and increases satiety was partially supported. Compared to the already low GI DWS pasta, the high protein pastas (FBPC and FBPI, but not FBF nor FBS) contributed to reduced acute postprandial and second meal glycaemia after an *ad libitum* meal, but not the fixed meal. Although FBPC and FBPI did not affect post-treatment (15-120 min) appetite, post-meal (140-200 min) subjective appetite after the fixed meal was lower for high protein pastas compared to control. Results showed that mechanisms associated with improved BG and appetite regulation were elicited by faba bean proteins. Moreover, from Pearson or Spearman correlation analyses, some correlation between BG and the hormones, as well as appetite and hormones were observed in both the post-treatment (0-120 min) and post-meal periods (140-200 min) (Table 5.8). In general, the effect of treatments on AUCs were not consistent with their corresponding effects over time.

Three post-treatment outcomes were affected by a single serving of pasta containing ~360 kcal. High protein pastas lowered BG (Table 5.3), lowered C-peptide (Table 5.5) and increased PYY compared to control (Table 5.7). Post-meal (140-200 min) outcome measures after the *ad libitum* meal were affected by high protein pastas and resulted in lower BG (Table 5.3) and increased PYY (Table 5.7), whereas the same pastas resulted in lower appetite and higher PYY compared to control after the fixed meal. More prominent effects were observed from FBPC rather than FBPI, suggesting that purification into isolates may remove synergistic effects among macronutrients that favour metabolic control.

Although high protein pastas improved PPG and aspects of glycaemic management, the percentage decrease in BG concentrations compared to control was low of ~4%. This might have been predicted because pasta using DWS already had low post-prandial glycaemic response and is classified as low to medium GI (GI = 32-65) compared to other starchy staples, such as potatoes (GI = 69-102), bread (GI = 66-80), and rice (GI = 43-68) (glucose as reference) [80-82]. FBPC and FBPI additions reduced post-prandial glycaemia perhaps because ~10 and 12 g protein was

added, respectively, to the ~13 g protein contained in the DWS pasta (Table 4.1). PPG was reduced by these same amounts in the carbohydrate fractions, making it difficult to attribute the modest effect on PPG to the protein alone. Meanwhile, FBF and FBS replaced 25% of the DWS carbohydrate, without increasing PPG, suggesting that their carbohydrate may be relatively low GI. It is also possible that antinutritional factors coming from faba bean proteins, such as α -amylase inhibitors prevented the breakdown of carbohydrates for digestion which can significantly reduce and/or slow glucose release into the bloodstream [52, 54]. Inhibition of α -amylase is attributed to phenolic compounds associated with protein substances of faba beans [53]. As mentioned, in low levels, amylase inhibitors may be a strategy for managing glycaemic response to starchy foods [52]. These results support that processing of pulses into powders retains their benefits for glycaemic control but only when further purified into concentrates and isolates.

During Exp. 1 and 2A, insulin concentrations were not significantly different among treatments (Table 5.4). In Exp. 2B, post-meal insulin was lower after FBPC consumption compared to FBF but was similar to control and the other pastas. This suggests that the observed BG response is insulin-independent but related to other factors, like slower gastric distention - a possible effect of PYY. Although there was a treatment effect on insulin in Exp. 2B, there was no difference in glycaemic response during the fixed meal. Moreover, in the post-treatment period, the reduced PPG response to the protein-enriched pastas was not correlated with any treatment effects on insulin, GLP-1 or PYY but the effect on C-peptide may be a factor (Table 4.1). As per HC draft guidelines for PPG claims, BG and insulin responses should be measured as iAUC. However, both showed no response to treatment when comparing iAUC, other than Exp. 2B for insulin but the response did not correspond to the effects on insulin concentration over time (Table 5.4).

In Exp. 1, consumption of FBPC reduced post-treatment C-peptide compared with DWS, FBF and FBS pastas. FBPI also reduced C-peptide compared with FBS, but the rest were similar. Moreover, in the post-treatment stage, C-peptide iAUC behaved similarly to the effects over time and was greater after consumption of FBPC compared to DWS, FBF and FBS, but similar to FBPI (Table 5.5). Since pancreatic β -cells split proinsulin into one molecule of active insulin and one molecule of inactive C-peptide [89], it was expected that in healthy subjects, insulin and C-peptide concentrations would be released at the same rate at 1:1 concentration ratios. A decrease in C-

peptide suggests that insulin formation was not increased by the protein additions. This supports HC draft guidelines for postprandial glycaemia that the decrease in blood glucose should not be associated with a disproportionate increase in insulin. Moreover, it is possible that plasma concentrations of insulin were maintained by other responses, reducing uptake and degradation by the liver and kidneys [90].

The effect of FBPC and FBPI on reducing BG and C-peptide was also apparent in the post-meal (second-meal) response, but only after the *ad libitum* meal. The effects were not consistent. Compared with DWS, none of the treatments affected BG or the selected hormones in response to the fixed meal. As mentioned, a fixed meal providing 12 kcal/kg was fed in Exp. 2B because it was expected that treatment effects would be more apparent without the effect of FI changes. Since the average of ~1220 kcal consumed in the *ad libitum* meals was not affected by treatment, the rationale for feeding the fixed meal intake of ~805 kcal was unnecessary. The lack of effect of treatments on FI was not predicated because the experiments were run concurrently and completed simultaneously due to presumed time constraints. Nevertheless, post-meal insulin, GLP-1, and BG concentrations were higher after an *ad libitum* meal compared to that of all the fixed meal responses, consistent with the 50% increase in pizza intake during *ad libitum* meal and the greater suppression of appetite reported compared with the fixed meal. Moreover, although no treatment effect on FI was detected, FI quantity was negatively correlated to post-meal BG, and positively correlated with post-meal insulin, C-peptide, GLP-1 and PYY (Table 5.8).

Although FBF and FBS did not elicit valuable improvements on glycaemic, appetite or metabolic control compared to conventional pasta, they exhibited similar low-medium GI properties while providing more quality nutrients, including complete essential amino acids, higher fibre, and more SDS and RS, rather than RDS (Appendix 16). Aforementioned, amino acid profiles of faba bean and DWS are complementary where bean flour provides lysine and DWS provides tryptophan and methionine [42, 45]. Moreover, there were ~1 to 2 g increases of dietary fiber per 85 g serving (Table 4.1) helping to reach HC's recommended daily requirements for 25 and 38 g of fibre for women and men, respectively, especially when consuming multiple servings per meal. Greater SDS and RS contents are desirable in helping slower release of BG and management of glycaemia. However, glycaemic and insulinaemic responses did not reflect this in the study, as

pasta was already low GI. Perhaps when substituted into higher GI starchy food products, faba bean flours have the potential to reduce PPG.

Subjective appetite after the fixed meal (Exp. 2B) but not the *ad libitum* meal (Exp. 2A), was affected by the treatment (Table 5.2), indicating that second meal effects of treatments are dependent upon the size of the second meal. In Exp. 2B, subjective appetite for FBPC and FBPI were significantly lower than control. There was no post-treatment appetite effect but FI was positively correlated to appetite in the present study ($r=0.41241$, $P=0.0004$, Table 5.8). Although VAS has an acceptable degree of reproducibility and is recommended by HC draft guidelines, they do not consistently correlate to energy intake in the second meal; thus, an appetite response does not necessarily translate into reduction of food intake later [20]. In Exp. 2A, it was expected that upon no effects being observed on FI, then treatment effects would not be observed on post-meal appetite either. However, in Exp. 2B treatment differences were found even if participants all ate the same amount of 12 kcal/kg based on their respective body weights. Fixed meal quantities eliminated variability in the appetite effects of the pastas due to food intake.

The latter results associated with the treatment effects on PYY concentrations that were negatively correlated to subjective appetite in Exp. 1 ($r=-0.23661$, $P=0.0151$) and 2B ($r=-0.29989$, $P=0.0199$; Table 5.8), but not Exp. 2A ($r=-0.23661$, $P=0.2352$). Thus, an increase in mean PYY concentrations was correlated to a decrease in mean appetite scores from Pearson or Spearman correlations but this result was not reflected for the measures over time. In all experiments, PYY concentrations were highest after FBPC and FBPI pastas, consistent with their effects on appetite after the fixed meal of Exp. 2B (Table 5.7). PYY is known to down-regulate appetite and promote satiety by reducing the rate of gastric emptying [91]. Although we did not directly measure gastric emptying rates in this study, the results suggest that the increased protein content of a single serving of pasta may contribute to increased satisfaction on a reduced energy meal consumed later in the day. These findings may aid in designing diet plans intended for body weight management. Underlying mechanisms of pulse proteins on the observed PYY response may be attributed to higher quantities of slow proteins coming from legumins, present at ~45% of the protein fraction of faba beans. Legumins are the plant equivalent to milk casein of animals, which are known to have a longer digestion period of 7 hours [43]. Thus, it is possible that the longer digestion time for the proteins in FBPC and FBPI required greater action from PYY throughout the study session.

It is less expensive to process faba bean flours into protein concentrate compared to that of isolate. Since both elicit similar metabolic responses, it may be more cost effective to use faba bean concentrate rather than the isolate. As well, more prominent effects were observed from FBPC. Thus, the food industry should focus on novel use of faba bean protein concentrates as a flour substitution for health benefits to eliminate extra costs associated with further purification into a protein isolate. Purification to isolate protein may remove beneficial macronutrients that work synergistically with protein to improve metabolic control. Similar to another study, pea protein and fibre together reduced PPG when included in noodles and tomato sauce, but such effects were not observed from adding pea protein or pea fibre flours alone [76]. This is valuable for the agri-food sector seeking novel ways to formulate low GI products for glycaemic control.

The lack of effect from the small pasta meal on FI two hours later was expected and consistent with many studies [2, 4, 27] showing that appetite reaches a modest trough quickly at 15-30 min post-treatment and climbs to fasting levels (approaching baseline values) before second meal consumption [77]. From one serving, the average caloric consumption of the pasta and sauce for lunch was 366.6 ± 0.93 kcal (Table 4.1), which is low for young adult males requiring 2500 to 3000 kcal per day according to HC energy requirements (Appendix 8). Thus, a third of this content consumed at lunch would require ~833 to 1000 kcal [92]. Moreover, there is evidence that upon food removal from the stomach, satiation does not occur. Hence, if the volume of pasta in the stomach was low, it is not expected that subjects could reach and/or maintain satiation over 120 min [19]. Therefore, the pasta quantity may have been insufficient for the subjects to sustain fullness and detect differences between the pastas at 120 min of Exp. 1.

These observed appetite response was consistent with similar studies from Anderson and colleagues investigating the effect of lentils, chickpeas, navy beans and yellow peas, which also used small treatment servings (Table 2.2). Two of these studies reported no effect on second meal food intake consumed between 120 and 135 min later after treatments consisting of 300 kcal and 200-300 kcal, respectively [2, 4]. Although this study followed the pasta serving size according to HC draft guidelines for satiety claims, other studies have reported otherwise, such as those of Mollard et al. [1, 3]. There was report of treatment effect on second meal food intake reduction in a meal consumed 260 min after the treatment, particularly by pasta with lentils, despite no appetite suppression. In these reports, one study provided 600 kcal of pulses and pasta (~63% more energy

provided in the treatment compared to the present study), while the other study provided pulses and pasta in excess to ensure satiation during the treatment. Moreover, 3 of 4 studies reported no effect on pre- or post-meal appetite [1, 3, 4]. Thus, evidence suggests that although appetite and FI responses were not consistent, larger treatment sizes or providing treatments in excess may aid in seeing a detectable treatment response on FI.

The mechanisms driving the beneficial responses observed in this study (reducing postprandial BG, promoting satiety and their associated metabolic responses), appear to be primarily attributed to faba bean proteins. Similarly, another study investigated the addition of yellow pea fractions in tomato sauce, particularly of fibre and protein. They reported that yellow pea protein isolate, but not the concentrate or the fibre fraction, reduced postprandial glycaemia post-treatment and post-meal, and reduced second meal FI after 30 min (but not after 120 min, Table 2.3) [77]. Thus, evidence suggests that using further processed faba bean flours as value-added ingredients have the potential to further improve glycaemic response of originally low GI foods, particularly for protein concentrates and isolates. This is possibly attributed to slower gastric emptying action of PYY and slower degradation of insulin, resulting in more efficient use. Dietary fibre content was higher in faba bean pastas (Table 4.1). Other studies have reported that the slower gastric emptying rate elicited by pulses may be attributed to a higher fibre content causing greater action by cholecystokinin (CCK), a satiety hormone, [93]; however, CCK was not analyzed in this study to confirm this.

From the present study, pasta has shown to be a good carrier of the benefits of pulse proteins. However, in comparison to other methods of pulse preparation, extrusion of faba bean products resulted in the lowest PER compared to baking and cooking, which may pose a challenge for receiving health benefits of faba beans through pasta [84]. Perhaps with a higher ratio of faba bean flours to semolina flour would allow for a greater percent decrease in BG, although texture quality and cooking yield are expected to decrease. It has been shown that 35% substitution of regular faba bean flours in DWS pasta and the use of high pasta drying temperatures induces lower glycaemic response *in vitro* [73, 74, 78], but has not yet been applied to faba bean fractions. Even though percent decrease in BG is small, this result may help guide the food industry to explore pulse flour applications to more low-medium GI carbohydrate foods; thus, help further attenuate postprandial BG. To improve nutritional quality of carbohydrate staples, further research in adding

pulse fractions to both low and high GI products and the use of novel processing methods are encouraged. These types of studies should focus on how processing impacts the technological function of novel pulse flours while examining the effects on physiological functionality.

In general, pastas were well-liked with a mean overall liking of 73.4 ± 0.77 out of full score of 100 (0 = Dislike very much; 100 = like very much). Overall liking of pasta samples was lower for the high protein pastas compared to the rest but was not different between FBF, FBS and control (Figure 5.7). Since pleasantness and tastiness were not significantly different, the liking for texture (increased firmness) reduced overall acceptance of high protein pastas (Figure 5.7). All pastas were cooked for the same amount of time (8 min) for consistency. The cooking time was chosen as it corresponds with most commercial instructions to produce al dente macaroni pasta (usually around 8-10 min) but may have left a harder texture in the high protein pastas compared to the others. If pastas were cooked for a longer period of time, the physicochemical properties may have been different and yielded different outcomes in this study, such as more easily digested starch and greater loss of protein. It is an important consideration for future studies to use the cooking instructions that would be specified on the product if anticipating commercialization. As well, pastas produced without further purifying the flours to protein concentrates and isolates received a similar liking to the staple (DWS) pasta. Therefore, this study demonstrates that although the high protein faba bean flours reduced the texture quality of the pastas, pastas were still well-received and had desirable palatability compared to conventional pasta, paving way for large-scale commercialization potential.

There were detectable treatment effects on energy levels and physical comfort but were inconsistent between the experiments (Table 5.9). In Exp. 1, energy levels were lower after FBPC and FBPI compared to control, which may be a result of slow proteins requiring more energy over time to metabolize. Although consumption of pulses can be associated with physical discomfort from fibre, physical comfort scores remain high for this study of was a high of 93 out of 100. Observed differences were quite small of a 2-3 scores out of 100 (Figure 5.8) and no detectable treatment effect or influence on FI. This provides further evidence to support the use of pulse flours in functional food design.

The majority of the requirements for HC draft guidelines for satiety and PPG health claims were satisfied in the present study design (Appendix 8, Appendix 9). This study partially supports PPG claim for pastas fortified with faba bean protein concentrates and isolate. As stated in the HC guidance document for PPG, concentrations of BG and insulin should both be reported. BG responses strongly supported the claim for high protein faba bean pastas, while insulin increase disproportionately to the decrease in BG as required by HC. Moreover, the design of this study did not focus solely on identifying a potential satiety health claim, as an energy free control was not included in the study.

6.2 Health Claims: Benefits and Limitations

As described in the literature review, HC has draft guidelines to encourage satiety and PPG claims. This study provides relevant information to lead to improvement in these guidelines. All of the key requirements for PPG claims were followed (Appendix 9). However, not all key requirements for satiety claims were followed (Appendix 8). The food was not tested twice as per requirements due to time and cost constraints. Study participants were not time-blinded as they had access to electronic devices, watches and timers for the study. Moreover, participants were not provided with an energy free control as one of the treatments. All other requirements for the study design were carefully followed and listed in Appendix 8.

One of the major challenges in designing this research study was following HC draft guidelines for satiety claims to get accurate scientific results whilst maintaining realistic food portions and time intervals between feeding times in the study (Appendix 8, Appendix 9). One of the limitations of the study was providing a serving size of pasta for lunch that was presumably much lower quantity than what young adult males would normally consume, making it unreflective of true meal size of an average adult. The portion of pasta and sauce following one serving size as per the Canadian Nutrient Facts Table was not reflective of the normal pasta serving quantity of a day-to-day lunch meal, especially not for a young, adult male. Thus, it is difficult to make accurate comparisons of these results to the real world. Furthermore, according to the HC draft guidelines for satiety claims, the time interval between meals is an important consideration and should be realistic when designing the experiment [20]. It would have been most representative of a day-to-day meal to provide pasta treatment as a lunch and pizza as a dinner. In this study, following a 10-

12 hour overnight fast and small mandatory breakfast, a small pasta lunch was provided which resulted in participants reporting to be almost at fasting levels of hunger by 120 min of their study sessions (Figure 5.1). Although required by HC to have at least 4 hours between test meals, waiting another 2-3 hours for another feeding time would not be viable (Appendix 8). Thus, it was a limitation to provide the pizza as an “afternoon snack” rather than a dinner meal as it is not reflective of regular feeding times and meal/snack quantities. Future research should consider this, while controlling for fasting levels.

Although this study provides direction for future study designs for satiety and PPG health claims, it has more positive considerations for a protein claim. The FBPC and FBPI provided 23 and 25 g protein in one 85 g serving of pasta, respectively, while DWS provided 13 g. Neither are balanced proteins but when combined provide a relatively balanced protein source with an Amino Acid Score of 0.9. The score combined with the quantity of protein gives a protein rating over 20, which qualifies for the claim “a source of protein”, whereas DWS pasta does not.

6.3 Other Limitations

Other limitations to the study exist. The study only assessed male response, but not female response to the pasta because of the time constraints in accounting for the hormonal changes due to the menstrual cycle if conducted on females. Although power calculations were used to determine the sample size of the experiments, it is possible that the study may be underpowered for hormones collected from IV participants and should account for greater dropout rates. Another possible confounder is in standardizing the pasta cooking times in the study to reflect in-home preparation of the pastas. For this study, all pastas were cooked for the same amount of time; however, this left the high protein pastas to have a less desirable texture than the others. Altering cooking times (ie. extending to beyond 10 min) may alter the physiological effects of the pastas but should be a consideration to represent realistic cooking times for the product.

6.4 Conclusion

In summary, pastas substituted with faba bean protein concentrate and isolate, but not regular nor high starch flours lowered postprandial BG, reduced second-meal appetite, and improved aspects of metabolic response, such as lowered C-peptide and increased PYY in both

Exp. 1 and Exp 2. Lack of effects observed for FI is consistent with similar studies and may be a result of the small pasta serving following HC draft guidelines for satiety claims. More realistic meal serving sizes are encouraged for future studies. Nonetheless, the study supports utilization of pulse protein fractions in starchy foods as a means to lower postprandial BG, even in already low GI foods. Moreover, utilization of pulse flours for fortification of grain products provides more wholesome source of essential amino acids. This study encourages the utilization of pulse flours, particular those high in protein to elicit benefits on glycaemic, appetite and overall metabolic control.

Chapter 7

7 Future Directions

The results of this study support the role of pulse flours and fractions as ingredients in functional foods (i.e. pasta) to improve glycaemic control. Our research shows that formulating foods with processed faba bean flours and further-purified fractions (ie. protein concentrates and isolates) has the potential to improve glycaemic response of originally low GI foods. This is possibly attributed to the action of faba bean proteins on reduction of gastric emptying related to PYY, rather than insulin-dependent. Although the high protein faba bean flours reduced the texture quality of the pastas, pastas were still well-received and had desirable palatability compared to conventional pasta, paving way for large-scale commercialization potential. As well, pulse flours produced without additional processing/purification steps (split bean and/or whole bean flours) should be viewed as novel, cheaper and sustainable low GI ingredients for functional food innovation. To improve nutritional quality of carbohydrate staples, further research in adding pulse fractions to both low and high GI products is encouraged. The utilization of pulse flours and fractions in novel food products are healthier than their conventional counterpart. The goal is to pave way for commercialization of tasty and familiar, yet healthy, food products as a strategy to prevent obesity, T2D and manage the health issues associated with excess weight gain.

Understanding structure-function properties of pulse flours is imperative, as they relate to both technological and physiological functionality. Subsequently, future studies should examine the effect(s) that processing methods have on a range of pulse types. These studies should consider how modifications in structure alters their utility in a range of food matrices (due to their physicochemical properties). This knowledge will allow food manufacturers to enhance processing methods and perhaps determine the most appropriate technologies to be used, depending on the food. As well, processing pulses into flours may alter the nutritional components (ie. protein content, SDS, RS, dietary fibre concentrations and micronutrients quantities). Therefore, analytical methods coupled with human *in vivo* trials are encouraged to gain a comprehensive understanding of how processing impacts different health outcomes. Designing the

human studies in accordance with HC draft guidelines for PPG and/or satiety claims will enhance consumer awareness about the benefits of pulse consumption.

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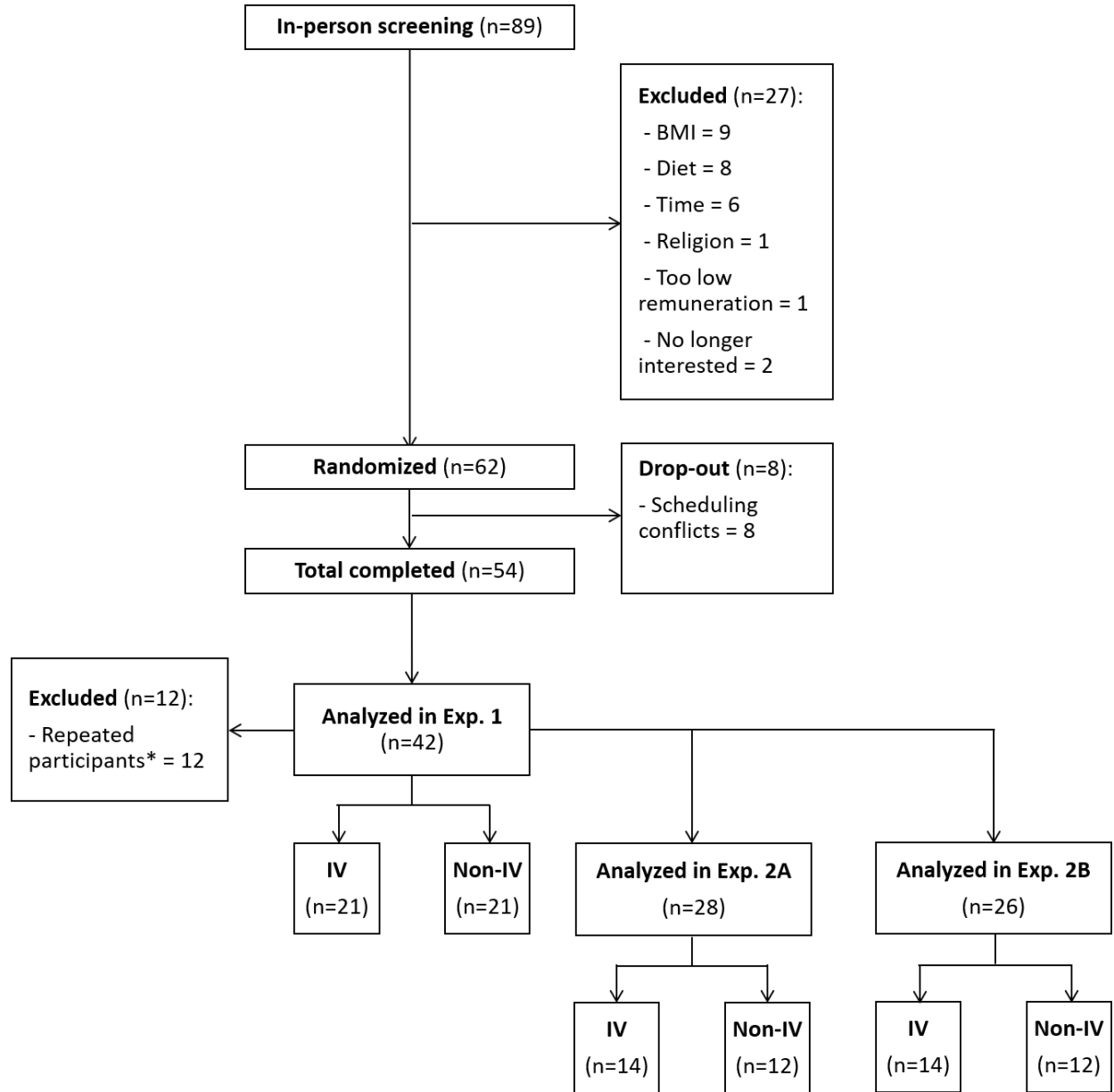
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Appendices

Appendix 1: CONSORT flow diagram



Appendix 2: Recruitment poster



Faba Bean Pasta Study

Department of Nutritional Sciences
University of Toronto

NOW RECRUITING!

Money compensation, travel expense, breakfast and lunch provided!

Looking for: Male (Age 20 – 30 yrs), non-smokers

Duration: 1 session per week for 5 weeks; Total = 5 sessions

If interested, please contact...

Email: fababeanpastastudy.utoronto@gmail.com Tel: 647-873-6720



Appendix 3: Recruitment poster with tabs



Faba Bean Pasta Study

NOW RECRUITING!

Money compensation, travel expense, breakfast and lunch provided!

Looking for: Male (Age 20 – 30 yrs), non-smokers

Duration: 1 session per week for 5 weeks; Total = 5 sessions

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647-873-6720

 fababeanpastastudy.utoronto@gmail.com
647-873-6720

Appendix 4: Study details email

Dear Name,

Thanks so much for contacting us and for your interest.

Below are details regarding the **Faba bean pasta study**. Please review carefully and let me know if this is something you are still interested in. We will proceed with a 30 min in-person screening conducted at the Fitzgerald building 150 College St. - Rm 333. If you have any questions regarding your eligibility, please do not hesitate to contact me.

Study Details:

Purpose: To investigate the effects of faba bean-fortified pasta on appetite, food intake and blood glucose and metabolic control.

Seeking 60 participants:

- male (age 20 - 30 yrs)
- healthy
- BMI: 18.5 - 24.9 kg/m² (Regular BMI)
- non-smoking
- no metabolic diseases
- regular breakfast consumer

Duration and session time:

- 1 session per week
- participants MUST be available for 5 weeks consecutively
- time per session = 3 h 20 min
- STUDY IS CONDUCTED ON WEEKENDS TOO!

Study layout: (breakfast and lunch provided)

- overnight fast: 10 - 12 hrs
- standard breakfast provided
- come in for the study at your selected date and time between 10am to 1pm
- consume pasta
- rest for two hours
- consume pizza lunch
- rest for one hour

Measurements taken:

- finger prick to measure blood glucose
- Optional: intravenous blood draw to measure peptides/hormones (small samples taken)

Scheduling:

Studies can take place during your availability. If you wish to do non-IV, the schedule is more lenient. If you wish to do IV, we must coordinate your schedule with that of the nurse availability.

Compensation (\$) for your time:

- \$40/session (finger prick only) + \$7 travel expense; Total = \$47 x 5 = \$235
- \$50/session (finger prick with IV) + \$7 travel expense; Total = \$57 x 5 = \$285

Location:

Fitzgerald building, Rm 305
University of Toronto
150 College St. 3rd floor

Thanks,
Catherine
Faba Bean Pasta Study
Department of Nutritional Sciences,
Dr. Harvey Anderson Lab
University of Toronto

Fitzgerald Building, 3rd Floor
150 College St
Toronto, Ontario. M5S 1A8

Appendix 5: Forms for recruitment

FORM – 3

Effects of faba bean fractions as ingredients in novel food products (pasta) on glycaemia, appetite and metabolic control

Information Sheet and Consent Form

Investigators:

Dr. G. Harvey Anderson, Principle Investigator
Department of Nutritional Sciences, University of Toronto

Dr. Hrvoje Fabek, Postdoctoral Fellow
Department of Nutritional Sciences, University of Toronto

Ms. Catherine Chan, MSc Candidate
Department of Nutritional Sciences, University of Toronto

Funding Source:

Funding for this project is provided by the Saskatchewan Pulse Growers.

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. 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.

The information obtained from this study may help us understand the potential of faba bean and its fractions as a value-added ingredient resulting in the control of appetite, food intake, blood glucose and in the prevention and management of obesity and type 2 diabetes. This study will have a total of 30 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 pasta meal five times (five sessions) one week apart. Four of the treatments will be pasta containing faba bean (FB) flour, FB protein concentrate, FB protein isolate, FB starch and one will be pasta (100% durum wheat) without FB flour, protein concentrate, protein isolate, or starch. A pizza lunch will also be provided 4 hours after eating treatments. Your appetite will be measured after eating the treatments. Each session will take up to 3.5 hours of your time.

Eligibility:

To participate in this study you must be a healthy male and between the ages of 20-30 years. You must be a nonsmoker and you cannot be taking any medications. You must also not be allergic to pulse products, such as lentils chickpeas and faba beans. The study will take place in the Department of Nutritional Sciences, rooms, 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 arrive at the FitzGerald Building (150 College Street, Toronto, M5S 3E2, third floor, room 334) between 10:00 a.m. and 1:00 pm after having breakfast. You will be asked to eat a standard breakfast (provided by us) at home on the day of the session following 10-12 hours of overnight fast (no eating for 10-12 hours before eating breakfast). The standard breakfast consisting of; 250 ml of 2% milk, 250 ml of Tropicana orange juice and cereal (honey nut cheerios), totaling 300kcal. 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.

Four hours after eating your standard breakfast (at each session), you will be asked to eat the pasta meal, give blood samples via finger prick and to complete questionnaires at the times outlined in the table.

At each session you will be asked to eat a pasta treatment 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 fill out visual analog scale (VAS) questionnaires measuring your appetite and physical comfort as well as the palatability (pleasantness) of the treatment and pizza meal. You will be served a pizza meal, either *ad libitum* or a fixed portion (12 kcal/kg body weight), 120 minutes after you eat the treatment.

Furthermore, you will be asked to provide a small drop of blood by finger prick 12 times during each session. Drawing blood at the selected time points poses no risk of injury and this is in conjunction with our earlier methods. Moreover, it is critical in allowing the research team to conduct the appropriate analyses. Blood will be sampled before eating the treatment (0 min) and then at 15, 30, 45, 60, 90 and 120 minutes after eating the treatment (pasta) meal and again at 140, 155, 170, 185 and 200 minutes after consumption of 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. Each session will last up to 3.5 hours of duration.

Time and Activity Schedule for Each Session; example

Time	Activity
8:00 a.m.	Consumption of standard breakfast at home

11:45 a.m.	Arrive at the laboratory
11:45 – 12:00 noon	Fill in questionnaires and collect baseline blood samples (0 minute)
12:00 - 12:10 p.m.	Eat the pasta meal (10 min).
12:15 – 2:15 p.m.	Fill in questionnaires and collect blood samples at 12:15pm, 12:30 pm, 12:45 pm, 1:00 pm, 1:15 pm, 1:45 pm, 2:15 pm
2:15 p.m. – 2:35 p.m.	Eat the pizza meal (20 min)
2:35 p.m. – 3:40 p.m.	Fill in questionnaires and collect blood samples at 2:35 pm, 2:50 pm, 3:05 pm, 3:20 pm and 3:35 pm
3:40 p.m.	End of session

* Notice: This is just an example. You can choose the breakfast time between 7.00 a.m. and 10.00 a.m.

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 any time without any consequences to you and you will be paid for sessions completed.

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 sample. The investigator will help you. At times there may be multiple sessions being run simultaneously and in order to make sure that you are not exposed to another person's study belongings, we will ask you to sit away from other study participants. We will be collecting your fasting finger prick blood samples by using disposable lancets. We will swab your finger with alcohol before and after each finger prick and will use a new sterile lancet each time.

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 fasting blood sugar results and if they are not normal you will be told and advised to talk to your doctor or you may refer to the Health and Wellness Centre located at the University of Toronto campus. 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 and this will be communicated to you by the e-mail provided upon signing the consent form.

Compensation:

You will be paid \$40 per session. You will also be given \$7 per session for travel (bus, subway). If you withdraw from the study before finishing or you are 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, or if you do not have one then we can relay information about the food you have consumed during the session to the Health and Wellness Centre at the University of Toronto, so take our phone number with you in order to ensure proper care is being provided.

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. Alternatively, we may also mail/e-mail it to you, or post it online upon request.

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.

Participant Name

Signature

Date

Witness Name

Signature

Date

Investigator Name

Signature

Date

FORM – 4
Effects of faba bean fractions as ingredients in novel food products (pasta) on glycaemia, appetite and metabolic control

Information Sheet and Consent Form

Investigators:

Dr. G. Harvey Anderson, Principle Investigator
Department of Nutritional Sciences, University of Toronto

Dr. Hrvoje Fabek, Postdoctoral Fellow
Department of Nutritional Sciences, University of Toronto

Ms. Catherine Chan, MSc Candidate
Department of Nutritional Sciences, University of Toronto

Funding Source:

Funding for this project is provided by the Saskatchewan Pulse Growers.

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. 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.

The information obtained from this study may help us understand the potential of faba bean and its fractions as a value added ingredient resulting in the control of appetite, food intake, blood glucose and in the prevention and management of obesity and type 2 diabetes. This study will have a total of 30 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 pasta meal five times (five sessions) one week apart. Four of the treatments will be pasta containing faba bean (FB) flour, FB protein concentrate, FB protein isolate, FB starch and one will be pasta (100% durum wheat) without FB flour, protein concentrate, protein isolate, or starch. A pizza lunch will also be provided 4 hours after eating treatments. Your appetite will be measured after eating the treatments. Each session will take up to 3.5 hours of your time.

Eligibility:

To participate in this study you must be a healthy male and between the ages of 20-30 years. You must be a nonsmoker and you cannot be taking any medications. You must also not be allergic to pulse products, such as lentils chickpeas and faba beans. The study will take place in the Department of Nutritional Sciences, rooms, 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 arrive at the FitzGerald Building (150 College Street, Toronto, M5S 3E2, third floor, room 334) between 10:00 a.m. and 1:00 pm after having breakfast. You will be asked to eat a standard breakfast (provided by us) at home on the day of the session following 10-12 hours of overnight fast (no eating for 10-12 hours before eating breakfast). The standard breakfast consisting of; 250 ml of 2% milk, 250 ml of Tropicana orange juice and cereal (honey nut cheerios), totaling 300kcal. 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.

Four hours after eating your standard breakfast (at each session), you will be asked to eat the pasta meal, give blood samples via finger prick and intravenous catheter (conducted by a registered nurse) and to complete questionnaires at the times outlined in the table.

At each session you will be asked to eat a pasta treatment 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 fill out visual analog scale (VAS) questionnaires measuring your appetite and physical comfort as well as the palatability (pleasantness) of the treatment and pizza meal. You will be served a pizza meal, either *ad libitum* or a fixed portion (12 kcal/kg body weight), 120 minutes after you eat the treatment.

Furthermore, you will be asked to provide a small drop of blood by finger prick 12 times during each session. Drawing blood at 12 time points poses no risk of injury and this is in conjunction with our earlier methods. Moreover, it is critical in allowing the research team to conduct the appropriate analyses. Blood will be sampled before eating the treatment (0 min) and then at 15, 30, 45, 60, 90 and 120 minutes after eating the treatment meal and again at 140, 155, 170, 185 and 200 minutes after consumption of the treatment. Also, at the start of each session, an indwelling intravenous catheter will be inserted in the antecubital vein by a registered nurse to take a sample of venous blood before eating the treatment (0 min) and then at 30, 60 and 120 minutes after eating the treatment meal and again at 140 and 200 minutes after consumption of the treatment. Approximately 54 mL of venous blood will be sampled at each experimental session

(8.5 mL per sample, plus approximately 0.5mL for flushing the line) for a total of 270 mL over the course of the 5 sessions (see measurement timings below). Blood samples will be analyzed for insulin, GLP-1, CCK, PYY and Ghrelin hormones. 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. Each session will last up to 3.5 hours of duration.

Time and Activity Schedule for Each Session; example

Time	Activity
8:00 a.m.	Consumption of standard breakfast at home
11:45 a.m.	Arrive at the laboratory
11:45 – 12:00 noon	Fill in questionnaires and collect baseline blood samples (0 minute)
12:00 - 12:10 p.m.	Eat the pasta meal (10 min).
12: 10 – 2:10 p.m.	Fill in questionnaires and collect blood samples at 12:20 pm, 12:35pm, 12:50pm, 1:05 pm, 1:35 pm and 2:05 pm.
2:10 p.m. – 2:30 p.m.	Eat the pizza meal (20 min)
2:30 p.m. – 3:30 p.m.	Fill in questionnaires and collect blood samples at 2:25 pm, 2:40 pm, 2:55 pm, 3:10 pm and 3:25 pm
3:35 p.m.	End of session

* Notice: This is just an example. You can choose the breakfast time between 7.00 a.m. and 10.00 a.m.

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 and you will be paid for sessions completed.

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 sample. The investigator will help you. At times there may be multiple sessions being run simultaneously and in order to make sure that you are not exposed to another person's study belongings, we will ask you to sit away from other study participants. We will be collecting your fasting finger prick blood samples by using disposable lancets. We will swab your finger with alcohol before and after each finger prick and will use a new sterile lancet 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.

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 fasting blood sugar results and if they are not normal you will be told and advised to talk to your doctor or you may refer to the Health and Wellness Centre located at the University of Toronto campus. 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 and this will be communicated to you by the e-mail provided upon signing the consent form.

Compensation:

You will be paid \$50 per session. You will also be given \$7 per session for travel (bus, subway). If you withdraw from the study before finishing or you are asked to withdraw, you will be paid for the sessions you have already finished.

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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, or if you do not have one then we can relay information about the food you have consumed during the session to the Health and Wellness Centre at the University of Toronto, so take our phone number with you in order to ensure proper care is being provided.

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Dissemination of findings:

A summary of results will be made available to you to pick up after the study is done. Alternatively, we may also mail/e-mail it to you, or post it online upon request.

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.

Participant Name

Signature

Date

Witness Name

Signature

Date

Investigator Name

Signature

Date

Investigator Name

Signature

Date

FORM - 5
BASELINE INFORMATION 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:

PHONE #: _____

E-MAIL:

HEIGHT: _____

WEIGHT: _____

BMI:

Participation in Athletics/Exercise:

ACTIVITY

HOW OFTEN?

HOW LONG? (HOURS)

ACTIVITY	HOW OFTEN?	HOW LONG? (HOURS)
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

Do you usually eat breakfast?

YES

NO

If YES, what do you usually eat?

Health Status:

Do you have diabetes?

YES

NO

Do you have any other major disease or condition?

YES

NO

If YES, please specify:

Are you taking any medications?

YES

NO

If YES, please specify: _____

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 (in the past 6 months) lost or gained weight?

YES

NO

If YES, please specify: _____

FORM - 6

Favism Questionnaire

(NOTE: Some studies have shown that a small percentage of people, primarily of Mediterranean, the Middle East, Southeast Asia, and North African descent, may have an enzyme deficiency that could cause symptoms, such as diarrhea, fatigue, back pain, and anemia, following faba bean consumption. In order to ensure your compliance with this study please answer the following questions)

Do you have any countries other than Canada in your heritage? YES NO

If YES, please list the countries (as far back as you are aware of) _____

Are you a regular consumer of pulses (lentils, chickpeas, faba beans, etc.)? YES NO

If YES, how often would you say you eat them in a month? _____

Are you currently taking acetaminophen, ibuprofen or aspirin? YES NO

If YES, are there any known side effects? _____

Have you ever been diagnosed with jaundice (yellowing of the skin or eyes)? YES NO

Have you had a fever in the last month (body temperature > 37°C)? YES NO

Have you noticed changes in your urine in the past 6 months (darker than usual)? YES NO

FORM - 7
Eating Habits Questionnaire

Choose the appropriate answer to best describe your personal situation.

1. How often are you dieting?

Never ____ rarely ____ sometimes ____ often ____ always ____

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

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

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

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

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

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

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

Not at all ____ slightly ____ moderately ____ very much ____

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

Never ____ rarely ____ often ____ always ____

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

Never ____ rarely ____ often ____ always ____

8. Do you have feelings of guilt after overeating?

Never ____ rarely ____ often ____ always ____

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

Not at all ____ slightly ____ moderately ____ extremely ____

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

0-1 ____ 2 - 5 ____ 6 - 10 ____ 11 - 20 ____ 21+ ____

FORM - 8
FOOD ACCEPTABILITY QUESTIONNAIRE

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).

	Enjoyment?	How often?
Milk (2%)	_____	_____
Orange juice (Tropicana)	_____	_____
Cereal	_____	_____
Faba bean (pulse)	_____	_____
Cheese pizza	_____	_____

At the end of each session, you will be provided with pizza.

Are you willing to eat three-cheese pizza only (Y/N) _____

Appendix 6: Baseline forms for study session

ID: _____
 DATE: _____
 SESSION: _____

**FORM - 9
 SLEEP HABITS AND STRESS FACTORS QUESTIONNAIRE**

Did you have a normal night's sleep last night?

YES NO

How many hours of sleep did you have?

At what time did you go to bed last night?

At what time did you wake up this morning?

Recount your activity since waking up:

TIME	ACTIVITY
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

Are you experiencing any feelings of illness or discomfort other than those from hunger?

Today: YES NO
 Past 24 hrs: YES NO

If yes, please describe briefly:

Are you under any unusual stress? (i.e. exams, reports, work deadlines, personal)

Today: YES NO
 Past 24 hrs: YES NO

If yes, please describe briefly:

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

YES NO

If yes, please describe briefly:

Have you had anything to eat or drink other than water for the past 11 hours?

YES NO

If yes, please describe briefly:

ID: _____
DATE: _____
SESSION: _____

FORM - 10
RECENT FOOD INTAKE AND ACTIVITY QUESTIONNAIRE

At 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 _____ than usual

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

Much LESS _____ Much MORE
than usual _____ than usual

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

Much LESS _____ Much MORE
than usual _____ than usual

Appendix 7: Visual analog scales

ID: _____
DATE: _____
SESSION: _____

FORM - 11

VISUAL ANALOGUE SCALES

PALATABILITY: TREATMENT

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 _____ VERY
at all _____ pleasant
pleasant

2. How tasty have you found the treatment?

NOT _____ VERY
at all _____ tasty
tasty

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

NOT _____ VERY
at all _____ much

ID: _____
DATE: _____
SESSION: _____

FORM - 12

VISUAL ANALOGUE SCALES

MOTIVATION TO EAT

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.

How strong is your desire to eat?

VERY weak _____ VERY strong

How hungry do you feel?

NOT hungry at all _____ As hungry as I have ever felt

How full do you feel?

NOT full at all _____ VERY full

How much food do you think you could eat?

NOTHING at all _____ A LARGE amount

How thirsty do you feel?

NOT thirsty at all _____ As thirsty as I have ever felt

ID: _____
DATE: _____
SESSION: _____

FORM - 13

VISUAL ANALOGUE SCALES

ENERGY AND FATIGUE

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.

How energetic do you feel right now?

NOT _____ VERY
at all energetic

How tired do you feel right now?

NOT _____ VERY
at all tired

ID: _____
DATE: _____
SESSION: _____

FORM - 14

VISUAL ANALOGUE SCALES

PHYSICAL COMFORT

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.

Do you feel nauseous?

NOT at all _____ VERY much

Does your stomach hurt?

NOT at all _____ VERY much

How well do you feel?

NOT well at all _____ VERY well

Do you feel like you have gas?

NOT at all _____ VERY much

Do you feel like you have diarrhoea?

NOT at all _____ VERY much

ID: _____
DATE: _____
SESSION: _____

FORM - 15

VISUAL ANALOGUE SCALES

PALATABILITY: PIZZA MEAL

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 pizza meal?

NOT _____ VERY
at all _____ pleasant
pleasant

2. How tasty have you found the pizza?

NOT _____ VERY
at all _____ tasty
tasty

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

NOT _____ VERY
at all _____ much

Appendix 8: Compliance to Health Canada's draft guidance document for satiety

Study design and considerations	It is recommended that the effect of the food be tested at least twice, before and at the end of the study period (5.2.1)	x
	Study participants should be time-blinded (5.2.9)	x
	It is recommended that antecedent levels of energy depletion and physical activity experienced by the study subjects be standardized prior to testing (5.2.10)	✓
	The study population should be adult individuals who are generally healthy (5.4.1)	✓
	Data on the measurements of satiety biomarkers (for example, gut hormones) can only be considered as supportive evidence (5.5.1)	✓
	It is recommended, whenever possible, to use a mean score comprised of all VAS scales used in a study (5.5.2)	✓
	The duration of an effect of foods on subjective satiety measures should be at least 3 hours for a snack, or 4 hours for a meal (5.5.4)	x
Characterization of test and reference foods	Include at least 3 experimental preloads: test food, control food or reference food, energy-free control (5.2.2)	✓x
	Amount of food tested and the reference food should match the serving size as stated in the Nutrition Facts table (5.3.1)	✓
	If the effect of the food tested is attributed to a specific added component, then this component should be identified (5.3.2)	✓
	The test food should be of equal or lower, but never of higher energy content (per serving) than the reference food (5.3.3)	✓
	The energy-free control, water, should match the test food in organoleptic characteristics only when the latter is in liquid form. For solid foods, the energy-free control preload could be plain water (5.3.4)	✓
Statistical analysis	The basis for sample size calculations should be the ability to detect at least 10% difference in satiety rating, with a statistical significance at $p < 0.05$ and a power of at least 80% (5.6.1)	✓
	Assessment of the satiety response should be done based on the total area under the curve (AUC) (5.6.4)	✓

Appendix 9: Compliance to Health Canada's Draft Guidance Document for the reduction in postprandial glycaemia

Study design and considerations	The study population should be adult individuals who are generally healthy (4.2.1)	✓
	Measurements should be taken for at least 2 hours, with higher frequency (ex: at 15 min intervals) in the first hour, and 30 minutes thereafter (4.3.3)	✓
Characterization of test and reference foods	The test food must be in the same food category or serve a similar dietary role as the reference food with equal or lower amounts of carbohydrate per serving (3.4)	✓
	The amounts of reference and test food given in the study must be consistent with its serving size and intended pattern of consumption (4.1.2)	✓
	The food should be given as usually prepared because of the effects that factors such as cooking, physical form (whole versus puréed) and particle size of food can have on the glycaemic response (4.1.2)	✓
Statistical analysis	Data on insulin concentrations following the consumption of the test food should be provided to show that the decrease in blood glucose concentrations is not accompanied by disproportionately increased levels of insulin, in comparison to the reference food (4.3.4)	✓
	The glycaemic and insulinemic responses should be measured as the incremental area under the response curves (iAUC) (4.3.5)	✓

Appendix 10: Subject characteristics for Experiment 1

#	Age (y)	Height (cm)	Weight (kg)	BMI ¹ (kg/m ²)
1	24	176	73.9	23.86
2	25	170.7	60.2	20.66
3	23	179.5	77	23.90
4	22	179.5	69.1	21.45
5	25	172	65.9	22.28
6	23	175.6	67.1	21.76
7	20	165.2	52	19.05
8	24	167.4	59.1	21.09
9	22	182	81.4	24.57
10	22	179.2	76.7	23.88
11	21	171.5	62.6	21.28
12	27	185	72.1	21.07
13	20	167	64.4	23.09
14	25	187	76.4	21.85
15	22	173	63.1	21.08
16	22	181	65.1	19.87
17	21	173	61.9	20.68
18	22	179.5	60.1	18.65
19	24	171	60.1	20.55
20	23	174	73.5	24.28
21	22	175	69.2	22.60
22	21	181.3	69.1	21.02
23	23	185	84.8	24.78
24	20	167.3	62.1	22.19
25	21	176.8	77.1	24.67
26	23	175	65.6	21.42
27	23	176	74.2	23.95
28	27	180	77.8	24.01
29	23	166.5	69	24.89
30	28	170.4	67	23.07
31	29	172	65.1	22.01
32	22	169	62.5	21.88
33	23	183.4	77.9	23.16
34	30	173	69.7	23.29
35	22	176.5	75.2	24.14
36	24	188.5	69.4	19.53
37	24	165	54.4	19.98
38	28	176	65.7	21.21
39	26	174	64.3	21.24
40	24	174.6	71.1	23.32
41	22	182.5	70.6	21.2
42	20	172	72.1	24.37
Average	23.38	175.45	68.47	22.21
SEM	0.38	0.93	1.10	0.26

¹ BMI, body mass index

Appendix 11: Subject characteristics for Experiment 1; post-treatment¹, IV only²

#	Age (y)	Height (cm)	Weight (kg)	BMI ³ (kg/m ²)
1	24	176	73.9	23.86
2	25	170.7	60.2	20.66
3	23	179.5	77	23.90
4	22	179.5	69.1	21.45
5	25	172	65.9	22.28
6	23	175.6	67.1	21.76
7	20	165.2	52	19.05
8	24	167.4	59.1	21.09
9	22	182	81.4	24.57
10	22	179.2	76.7	23.88
11	21	171.5	62.6	21.28
12	27	185	72.1	21.07
13	20	167	64.4	23.09
14	25	187	76.4	21.85
15	22	173	63.1	21.08
16	22	181	65.1	19.87
17	21	173	61.9	20.68
18	22	179.5	60.1	18.65
19	24	171	60.1	20.55
20	23	174	73.5	24.28
21	22	175	69.2	22.60
Average	22.81	175.43	67.19	21.79
SEM	0.39	1.28	1.63	0.37

¹ Post-treatment measures taken from 0-120 min of each study session

² IV, Intravenous; this list only includes participants who provided intravenous blood samples for gut peptide and hormone measures

³ BMI, body mass index

Appendix 12: Subject characteristics for Experiment 2A; *ad libitum*¹

#	Age (y)	Height (cm)	Weight (kg)	BMI ² (kg/m ²)
1	24	176	73.9	23.86
2	25	170.7	60.2	20.66
3	23	179.5	77	23.90
4	22	179.5	69.1	21.45
5	25	172	65.9	22.28
6	23	175.6	67.1	21.76
7	20	165.2	52	19.05
8	24	167.4	59.1	21.09
9	22	182	81.4	24.57
10	22	179.2	76.7	23.88
11	21	171.5	62.6	21.28
12	27	185	72.1	21.07
13	20	167	64.4	23.09
14	25	187	76.4	21.85
15	21	181.3	69.1	21.02
16	23	185	84.8	24.78
17	20	167.3	62.1	22.19
18	21	176.8	77.1	24.67
19	23	175	65.6	21.42
20	23	176	74.2	23.95
21	27	180	77.8	24.01
22	23	166.5	69	24.89
23	28	170.4	67	23.07
24	29	172	65.1	22.01
25	22	169	62.5	21.88
26	23	183.4	77.9	23.16
27	30	173	69.7	23.29
28	22	176.5	75.2	24.14
Average	23.50	175.35	69.82	22.65
SEM	0.51	1.18	1.43	0.28

¹*Ad libitum* pizza meal served at 120 min into the study session for all listed participants

²BMI, body mass index

Appendix 13: Subject characteristics for Experiment 2A; *ad libitum*¹, IV only²

#	Age (y)	Height (cm)	Weight (kg)	BMI ³ (kg/m ²)
1	24	176	73.9	23.86
2	25	170.7	60.2	20.66
3	23	179.5	77	23.90
4	22	179.5	69.1	21.45
5	25	172	65.9	22.28
6	23	175.6	67.1	21.76
7	20	165.2	52	19.05
8	24	167.4	59.1	21.09
9	22	182	81.4	24.57
10	22	179.2	76.7	23.88
11	21	171.5	62.6	21.28
12	27	185	72.1	21.07
13	20	167	64.4	23.09
14	25	187	76.4	21.85
Average	23.07	175.54	68.42	22.13
SEM	0.55	1.82	2.22	0.41

¹*Ad libitum* pizza meal served at 120 min into the study session for all listed participants

²IV, Intravenous; this list only includes participants who provided intravenous blood samples for gut peptide and hormone measures

³BMI, body mass index

Appendix 14: Subject characteristics for Experiment 2B; fixed meal¹

#	Age (y)	Height (cm)	Weight (kg)	BMI ² (kg/m ²)
1	25	171	67.2	22.98
2	20	167.5	65.8	23.45
3	22	173	63.1	21.08
4	22	181	65.1	19.87
5	21	173	61.9	20.68
6	22	179.5	60.1	18.65
7	24	171	60.1	20.55
8	23	174	73.5	24.28
9	22	174.4	73.7	24.23
10	23	171	68.6	23.46
11	22	175	69.2	22.60
12	25	177.8	74.8	23.66
13	21	182	67.2	20.29
14	25	171.5	60	20.40
15	20	157	59	23.94
16	22	169	62.2	21.78
17	30	175	70.1	22.89
18	24	175	71	23.18
19	24	188.5	69.4	19.53
20	24	165	54.4	19.98
21	28	176	65.7	21.21
22	26	174	64.3	21.24
23	24	174.6	71.1	23.32
24	22	182.5	70.6	21.2
25	20	172	72.1	24.37
26	23	178	78.9	24.90
Average	23.23	174.17	66.89	22.07
SEM	0.47	1.20	1.13	0.35

¹Fixed quantity pizza meal served at 120 min into the study session for all listed participants

²BMI, body mass index

Appendix 15: Subject characteristics for Experiment 2B; fixed meal¹, IV only²

#	Age (y)	Height (cm)	Weight (kg)	BMI ³ (kg/m ²)
1	25	171	67.2	22.98
2	20	167.5	65.8	23.45
3	22	173	63.1	21.08
4	22	181	65.1	19.87
5	21	173	61.9	20.68
6	22	179.5	60.1	18.65
7	24	171	60.1	20.55
8	23	174	73.5	24.28
9	22	174.4	73.7	24.23
10	23	171	68.6	23.46
11	22	175	69.2	22.60
12	25	177.8	74.8	23.66
Average	22.58	174.02	66.93	22.13
SEM	0.43	1.12	1.50	0.54

¹ Fixed quantity pizza meal served at 120 min into the study session for all listed participants

² IV, Intravenous; this list only includes participants who provided intravenous blood samples for gut peptide and hormone measures

³ BMI, body mass index

Appendix 16: Concentrations of starch and its fractions in faba bean pasta

Sample	Starch ¹	Amylose*	TDF*	RDS*	RS*	SDS*
DWS ²	73.3±1.8	32.9±0.4	4.6±0.0	81.2±0.3	1.8±0.1	17.1±0.4
FBF	62.9±3.4	31.8±0.4	6.1±0.2	63.3±1.2	4.3±2.3	32.4±3.5
FBS	69.6±0.4	31.4±0.4	5.1±0.1	64.5±0.7	8.8±1.1	26.7±0.6
FBPC	56.9±0.9	30.4±0.2	6.7±0.0	70.6±4.3	3.9±3.8	25.5±0.5
FBPI	52.2±1.1	32.8±0.1	5.6±0.0	63.8±0.4	2.4±0.2	33.8±0.5

¹DWS, 100% durum wheat semolina pasta;

FBF, 25% faba bean flour pasta;

FBS, 25% faba bean high starch pasta;

FBPC, 25% faba bean protein concentrate pasta;

FBPI, 25% faba bean protein isolate pasta

²RDS, SDS and RS stand for readily digestible-, slowly digestible-, and resistant- starch whereas TDF represents total dietary fibre.

Starch and TDF are presented as % of seed meal whereas all others are presented as % of total starch in the seed meal

³Data was collected from University of Saskatchewan