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Master thesis in Food Technology and Nutrition

Effects of plant polar lipids on postprandial glycemic regulation in healthy humans

by

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Abstract

In light of diabetes becoming a global discussion topic, some steps ought to be taken to help reduce the number of individuals suffering from the disease. Keeping good glycemic control through a healthy diet helps prevent diabetes and cardiovascular disease. Plant lipids are increasingly being studied for their postprandial effects on humans. Using a randomized cross-over dose-effect design based on one previous study, which found that high amounts of polar lipids (12g in 21g oil) may beneficially affect postprandial blood glucose and insulin concentrations, this study tested whether a lower level of polar lipids can have the same effect on glycemic regulation. For this study, 17 healthy subjects aged 20-40 years with BMIs ranging from 19 to 28 kg/m^2 were recruited. In random order, five different breakfasts were consumed by participants. Three of the breakfasts contained variable amounts of a preparation rich in plant polar lipids (5g, 10g, and 15g) consumed with white wheat bread. In addition, one breakfast contained white wheat bread without added lipids, and another breakfast consisted of rapeseed oil (15g fat) and white wheat bread; these last breakfasts were included as reference meals. In contrast to the previous study, no significant differences between the breakfasts were found in terms of their effect on postprandial glycemic regulation.

The study of the effects of polar lipids on this function is still relatively new. To draw a more solid conclusion, there is a need for more studies to explore polar lipids' ability to regulate postprandial glycemic responses. The results of this study may provide some new directions for future research, such as increasing the number of participants, studying the effects of different sources of polar lipid and trying to use different ways to prepare plant polar lipid-based food.

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It has been a privilege to meet everyone I have mentioned. And they helped me to become a better person. Because of them, science becomes even more interesting to me, and they inspire me to learn more about it.

1 Introduction

1.1 Background

The diabetes epidemic is the biggest global health challenge of the 21st century. A high prevalence of diabetes has made it one of the top 10 causes of death in recent decades. (World Health Organization, 2021). Diabetes type 2 is commonly exacerbated by hypertension, hyperlipidemia, and obesity. It has been suggested that good glycemic regulation is key to preventing diabetic complications (Milosevic & Panin, 2019). Studies are beginning to suggest that postprandial changes in glycemic and blood lipid profiles could exacerbate atherosclerosis by promoting vascular disease. Increasing postprandial blood glucose levels correlate with an increased risk of cardiovascular events (Violi et al., 2015). Therefore, the progression of diabetes and atherosclerosis can be attenuated and prevented by regulating postprandial glucose levels.

Plant lipids are shown to have merits in human metabolic regulation, especially known for promoting lower total plasma cholesterol and LDL cholesterol, which will be illustrated more in the next section. Also, excess in blood cholesterol is the main cause of coronary heart disease (Epstein et al., 1989). Additionally, studies have found that the intake of plant lipids could lower liver lipids and alleviate hyperlipidemia.

Recently, it has been demonstrated that the intake of a relatively high amount of oat polar lipids (12g) dissolved in 30g of total oat oil could significantly affect people's postprandial glycemic regulation, shown by reducing the glucose and insulin responses (Hossain et al., 2021). Here, a randomized cross-over dose-response meal study was conducted to investigate if a lower total amount of lipids with a higher ratio of polar lipids can have the same effect on glycemic regulation found in that previous study. White bread-based breakfasts with three different concentrations of polar lipids were prepared, while plain white bread and white bread with rapeseed oil breakfast were used as reference meals. Seventeen healthy subjects were included in the study and their blood glucose and insulin concentrations were investigated postprandially after the five different types of breakfasts.

1.2 Research objective

The thesis project aimed to investigate how different doses of one type of plant polar lipid affect glycemic regulation, exploring the minimum amount of polar lipids that could help to regulate postprandial blood glucose and insulin responses, without leading to overconsumption of fat. Test subjects were required to consume five test breakfasts in the lab, and their blood glucose and insulin concentrations were measured at fasting and at certain time intervals after the meal. The result of this study may be useful information for the eventual development of polar lipid-based diabetes preventive food products.

2 Theoretical background

2.1 Plant lipids

Plants contain a variety of lipids. Dietary phospholipids and phytosterols can be found in leafy and fruit components of plants, while triglycerides are abundant in seeds and nuts (Gibney & Britain, 2009). Dietary lipids from plant origin can have influence on lipidemia. Plant-based foods rich in phytosterol can help to decrease blood cholesterol by preventing cholesterol absorption from the gut (Gibney & Britain, 2009).

In the last century, loads of research have shown plant stanols and plants sterols' ability to modulate the lipid profile, decreasing plasma cholesterol. Pollak (1953) and Lees et al. (1977) both found their ability of lowering plasma cholesterol with 5-10g and 3g of concentrated plant sterol respectively. And later on, Ostlund et al found the same functionality of plant stanol, however, they pointed out that it must be soluble enough both in carrier lipids and in small intestinal to be used by the body. The solubility of plant stanol in margarine is 1/3 or less than that of plant sterol (Ostlund et al., 1999).

Apart from the effect of plant lipids on lowering cholesterol, recent attention has focused on their effect on glycemic control. As a result of comparing the subjects' glycemic profiles before and after meals in a cross-sectional study, Violi et al. demonstrated that adding 10 grams of extra virgin olive oil (EVOO) to a lunch of 25 healthy individuals could decrease postprandial glucose levels and increase insulin levels (Violi et al., 2015). With the same study method, Carnevale et al. concluded that EVOO has a positive effect on the postprandial glycemic profile in patients with impaired fasting glucose (Carnevale et al., 2017). Ohlsson et al. claimed that intake of 35 g of oat lipids as part of a breakfast can significantly lower blood glucose during the 5 hours after consumption, compared with milk fat, as well as an increase in the intestinal hormones CCK, PYY, GLP-1, and GLP-2 (Ohlsson et al., 2014). Hossain et al. compared the effect of different concentrations of oat polar lipids in a breakfast and concluded that 30g oat oil containing 12g polar lipids can significantly decrease postprandial glucose and insulin responses compared to reference meals containing bread and 30g rapeseed oil or plain white wheat bread. Oat polar lipids also increased the concentration of gut hormones GLP-1 and PYY (Hossain et al., 2021).

2.1.1 Phospholipids

Phospholipids are essential components of biological membranes in plants and animals due to their amphiphilic properties. And this characteristic is derived from their two different types of chemical groups. One party is two nonpolar (hydrophobic) long-chain fatty acyl tail groups, one of which is normally unsaturated. Another part is the polar hydrophilic functional head group. Those two parts are linked together by an alcohol (glycerol) molecule (Li et al., 2015).

Obesity, diabetes, and cardiovascular disease can develop as a result of lipid accumulation in the liver. Various animal studies have demonstrated that dietary

phospholipids could protect the hepatocyte from apoptosis and fibrosis in the alcohol-damaged liver by reducing lipid peroxidation and oxidative stress (Cohn et al., 2008). And they could also reduce plasma and liver lipids in dietary-induced fatty liver. Buang et al. found that dietary phospholipids can reduce triacylglycerol accumulation in orotic acid-induced fatty liver in rats by stimulating triacylglycerol oxidation and inhibiting triacylglycerol synthesis in the liver. The amount added to the diet of these animals ranged from 2 to 6% by weight (Buang et al., 2005). Additionally, both in vitro and in vivo studies have consistently shown that phospholipids inhibit the intestinal absorption of cholesterol (Cohn et al., 2010). This appears paradoxical to what we know about phospholipids' role in solubilizing and absorbing fat by forming complex aggregates (mixed micelles) with bile salts, and then forming lipoproteins (Gibney & Britain, 2009). However, excess phospholipids may lead to oversized or inappropriate mixed micelles, which cannot be processed effectively by luminal enzymes (Cohn et al., 2008). In this situation, phospholipids are entitled to an antisteatotic effect to reduced absorption of dietary lipids or reabsorption of biliary lipids.

Previous studies have shown that dietary phospholipids are beneficial to plasma and liver lipid metabolism, which may also could indirectly prevent atherosclerosis (Cohn et al., 2008).

2.1.2 Lecithin

The most common type of dietary phospholipids is Phosphatidylcholine (lecithin). In accordance with what has been mentioned above, animal studies have shown that dietary lecithin could reduce plasma or hepatic lipid levels significantly. Polichetti et al. have conducted experiments in hypercholesterolemic rabbits fed a soy lecithin-rich diet, showing that this phospholipid could decrease hepatic cholesterol and triacylglycerol significantly compared with a soybean triacylglycerol diet, an affect explained by reducing the β-VLDL and promoting the secretion of bile cholesterol (Polichetti et al., 2000). In a twelve-week study with rats given a high-fat diet and lecithin supplementation, Ho Sung lee et al demonstrated that lecithin could prevent weight gain and lipid accumulation while alleviating hyperlipidemia by reducing plasma triglycerides, cholesterol, and leptin levels (Lee et al., 2014).

Plant lecithin was also shown to affect the bioavailability of fatty acids when it is mixed with other lipids. According to Couëdelo et al., the flaxseed oil emulsified with soy lecithin could increase the bioavailability of alpha-linolenic acid (ALA) and the secretion of large chylomicrons by enhancing lipolysis of the oil (Couëdelo et al., 2015). Another similar study came to the same conclusion. Geurden et al. found in carp that soyabean lecithin mixed with soyabean oil could increase apparent lipid digestibility and increase postprandial plasma levels of TAG, indicating that this combination can stimulate the absorption of TAG during digestion (Geurden et al., 2008).

Also, lecithin supplementation is capable of decreasing gastric emptying rate and food intake in rats, likely due to the increase in the secretion of cholecystokinin (CCK)

(Robert et al., 2020). Another study has demonstrated that lecithin could lower blood total cholesterol and LDL cholesterol levels in hypercholesterolemic patients by 42.00% and 56.15% respectively by supplying them with daily consumption of soy lecithin for two months (Mourad et al., 2010). Similar results were found in experiments conducted by Spilburg et al as well (Spilburg et al., 2003).

2.1.3 Digestion of lipids

According to Nordic Nutritional Recommendations, the energy contribution of adults' total fat should be around 25-40%, which is around 50 ~100 g (Nordic Council Of Ministers, 2014). Typically, the daily intake of phospholipid is around 2-8 g, accounting for 1-10% of total fat intake.

The digestive process of dietary lipids occurs mainly in three parts of the body: the stomach, duodenum, and ileum. To begin with, lipids are catabolized by a gastric mechanical movement and crudely emulsified by chyme, which is an emulsion produced by the stomach. Following this, lipolysis, enzyme-catalyzed hydrolysis, happens in the duodenum, releasing fatty acids from emulsified lipids with lipases. Phospholipids are decomposed by lipases, cleaving bonds between a fatty acid and the glycerol backbone (Gibney & Britain, 2009). And the products of lipolysis are solubilized with the help of bile salts. In the end, the substances are absorbed in enterocytes and epithelial cells of the small intestine or ileum (Gibney & Britain, 2009). Approximately 90% of dietary phospholipids are absorbed into enterocytes and resynthesized, forming the surface layer of chylomicrons (Robert et al., 2020).

2.2 Glycemic regulation

2.2.1 Blood glucose regulation

Blood glucose concentration (glycemia) is homeostatically regulated within a relatively narrow range in healthy individuals, not exceeding 10 mmol/L normally (Hinson, 2010). It is well controlled by peptide hormones (Gibney & Britain, 2009). Even after overnight fasting, the value will seldomly fall below 5mM (Gibney & Britain, 2009).

In long periods of starvation, the body will form glucose from noncarbohydrate sources by gluconeogenesis, which occurs in the liver and kidneys. The substrates could be pyruvate, lactate, glycerol from adipose tissue, and amino acids from the body's proteins (Gibney & Britain, 2009). When the blood glucose falls below 5 mM, glucagon and glucocorticoid are secreted to deliver signals to the tissues to boost blood glucose concentration (Gibney & Britain, 2009). Glucagon is secreted from α cells of the endocrine pancreas as a peptide hormone. The primary function of glucagon is to promote the breakdown of glycogen into glucose (glycogenolysis) in the liver to raise blood glucose levels, and stimulate gluconeogenesis, which is the formation of glucose, mainly from amino acids. Furthermore, it stimulates lipolysis, the breakdown of triglycerides into free fatty acids in adipose tissue (Hinson, 2010). Aside from glucagon,

growth hormone, catecholamines and glucocorticoids are also responsible for regulating too low blood glucose levels (Hinson, 2010).

Insulin is opposed to glucagon, when blood glucose increases after food intake, peptide hormones amplify the response of beta-cells of the endocrine pancreas, stimulating the release of insulin from secretory granules on the cell membrane. And insulin will facilitate glucose transport into adipocytes and muscle cells by increasing the activity of GLUT4 at the plasma membrane level (Hinson, 2010). In this way, insulin not only has an impact on stimulating cells to absorb glucose and convert it to glycogen, but it also affects protein and fat metabolism, stimulating protein synthesis and lipogenesis (Hinson, 2010).

Some diseases can develop if there is a disorder of blood glucose regulation. One of the most common ones is diabetes. Two types of diabetes result in high blood glucose concentrations. One type of them is type 1 diabetes, which is caused by the destruction of beta cells in the pancreas, failing to secrete insulin. And this is usually due to the inflammation of pancreas linked to genetic inheritance. To alleviate inflammation, the immune system begins attacking beta cells leading to destruction (Hinson, 2010). All the mechanisms responsible for correcting hypoglycemia are active in this type of diabetes. The presence of excess ketone bodies from lipolysis, along with an increased concentration of free fatty acids, can lead to ketoacidosis in this condition. Type 2 diabetes is much more common than type 1 diabetes, and it is more likely to be diagnosed in obese individuals. Although insulin can be secreted normally or excessively in this case, the insulin receptors are insensitive, which means more insulin is needed to achieve the same effect compared with healthy individuals (Hinson, 2010). Another disease associated with out-of-balance hormones could be impaired glucose tolerance caused by the overproduction of growth hormone or cortisol (Hinson, 2010).

2.2.2 Insulin regulation

In the human body, insulin secretion plays a central role in maintaining glucose homeostasis. There are two mechanisms responsible for stimulating insulin secretion from beta cells, either by triggering its secretion pathway or amplifying its secretion pathway (Gupta, 2021).

Insulin is essential for the metabolism of carbohydrates, proteins, and lipids. Monosaccharides, fatty acids, amino acids, and glucose are closely linked to insulin secretion. These nutrients can work as secretagogues to stimulate beta cells to modulate the rate of insulin secretion (Gupta, 2021). Hormones can also regulate insulin secretion along with nutrients.

Glucagon-like peptide-1(GLP-1), as an incretin hormone, enhance the release of insulin in a glucose dependent manner when blood glucose concentrations are elevated and inhibiting the release of glucagon from the alpha cells. Also, it works together with another incretin, gastric inhibitory peptide (GIP), to decrease the gastric emptying rate

and intake of food. Cholecystokinin (CCK) stimulates the secretion of insulin by protecting the beta cells from apoptosis when it is above physiological concentration (Gupta, 2021).

On the contrary, estrogen, somatostatin and ghrelin can decrease insulin secretion from beta cells, as well as leptin, which suppresses hunger and fat storage in adipose tissue (Gupta, 2021).

As mentioned before, the metabolic effect of insulin is mainly reflected in three aspects: promoting uptake and storage of glucose, protein formation, and fat storage. When it comes to glucose metabolism, insulin will accelerate the uptake of glucose by increasing glycogen synthesis and inhibiting its breakdown in muscle. In addition, it will enhance glycogen formation and inhibit glycogenolysis and gluconeogenesis in the liver. When it comes to protein metabolism, insulin facilitates amino acid absorption, promotes protein synthesis, and inhibits protein degradation in muscles, adipose tissue, liver, etc. Furthermore, it inhibits the breakdown of amino acids into glucose, which leads to less liver production of urea. When it comes to fat metabolism, it boosts triglyceride storage in the body by activating extracellular lipoprotein lipase and inhibiting intracellular lipase. Also, the esterification and storage of fatty acids in the adipose tissue are increased. Furthermore, insulin inhibits the conversion of fatty acids into ketones, while increasing the production of triglycerides, cholesterol, and very low-density lipoproteins in the liver (Hinson, 2010).

3 Methodology

3.1 Study design and procedure

3.1.1 Test subjects

This study included 17 healthy test subjects, ten of whom were female and seven of whom were male. Aged 25.6±0.86 and 24.57±0.75 years old respectively and having a BMI of 22.16±0.79 and 22.51±1.03, respectively (means±SD). All subjects were in good health and met the following inclusion criteria, which were: age between 20-40 years old and normal weight (body mass index (BMI) between 19-28 kg/m^2, calculated using the following formula 65/ (1.65*1.65)) and had normal fasting blood glucose concentrations (<6.1 mmol/L). No known metabolic conditions or other diseases that could have affected the study results were allowed. All subjects consumed an omnivorous diet and were required to refrain from using antibiotics or probiotics for at least one month before the study, and the use of those products was also prohibited during the study.

An information session was held for all subjects one week before the study began. Informed consent was obtained from the subjects before completing questionnaires about their diets and exercise habits. To commence the study, ethical approval was obtained from the Reginal Ethics Review Board in Lund.

3.1.2 Experimental procedure

In this study, a randomized cross-over dose-response design was used. A schematic overview of the experimental design of the meal study can be seen in Figure 1.

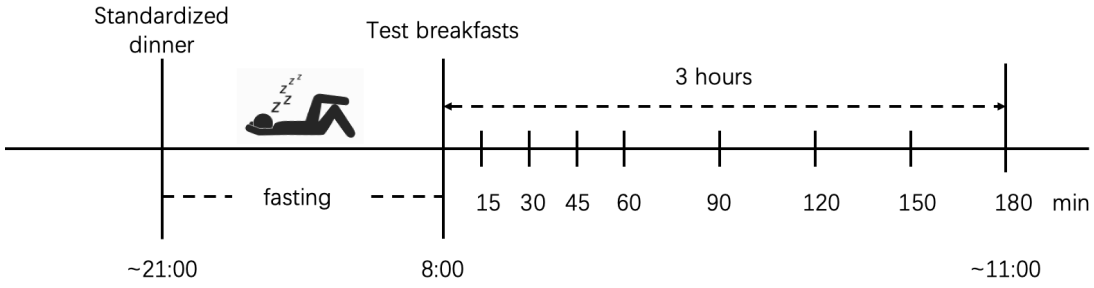


Figure 1*. Schematic overview of the experimental design (blood glucose and insulin (except at 15 min) were measured at the defined timepoints in the postprandial period.)*

Five different types of breakfast were prepared for each subject in this study, resulting in a total of five trial days per subject. The order of intake of different breakfasts was randomized. Randomization was accomplished by assigning a random code to each subject, which was then used throughout the whole experiment. We then randomly allocated five different types of breakfast to one subject in five trials and repeated the procedure for each subject. As a result, a list of the subject's experimental order could be obtained. Every trial day for a test person was separated with an interval of one week.

To reduce factors that are known to affect the experimental variables, all subjects were required to consume an identical dinner at 6 pm the day before their trial days. Alcohol and all types of strenuous physical exercise should be avoided as well. Commercial white bread was prepared for all subjects as their standardized evening meal at 9 pm, to avoid over-fasting and keep the same condition before they came to the clinical room. The amount of bread that they consumed at this late evening meal should be the same all the time. After 9 pm, they should keep fasting until the next day's test breakfasts were served. Also, they were strongly recommended to keep a similar breakfast and lunch one day before the trial day. For their meal, all high-fiber foods were forbidden, the forbidden food list could be found in Appendix 3. A food diary notebook was provided to all subjects to help them keep track of their mealtime and meal amounts.

On the day of the trial, subjects arrived at the clinical site at Kemicentrum, Lund University, around 7.45 am. They were requested to be fasting since the standardized evening meal last night. Running or other strenuous exercise was not allowed in the mornings at the study days. After they arrived, the first finger-prick capillary blood sample was taken and counted as the result of time point 0 min. Around 8.00 am, prepared breakfasts were served to everyone, and the clock started. Three or four test persons participated each day, and the breakfast was commenced with 3 min in between each subject. The breakfast should be finished within 10-12 minutes. Then, the blood

glucose concentrations were determined at the time points 15, 30, 45, 60, 90, 120, 150, and 180 min. As shown in Figure 1, the insulin concentration measurement schedule is similar to the one for blood glucose, except for the 15-minute time point. The whole trail was around 3.5 hours. During the experiments, subjects were required to remain in the room quietly and to eat only the breakfast that was provided.

3.2 Test breakfast

Table 1 shows the formulation of five different types of test breakfasts, which subjects were served during the trials. All of them should provide 50 g of available starch and 15 g of lipids. 5 g, 10 g, and 15 g of polar lipids (83% lipid formulation) were compensated with rapeseed oil (91.6%) to achieve the total lipid content of 15 g. The nutritional composition of different breakfasts is shown in Table 2. Rapeseed oil contains a low concentration of saturated fatty acids and a high level of unsaturated fatty acids, making it a healthy oil for use as compensation. Polar lipids with rapeseed oil were mixed with 10 ml of water. The lipid-water mix was put between slices of white wheat bread (120 g). 250 ml water was served with the meal. Rapeseed oil was put in between the bread slices directly as liquid and served with 260 ml of water. Plain white wheat bread without any lipids was included as a reference breakfast. Some breakfast images could be seen in Appendix 1. Subjects consume the breakfast without knowing which type of products they had, e.g., in a blinded manner.

Table 1. *Recipes for five breakfasts*

Test breakfast	**15G**	Polar lipids (15g) + white wheat bread	120 g white wheat bread + 18 g polar lipid product (15g polar lipids) + 10 ml water + 250 ml water
	10G	Polar lipids (10g) + white wheat bread	120 g white wheat bread + 12 g polar lipid product (10g polar lipids) + 5.5 g rapeseed oils (5 g fat) + 260 ml water
	5G	Polar lipids (5g) + white wheat bread	120 g white wheat bread + 6 g polar lipid product (5g polar lipids) + 10.9 g rapeseed oils (10 g fat) + 260 ml water
Reference breakfast	**RSO**	Rasp + white wheat bread	120 g white wheat bread + 16.4 g rapeseed oils + 260 ml water
	NL	White wheat bread	120 g white wheat bread + no oils + 260 ml water

NL, white wheat bread without polar lipids; RSO, white wheat break added with rapeseed oil; 5G, white wheat bread added with 5 grams of polar lipids; 10G, white wheat bread added with 5 grams of polar lipids; 15G, white wheat bread added with 5 grams of polar lipids. Total amounts of ?? was included in all breakfast with lipid supplementation.

Table 2. *Nutritional composition of the bread-based breakfast*

	15G	**10G**	**5G**	**RSO**	**WWB**
Carbohydrate (g)	50	50	50	50	50
Fats (g)	15	15	15	15	0
Polar lipids (g)	15	10	5	0	0

NL, white wheat bread without polar lipids; RSO, white wheat break added with rapeseed oil; 5G, white wheat bread added with 5 grams of polar lipids; 10G, white wheat bread added with 5 grams of polar lipids; 15G, white wheat bread added with 5 grams of polar lipids.

3.3 Physiological test parameters

3.3.1 Blood glucose

The blood glucose concentrations were determined by the HemoCue equipment with capillary blood samples. Subjects' blood was drawn from finger pricks from Haemolance Plus-normal flow and collected in a glucose cuvette. Cuvettes were put outside from the refrigerator half an hour in advance to be used. Once the cuvette is full and no air bubbles could be seen, they were immediately put in the HemoCue Glucose 201^{+} analyzer, which would show the result in one minute. Every day before used, the HemoCue Glucose machine was verified for accuracy with a standard plasma solution.

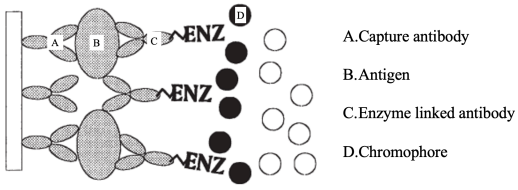
3.3.2 Serum insulin

The serum insulin concentrations were analyzed by a solid phase two-site enzyme immunoassay kit. Three big drops of blood (0.5 ml) were collected in BD Microtainer SST tubes. Tubes with blood were then standing at room temperature to clot for around 30 minutes, whereafter they were centrifuged (5 mins, 5000 rpm, 25 ℃). The serum was then pipetted into 5 ml tubes and stored in a freezer at – 40 ℃ until analysis.

Insulin concentrations were detected with the Enzyme-Linked Immunosorbent Assay (Elisa) method, specifically, direct sandwich Elisa. It is a powerful technique used to detect antibodies or antigens in biological fluids, with high specificity, flexibility, and sensitivity, and plays a major role in the field of clinical analysis (Masoodi et al., 2021).

The ELISA method involves adding and reacting the reagents successively. As its name implies, sandwich Elisa uses two different antibodies for the same target antigen, which is insulin in our case. Each antibody binds to a different epitope of this antigen (insulin) and the two types of antibodies are capture antibody and enzyme-linked antibody. Capture antibody specific for the target antigen is immobilized on a solid phase, usually a microtiter plate with 8×12 microtiter well, by insulin kit manufacture. Then, a serum sample containing the antigen (insulin) is added. After that, enzyme-linked antibodies are added to the well. Substrate (Chromogen) specific to the enzyme is added to the wells (Walker & Crowther, 2009). Finally, the reaction is stopped by stop solution to

inhibit color change, and their color is quantified in a spectrophotometer at 450 nm. Figure 2 shows the complete “sandwich” form. And some pictures of running Elisa's experiments can be found in Appendix 2.



(Walker & Crowther, 2009)

***Figure 2**. Direct sandwich insulin*

3.4 Calculation and statistical methods

Data of blood glucose and serum insulin were expressed as mean values±standard error of the mean (SEM). All graphs were plotted in GraphPad Prism, which was used to calculate the incremental area under the curve (iAUC) for blood glucose and insulin concentration (0-180 min) as well. The calculations of iAUC were performed with a trapezoid model for all test subjects using their fasting values as baselines. Areas below the baselines was ignored. The positive iAUC data were used to identify possible significant differences among the products.

Possible differences were determined by the ANOVA test (general Linear Model) using the statistical software MiniTab. When $P < 0.05$, we can see the significant differences between groups. Before running an ANOVA test, all results were analyzed with the Anderson-Darling method to determine their distribution. If they did not follow a normal distribution, the box-cox transformation was used to transform them to be normally distributed.

4 Results

4.1 Blood glucose

The average incremental changes in blood glucose concentrations are depicted in Figure 3. All values exhibit the same trend, with rapid increases during the first 30 minutes and steady declines to almost zero at 180 minutes. The mean peak values of all test breakfasts can be observed at a time point of 30 min, with 10G having the lowest peak blood glucose response, 2.27 mmol/L, and NL having the highest peak value, 2.87 mmol/L.

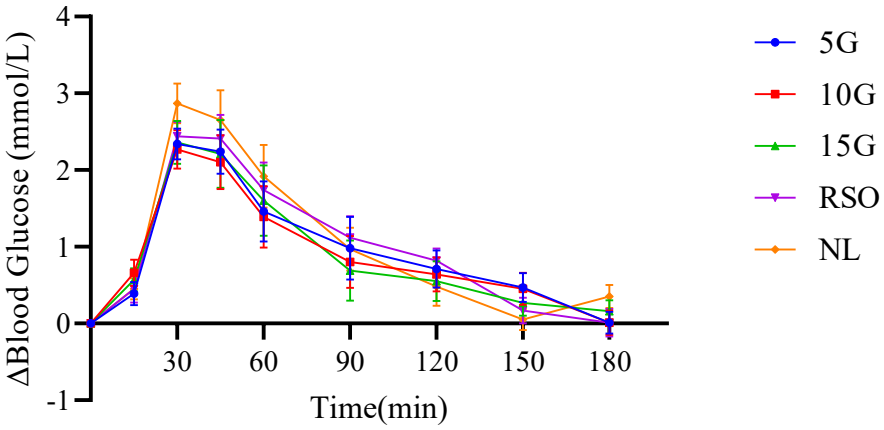


Figure 3. *Incremental changes in serum blood glucose concentrations after test breakfasts. Values are means ± SEM, n = 17 healthy subjects. NL, white wheat bread without added lipids; RSO, white wheat break added with rapeseed oil; 5G, white wheat bread added with 5 grams of polar lipids; 10G, white wheat bread added with 5 grams of polar lipids; 15G, white wheat bread added with 5 grams of polar lipids.*

The calculation of the average incremental area of blood glucose response of different test breakfasts can be found in Table 3. According to the ANOVA test, there were no significant differences in subjects' fasting blood glucose values. Also, no significant differences were found among the five different polar lipid levels, or between NL and the high lipid products.

Table 3. *Fasting blood glucose concentrations and incremental area of blood glucose after consumption of test meals at breakfast.*

Test variables	5G	10G	15G	RSO	NL
Fasting blood glucose (mmol/L)	5.23 ± 0.15 [a]	5.1 ± 0.06 [a]	5.12 ± 0.08 [a]	5.04 ± 0.10 [a]	5.02 ± 0.13 [a]
Blood glucose iAUC = 0–180 min (mmol*min/L)	183.4 ± 36 [a]	174.6 ± 36.6 [a]	168.8 ± 40.1 [a]	193.3 ± 26.4 [a]	188.2 ± 28.7 [a]

1 Data are presented as means ± SEM, n = 17 healthy subjects. Values in the same row with different superscript letters are significantly different, p < 0.05 (ANOVA, followed by Tukey's test). iAUC, incremental area under the curve.

4.2 Insulin concentration

Figure 4 illustrates the incremental changes in insulin concentration. In a similar way to blood glucose concentrations, insulin concentrations of all groups rise dramatically during the first 30 or 45 minutes, followed by a continuous decrease throughout the rest of the test period. As can be seen in the graph, 15G showed a tendency (not significant) towards lowest insulin response between 0-60 minutes.

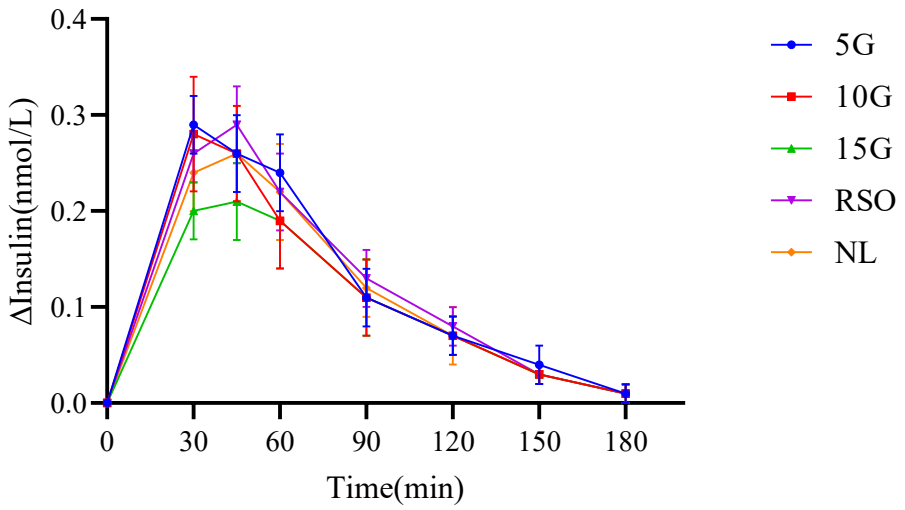


Figure 4. *Incremental changes in insulin concentrations after test breakfasts. Values are means ± SEM, n = 17 healthy subjects. NL, white wheat bread without added lipids; RSO, white wheat break added with rapeseed oil; 5G, white wheat bread added with 5 grams of polar lipids; 10G, white wheat bread added with 5 grams of polar lipids; 15G, white wheat bread added with 5 grams of polar lipids.*

Table 4 shows the result of the fasting insulin concentrations and iAUCs. According to the ANOVA test, there were no significant differences in subjects' plasma insulin concentration at fasting or depending on breakfast products. However, according to mean values, 15G resulted in the smallest postprandial iAUC during 0-180 min, which is 18.52 ± 3.84 nmol*min/L, while 5G resulted in the biggest area, which is 23.58 ± 2.85 nmol*min/L. However, no significant differences are found between these 2 curve segments, or with respect to the other meals tested.

Table 4. *Fasting insulin concentrations and iAUC after consumption of test meals at breakfast.*

Test variables	5G	10G	15G	RSO	NL
Fasting insulin concentration (nmol/L)	0.03 ± 0.01 [a]	0.03 ± 0.00 [a]	0.02 ± 0.00 [a]	0.03 ± 0.01 [a]	0.02 ± 0.00 [a]
Insulin iAUC = 0–180 min (nmol*min/L)	23.58 ± 2.85 [a]	21.89 ± 4.05 [a]	18.52 ± 3.84 [a]	22.47 ± 2.92 [a]	21.02 ± 4.21 [a]

1 Data are presented as means ± SEM, n = 17 healthy subjects. Values in the same row with different superscript letters are significantly different, p < 0.05 (ANOVA, followed by Tukey's test). iAUC, incremental area under the curve.

5 Discussion and Conclusion

5.1 Discussion

This study aimed to examine if different concentrations of polar lipids of plant origin exert a dose response effect on healthy individuals' glycemic regulation. For this purpose, blood glucose and insulin concentrations were determined in the postprandial period after the consumption of different amounts of polar lipids incorporated in breakfast meals. A control meal with a low polar lipid oil (RSO), and a reference bread meal without lipid (NL) supplementation were also included. Contrary to expectations based on previous studies, this study did not find significant dose response effects of the plant polar lipids or differences between the polar lipid and non-polar lipid preparations included in the study. However, a trend towards reduced postprandial insulin concentration was observed after 15G compared with the other lipid-containing meals.

Consequently, this result does not match the result of the experiments mentioned in the theoretical background. Despite polar lipids having no significant effect on blood glucose and insulin concentration in this study, from the curves mentioned in results it is notable that in the insulin curve, 15G elicited a lower peak value than 5G. And according to iAUC calculations, polar lipids lowered the insulin response by 22% between 5G (23.58) and 15G (18.52) during 0-180 min ($P<0.1$).

Possibly, the non-significant results could be attributed to a lower total fat content in the meals used in this study compared with the previous research (15g in our project vs. 33g in Hossain's (2021) project). Other studies observing effects on postprandial glycemic regulation have mixed the polar lipids-enriched preparation with the oil from the same plant source before its consumption by the volunteers. Therefore, it could be speculated that when homologous oils are mixed with polar lipids, the polar lipids can be more effectively solubilized or dispersed, enabling them to be more easily digested and absorbed in the small intestine, a phenomenon that may facilitate glycemic regulation. However, no mechanism can be currently proposed for this hypothetic modulation.

In addition, the structure of the fatty acids present in the test lipid is a quite crucial factor of its functionality. Since other studies have not indicated the fatty acid composition of the polar lipids that show regulating action on blood glucose and insulin concentrations, it may be that the polar oils used in this study and other studies have different chemical structures (different types of fatty acids), which may lead to dissimilar results.

Furthermore, the way we prepared the breakfasts containing polar lipids was different from that of other studies. In both Hossain's (2021) and Ohlsson's (2014) studies, the polar lipids containing breakfasts were drinks, whereas we prepared sandwiches,

resulting in a more complex food matrix that can affect the absorption of nutrients, which may explain differences in the blood glucose response recorded in our study.

Another possible explanation for these discrepant results might relate to the characteristics of the participants. Humans differ genetically. They also vary greatly in their lifestyle, background diet, health, physical activity and many other ways. Due to the fact that everything cannot be standardized in a study when participants live in their own homes, it is far more difficult to control their diet every day, which may affect the test results. Firstly, the group of subjects in this study was different from that in the previous study conducted by Hossain (2021) which consisted of volunteers of long-term residence in Sweden. Here, only 3 out of the 17 participants in this study were native Europeans. The rest were immigrants who have lived in Sweden for about 1 year and 7 months on average. Migration may affect bacteria in the gut, which can affect the absorption of nutrients or hormone secretion. Secondly, although we have taken sufficient precautions to prepare for the pre-experiment meeting and diet record diary, the actual participants' compliance is unknown, and their sleep quality, dietary habits and exercise habits may all affect the physiological test results.

These are all factors that may have interfered with the results in this study.

As mentioned before, polar lipids tended to decrease the postprandial insulin response ($P<0.1$ between 15G and 5G for insulin), although no statistically significant differences with the reference meals were found here. However, the potential mechanism behind the polar lipid insulin regulating action is not clear yet.

As a requirement, the fat contents in all meals tested were the same. It is likely that 5G and 10G did not show the same effect as 15G because they were mixed with rapeseed oil before being consumed by the subjects. The high ratio of triglycerides (TAG) contained in rapeseed oil led to higher levels of plasma fatty acids, which can stimulate the secretion of insulin (Gupta, 2021).

Based on what was stated in the theoretical background (**2.1.2 Lecithin**), another suggested hypothesis of the result can be that when polar lipids are mixed with other oils before consumption, the absorption of TAG in the oil will increase compared to a meal that is without polar lipids dispersed previously. A possible explanation for this phenomenon is that polar lipid may emulsify the TAG in the oil, which can increase the rate of absorption of it in the body, resulting in higher plasma fatty acid levels. Therefore, groups 5G and 10G had higher insulin concentrations. However, the postprandial concentration of fatty acids was not measured in this study, and more research are needed to prove these hypotheses.

A potential effect of plant polar lipids on the release of intestinal hormones was not analyzed in this study, so the effects of intestinal hormones could not be determined.

5.2 Conclusion

The present results do not support a significant effect of the plant polar lipid investigated, nor of rapeseed oil alone. This contradicts observations from previous studies investigating the effect of other polar lipids. However, to draw more solid conclusions the study should be repeated with changes in the study design, by either trying to use a more homologous group of subjects as in the study of Hossain (2021) or increasing the number of participants to minimize the influence of individual differences. Furthermore, we could conduct experiments with different polar lipid sources to find out how their differences in composition or chemical structure can affect postprandial glycemic control. It is also important to pay attention to the preparation of the test products and the food matrix, as this could affect the results.

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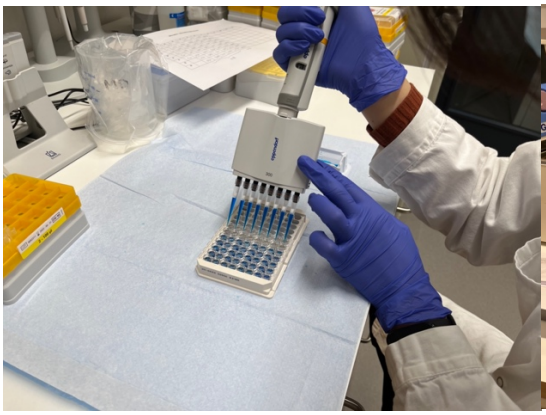
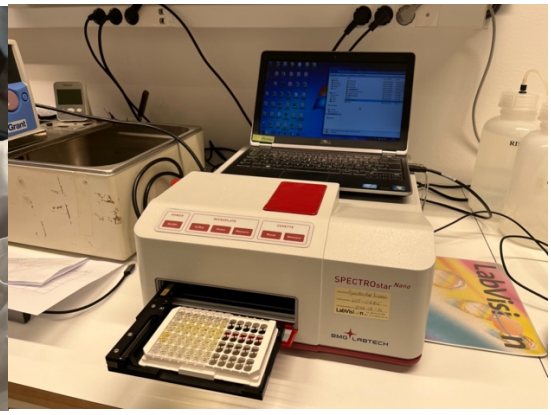
Appendix

Appendix 1. Sample breakfasts for subjects

Appendix 2. Elisa experiments





Appendix 3. High fiber foods to avoid the day before the trial day:

1. Bread that contains whole grain kernels. Dietary fiber, and whole grains (such as pumpernickel, Danish rye bread, crispbread, and whole meal bread)
2. Muesli
3. Beans and lentils (such as brown beans, black beans, white beans: chili con carne, kidney beans, soybeans, and red lentils)
4. Peas (such as green peas, green beans, yellow peas: pea soup, chickpeas: hummus, falafel)
5. Jerusalem artichoke
6. Bran (such as wheat bran, oat bran, etc.) i.e.: do not eat fiber supplements.
7. Cabbage (Brussels sprouts, kale, red cabbage, broccoli, cauliflower, cabbage: cabbage pudding, cabbage dolma)
8. Also, avoid onions in every form
9. Whole grain pasta and such