

The Impact of Soluble Dietary Fibre on Blood Glucose, Insulin
and Gut Hormones in Obese Human Subjects

by

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ABSTRACT

The incidence of obesity and type 2 diabetes mellitus (T2D) has been on the rise and the evidence suggests that soluble dietary fibres may be helpful for obesity management and T2D prevention. Polyglycoplex (PGX), a novel, viscous polysaccharide, has been shown in several studies to promote weight loss and exert positive effects on postprandial glycemia and satiety hormone secretions. Furthermore, recent evidence suggests a role of dietary fibres in appetite glycemetic control, which may be partly mediated by gut hormones such as glucagon-like peptide-1 (GLP-1), gastric inhibitory polypeptide (GIP), amylin, leptin, ghrelin, peptide YY (PYY) and pancreatic polypeptide (PP). These substances may aid weight loss and the maintenance of a constant plasma insulin level, which is important for obese and T2D patients.

Healthy, overweight individuals (Body Mass Index (BMI) > 27 kg/m²) were recruited for this study. Participants were randomized for 13 weeks to receive supplements, containing either 750 mg of PGX (Treatment group, n=76) or 750 mg of cellulose (Placebo group, n=72). The participants were asked to take six capsules with water before each meal. At baseline (week 0) and at week 13 of the study, after 12 hours of overnight fasting, participants consumed a test meal. Participants did not consume

PGX or placebo capsules prior to breakfast on the days of a meal tolerance test. Blood samples were collected at time 0, and at 15, 30, 45, 60, 120, and 240 min after the test meal. Although previous studies carried out on the granular form of PGX suggest that it may promote acute effects of weight loss and reductions in postprandial glycemia, in this specific study, capsulated PGX did not show a statistically significant effect on body weight, BMI, blood glucose, insulin, or other hormones, in comparison with placebo. Nevertheless, PGX had statistically significant improvements for GIP and showed some positive trends in changes of leptin over ghrelin through the cross-sectional analysis. A recent study showed that PGX capsules may be more suitable for maintaining body weight and blood glucose levels due to its delayed effects and convenience.

PREFACE

Certificates of Approval

The research involving human subjects that is reported in this thesis was performed with ethics approval from University of British Columbia Clinical Research Ethics Board (Certificate # H06-03930).

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LIST OF ABBREVIATIONS

AUC	Area Under the Curve
BMI	Body Mass Index
CHD	Coronary Heart Disease
CVD	Cardiovascular Disease
EDTA	EthyleneDiamineTetraacetic Acid
FDA	Food and Dug Administrations
GIP	Gastric Inhibitory Peptide (or Glucose-dependent insulintropic peptide)
GLP-1	Glucagon-Like Peptide-1
HDL	High Density Lipoprotein
IQR	InterQuartile Range
LCT	Long Chain Triglycerides
LDL	Low Density Lipoprtoein
MCT	Medium Chain Triglycerides
PGX	Polyglycoplex
PP	Pancreatic Polypeptide
PYY	Peptide YY
SCFA	Short Chain Fatty Acids
T2D	Type 2 Diabetes Mellitus

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CHAPTER 1: GENERAL INTRODUCTION

In the past four decades, evidence of the beneficial effects of dietary fibres in chronic diseases such as diabetes, heart diseases, and certain type of cancer has accumulated (Zhang et al., 2006; Lopez et al., 2007; Bingham et al., 2003). Reports of various government agencies have indicated a great interest in the specific effects of dietary fibres on general health. The Nutrition Labeling and Education Act (NLEA) of 1990, providing rules regarding health claims used on labels, has characterized the relationship between food, food components, dietary ingredients or dietary supplements, and risk of diseases (Food and Drug Administration, 1998). The adequate intake of total dietary fibre for children, adolescents, and adults was set to 14 g dietary fibre / 1000 Kcal, by the U.S. Department of Agriculture (USDA, 2005). In many developing countries, fibre is recognized as a shortfall nutrient that is low in the daily diet, whereas, a majority of Western populations do not meet the recommended intake of dietary fibres. Further, epidemiological and intervention studies suggest that the intake of certain fibres may delay glucose uptake and attenuate insulin response (Murakami et al., 2007). Also, dietary fibres are effective in intestinal regularity. The gastrointestinal tract is highly sensitive to dietary fibre, and the consumption of fibre seems to relieve and prevent constipation (Marlett et al., 2002). Dietary fibres have a number of important

health benefits including promoting healthy laxation, reducing the risk of T2D, and decreasing the risk of coronary heart diseases (U.S. Department of Agriculture, 2004).

Both observational and clinical studies suggest that the intake of certain fibres may be useful in controlling body weight (Lindstrom et al., 2006). The 2005 dietary guidelines for Americans (USDA) stated that a higher fibre content of food, particularly whole grains, may help individuals feel full with less calories (USDA, 2005). In the report defining the term high fibre diets were said to delay stomach emptying, which increases the time for energy and nutrients to be absorbed from the digestive tract. Additionally, several important review articles provide direct support for high fibre intake and satiety/weight control. According to the IOM (2002) report, even though the finding that the overall data on dietary fibre intake is negatively correlated with BMI is suggestive of a role of fibre in weight control, the studies designed to determine how fibre intake might influence overall energy intake have not shown a major effect (Lindstrom et al., 2006). Epidemiological studies indicate that dietary fibre intake may also protect against the development of obesity. In studies including more than 100,000 subjects and four prospective cohort studies, also including 100,000 subjects, a positive association was found between fibre intake and obesity (Anderson, 2008).

As mentioned above, fibre intake tends to delay gastric emptying and create a sense of fullness, since increased fibre intake is associated with an increase in the satiating gut hormones. PGX (PolyGlycopleX; α -D-glucurono- α -manno- β -D-manno- β -D-gluco; β -L-gulurono- β -D-mannurono; β -D-gluco- β -D-mannan; Inovobiologic Inc., Calgary, Canada) is a unique, high-viscosity functional fibre that can be used in functional foods, and dietary and health supplements (Abdelhameed et al., 2010; Harding, et al., 2010). The efficacy of PGX has been the subject of over a dozen human clinical studies conducted on a number of subjects over several weeks in Canada, Australia, and France. PGX has also been clinically proven to correct appetite and helpful for losing weight, lowering cholesterol, and controlling and balancing blood sugar levels (Reimer et al., 2010; Lyon, & Reichert, 2010). In contrast to other fibres, PGX holds its highly viscous properties despite the influence of stomach acid and digestive enzymes. Viscosity is an important factor for the dietary fibre (Lyon, & Reichert, 2010), but the PGX viscosity develops slowly after mixing with matrices (e.g., water, food, or other liquids), with maximum viscosity reached after 60 to 90 min, since rapid increase in viscosity could be unpalatable and possibly resulting in esophageal obstruction. Also, dietary fibre that develops viscosity in the gastrointestinal tract could be capable of addressing various aspects of food intake control. PGX, in addition to

different food matrices is also highly effective in lowering the glycemic index of a food in a dose-responsive manner (Lyon, & Reichert, 2010). Although a variety of dietary fibres have been shown to alter satiety hormone gene expression and secretion, in a randomized, double-blind, placebo-controlled 21-day clinical study, PGX revealed a reasonable efficacy in increasing fasting PYY levels, and significantly decreased fasting ghrelin and insulin levels (Reimer et al., 2010). PGX has been proven safe in a human tolerance study (54 subjects) approved by French Health Authorities and in another genotoxicity study showing the safety of PGX (Marone et al., 2009). PGX is safe to use, without any serious side-effects, and was recognized to be Self-Affirmed GRAS (Generally Recognized As Safe) and a Self-Affirmed Medical Food GRAS.

CHAPTER 2: HYPOTHESIS

The relationship of fiber intake and its effects on the metabolism is quite complex, which may explain why many of the fiber-health studies do not show clear relationships between fiber intake and the outcomes, such as in prevention of type-2 diabetes, obesity, and weight loss. In a number of clinical trials evaluating the effect of PGX consumption against placebo (cellulose), both fasting and postprandial blood glucose were reduced. A few studies demonstrated the promotion of satiety and insulin secretion through increasing plasma concentrations of GLP-1, PYY, PP, and leptin, by suppressing GIP, ghrelin, and amylin levels. Consequently, the hypothesized relational effect of PGX consumption on weight loss or obesity is of high interest. Thus, it is hypothesized that that prototypes PGX with high viscosity and water –holding capacity in the capsulated form would result a significant outcomes in the prevention of weight loss or obesity under the prescribed dose (i.e. 750 mg PGX /dose ; maximum 6 capsules/day) selection and trial parameters. It is also hypothesized that prototype capsule containing PGX with MCT would result somewhat significant results compared to the granular form of the PGX studied in number of previous studies.

CHAPTER 3: OBJECTIVES

Since the majority of evidence from PGX-related clinical trials partially supports the hypothesis that PGX consumption aids in weight loss or obesity, an objective of this study is to further investigate the effects of PGX on weight loss and levels of postprandial glucose, insulin, and especially gut peptides/hormones in overweight subjects in a randomized, double-blind 13-week clinical trial. An additional objective of this study is to determine the relative effect of the capsule form of PGX with MCT treatment, in comparison to the granular form. The outcome of this trial may be helpful in preventing and managing overweight syndromes and controlling postprandial blood glucose levels.

CHAPTER 4: LITERATURE REVIEW

4.1 Obesity

Obesity is a disease caused by an excess accumulation of body fat which may adversely affect health outcomes (Bray, 2007). Within the last few decades, obesity has become a global health issue affecting millions of people worldwide (Hedley, 2004). In 2005, 400 million adults were estimated to be obese (World Health Organization, 2006). Moreover, obesity, once thought to be a problem only in Western countries, is now becoming a problem in emerging and developing countries (Wang & Lobstein, 2006). The percentage of Canadians who are overweight or obese has increased substantially in recent years. According to the 2004 Canadian Community Health Survey (CCHS), 23.1% of Canadians aged 18 or older (an estimated 5.5 million adults) were obese. The percentages of 25 to 34-year-olds that were obese more than doubled, rising from 8.5% in 1978/79 to 20.5% in 2004 (Statistics Canada, 2010). The most dramatic increases were among people younger than 35 and 75 or older. Body Mass Index (BMI) is often used to define obesity, which is calculated as weight/height^2 (Kg/m²). Generally, BMI groups are categorized as <18.5 (underweight), 18.5-24.9 (normal), 25.0-29.9 (overweight), 30.0-34.9 (obesity class 1), 35.0-39.9 (obesity class 2) and 40 (obesity

class 3) (Bray, 2007). Obesity may also be measured by waist circumference and body composition, using several different analytical techniques.

4.1.1 Obesity: prevalence and causes.

Obesity is associated with increased mortality from cardiovascular disease (CVD), kidney failure, diabetes, and different types of cancer. According to Statistics Canada (2004), obesity rates (BMI > 30 Kg/m²) in Canada have risen from 14% in 1978 to over 23% in 2004. In the US, obesity is estimated to cost the health care system \$117 billion annually (U.S. Department of Health and Human Services, 2001). The exact cause of obesity is not known, though it is likely an interplay of genetic and environmental factors. The key environmental factors include: excess energy intake and/or decreased activity (Bray, 2007).

In general, rising rates of obesity have been related to increased consumption of sugar formulated beverages and energy dense foods. Usually, the food choices by obese people are defined in terms of abnormal activities in biology, behavior, and physiology (Drewnowski, 1998), where the cravings for obesogenic foods are driven by central metabolic events. Some examples of these include an imbalance of serotonin and dopamine, increased leptin levels, and the functioning of the endogenous opiate peptide system.

4.1.2 Health risks of obesity.

Obesity places a large burden on the healthcare system and on society, with cost estimates of between 5.3-7.0% of the annual medical expenditures of the health budget in the US and Canada (Bray, 2007). Age-standardized results indicate that 29.7% of Americans aged 18 or older were obese in 1999-2002, which is significantly higher than the 2004 figure for Canada (23.1%). Most of the difference was attributable to the situation among women. While 23.2% of Canadian women were obese, the figure for American women was 32.6% (Wang, & Lobstein, 2006). Somewhat similar trends have been observed worldwide. Generally, overweight people are at higher risk of disability at work, coronary heart disease (CHD), and more likely to be on long-term medication than their normal-weight counterparts (Bray, 2007).

Obesity is associated with other chronic diseases such as stroke, hypertension, and type-2 diabetes (T2D) mellitus (Bray, 2007). It was reported in one study that 79% of diabetic patients were overweight and of these 46% had BMIs that were higher than 30 kg/m² (Bray, 2007). In a recent survey, the numbers are slightly increased to 82.7% of diabetics being overweight and 54.8% having BMIs of higher than 30 kg/m². Although the risk of diabetes was the lowest among individuals with BMIs below 22 kg/m², the risk of T2D increased with the degree and duration of being overweight

(Bray, 2007). For example, at a BMI higher than 35 kg/m², the relative risk of T2D increased 40-fold with increasing BMI (Bray, 2007).

4.2 Dietary Supplements and Obesity Treatment

4.2.1 Medium chain triglycerides.

A number of reviews have addressed the approaches and methods for treating overweight and obesity (Daniels, 2005; Seidell, 2005; Swinburn, 2004; Bray, 2007). In this regards, the dietary approaches, including dietary supplements, have been examined for their efficacy in treating obesity. Most notably, dietary fibre and MCT have been demonstrated to be helpful for weight loss in several animal and human studies (Bray, 2007; Hashim & Tantibhedyangkul, 1987; Tsuji et al., 2001; Papamandjaris et al., 2000). It is generally known that MCT, which typically consist of fatty acids with chain lengths of <10 carbon atoms is processed by the human body somewhat differently from long chain triglycerides (LCT) (Babayan, 1987). It has also been shown that MCT consumption produces a greater rise in fat oxidation, compared to that of LCTs, which is believed to be linked to lower initial body weight and greater loss of subcutaneous adipose tissue in a study of energy expenditure (EE) and body composition in 19 healthy overweight men aged 44.5 ± 2.5 years with BMI of 27.8 ± 0.29 kg/m² (St-Onge, & Bourque et al., 2003; St-Onge & Ross et al., 2003). A decrease in body weight ($p <$

0.05) by 10.3 ± 0.25 kg with MCT consumption was reported, in comparison to a decrease to 0.62 ± 0.29 Kg with LCT consumption (St-Onge & Bourque et al., 2003). It was also concluded that MCT consumption may stimulate EE and fat oxidation to a lower extent in men of greater bodyweight, compared to men with lower bodyweight. From reports in the literature on the positive effects on thermogenesis and fat deposition with MCT consumption, MCT may be a helpful adjunct to weight loss diets. Moreover, some reports suggest that MCT increases satiety, when compared to LCT (St-Onge & Ross et al., 2003). The positive effect of MCT on energy balance and health benefits have been often reported in the literature due to their fast metabolism, lack of deposition into adipose tissue, and rapid transit into the human body, which helps to increase energy expenditure. A recent, detailed review describes the benefits of MCT in promoting weight loss, since MCT may increase fat oxidation and energy expenditure, to alter body composition (Miriam, 2010). In another article, the authors revealed that weight-loss diets that include consumption of MCT lead to a greater rate of weight and fat mass loss, when compared to olive oil (St-Onge & Bosarge, 2008). Furthermore, the effect of MCT on weight loss and insulin sensitivity in a group of moderately overweight, free-living T2D Chinese subjects was reported (Han et al., 2007), which was attributed to an involuntary reduction in energy intake ($P < 0.05$ in repeated

measures). Although limited use of MCT intake is recommended by health concerned authorities, a number of studies have reported on the safety of MCT use in the diet at appropriate doses. Since MCT has been recognized as a potential weight-lowering agent, it is relevant to understand its negative impact on CVD risks. Hu et al. (1999) reported that MCT does not increase the risk of coronary diseases, whereas, LCT has a tendency for considerable risk. St-Onge & Bosarge et al., (2008) summarized that MCT consumption, as part of a weight loss diet, does not lead to an adverse metabolic profile (glucose, insulin, and blood pressure), when compared to olive oil.

Dietary fibre might be also beneficial in maintaining public health.

Epidemiological data suggests that higher consumption of dietary fibre may be associated with a lower prevalence of overweight or obesity (Zanovec et al., 2010).

4.2.2 Dietary fibres and their functions.

Dietary fibre, a plant-derived complex carbohydrate, is mainly associated with non-digestible, but fermentable carbohydrates mixtures, which are neither hydrolyzed nor absorbed in the upper gastrointestinal tract (Howlett et al., 2010).

The main function of insoluble fibre could be attributed to its passive water-holding capacity and non-digestibility that might help to enhance the bulk and minimize the transit period of the stool through the intestinal tract (Casterline et al., 1997).

Insoluble fibres also stimulate the growth of colonic micro flora that is helpful in increasing biomass. Soluble fibre is completely or partially fermentable in the large intestine, via the action of colonic bacteria, to produce short chain fatty acids (SCFA) such as butyrate, propionate, and acetate (Wong et al., 2006; Velázquez et al., 2000; Wright et al., 1990). The SCFA positively contribute toward a number of health benefits, from colonic health to cardiac health, via well-established biochemical pathways (Wong et al., 2006; Velázquez et al., 2000; Wright et al., 1990). In summary, the soluble fibre enhances the viscosity of the stomach contents, reduces absorption of fatty acids, regulates blood sugar in the body, and helps in reducing cardiac risk factors such as triglycerides, cholesterol, and reactive proteins (Topping, 1991). A generous intake of dietary fibre could also reduce risk for CHD, hypertension, obesity, diabetes, stroke, certain gastrointestinal disorders, and improve blood glucose control in diabetes, promote regularity, help in weight loss, and improve immune functions (Whelton et al., 2005; Steffen et al., 2003; Liu et al., 1999; Montonen et al., 2003; Lairon et al., 2005). Presently, consumers are also more aware of the fibre benefits due to many human research clinical trials on the health benefits of fibre and the variety of available fibre products in the market. Therefore, a balanced diet should contain both soluble and insoluble dietary fibres. Since dietary fibres provide similar benefits to both minors and

adults, the recommended dietary fibre intake for adults is 14 g/1000 kcal. Using the energy guidelines of 2000 kcal/day for women and 2600 kcal/day for men; however, the recommended daily dietary fibre dose is 28 g/day and 36 g/day for women and men, respectively (U.S. Department of Agriculture, 2005). This includes the non-starch polysaccharides, analogous carbohydrates, lignin, and associated components (DeVries & Rader, 2005).

The purpose of this section is to outline the effects of dietary fibres on health. Plenty of reports exist on the disease prevalence and events, that are often summarized from epidemiological studies; however, few reports are focused on the effect of the composition of high fibre foods or food sources of fibre. Numerous reports are also available relating the effect of fibre supplements on serum lipids, weight management, postprandial glycemia, or gastrointestinal functions (Keenan et al., 2002; Petruzzello et al., 2006; Anderson et al., 2004; Brown et al., 1999; Cummings, 2001).

CVD, such as CHD, stroke, and hypertension are the leading cause of morbidity. Moreover, high levels of dietary fibre ingestion are associated with significantly lower prevalence rates for the aforementioned diseases. Soluble or viscous fibres may promote significant positive effects on hypocholesterolemic status (Kirby et al., 1981). Extensive studies with guar gum has focused on several diseases. Psyllium and oat beta-glucan

are also widely used sources of soluble fibre and the FDA has approved health claims related to protection from CHD for soluble dietary fibre (U.S. Department of Health and Human Services FDA, 1997; 1998). Some information is also available for konjac mannan (glucomannan), suggesting its significant hypocholesterolemic effects (Chen et al., 2003). Whereas, gum arabic, partially hydrolyzed guar gum, and methylcellulose seem to show limited hypocholesterolemic effects (Haskell et al., 1992; Yamada et al., 1999; Anderson & Floore, et al., 1991). According to several studies, especially with hypertensive subjects, an increase in consumption of dietary fibre is usually accompanied by a reduction of systolic and diastolic blood pressure. In addition to the above, dietary fibre has favorable effects on other factors such as body weight, visceral adiposity, insulin sensitivity, and inflammatory markers (Delzenne & Cani, 2005; Davy & Melby, 2003; Bo et al., 2006).

The role of dietary fibres in diabetes prevention and management has been extensively studied, in the wake of the alarming worldwide increase in diabetes, due to the presence of metabolic syndrome (Zimmet, 2010). High levels of dietary fibres, leading to decreased postprandial glycemia, insulinemia, and enhanced insulin sensitivity, could prevent the metabolic syndrome, a group of abnormalities including insulin resistance, dyslipidemia, and hypertension (Anderson, 2008). General nutrition

guidelines, published by nine different influential agencies, also recommended the beneficial effects of increased fibre intake on weight management, with specific mention that the intake of whole grain or cereal fibres can protect against the development of obesity. Cross-sectional studies have shown that men and women with the highest level of fibre consumption have a lower relative risk for obesity of 0.77 (95% CI), compared to those with lower fibre intake levels (Anderson, 2008). The effect of dietary fibres on satiety and energy intake is poorly understood. Several studies have examined changes in orexigenic or onorexigenic hormones, and more than 20 gut hormones involved in regulation of eating style and behavior (Anderson, 2008; Haber et al., 1977). Nevertheless, a systematic approach is needed to estimate the connection between key gut hormones and different types and/or formulations of fibres, to provide useful information on these issues.

Gastrointestinal functions of dietary fibre on human health are widely reported in the literature, showing the effect of dietary fibres on the entire gastrointestinal tract. Soluble fibres usually delay gastric emptying and the digestion of food through the small intestine, whereas, insoluble fibres seem to produce an intestinal slurry and are especially active in increasing fecal mass and promoting regularities (Cummings, 2001). Some fibres bind bile acids and impede micelle formation, which increases fecal

excretion of bile acids and cholesterol (Kirby et al., 1981). In the colon, fermentable fibres may act as prebiotics to promote health-promoting bacteria such as bifidobacteria and lactobacilli (Roberfroid, 2005). Therefore, the use of fibre-rich food is recommended for a large variety of gut disorders such as gastro-esophageal reflux disease, duodenal ulcers, inflammatory bowel disease, irritable bowel syndrome, constipation, hemorrhoids, and colorectal cancer (El-Serag et al., 2005; Aldoori et al., 1997; Tsai et al., 2004; Aldoori, 1997). Among these, irritable bowel syndrome is one of the most common gastrointestinal functional disorders worldwide with various pathogenetic factors, including abdominal pain or discomfort, diarrhea, constipation, and bloating (Smith, 1974). Guar gum and, possibly other soluble fibres are associated with low levels of gastric acid production, which may protect from duodenal ulcers or irritable bowel syndrome (Harju, 1984; Giannini et al., 2006). Most prebiotics are non-digestible carbohydrates, which are fermented and stimulate growth of bifidobacteria in the colon, resulting in the formation of SCFA (Wright et al., 1990). Bifidobacteria protect against intestinal infections, lowering the pH for formation of acids after assimilation of carbohydrates, reduce the number of potentially harmful bacteria, produce vitamins and antioxidants, activate the intestinal functions, assist in digestion and absorption, stimulate activity to prevent and treat constipation, stimulate the

immune response, and potentially reduce the risk for colorectal cancer (Wu et al., 2007).

Dietary fibres have also been used in the treatment of child obesity. In a cross-over study of obese children, 15 g/day dietary fibre, which is added to a high-calorie diet even showed a greater mean weight loss despite of the calorie content, compared to the non-fibre treatment period (Gropper & Acosta, 1987). Thus, dietary fibres are certainly one of the recommended functional ingredients, promoting positive effects in maintaining good health and preventing many of the metabolic syndromes.

In general, various types of plants are rich in water-soluble fibre. Psyllium, guar gum (intact or partially hydrolyzed), pectin, defatted fenugreek seed powder, and glucomannan from konjac root all provide rich sources of fibre, and some seaweeds provide fibres such as carrageenan and alginate. These types of dietary fibre are usually associated with lowering cholesterol levels, reducing postprandial blood sugar levels, and thus promoting weight loss. When consumed with enough water prior to meals, these fibres bind to the water in the stomach and small intestine to form a gelatinous, viscous mass, which decreases the absorption of calories by slowing down the absorption of plasma glucose and inducing a sense of satiety (Carabin et al., 2009). In such cases, the target population is expected to be individuals who need to regulate their energy intake. Satiety is inferred as a decrease in hunger after consumption of fibre-rich

foods, leading to a reduction in energy intake (Brand-Miller et al., 2010). It is generally accepted that an increase in satiety might be a favorable physiological effect. Therefore, in some weight loss clinical studies, fibre supplements were found to reduce absorbed calories by a factor of 50 to 200 calories, on a daily basis, which corresponds to a 5 to 20 pound weight loss over the course of a year. Dietary fibre supplements may exert a dose-dependent effect in lowering body weight, and levels of cholesterol and blood glucose. A number of clinical studies have repeatedly suggested that postprandial blood sugar levels decrease as soluble fibre viscosity increases (Lyon, & Reichert, 2010; Jenkins et al., 2000). These relationships have been confirmed in several physiological benefits brought by soluble fibres, such as diminished appetite, decreased serum cholesterol, increased insulin sensitivity, improved bowel movements, and significant weight control.

4.2.3 Polyglycoplex.

The Polyglycoplex (PGX) is a unique and novel polysaccharide with high viscosity, manufactured with proprietary technology under good manufacturing practice (GMP). The product is constituted from three, highly purified water-soluble glucomannans from konjac powder, and sodium alginate and xanthan gum.

Glucomannans [β -D-(1-4)-linked linear polymer of glucose to mannose is substituted

with acetate every 9 to 19 sugar units] are well known for their fermentation in the large intestine. Alginates and xanthan gums are algal polysaccharides and a polysaccharide produced by the bacterium *Xanthomonas campestris*, respectively. These compounds are used in the food industry as stabilizers or thickening agents (Abdelhameed et al., 2010; Harding et al., 2010). PGX is a mixture of molecules with the highest viscosity and water-holding capacity among currently known dietary fibres, and the three components (konjac mannan, sodium alginate, and xanthan gum) act synergistically to form strong interactions through non-covalent bonds, to increase the viscosity level 3 to 5 times higher than any known individual polysaccharide material (Harding et al., 2010; Lyon, & Reichert, 2010). For example, PGX is 3-fold as viscous as guar gum and nearly 8-fold as viscous as the psylliums, which are also soluble type of fibres. The overall formula of PGX could be derived as α -D-glucurono- α -D-manno- β -D-manno- β -D-glucan, α -L-gulurono- β -D manunuronan, β -D-gluco- β -D-mannan, α -D-glucurono- α -D-manno- β -D-manno- β -D-manno- β -D-gluco, α -L-gulurono- β -D-mannourono, β -D-gluco- β -D-mannan (Figure 4.1).

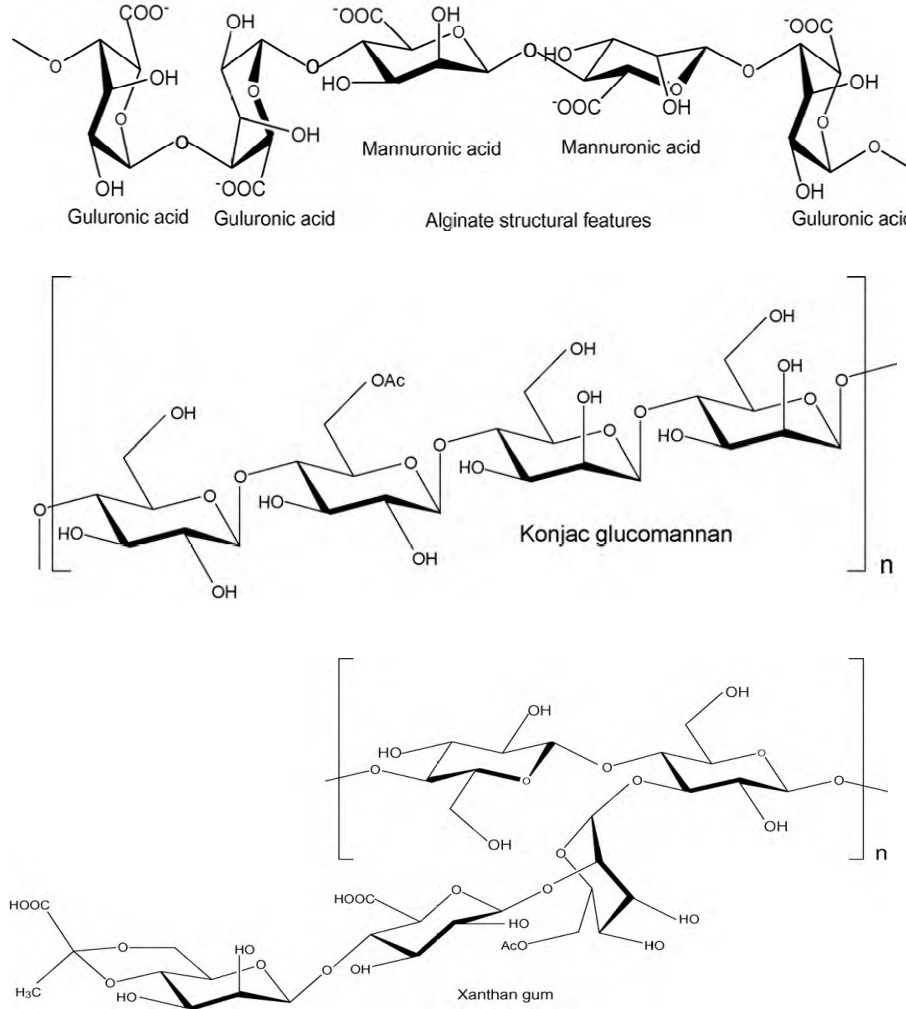


Figure 4.1: Schematic diagram of molecular structure of PGX components (Harding et al., 2010)

To achieve the maximum benefit of dietary fibre, the recommendation is to consume between 20 and 35 g of dietary fibre, though consuming the required amount from daily food sources would be difficult. Individuals who suffer from insulin resistance, diabetes, or obesity epidemic could benefit by taking PGX as a supplement, because of its unique properties (i.e., high viscosity). In a recent review, the authors

claimed that 5 g of PGX is capable of absorbing nearly 1 L of water, which is numerically equal to 5 or 6 times as much of any other dietary fibre (Lyon, & Reichert, 2010). This provides an opportunity to consume an extra amount of food material to improve satiety without consuming unnecessary calories. PGX also helps in lowering the absorption of carbohydrates, which has been reported to have beneficial effects to the glycemic index. These unique characteristics of PGX may also make it useful for lowering cholesterol, and maintaining a healthy blood glucose balance, to substantially improve the daily use of dietary fibre in common diets. PGX significantly lowers postprandial blood glucose (after a meal) by nearly 20% and also reduces insulin secretion by 40%. This corresponds to an improvement of the whole body insulin sensitivity index by nearly 50%, which is a significant accomplishment. In addition, PGX is suggested to have effects in lowering triglyceride levels. These characteristics, along with its excellent safety profile, place PGX as a strong supplement on the list of functional food products for diabetics. In a series of related publications, PGX is claimed to offer benefits in therapeutic applications related to diabetes, obesity, elevated blood lipids (high cholesterol), heart disease, and a cluster of disorders collectively known as metabolic syndrome (Lyon, & Reichert, 2010). Although the gel-forming fibres (partially soluble or insoluble) with their higher viscosity have been often studied

for their ability to reduce CVD risk factors, particularly in overweight and obese subjects, only a few studies have examined the usefulness of soluble fibre and mixtures for controlling and reducing body weight, with variable results. Similarly, in a PGX trial, significant weight loss was observed in both men and women participants, and the results showed a reduction in several risk factors associated with moderate obesity (Lyon, & Reichert, 2010). PGX contains 87.4% dietary fibre, of which 81.8% is a soluble fibre (Brand-Miller et al., 2010).

In addition, the PGX complex has been well studied for its safety. In a recent, 90-day rodent feeding study, 10 male and 10 female Sprague Dawley rats were fed viscous PGX dietary fibre (Matulka et al., 2009). The authors found no observed adverse effect level (NOAEL) for PGX at 5% of the diet, which corresponds to an average daily intake of 3129 and 3799 mg/kg bw/day in male and female rats, respectively. No noticeable difference in mean organ weight, organ to body weight, or organ to brain weight values were seen between control and treated animals (Jenkins et al., 2000). In another human study (total of 54 participants), supplementation of the diet with the functional PGX fibre was evaluated. The results revealed that PGX is well tolerated as part of a regular healthy diet with limited adverse effects. The study was designed to examine the tolerance of PGX ingestion for 21 days, to a maximum dose

level of 10 g/day in both healthy male (42) and female (47) participants with a mean age of 31.6 ± 10.5 years (Carabin et al., 2009). Moreover, Marone et al., (2009) presented a genotoxicity study of PGX that reported no mutagenic effects when using a bacterial reverse mutation and mouse micronucleus assay. The absence of genotoxic activity of PGX in the bacterial reverse mutation assay and mammalian erythrocyte micronucleus test of murine peripheral blood cells was confirmed along with a noted adaptation for the viscosity of PGX in both procedures, which was considered to be within the normal range for use in the food industry.

Research carried out at the University of Toronto revealed that PGX significantly decreases the glycemic index in a dose-dependent manner with commonly consumed foods such as cornflakes, rice, white bread, and granola (Matulka et al., 2009). In the randomized controlled trial, two groups of 10 healthy subjects were assigned to each group (Group 1: 5 male, 5 female; 35.6 ± 13.2 years; 24.6 ± 2.1 kg/m²; and Group 2: 3 male, 7 female; 33.5 ± 11.1 years; 26.3 ± 5.2 kg/m²). Varied contents of PGX (0, 2.5, 5, and 7.5 g) were added to glucose drink (Group 1) or to white bread and margarine (Group 2) and fed to subjects. Blood glucose concentrations were measured at different durations after the start of the meal. The results indicated that the addition of a 7.5 g dose to glucose significantly ($p < 0.001$) reduced the blood glucose concentration, and

the 5 and 7.5 g doses added to white bread + margarine were also significant ($p < 0.001$).

In another recent trial, 7.5 g of PGX novel fibre supplement was shown to reduce the blood glucose response over a two-hour period by 50% ($p < 0.001$), and was capable of reducing the postprandial response by up to 28% ($p < 0.001$) (Brand-Miller, Atkinson, Gahler, Kacinik, Lyon & Wood, 2010). The trial was conducted with 10 subjects from a pool of 16 (8 males and 8 females; age 24.4 ± 2.6 years) and granular PGX supplement was given at four doses (0, 2.5, 5.0, and 7.5 g) with breakfast. In a second study, granular (5 g) and capsule (4.5 g) forms of PGX were given in single doses at -60, -45, -30, -15, and 0 before and +15 minutes after a bread meal. In a third study, capsules of PGX (at increasing doses: 1.5, 3.0, 4.5, and 6 g) were fed with evening meal to measure the effects on glucose tolerance at breakfast. The overall conclusion from the study was that PGX has biologically important, dose-related effects on acute and delayed postprandial glycaemia.

PGX has also been reported to have significant effects on obesity treatment.

Recent studies suggest how PGX might promote weight loss and reduce the related risk factors for developing obesity. In a randomized, double-blind study, 5 g of PGX was added to a meal replacement, resulting in some appetite suppression, compared to a low and a medium viscosity dietary fibre. The authors also suggested that the viscosity of

the fibre might have an impact on satiety by promoting the secretion of anorexigenic gut peptide hormones such as peptide YY, glucagon-like peptide-1 (GLP-1) and cholecystinin (Reimer et al., 2010). In an original article, Reimer et al. showed an increase in plasma PYY levels following the supplementation with functional PGX fibre in healthy adults, over the 21-day trial. PGX has also been reported to reduce the risks of metabolic syndrome, including waist circumference, improvement in insulin sensitivity, lowering of cholesterol, and fasting and postprandial blood glucose levels among human subjects (Reimer et al., 2010). Several other research studies have also clearly indicated the positive effects of PGX on SCFA production, the influence on appetite-regulating hormones for appetite control, and the stabilization of blood glucose and insulin levels (Carabin et al., 2009). In a retrospective 14-week study, the useful effect of novel PGX fibre was shown on lifestyle changes, short-term weight loss, and its associated risk factors in obese and overweight adults. A total of 29 sedentary overweight or obese adults (23 female; 6 male), ages 20-65 years, with BMIs ranging from 25-36 kg/m² participated (Lyon, & Reichert, 2010). Five grams of PGX with 500 mL of water was ingested 5-10 min before each meal, 2-3 times per day, for a consecutive 14 weeks, and compared to baseline. The results showed a significant reduction ($p < 0.05$) of -5.79 ± 3.55 kg, -12.07 ± 5.56 cm, and $-2.43 \pm 2.39\%$ in weight, waist

circumference, and percentage body fat, respectively. Total and LDL plasma cholesterol reduction was also significant ($p < 0.05$) for the PGX group.

4.3 Blood Glucose, Insulin, and Gut Peptides

4.3.1 Insulin, glucagon, and blood glucose regulation.

Insulin and glucagon are two major pancreatic hormones, which are secreted from the pancreas for glycemic control. Insulin is encoded by the INS gene and mainly expressed in the beta cells of the pancreas (Bell et al., 1980), plays an important role in glucose uptake from the blood, and stores excessive glucose as glycogen in the liver and muscle (Odegaard & Chawla, 2008). A summary is listed in Table 4.1. Usually, insulin stimulates the synthesis of glycogen, fatty acids, amino acids, and potassium uptake, and decreases proteolysis and lipolysis (Lomberk & Urrutia, 2009). The first-phase response of insulin (nearly 10 min after meal intake) can be an important biomarker for T2D, as the level of first-phase insulin secretion among T2D patients is much lower than in normal healthy people, due to their pancreatic functioning (Caumo & Luzi, 2004; Cutfield, et al., 2000; Bertram et al., 2007). Insulin secretion is regulated through a oscillation system, instead of through continuous secretion, with insulin release oscillating with a period of 4-6 min. This avoids continuous insulin secretion, which could induce down-regulation of insulin receptors (Bertram et al., 2007). Consequently,

mimicking the oscillation function by injecting insulin is difficult for T2D patients, so that enhancing insulin secretion in a natural way would be ideal. Alternatively, glucagon is released from the alpha cells during hypoglycemia, to stimulate glycogen breakdown and increase blood glucose levels. Glucagon secretion also suppresses insulin secretion to prevent the uptake of blood glucose (Kawamori et al., 2009).

Table 4.1: Descriptive summary of studied peptides and hormones

	Gene/Cell/ Location/ Half Life/ Receptor	Characteristics	Physiological Functions
<i>Insulin</i>	INS Beta Cell Pancreas 70 min IRS-1	<ul style="list-style-type: none"> • Oscillations • Insulin sensitivity is low among T2D patients 	<ul style="list-style-type: none"> • Glucose uptake • Stores glucose as glycogen
<i>GLP-1</i>	Proglucagon L Cell Ileum/Colon 2-5 min GLP-1 R	<ul style="list-style-type: none"> • Degraded by an enzyme DPP-4 • Stimulates Insulin • Inhibits Glucagon 	<ul style="list-style-type: none"> • Stimulates satiety, beta cell mass • Inhibits gastric emptying, food intake, etc.
<i>GIP</i>	GIP K Cell Duodenum/Jejunum 7-8 min GIP R	<ul style="list-style-type: none"> • Resistant among T2D patients 	<ul style="list-style-type: none"> • Stimulates postprandial insulin secretion

4.3.2 Incretins.

Incretins are important gut hormones (i.e., GLP-1 and GIP), which assist the postprandial insulin secretion (Svendsen, et al., 2009). GLP-1 is mainly secreted by L-cells in the ileum and colon, and encoded by proglucagon genes. Proglucagon genes encode other hormones such as glucagon in the pancreatic alpha cells and GLP-1 in the L-cells (Tolhurst et al., 2009). A summary of the gut hormones and their characteristics is shown in Table 4.2.

Table 4.2: Descriptive summary of studied gut hormones

	Gene/ Cell / Location/ Half Life/ Receptor	Characteristics	Physiological Functions
<i>Amylin</i>	S 20 G Beta Cell Pancreas 5-13 min RAMP 1-3	<ul style="list-style-type: none"> • More common among T2D patients • Secreted with insulin 	<ul style="list-style-type: none"> • Enhances beta cell apoptosis • Decreases plasma glucose and insulin demand
<i>Ghrelin</i>	GHRL P/D1 cell Stomach 22-26 min GHS-R	<ul style="list-style-type: none"> • Hunger Hormone • Increases before meal • Decreases after meal 	<ul style="list-style-type: none"> • Increases satiety • Decreases insulin
<i>Leptin</i>	Ob (lep) Adipose Tissue 20-30 min LEPR	<ul style="list-style-type: none"> • Obese people are resistant 	<ul style="list-style-type: none"> • Decreases energy intake • Increases energy expenditure
<i>PYY</i>	PYY L Cell Ileum/Colon ~ 10 min Y2R	<ul style="list-style-type: none"> • Discrete population in the brain 	<ul style="list-style-type: none"> • Decreases appetite • Increases digestion efficiency and nutrient absorption
<i>PP</i>	PP Cell Pancreas PPYR	<ul style="list-style-type: none"> • Structural homology with NPY and PP • Increased in T2D patients 	<ul style="list-style-type: none"> • May decrease appetite • Self-regulate pancreatic secretions

GLP-1 binds the GLP-1 receptor located in the pancreatic beta cells, which also has a low affinity for GIP and glucagon, to exert several physiological functions (Wren & Bloom, 2007). For example, when the same amount of glucose is available, oral glucose intake stimulates insulin secretion more than does injecting glucose intravenously, due to secretions of incretin hormones in the gut. When GLP-1 binds to the receptor, it activates an adenylyl cyclase pathway and a number of protein kinases (Ramos et al., 2008). Subsequently, it potentiates glucose-induced closure of the ATP-dependent potassium channels, which stimulates membrane depolarization and an influx of calcium ions. Finally, it facilitates the exocytosis of insulin-containing secretory granules and releases insulin (Lomberk & Urrutia, 2009). GLP-1 also enhances satiety, beta cell reproduction, decreases glucagon secretion, and inhibits stomach acid secretion, gastric emptying and food intake, which could be beneficial for obese and diabetic patients (Knop et al., 2009). One of the major problems with GLP-1 is that its half-life is usually around 2 min, because of the degradation by dipeptidyl peptidase-IV (DPP-4), which is found in many cells (Deacon et al., 2008). To increase plasma GLP-1 levels, the DPP-4 activity must be inhibited. Nevertheless, DPP-4 also has a significant role in cancer and tumor suppression through immune regulation, signal transduction, and

apoptosis, so that the balance in activities is critical (Wesley, 2004). Incretin mimetics, such as Exenatide (Byetta) could be a new way to treat T2D. These agents, originally found in the Gila monster, are GLP-1 agonists, but are not degraded by DPP-4 (Gentilella et al., 2009).

4.3.3 Other gut-derived peptides.

Amylin: Amylin is co-secreted with insulin and helps to decrease glucose disappearance from plasma, and decrease food intake, gastric emptying, and the digestive enzymes (Otto-Buczowska et al., 2008; Ratner et al., 2004). Such physiological effects would help to reduce the insulin demand. Nevertheless, the accumulation of amylin can form amyloid, which stimulates apoptic cell death of the insulin-producing beta cells in the pancreas, resulting in a reduction of insulin secretion (Lorenzo, et al., 1994; Neumann, et al., 2008). For 80 years, insulin was the only pharmaceutical product for type-1 diabetes patients, until an amylin synthetic analogue was successfully introduced in the American market in 2005. The product, called Symlin, can be prescribed for diabetic patients who have poor glycemic control with insulin therapy (Edelman et al., 2008). Amylin is mainly encoded by the S20 gene by pancreatic beta cells, which is also known to be increased in T2D patients (Pittner, et al., 1994). Amylin is also known as islet amyloid polypeptide and it binds specific types of

receptors called RCMP, that contain calcitonin receptors (calcitonin is a thyroid hormone that lowers the calcium level in the blood plasma and inhibits the resorption of bone (Hay, et al., 2004).

Leptin: Leptin is mostly found in white adipose tissues and encoded by the Ob (lep) gene (Brennan & Mantzoros, 2006). Leptin is well known to suppress energy intake and enhance energy expenditure, and is capable of binding several receptors, such as LEPR in the hypothalamus, which is considered as an appetite control center (Ragin, et al., 2009; Palou, et al., 2009). When leptin binds the LEPR receptor, it decreases satiety stimulating substances such as neuro-peptide Y (NPY) and Agouti-related protein (AgRP). Thus, leptin may be used as a biomarker for body fat, since it is widely found in adipose tissues, especially among obese people. Nevertheless, obese people are resistant to leptin and may not be able to receive the beneficial physiological effects of leptin (Weigle, et al., 1997). A very low calorie, short-term diet has been reported to decrease the amount of leptin, whereas over feeding had no effect on leptin (Dubuc, et al., 1998). Ogawa et al. (1999; 2002) developed a new type of mice called Skinny Mice in Japan, which have 10-times more leptin than average mice. The mice have several interesting physiological characteristics, such as less adipose tissue, low blood glucose, high blood pressure and better insulin sensitivity.

Ghrelin: Ghrelin is acknowledged to be a hunger hormone, since it stimulates hunger and it increases before food intake, and decreases after meal consumption. Ghrelin is mainly expressed by P/D1 cells and epsilon cells, which are commonly found in the stomach and pancreas, respectively (Inui, et al., 2004). Ghrelin is identified as promoting food intake, fat mass, NPY production, and the consumption of addictive substances like drug and ethanol. Ghrelin also decreases the insulin production, and extremely high amounts of ghrelin has been observed in anorexic people. Also, obese people tend to have relatively high amounts of ghrelin, compared to skinny and lean people (Field, et al., 2008). Furthermore, the bariatric process has been reported to decrease the levels of ghrelin, and short-sleep may increase ghrelin. To suppress the amount of circulating ghrelin, several types of antibodies have been developed in a ghrelin vaccine, for possible commercial use in the near future. Ghrelin has several important physiological roles in human metabolism, and also decreases stress-induced depression (Santos, et al., 2006; Lutter, et al., 2008). It is also identified as a neutrophin in the hippocampus area, and improves memory and learning skills (Jones, 2003).

Peptide-Tyrosine-Tyrosine (PYY): PYY is an important satiety-suppressing gut peptide, widely found in L-cells in the ileum and colon, and also has a discrete population in the brain (Field, et al., 2008). PYY binds Y2R, an NPY receptor, and

decreases gastric motility, gut emptying, and satiety, and also increases digestion efficiency and water and electrolyte absorption in the large intestine (Field, et al., 2008). The consumption of protein and iso-flavones have also been reported to increase PYY levels (Weickert, et al., 2006). In a study of human subjects, infusion of PYY was found to decrease by 30% during food intake at a buffet lunch; however, no significant satiety reduction was seen in Y2R knockout mice (Wren & Bloom, 2007; Field, et al., 2008; Batterham, et al., 2003). In a separate animal study, the fat mass and body weight increased in Y2R knockout mice, suggesting the need for more studies before the actual physiological functions of PYY in humans can be determined (Field, et al., 2008).

Pancreatic Polypeptide (PP): PP is generally expressed in the pancreas and has a structural homology with NPY and PYY (Field, et al., 2008). Circulating levels of PP in humans are rather low in the morning, reaching the maximum level prior to sleep. In any case, the amount of food and the caloric intake have significant effects on PP levels, rather than time factors. Protein meals, fasting, exercise, and acute hypoglycemia are all known to increase PP levels, though somatostatin secretion and intravenous glucose intake can lower the amount of PP secretion. PP binds to the receptor, called PPYR, and contributes to appetite reduction; however, this function is still controversial since PP is

not correlated to BMI (Field, et al., 2008). Furthermore, PP self-regulates pancreatic secretion activities and is readily found in T2D patients.

4.3.4 Dietary fibres, hormones, and glycemic control.

In recent years, dietary fibres have been of tremendous interest with consumers due to their potential to provide diverse health benefits. A healthy diet is recognized as one of the most effective and safest approaches for preventing lifestyle diseases. Evidence suggests that the consumption of certain types of dietary fibres may help prevent and treat obesity, as well as type-2 diabetes. Soluble dietary fibre, such as inulin and guar gum, are known to delay blood glucose absorption through gel formation, which traps carbohydrates and allows them to be fermented in the colon with SCFA production. In turn, this may contribute to hypercholesterolemia by attenuating cholesterol synthesis. Thus, soluble dietary fibres may be helpful in decreasing total cholesterol and LDL levels in humans (Weickert & Pfeiffer, 2008). Insoluble dietary fibre, such as cellulose, may balance the intestinal pH and alleviate constipation by speeding the passage of food and adding bulk to the stool (Gray, 1995). The properties and health benefits have been described previously, in that both soluble and insoluble fibres are necessary for the diet, and helpful in contributing to weight loss. Nevertheless, clear evidence for the link between dietary fibre appetite control and obesity is still

lacking. Dietary fibres have been reported to be capable of stimulating incretin production that suggests a solution for obesity and T2D management (Massimino, et al., 1998; Reimer & McBurney, 1996; Zhou, et al., 2008; Juvonen, et al., 2009).

According to Massimino et al. (1998), high fermentable dietary fibres increase ileal proglucagon mRNA and intestinal GLP-1 concentrations among healthy dogs. Furthermore, the integrated area under the curve (AUC) for plasma GLP-1 and insulin were greater than that of low fermentable fibre diet (Massimino, et al., 1998). Reimer and coworkers (1996) reported that a dietary fibre mixture increased SCFA, and acetate, propionate, and butyrate in the cecum and colon, which stimulated mucosal adaptation among rats. SCFA also altered proglucagon messenger RNA and enhanced the release of GLP-1, and the average plasma level of insulin increased as well (Reimer & McBurney, 1996). Moreover, Zhou et al. (2008) concluded that resistant starch could significantly increase both GLP-1 and PYY levels, and decrease insulin secretion among rats. The authors also suggested that fermentation of resistant starch increases the levels of butyrate, propionate, and acetate, which may directly stimulate proglucagon gene expression and PYY promoter activity (Zhou, et al., 2008). Juvonen et al. (2009) reported that beta-glucan, a viscous dietary fibre found in oat bran, has a positive influence on gastrointestinal hormonal responses among healthy humans, with

the beta-glucan increasing insulin, GLP-1, and PYY, and decreasing ghrelin significantly (Juvonen, et al., 2009).

CHAPTER 5: MATERIALS AND METHODS

5.1 Study Population

Subjects were recruited from the lower mainland of British Columbia through local newspapers, posters, and a clinic website. Potential subjects were pre-screened via telephone or by an online screening questionnaires, based on inclusion and exclusion criteria:

Participant Inclusion Criteria:

1. Ability to provide written informed consent
2. Male or female
3. Age between 20-45 years
4. BMI 27-35 kg/m²
5. Ability and willingness to complete dietary diaries and questionnaires
6. A negative result on the screening pregnancy test and agreement to use an acceptable form of birth control during the trial.

Participant Exclusion Criteria:

1. More than 3 kg weight gain or weight loss in the past three months
2. Pulmonary, hepatic, renal disease, or known diabetes
3. Heart attack or stroke within the last six months
4. Systolic blood pressure \geq 130 mmHg or a diastolic blood pressure \geq 85 mmHg combined with three or more risk factors

5. Total cholesterol > 6.2 mmol/L, >LDL 4.0 mmol/L, or triglycerides > 3.0 mmol/L and not on pharmacotherapy
6. Substantial neurological or psychological illness within the last six months, including depression-necessitating hospitalization
7. History of weight loss surgery such as gastric banding or gastric bypass
8. Concomitant use of medication or supplements that have the potential to significantly alter body weight or appetite [i.e., ephedra, synephrine, green tea extracts, bulk fibre laxatives (e.g., psyllium, glucomannan), diuretics, orlistat, sibutramine, antidepressants (other than those which do not typically alter appetite), neuroleptics, cholesterol lowering medication, nicotine substitutes, and hypoglycemic medication and others at the discretion of the physician]
9. Known eating disorder
10. Concomitant use of medication or supplements that have the potential to significantly alter body weight or appetite
11. Substance abuse: tobacco including those who have quit smoking in the last 12 months, alcohol (>2 drinks a day), Cannabis sativa or other controlled substances use, diuretics or laxatives
12. Pregnancy, breast-feeding, or oral contraceptives started in the last six months prior to the start of the trial
13. Gastric and/or esophageal strictures; history of bowel obstruction
14. Other conditions such as medical, social, or geographic, which in the judgment of the investigator would not allow safe completion of the protocol and/or safe administration of the investigational product

An information session was held by the investigators to answer any questions or concerns that participants might have regarding the study. A consent form was provided to those interested in participating in the study, who were encouraged to review it with

their family and family doctor. A more detailed screening visit to review the exclusion and inclusion criteria was scheduled for those participants who were still interested in participating in the study. Study approval was obtained from the University of British Columbia Clinical Ethics Research Board, and written informed consent was collected from all participants prior to the study.

5.2 Study Design, Products, and Procedure

The clinical study was conducted between September 2008 and June 2009, using a double-blind, randomized, placebo-controlled design. Participants attended an early morning clinic, following a 10-12 hour overnight fast. After obtaining written consent from each participant, the height, weight, hip, and waist circumference was measured using standard procedures. A cannula was placed into the arm and a fasting blood sample was collected. Participants completed a four-hour meal tolerance test (details follow) and then randomly assigned to one of two groups (Placebo or Treatment) for 13 consecutive weeks.

5.2.1 Supplementation.

Participants were provided with the supplements in capsule form. Both the treatment (PGX) and the placebo capsules were composed of dietary fibre, oil, beeswax, and lecithin. The capsules were identical and indistinguishable in terms of size, shape,

color, appearance, and nutritional content. Treatment capsules were composed of 750 mg of PGX fibre, 600 mg of MCT, 51 mg of beeswax and 24 mg of lecithin. The Placebo capsules consisted of 750 mg of cellulose, 600 mg of soybean oil, 51 mg of beeswax and 24 mg of lecithin. The investigators did not have access to the randomization (treatment) code, unless a serious adverse event occurred. The participants were provided with the supplements in plastic containers, with each container having 126 capsules. Participants were given a two-week supply (252 capsules) all at once. The labels of every container included: contact information, trial number, study treatment number, participant number, visit number, directions for use, caution statement, lot number, expiry date, and address of manufacturer.

5.2.2 Dose selection and trial parameters.

The first week was a run-in phase to allow participants to adapt to the full dosage of the product. The participants were instructed to start by taking 2 capsules, 15 min before each meal with water, and to increase the dosage by 1 capsule with each meal every 2 days, until a maximum of 6 capsules were being consumed with each meal. If any participant developed discomfort, side-effects, or too much appetite suppression, they were advised to remain at, or drop down to, a minimum of three capsules per meal. If any participant was unable to consistently tolerate a minimum of three capsules with

each meal, they were asked to withdraw from the study. Following the first week, participants visited the clinic to have their anthropometric measurements taken and to discuss any questions or concerns they might have. Thereafter, participants visited the clinic every two weeks during the weight loss phase and asked to return non-consumed supplements, as well as to receive a new supply of supplements for the next two weeks. Anthropometric measurements were recorded and any physical discomfort or appetite was discussed according to the timeline shown in Figure 5.1. After 13 weeks on treatment, participants returned to the clinic and were asked to provide a fasting blood sample, and completed another meal tolerance test.

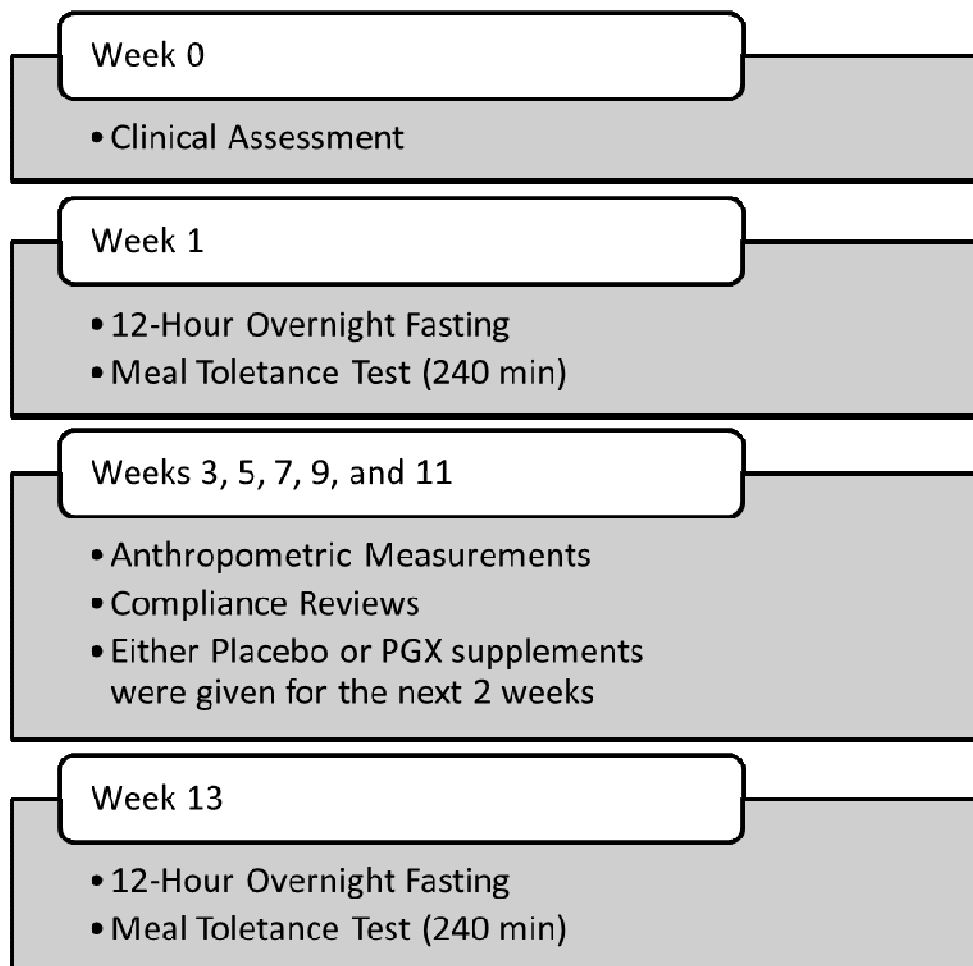


Figure 5.1: The systematic plan and schedule for the clinical trial

5.3 Meal Tolerance Tests

The procedure for the meal tolerance test was as follows. A fasting blood sample was collected into an evacuated tube containing EDTA. The participant was then instructed to consume the test meal within 15 min. The test meal consisted of: a

McDonald's pancake with syrup and orange juice; comprised of 9 g of protein, 14 g of fat, 132 g of carbohydrate, and 3 g of dietary fibre (700 kcal in total). Blood samples were collected at 15, 30, 45, 60, 120, and 240 min after the fasting blood. Participants were encouraged to drink water during the test period. The same amount of water was provided to each participant during the blood collection at week 13 as was provided at week 0 (Baseline).

5.4 Anthropometrics, Dietary, and Exercise Advice

Participants were provided with written advice regarding portion control and reduced calorie food choices. Daily physical activity was encouraged and a handout describing appropriate exercise was provided.

5.5 Compliance

Participants were requested to comply with instructions at the beginning of the study and were assessed for compliance throughout the trial. All participants were required to visit the clinic every two weeks, and to return their unconsumed products to the investigators of the clinical trial. Compliance was calculated based on a physical count of returned supplementation products. The following formula was used for the estimation:

$$\text{Compliance (\%)} = \frac{\text{Number of capsules actually consumed Since last capsule count}}{\text{Number of capsules that should be theoretically consumed during the assigned period}} \times 100$$

5.6 Premature Withdrawal and Discontinuation Criteria

"Drop-outs" were defined as those participants who discontinued the trial for reasons not related to the treatment. "Withdrawals" were defined as those subjects who discontinued the trial due to treatment-related reasons. In case a participant was prematurely withdrawn from the study, the latest data on the participant was collected to fully support the final intent-to-treat analysis and the last weight before termination was recorded for both drop-outs and withdrawals. Appropriate documentation was filed and entered into the study database. Also, both dropouts and withdrawals had similar close-out exams (weight, waist circumference, and final blood work) at the completion of the study procedures, and final visit forms.

Date of the last supplementation dose and reason for discontinuation was recorded for all drop-outs and withdrawals. Concomitant diagnosis (i.e., medical history) and concomitant therapy use was also documented, for participants who discontinued due to an adverse event. All adverse events and relevant information, which occurred when the participant had discontinued from the study, were followed

until the event was resolved or until the follow-up was considered sufficient, and recorded into the study database. Unused capsules were collected from participants, and compliance was assessed through the capsule counts. Nevertheless, all random dropouts and withdrawals were replaced with other participants to ensure that sufficient data was collected from the pre-set number of participants.

5.7 Laboratory Biochemical Analysis Methods

Blood collected for glucose determination was immediately centrifuged after collection and the plasma samples were transferred into separate tubes. The plasma samples were immediately analyzed using the YSI 2300 STAT Plus™ at the Canadian Centre for Functional Medicine. Whole blood samples for gut hormone and insulin analysis were collected into tubes containing EDTA, DPP-4 inhibitor (LINCO), protease inhibitor cocktail (Sigma), and serine protease inhibitor (Pefabloc SC, Roche). These inhibitors protect GLP-1, amylin, and ghrelin from immediate degradation. For each test tube, 0.238 mg (17 mg/mL) of Diprotin A (ILE-PRO-ILE) (MP Biomedicals) and 14 μ L of water was mixed with 8.75 mg (0.5 mg/ μ L) of Pefabloc SC and 17.5 μ L of water. After mixing these inhibitors, 70 μ L of protease inhibitor cocktail was added into each tube. Blood samples were centrifuged within 30 min, plasma was removed, and

samples were stored at -80 °C until analyzed. The plasma samples underwent no freeze-thaw cycles.

The analyses of the gut peptides were conducted at the University of Calgary using a multiple gut peptide analysis kit. The plasma samples were centrifuged at 3,000 g for 10 min prior to the analysis and the multiplex assay kit (LINCO) was used for the simultaneous quantification of GLP-1, GIP, peptide tyrosine-tyrosine (PYY), ghrelin, pancreatic polypeptide (PP), leptin, insulin, and amylin from the blood plasma samples. In addition, the total cholesterol, HDL cholesterol, and triglycerides were measured for fasting plasma samples collected at weeks 0 and 13 at BC Bio-Labs located in Surrey, BC.

Regarding quality control, two different solutions (Millipore) were used to maintain the accuracy of the measurements. In comparison with these solutions, the accuracy of each peptide was calculated (shown in the original protocol). The accuracy of all peptides was above 85%. The details of the quality control can be found in Cat. No HGT-68K (Millipore, USA).

5.8 Statistical Analysis

Concentration of all plasma hormones, body weight, height, BMI, and various laboratory values, before and after treatment, were computed statistically using SAS software (version 9.2; SAS Institute, Cary, NC) (Shiang, 2004). General clinical evaluation values were expressed as Mean +/- SEM and calculated using analysis of Wilcoxon-Mann rank test, depending on the normality of the distribution. The concentration of the different hormones and peptides are presented as median and interquartile range (IQR), unless otherwise noted. A p value <0.05 was considered significant. Statistical tests were performed on the change from baseline AUC scores, and to express the relationship, variables were log-transformed prior to the AUC calculation, with inclusion of the baseline as covariates.

The Wilcoxon-Mann test also known as Wilcoxon Signed-Rank Test (numerically equivalent to the Mann-Whitney U test) is a non-parametric analog to the independent two samples t-test and can be used to test whether the location of the measurement is equal to a pre-specified value. Also, this is most common method for determining whether there is a difference between two distributions. The basic requirements are that the data must be ordinal or continuous measurements and the two samples must be independent. Furthermore, this test can also be performed if only rankings are available.

In particular, it tests the null hypothesis (H_0), which the two distributions are identical against its alternative hypothesis. In this case, the two distributions differ only with respect to the median. This test assumes that the observations within each sample are identically distributed, independent, and the shapes and spreads of the distributions are identical (Pappas & DePuy, 2004).

The advantage of non-parametric methods over asymptotic methods is that they remain valid for very small sample size, and for data that are heavily tied. These non-parametric methods can be referred to as distribution-free method because they do not assume an underlying parametric distribution for the data. The non-parametric methods often require the use of interval- or ratio-scaled data. They provide a different series of statistical methods which require no or very limited assumptions to be made about the data.

Wilcoxon-Mann Scores (S) also called Rank Sum assigned to the observations could be described as follows:

$$S = \sum_{j=1}^N c_j w_j$$

Wherein assigned scores w_j to the observation based on ranks, c_j is an indicator denoting the class (0 or 1) to which the j^{th} observation belong (Hollander & Wolfe, 1973).

In Wilcoxon-Mann Rank Test, the sampling distribution (T) for identical populations under the null hypothesis (H_0 ; two populations are identical) could be defined as:

$$\text{Mean} = \mu_T = \frac{n_1 (n_1 + n_2 + 1)}{2}$$

$$\text{Variance} = V_T = \frac{n_1 n_2 (n_1 + n_2 + 1)}{12}$$

$$\text{Test Statistic} = z = \frac{T - \mu_T}{\sqrt{V_T}} \quad \{\text{asymptotically } N(0,1) \text{ distribution}\}$$

SAS codes for Wilcoxon-Mann Rank Test used are as follows wherein NPAR1WAY procedure could be invoked using the following statements:

PROC NPAR1WAY WILCOXON;

CLASS variable;

VAR variable;

EXACT WILCOXON;

The NPR1WAY procedure computes exact p-values for the simple linear rank statistics based on the Wilcoxon–Mann score (S), provided the data are classified into two or more levels (i.e. two or more sample tests). The outcome of the NPR1WAY procedure is the exact one- sided and two-sided p-values for each statistic specified in the statement. Mathematically, the one- sided (p1) and two- sided (p2) p-value can be expressed as

$$p1 = P(\text{Rank Statistic} \geq S) \text{ if } S > \text{Mean}$$

$$p1 = P(\text{Rank Statistic} \leq S) \text{ if } S \leq \text{Mean}$$

$$p2 = P(|\text{Rank Statistic} - \text{Mean}| \geq |S - \text{Mean}|)$$

Wherein the S is the observed rank statistic, Mean is the expected value of the test statistic values. There is proof to suggest that the two population medians differ if the p-value equals less than the specified level.

The values of the Sign Test confirmed and concluded the statistically significant difference between the observed PGX treatment and placebo control group. The Sign Test values were used as the SAS output yields the results for both the Wilcoxon-Mann

Signed Rank Test and the Sign Test without use of any options (SAS/STAT User's Guide under the NPAR1WAY procedure; 1990).

In this study, the NPAR1WAY method is performed to test the null hypothesis whether there is any difference in the participant response status and the option specifies that Wilcoxon-Mann scores are used in the computation. Also, it is possible to precisely calculate AUC (Area Under the Curve) in SAS for a binary classifier rank ordered data, since AUC is also closely related to the Wilcoxon-Mann Rank-Sum Test. Usually, the AUC is the area under receiver operating characteristic (ROC) curve and is an important measurement on the accuracy of a binary classifier (Yan et al., 2003). To calculate AUC for the SAS data set of PGX treatment and placebo control, at first it was rank ordered by a binary classifier such as linear logistic regression), wherein the binary outcome Y and the rank order measurements could be obtained. Aforementioned PROC NPAR1WAY method was used to obtain Wilcoxon-Mann Rank Sum statistics and from there it was possible to obtain an accurate measurement of AUC for this data (Cortes & Mohri, 2004).

The relationship between AUC and Wilcoxon-Mann Rank Sum test statistics is as follows:

$$AUC = (W - W_0) / (N_{\text{at baseline}} * N_{\text{at end of trial}}) + 0.5$$

Wherein, $N_{\text{at baseline}}$ and $N_{\text{at end of trial}}$ are the frequency of class Variables (PGX and Placebo) at baseline (0 week) and at the end of the trial (13 weeks), and W_0 is the expected Sum of Ranks under H_0 : Randomly ordered (null hypothesis), and W is the Wilcoxon-Mann Rank Sums.

CHAPTER 6: RESULTS AND DISCUSSION

6.1 Results

6.1.1 Participants and follow-up.

The participants that were selected for this trial, after the appropriate screening according to the exclusion-inclusion criteria, have been summarized previously. After the online survey and the pre-clinical assessments, 148 participants were accepted for the clinical trial. All selected participants were randomly assigned (72 to the placebo group and 76 to the PGX treatment group). Premature withdrawal and discontinuation criteria were applied to those who chose to discontinue the trial for reasons related to the treatment or for unrelated reasons. A total of 11 participants (placebo 2; PGX 9) fell under these criteria. Therefore, 70 participants in the placebo group and 67 participants in the PGX group completed the trial. Although compliance was above 90% in both groups, ultimately, 42 participants finished the blood analysis test in the placebo group, and 46 participants completed the blood analysis test in the PGX group. The statistical analysis of the final results included the blood analysis of these participants. The characteristic baseline was drawn and the significance of the results was compared

between the two groups. A general flow chart of participants in the study is shown in

Figure 6.1.

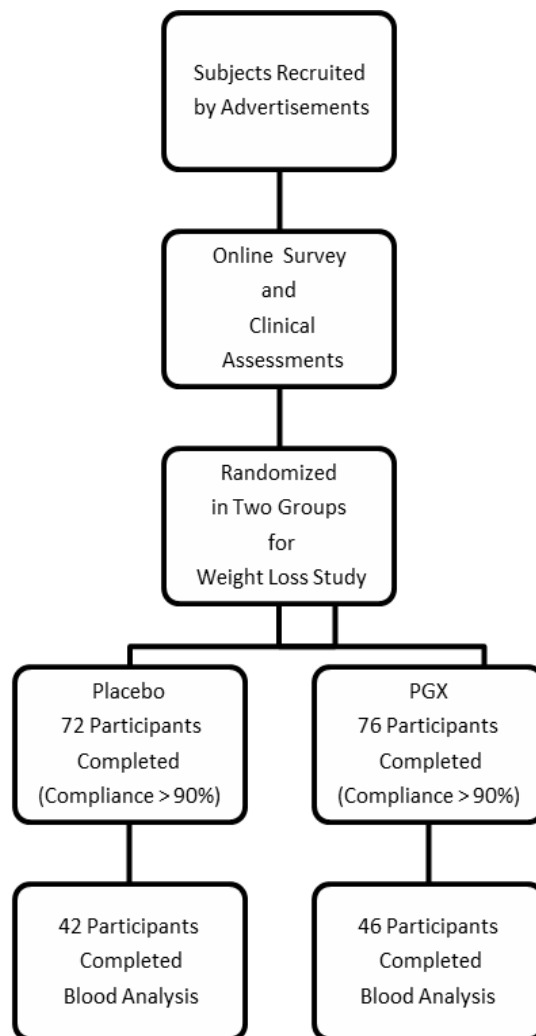


Figure 6.1: Study participation and follow-up

6.1.2 Participant characteristics at baseline.

Baseline characteristics of participants are presented in Table 6.1, which shows the lack of difference between the groups. Nearly 86% of the participants were female.

The placebo control group (n= 72) had a mean age of 39.2 ± 5.3 y and BMI of 31.3 ± 3.3 kg/m². The PGX treatment group (n=76) had a mean age of 37.3 ± 5.7 y and BMI of 31.8 ± 2.9 kg/m². Mean waist and hip circumference was 99.4 and 111.4 cm and 100 and 110 cm for the placebo group and the PGX group, respectively.

Table 6.1: Participant characteristics at baseline*

Characteristics	Placebo (n = 72)	PGX (n = 76)
	<i>Mean ± S.D</i>	
Age (years)	39.2± 5.3	37.3 ± 5.7
Height (cm)	166.3± 7.3	164.7 ± 9.8
Weight (kg)	87 ± 12.9	86.6 ± 13.7
BMI (kg/m ²)	31.3 ± 3.3	31.8 ± 2.9
Waist Circumference (cm)	99.4± 10.8	100 ± 9.9
Hip Circumference (cm)	111.4 ± 8.6	110 ± 6.0
Total Cholesterol (m mol/L)	5.1 ± 0.9	5.0 ± 0.9
HDL Cholesterol	1.5 ± 0.4	1.3 ± 0.3
LDL Cholesterol	2.9 ± 0.9	3.0 ± 0.8
Total Cholesterol: HDL Ratio	3.7 ± 1.4	4.0 ± 1.3

* Female; n (%): Placebo, 62 (86 %); PGX, 65 (86 %)

6.2 Data Analysis

6.2.1 Anthropometrics.

At 13 weeks, no significant difference was seen in weight change from baseline between the placebo and the PGX groups (-1.1 ± 7.6 vs. -0.6 ± 6.8 kg; $p < 0.24$).

Likewise, no significant difference was seen in the change from baseline for BMI (-0.4 ± 0.8 vs. -0.3 ± 0.8 kg/m²; $p < 0.26$), waist circumference (-2.6 ± 4.2 vs. -2.0 ± 2.7 cm; $p < 0.23$), hip circumference (-1.5 ± 2.0 vs. -1.0 ± 1.7 cm; $p < 0.13$) (see Table 6.2).

Table 6.2: Bio-physical characteristics of the participants at week 0 (Baseline) and at week 13, and their variation from baseline along with statistical significance

Parameters	n	Week 0 (Baseline)	Week 13	Change from baseline	p-Value
		<i>Mean ± S.D</i>			
<i>Weight (kg)</i>					
<i>Placebo</i>	72	87.0 ± 12.9	86.0 ± 13.5	-1.1 ± -7.6	0.24
<i>PGX</i>	76	86.6 ± 13.7	85.9 ± 13.9	-0.6 ± -6.8	
<i>BMI (kg/m²)</i>					
<i>Placebo</i>	72	31.3 ± 3.3	30.9 ± 3.4	-0.4 ± 0.8	0.26
<i>PGX</i>	76	31.8 ± 2.9	31.5 ± 2.9	-0.3 ± 0.8	
<i>Waist Circumference (cm)</i>					
<i>Placebo</i>	72	99.4 ± 10.8	96.7 ± 11.5	-2.6 ± 4.2	0.23
<i>PGX</i>	76	100 ± 9.9	98 ± 10	-2.0 ± 2.7	
<i>Hip Circumference (cm)</i>					
<i>Placebo</i>	72	111.4 ± 8.6	109.9 ± 8.7	-1.5 ± 2.0	0.13
<i>PGX</i>	76	110 ± 6.0	108.9 ± 6.3	-1.0 ± 1.7	

6.2.2 Hormones.

AUC was calculated for glucose, insulin, GLP-1, GIP, ghrelin, leptin, amylin, PYY, and PP at the baseline and at week 13. The change in AUC from baseline to week 13 for each of these was also calculated. The data is presented as medians (IQR) in Tables 6.3 and 6.4. With the exception of GIP, no significant difference was found in AUC at 13 weeks for these hormones, in the change from baseline, between the placebo and PGX groups ($P < 0.05$). A significantly greater reduction was found in GIP from baseline and at 13 weeks in the PGX group, compared with the placebo group (7 vs. 58: $p < 0.01$). Changes in blood hormones and other gut peptides were practically non-significant incremental changes over time, in the comparison between PGX and control groups. Both ghrelin and leptin concentrations at 13 weeks were not significant when compared to the baseline, for PGX and the control (see Appendix A). Nevertheless, the change in concentration of leptin to ghrelin ratio with ingestion time at baseline and after 13 weeks, in both placebo and the PGX treatment group, provides interesting information on the behavior of ghrelin and leptin trends. Figure 6.2 shows the variation in the ratio of leptin to ghrelin, where the ratio at baseline and after 13 weeks remained almost the same until 30 min after ingesting either placebo or PGX treatment. The leptin to ghrelin ratio tended to be lower at 13 weeks, for 45 to 120 min after ingestion, which

was somewhat more pronounced in the PGX treatment group compared to the placebo group. Furthermore, the cross-sectional relationship between leptin and ghrelin could explain the overall phenomenon (Figure 6.3). The changes in actual plasma ghrelin are plotted against the changes in the actual plasma leptin levels at different times after ingestion (0 to 240 min) with a clear difference from the baseline (0 week) to after 13 weeks (trial duration) for both the placebo and the PGX group. A positive cross-sectional relation in the PGX group suggests that, even at relatively smaller decreases in change in leptin values, a significant increase was seen in the corresponding change in plasma ghrelin values. In contrast, in the placebo group, the changes in leptin levels were least significant at the corresponding changes in the plasma ghrelin values.

Table 6.3: Analysis of different biochemical parameters for the participants of both placebo and PGX groups and their statistical significance

	Glucose	Insulin	GLP-1	GIP
	Arbitrary Units			
Placebo				
Baseline AUC				
Median (IQR)	218 (83, 308)	333 (279, 434)	17 (5, 23)	292 (214, 366)
Week 13 AUC				
Median (IQR)	210 (106, 355)	354 (252, 438)	20 (8, 39)	271 (229, 417)
Change from baseline				
Median (IQR)	4 (-161, 86)	7 (-443, 277)	3 (-3, 22)	7 (-65, 109)
PGX				
Baseline AUC				
Median (IQR)	214 (86, 394)	356 (303, 426)	21 (10, 31)	344 (214, 429)
Week 13 AUC				
Median (IQR)	225 (104, 304)	343 (276, 419)	19 (12, 36)	282 (194, 338)
Change from baseline				
Median (IQR)	-20 (-60, 37)	-29 (-103, 62)	0 (-10, 15)	-58 (-145, 28)
P-value for difference	0.65	0.36	0.41	<0.01

Table 6.4: Analysis of different peptides for the participants of both placebo and PGX groups and their statistical significance

		Ghrelin	Leptin	Amylin	PYY	PP
		Arbitrary Units				
Placebo						
Baseline AUC						
<i>Median (IQR)</i>		111 (66, 152)	31 (18, 65)	264 (189, 316)	N/A	249 (143, 372)
Week 13 AUC						
<i>Median (IQR)</i>		130 (83, 169)	40 (12, 69)	272 (200, 319)	N/A	209 (105, 338)
Change from baseline						
<i>Median (IQR)</i>		6 (-45, 52)	1 (-20, 19)	17 (-94, 88)	N/A	-20 (-157, 40)
PGX						
Baseline AUC						
<i>Median (IQR)</i>		135 (66, 162)	18 (6, 48)	270 (213, 350)	N/A	246 (139, 414)
Week 13 AUC						
<i>Median (IQR)</i>		113 (50, 176)	23 (4, 64)	259 (188, 325)	N/A	192 (129, 297)
Change from baseline						
<i>Median (IQR)</i>		12 (-56, 53)	3 (-28, 44)	-16.9 (-91, 55)	N/A	-31 (-161, 87)
<i>P value for difference</i>		0.86	0.71	0.26	N/A	0.8

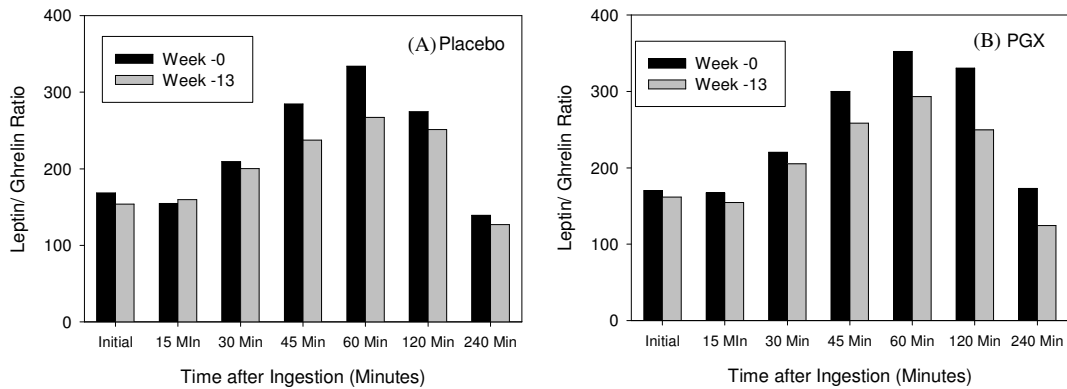


Figure 6.2: Change in concentrations of leptin to ghrelin ratio with ingestion time at the baseline (week 0) and after 13 weeks in both (A) placebo (control), and (B) PGX treatment groups.

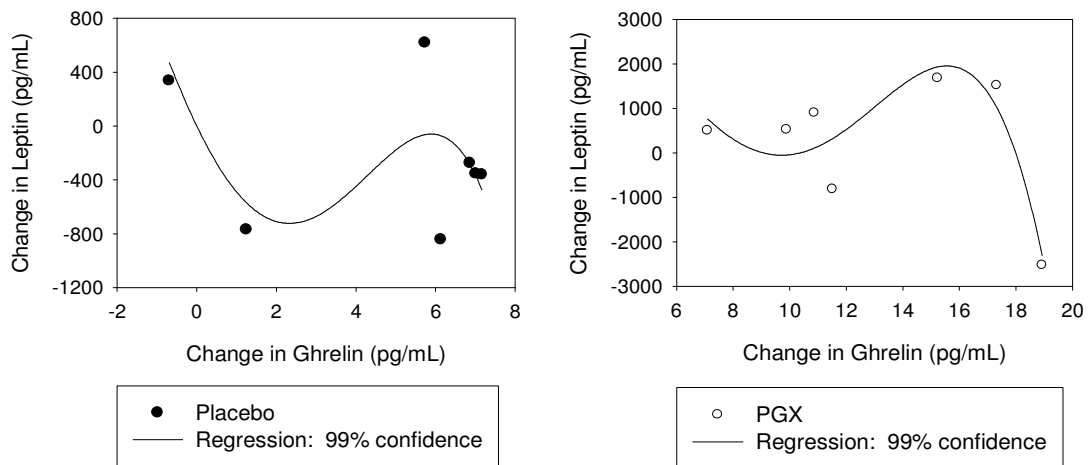


Figure 6.3: Cross-sectional relationship graph between the change in leptin and ghrelin concentrations with ingestion time during the whole duration of clinical trial (at week 0 to week 13) in both (left) placebo (control), and (right) PGX treatment groups.

6.2.3 Compliance and adverse events.

The compliance with the provided instructions was checked periodically during the study as described in compliance details (section 2.5) of this report, and the average compliance for both the placebo and PGX treatment group was > 90 %. This suggests that tolerance to the supplementation products was adequate. No serious adverse events were reported throughout the study and the participants successfully completed the clinical trial. In a recent, controlled study of PGX tolerance in France, some minor gastrointestinal (GI) symptoms were reported but not sufficiently severe to warrant user withdrawal (Carabin et al., 2009).

6.3 Discussion

Although the results of the study are not encouraging, some of the observed trends support the previous clinical studies on PGX. The findings reported here suggest that supplemental PGX capsule intake produced an effect that was not statistically different from that of the placebo. In evaluating the range of gut peptides and hormones for efficacy of the PGX fibre in capsule form, only GIP levels were reduced significantly during the 13-week, placebo-controlled randomized clinical trial. Even

with the large number of statistical comparisons, this outcome was the only finding of significance.

Many studies have suggested positive efficacies of PGX dietary fibre, and a range of analytical findings have been listed and discussed in earlier sections. This study has been unique, as it is the first to use the capsule form of PGX for supplementation, before the recent report of Brand-Miller et al. (2010). In all earlier studies, the granulated powder form of PGX (Carabin et al., 2009) was used in water, or PGX fibre was used in preloaded drinks (Lyon, & Reichert, 2010). In some studies, PGX was premixed in a solid matrix (breakfast cereals or bread) (Jenkins et al., 2000). Earlier studies have found that the highest dose of the granulated powder form of PGX (7.5 g) lowered blood glucose responses by 50%, when dissolved in water and taken with a carbohydrate meal at breakfast. The effectiveness of the granulated powder form of PGX was also related to timing, in studies where consumption was monitored after 15 min of the beginning of the meal, and glycemia was effectively reduced when PGX was consumed with a meal (nearly 28% improvement). This effect was not observed when PGX was ingested 45 or 60 min before the meal (Jenkins et al., 2000). According to Brand-Miller et al. (2010), PGX capsule consumption did not show any acute effects in lowering plasma glycemia, but had a secondary meal effect that could be comparable

to improved glucose tolerance at the beginning of the ingestion, with respect to the previous evening meal. Thus, the results here, with capsule supplementation, could still indicate that the effectiveness of capsule PGX may depend on the dose or on the timing of the consumption.

Some quality control issues in the production of PGX capsules, such as proper pH and pressure of PGX inside capsules, could help to explain the findings. These issues were addressed by Brand-Miller, et al. (2010), who also reported some second meal effects. PGX supplementation in the granulated powder form suggests that the timing of ingestion is not critical, since intake either 15 min before or after the start of meal was effective. Possibly, for the effect on acute postprandial glycemia, the capsule PGX could provide a small, delayed postprandial glycemia.

In addition, the beneficial functional effects of PGX were highly dependent on the food matrix. The supplementation of PGX in capsule form turned out to have a non-significant effect, compared to the granulated powder form as a supplement to the food matrix. PGX could be just as effective when sprinkled on food materials as when dissolved in water. Moreover, no significant reduction in insulin levels was observed with the capsule form of PGX, unlike earlier studies. When the granulated powder form of PGX was used, fasting insulin levels decreased from 89.4 ± 44.8 pmol/L to $65.0 \pm$

33.2 pmol/L (at the $p < 0.05$ level). In the present study, the non-significant reduction in insulin and postprandial glycemia levels could have been due to unchanged levels of GLP-1 type gut hormone ($p = 0.41$) from the baseline (week 0) until week 13. The increased activity of GLP-1 type hormone is usually responsible for enhancing insulin sensitivity and decreasing postprandial glucose that, in turn, accompanies weight loss. The precise reason for the spontaneous reduction in GIP ($p=0.01$), which is also related to postprandial insulin secretion, is not entirely clear. GLP-1, which is mainly secreted by L-cells in the ileum and colon, preferably binds the GLP-1 receptor in the pancreatic beta cells and has a reasonably low affinity for GIP. Perhaps a poor inhibition of DPP-4 activity, caused an increase in plasma GLP-1 levels to be undetected during the trial, which would explain the GIP levels being unaffected by GLP-1 secretion and its binding with beta cells. On the other hand, the reduction in the fasting ghrelin level, a hunger hormone that stimulates food intake and promotes adiposity, was somewhat greater in the PGX group compared to the placebo group, though statistically not significant. Also, a slight reduction in insulin would be expected, since ghrelin has been reported to lower the insulin production. In the previous acute meal studies, a fair reduction in the fasting ghrelin level with wheat dietary fibre was reported, but no pronounced effect on ghrelin was seen from oat fibre or cereal fibre (Batterham, et al.,

2003; Weickert & Pfeiffer, 2008). Recent evidence suggests that circulating ghrelin might perform in concert with leptin as a signal of adiposity in the central nervous system. Therefore, any treatment for obesity that improves the activity of leptin in the central nervous system should reduce food intake and circulating leptin, without increasing the appetite caused by a restriction of dietary energy. Ghrelin rapidly stimulates food intake in human subjects (Gray, 1995), and dietary-related weight loss studies have also indicated a coordinated decrease in plasma levels of leptin with an increase in plasma levels of ghrelin (Massimino, et al., 1998). A possible explanation for the different outcome in this PGX capsule treatment study could be the inclusion of MCT as an ingredient in the PGX treatment, which could provide a considerable stimulus to additional insulin secretion. Nevertheless, we speculate that the failure of AUC gerhlin to rise significantly may be an indicator of enhanced leptin action, which probably suppresses the gherlin mRNA expression in the stomach with only a slight decrease in body weight.

The primary reason for the non-significant change in amylin levels during the trial likely depends on the alteration in insulin level, since amylin is co-secreted with insulin. Although few studies have examined the correlation between amylin and overweight, some studies have demonstrated that amylin concentrations are associated

with the degree of overweight, revealing that the basal amylin concentration is usually higher in obese, rather than lean human subjects (Reimer & McBurney, 1996; Zhou, et al., 2008). The data presented here also shows a reduction in insulin (median -29; IQR, -103 to 62) and amylin (median -16.9; IQR, -91 to 55) concentrations with PGX treatment, indicating an indirect role of amylin in inhibiting insulin secretion, which has been reported in animal models (Juvonen, et al., 2009).

Furthermore, the composition of the soft gel capsule material could have inhibited the effectiveness of the PGX ingredient. Also, since the time needed for the disintegration of the capsule shell material after ingestion was unknown, a longer study duration, refinement of dosages, and improved timings could have been useful with a well characterized, clinically neutral and natural capsule shell material. Generally, the PGX active treatment, consisting of 600 mg of MCT with 750 mg of PGX fibre, is well-recognized for weight loss and weight management. At first, these components were thought to have a synergistic effect as a combination of PGX and MCT, as both ingredients have been extensively reported in weight loss diets. Nevertheless, since no synergistic action was observed in this study, the effect of MCT appeared to be inert, even in the presence of PGX fibre. To explore whether or not the properties of PGX induced a negative interaction with MCT, suppressing its beneficial function, would

require further testing. Also, because of the higher water affinity of PGX, it could have absorbed the MCT oil and restricted its functional profile for energy expenditure, which may have influenced the effect of MCT on thermogenesis. At this time, these explanations are highly conjectural.

Of importance is the preparation technique for the supplements used in this trial, which were significantly different from those reported by Brand-Miller et al. (2010). Specifically, Brand-Miller et al. used a heat treatment and applied pressure during the filling of the supplementation capsules. Although the effect of preparation on the product is not known, the exact physical state of the active ingredients may have an effect on the clinical outcomes. In the study by Brand-Miller et al., the heat treatment and pressure may have led to the optimal viscosity required for PGX efficacy. Also, the authors clearly mentioned that the granular form of PGX dose-dependently reduced glycemia by up to 50% ($P < 0.001$), but the same results were not observed, when capsules were ingested with breakfast. Capsules containing 3, 4.5, and 6 g PGX were reported to reduce glycemia at breakfast by up to 28% ($P < 0.001$) when consumed with the evening meal. Therefore, PGX affected acute postprandial glycemia when consumed in a granule form, and affected delayed (second meal) postprandial glycemia at breakfast, when taken as capsules with the evening meal. Although several types of

soluble and insoluble fibres have been evaluated (Brighenti et al., 2006; Weickert et al., 2005; Jenkins et al., 1990), demonstrating the secondary meal effect, because of the higher viscosity of PGX, the effect was somewhat more pronounced. Jenkins et al. (1978) reported that improvements in glucose and metabolism are directly proportional to the higher viscosity of the fibres. Taking these findings into consideration, the results of the present clinical trial could be seen as an extension of Brand-Miller et al.'s (2010) study. In general, the results suggest some support that the effectiveness of PGX may depend on the dosage, the timing of ingestion, and the physical properties of the ingested matrix.

The effects of the treatment on dietary intake, with continuous glucose monitoring, was not explored in this clinical trial, because the focus of the study was to assess the efficacy of PGX capsule, and in combination with MCT to explore additional synergic effects. This omission could be taken as a limitation for interpreting the clinical data. Clinical data obtained from a probe inserted under the skin to measure the glucose concentration and from interstitial tissues by a continuous glucose monitoring system (CGMS) iPro would allow for measurements of blood glucose every 5 min over the test period (Ryan et al., 2009). In the future, such data could provide more accurate

assessments of blood glucose concentrations than could conventional methods, and could be the focus of future clinical trials.

CHAPTER 7: CONCLUSION AND RECOMMENDATIONS

Lifestyle-related diseases such as T2D and obesity, follow a socio-economic gradient, and have risen in prevalence dramatically over the past several decades. Nevertheless, a high level of dietary fibre intake has beneficial effects on health, and increasing evidence suggests that certain types of dietary fibres might help prevent obesity and T2D. In the present clinical trial, the consumption of PGX was compared with a placebo (cellulose), with the idea that it would reduce both fasting and postprandial blood glucose, and promote satiety and insulin secretion by increasing the plasma concentrations of GLP1, GIP, PYY, PP, leptin, by suppressing levels of ghrelin and amylin. As discussed in Chapter 1, Polyglycoplex (PGX) is a highly viscous blend of soluble non-starch polysaccharides containing three complementary dietary fibres: konjac mannan (also known as glucomannan), sodium alginate, and xanthan gum. Recent human clinical trials have shown the positive effect of PGX on appetite control, cholesterol lowering, and stabilization of blood glucose and insulin levels. The aim of the present trial was determine the evidence for the influence of dietary fibre on appetite control and obesity.

Although the causes of obesity may include some combination of excessive energy intake, inactivity, and genetics, these factors all result in an imbalance between energy intake and expenditure. Moreover, the evidence suggests that the role of fibres in appetite control might be mediated, in part, by gut hormones.

This study has some potential limitations. The present 13-week, randomized double-blind clinical study, with capsulated supplementation containing either 750 mg of PGX or 750 mg of cellulose (placebo), differs distinctly from earlier PGX-supplemented clinical trials, where participants consumed liquid or powder matrices. This study was the first clinical trial using the capsulated form of PGX with MCT. The inclusion of MCT as an active treatment (600 mg MCT, mainly from coconut oil) showed no synergistic action or statistically significant effect of the active treatment, when compared to the placebo. A complete physical absorbance of MCT oil, due to the higher water affinity of PGX, could also explain the lack of effect. Furthermore, the use of BMI percentile measurements, to classify overweight subjects, considered as a good measure of obesity, is limited in being able to indirectly measure the fat mass. Since, restrictions in dietary fat intake could produce weight loss, by increasing the sensitivity of the central nervous system to leptin, this would allow energy intake, adipose mass, and leptin levels to fall without an increase in appetite. The effectiveness of the

supplementation doses in the present clinical study could be attributed to a limited, time-dependent delayed postprandial glycemia, or significantly dependent on the matrix used in the capsules. In fact, the capsule form of PGX supplementation turned out to be not significantly effective, when compared to the granulated powder form of PGX.

Although a growing body of evidence indicates that loss of fat mass causes an increase in circulating levels of ghrelin, which coincides with decreasing leptin levels, the cross-sectional relationship between ghrelin and leptin, based on the data presented here, was only moderate. The ability of PGX to reduce postprandial glycemia could be positively correlated to its potential role in obesity prevention, weight management, and adolescents.

Despite non-significant results from the present clinical trial, using supplementation with PGX in capsules, the study had a number of strengths. A large number of subjects were involved, and the randomized, double-blind design produced precise and transparent results, allowing for a clear interpretation of findings. The aim was to determine the effectiveness of PGX fibre when used in a form and in a matrix that was not granular or liquid/solid. The PGX contained 87.4% dietary fibre, of which 81.8% was soluble fibre. It is currently available as one of two physical forms: granular PGX, which is readily dissolved in water and can be sprinkled on food prior to

consumption, or soft-gelatin capsules, which can be swallowed with meals.

Overall, the study explored the effectiveness of evidence-based products that have shown a high level of compliance and adequate tolerance. In future research, new innovative delivery methods and different physical forms should be considered when examining the possible beneficial health effects of PGX or other dietary fibre supplements to help the obese population.

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**APPENDIX: PATTERN OF CHANGE IN BLOOD GLUCOSE, INSULIN
AND GUT HORMONES CONCENTRATIONS WITH INGESTION TIME
AT WEEK 0 AND AFTER 13 WEEKS**

Variation in blood glucose concentrations with ingestion time at the beginning (baseline – week 0) and after 13 weeks for placebo and the PGX treatment group are shown in Figure A.1. No significant difference was seen among the treatments at any time during the trial period. In both cases, blood glucose concentrations reached a maximum within 45 min of intake, and then gradually decreased to normal levels. A similar trend was observed with plasma insulin; however, its level was maintained until 120 min after intake before leveling to the initial values (Figure A.2). While GLP-1 concentration showed a variation from week 0 to week 13, in the placebo and the PGX treatment group (Figure A.3), the values were still not significantly different. Also, the observed pattern of change was somewhat higher in the placebo group, compared to the PGX group. The concentration of the gut peptide, GIP, was also found to increase after 13 weeks, reaching a maximum at 60 min after ingestion, for both the placebo and the PGX group, with no difference between treatments (Figure A.4). While the trend of the amylin concentration followed a similar pattern to that of plasma insulin (Figure A.5), a noticeable dependence was observed on the insulin plasma concentration. On the other

hand, PYY (Figure A.6) and PP (Figure A.7) concentrations increased rapidly until 30 min, and then decreased to a certain level with increasing time. The concentrations of PYY were higher after 13 weeks of the clinical trial, for both the placebo and the PGX treatment group. Nevertheless, no difference was observed in the PP concentration even after 13 weeks of the trial.

Although most of the studied hormones and gut peptides initially showed an increasing trend after ingestion, in both placebo and PGX groups, ghrelin and leptin concentrations decreased after ingestion of the placebo or the active PGX treatment. The concentration of ghrelin showed an initial decrease, until 60 min after ingestion of either placebo or PGX treatment, and then tended to increase up to the initial concentration level (Figure A.8). The results show even higher concentrations after the 13 week trial period, when compared to the initial values at week 0 (baseline). The leptin concentration also showed a similar trend, though the results were not statistically significant (Figure A.9).

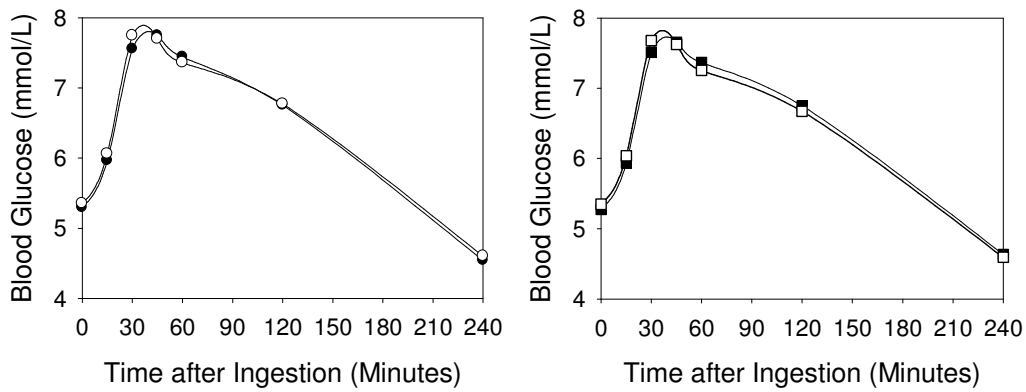


Figure A.1: Pattern of change in blood glucose concentrations with ingestion time at week 0 (filled) and after 13 weeks (empty) {*Placebo* (left), and *PGX* (right)}.

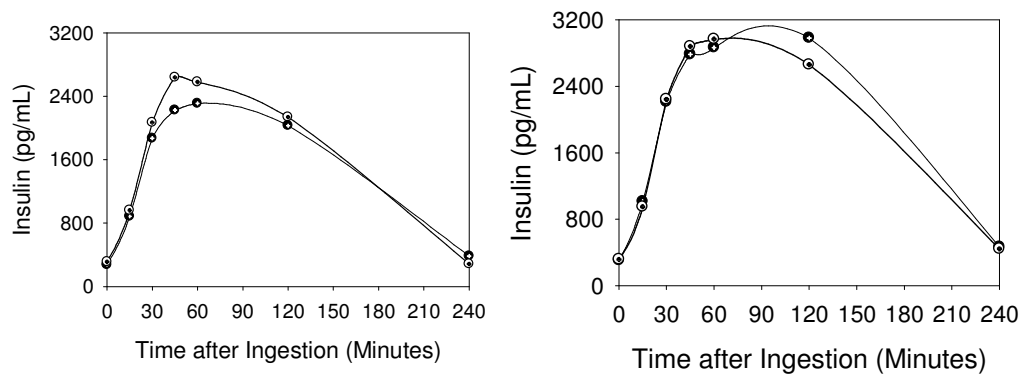


Figure A.2: Pattern of change in insulin concentrations with ingestion time at week 0 (empty) and after 13 weeks (filled) {*Placebo* (left), and *PGX* (right)}.

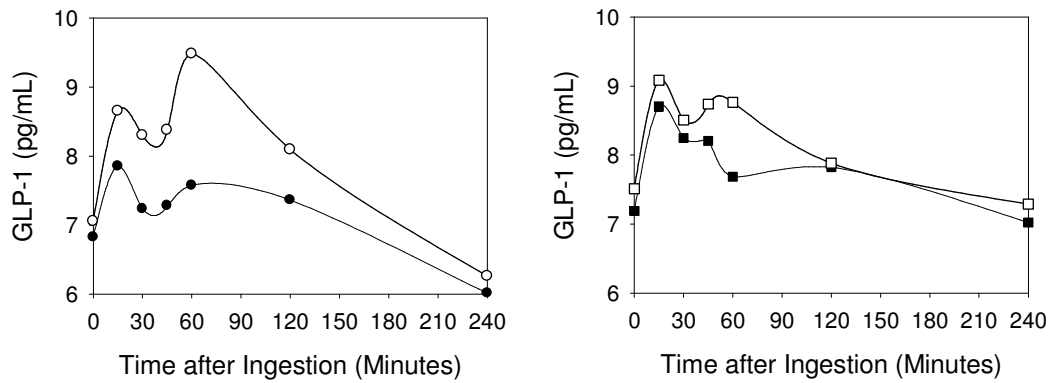


Figure A.3: Pattern of change in GLP-1 concentrations with ingestion time at week 0 (empty) and after 13 weeks (filled) {*Placebo (left), and PGX (right)*}.

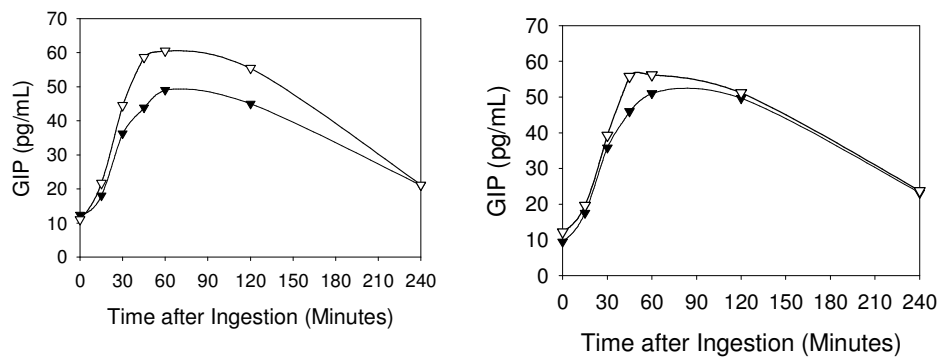


Figure A.4: Pattern of change in GIP concentrations with ingestion time at week 0 (empty) and after 13 weeks (filled) {*Placebo (left), and PGX (right)*}.

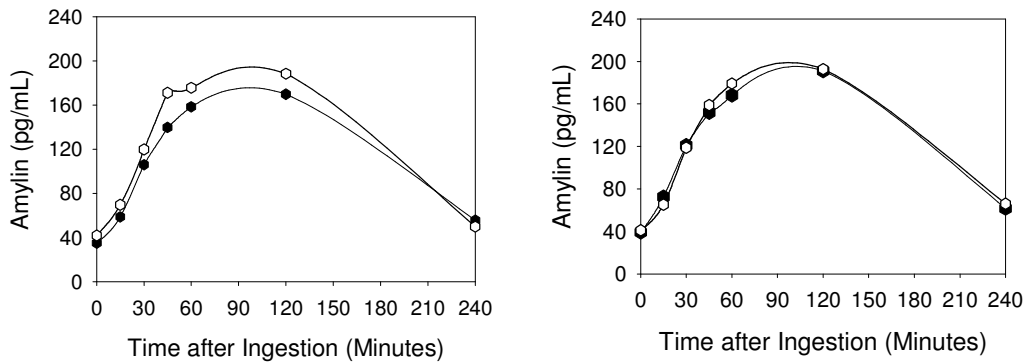


Figure A.5: Pattern of change in amylin concentrations with ingestion time at week 0 (empty) and after 13 weeks (filled) {*Placebo (left), and PGX (right)*}.

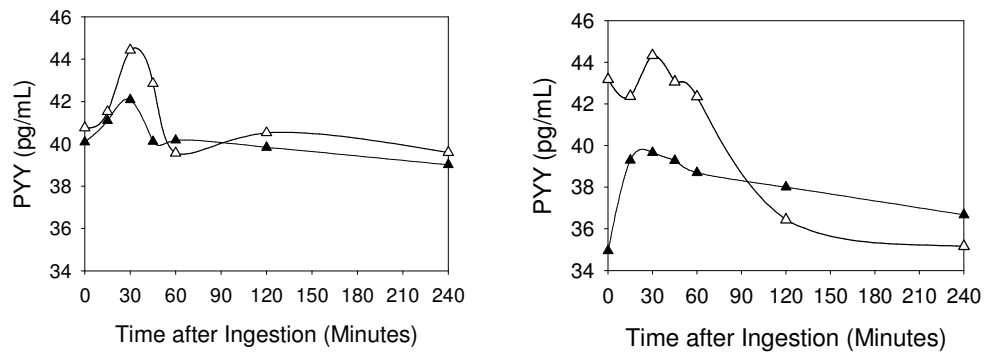


Figure A.6: Pattern of change in PYY concentrations with ingestion time at week 0 (empty) and after 13 weeks (filled) {*Placebo (left), and PGX (right)*}.

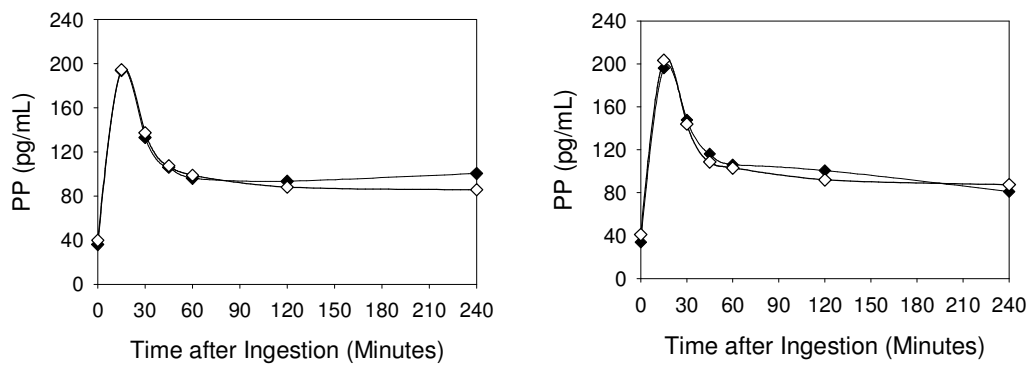


Figure A.7: Pattern of change in PP concentrations with ingestion time at week 0 (empty) and after 13 weeks (filled) {*Placebo (left), and PGX (right)*}.

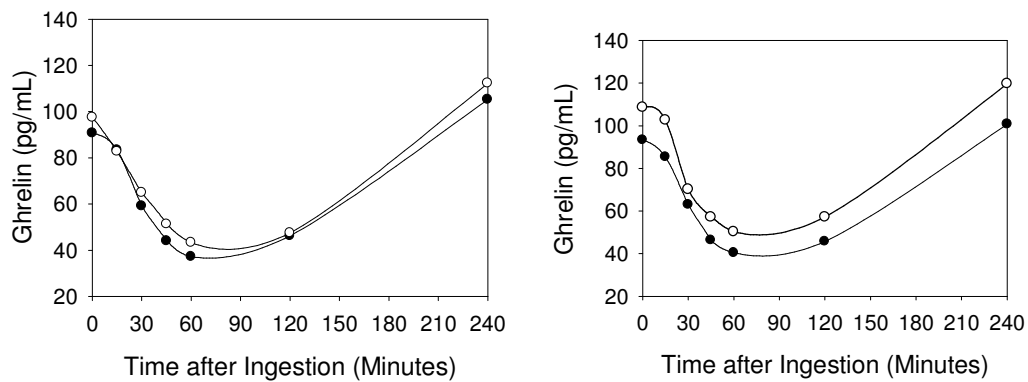


Figure A.8: Pattern of change in ghrelin concentrations with ingestion time at week 0 (empty) and after 13 weeks (filled) {*Placebo (left), and PGX (right)*}.

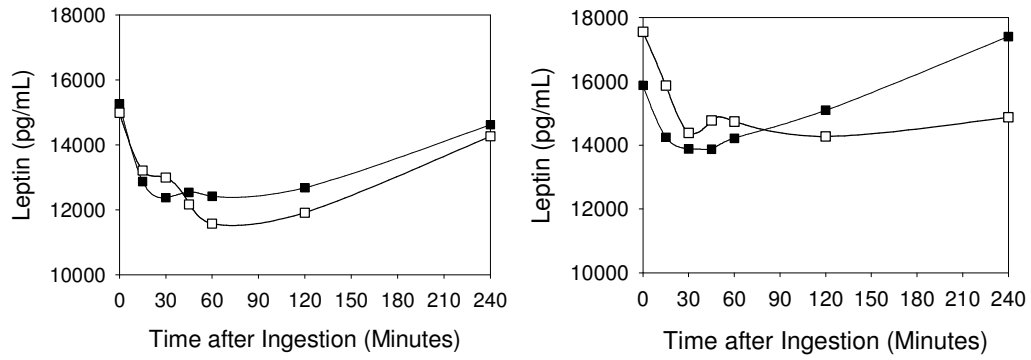


Figure A.9: Pattern of change in leptin concentrations with ingestion time at week 0 (empty) and after 13 weeks (filled) {*Placebo (left), and PGX (right)*}.