LUND UNIVERSITY

LTH

FACULTY OF ENGINEERING

The assessment of the acute and second meal postprandial impact of plant polar lipid on glucose concentrations and satiety in healthy young adults

Master thesis Food technology and Nutrition
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Faculty of Engineering, LTH, Lund University

May 2023

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Abstract

Lifestyle modifications could be crucial in preventing and managing type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVD), including diet and postprandial glycaemic control. Previous studies on the effects of polar lipids (PLs) on postprandial metabolic responses at Lund University have shown conflicting results. To reconcile these findings and contribute to the existing literature, this study investigated the acute and second meal postprandial responses of blood glucose and subjective appetite parameters in healthy young adults consuming different concentrations of another plant PL preparation. A single-blind, randomised crossover study compared two doses of PL with reference samples. Twenty subjects consumed four solid test meals on different occasions, consisting of a standardised portion of white wheat flour bread supplemented with: i. 8 g PL (PLL), ii. 17 g PL (PLH), iii. 17 g commercial reference oil (RSO), iv. no added lipids (NL). Total fat content was equivalent in all meals. The test meals were given as breakfast, followed by a standardised lunch.

The results showed a significant reduction in postprandial blood glucose concentrations after breakfast with lipid-prepared test meals. PLH decreases blood glucose concentrations by 42% compared to NL, while RSO and PLL reduce blood glucose by 20% and 26%, respectively. Notably, a dose-response effect was observed, with PLH having a more significant effect on postprandial blood glucose concentrations than PLL, RSO and NL. Significant improvements in appetite were observed before the start of the second meal. These results were consistent with Hossain et al.'s (2021) randomised crossover study, strengthening evidence of the beneficial effects of plant PLs on postprandial glucose response and appetite regulation. Nevertheless, future investigation should focus on determining the minimal dose-response effect of PL and understanding the mechanisms underlying the effect of PL on blood glucose concentrations and appetite regulation.

Keywords: postprandial blood glucose, plant polar lipid, type 2 diabetes mellitus, appetite regulation, dose-response effect.

Popular science

'Effects of a plant polar lipid on glucose concentrations and Satiety in healthy subjects'

Outcomes in a clinical study indicate that supplementing a breakfast meal with plant polar lipid X can reduce glucose responses both directly after the meal and after a second meal consumed 3.5 h thereafter.

These results show a promising preventative strategy for lifestyle-related diseases such as type 2 diabetes and obesity. The prevalence of these diseases is rising rapidly, resulting in global healthcare problems. In 2019, 9.3% of the world's population suffered from diabetes, and it is expected to rise to 10.9% by 2045; wow! Also, such diseases have intense consequences, e.g. cardiovascular diseases and early death. It is known that following a healthy lifestyle and controlling postprandial blood glucose levels are essential to counteract or prevent these diseases; however, specific preventative strategies are still urgently needed. Studying the effect of plant polar lipids is becoming more attractive due to their suggested metabolic effects.

In this diploma work, we investigated the effect of two different doses of a polar lipid of plant origin (PL X), on blood glucose levels and satiety sensations. The lipids were incorporated in a spread (blended with glucose, cacao and water) and served on white wheat bread. The results were compared with the effects of a commercial reference oil and controlled with a negative sample containing no lipids, all included 43 g of available carbohydrates. A randomised cross-over single-blind study was performed on 20 healthy subjects. Requirements were; aged 20 - 40 years, BMI 19-28 kg/m2, non-smoker and no metabolic disorder or food allergy. A typical experiment day started with a breakfast meal, including one of the test products, and blood glucose levels were measured for up to 3.5 hours. Then, a standardised lunch meal was given, composed of mashed potato and vegetarian meatballs. Blood glucose measurements continued up to 2.5 hours after lunch. Meanwhile, subjects filled in a (100 mm) scale with questions about their fullness, hunger and desire to eat.

In line with previous studies, the results indicated an improved postprandial acute and second-meal blood glucose response following a supplemented breakfast meal with PL X. Additionally, satiety sensations were improved by PL X right before lunch. These results imply that PL X helps

control postprandial blood glucose responses, prolong satiety feelings and thereby might help control food intake and prevent obesity and type 2 diabetes. However, studying the effect of PL X on blood glucose levels and satiety is relatively new, so there is a need for further studies to reinforce the results of this study and to find the underlying mechanisms.

Preface

In front of you lies the master thesis 'The assessment of the acute and second meal postprandial impact of plant polar lipid on glucose concentrations and satiety in healthy young adults'. It was written by Wilmie and Susie, two Dutch students who pursued a master's in Food Technology and Nutrition at Lund University, Sweden. Researching and writing this thesis was our last task before fulfilling the graduation requirements and thus completing the master's. The thesis project was performed from January 2023 until June 2023.

While studying for our Master's, we were free to choose some courses. Here it happened that we chose the same course, 'Food and Diet - physiological effects and consequences', led by Anne Nilsson and Juscelino Tovar. We both really enjoyed the lectures, have learned a lot, and they sparked an interest in the nutritional aspect of our study. For these reasons, we wanted to pursue a thesis project at the nutritional department to learn more about how food ingredients can prevent/treat diseases and how to set up a human research study since we had yet to do this before. During our thesis study, we worked with various people, which made it sometimes difficult to plan and work structure. However, it did teach us to be flexible. The experimental phase of our study was intense for both of us, having early and long working days. It made us realize that performing human studies is hard work, nevertheless rewarding. In addition, we have learned more about statistical analysis and how to interpret the results linked to expectations.

First, we want to thank our supervisors, Anne Nilsson and Juscelino Tovar, for giving us the opportunity to perform a master's thesis project at your department and guiding us through the study. Your expertise and interest helped us gain the proper knowledge for our research. In addition, we want to thank all participants of our study. With your commitment, we were able to get such complete results. Finally, we would like to thank all of our friends and family for their support, and of course, you, the reader; we hope you enjoy reading our thesis project.

Houdoe en bedankt!

Wilmie van den Elzen and Susie Ketelaars

Lund, Sweden, 15/05/2023

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List of Abbreviations

Abbreviation	Definition
ANOVA	Analysis of Variance
AUC	Area Under the Curve
BMI	Body Mass Index
CCK	Cholecystokinin
CVD	Cardiovascular Disease
EFSA	European Food Safety Authority
EFA	Essential Fatty Acid
EPA/AA	Eicosapentaenoic Acid/Arachidonic Acid
GIT	Gastrointestinal Tract
GLP-1	Glucagon-Like Peptide 1
HDL	High-density Lipoprotein
iAUC	Incremental Area Under the Curve
LDL	Low-density Lipoprotein
MUFA	Monounsaturated Fatty acid
NL	No Lipid Sample
NNR	Nordic Nutrition Recommendations
PC	Phosphatidylcholine
PL	Polar Lipid
PLH	Polar Lipid High
PLL	Polar Lipid Low
PUFA	Polyunsaturated Fatty Acid
PYY	Pancreatic Peptide YY
RSO	Reference Sample Oil
SFA	Saturated Fatty Acid

SEM	Standard Error of Mean
T2DM	Type 2 Diabetes Mellitus
TFA	Trans Fatty Acid
TAG	Triacylglycerol
USFA	Unsaturated Fatty Acid
VAS	Visual Analog Scale

Introduction

1.1. Background

Diabetes prevalence worldwide is increasing rapidly and is currently at an epidemic stage, resulting in global health care problems. According to the International Diabetes Federation Diabetes Atlas 9th edition, in 2019, 9.3% (or 463 million people) of the world's population suffered from diabetes [1]. These numbers are expected to rise to 10.2% by 2030 and 10.9% by 2045. Of these numbers, 90% account for type 2 diabetes mellitus (T2DM) which is a non-insulin dependent metabolic disorder defined as the state of insulin resistance and hyperglycemia [2]. Over time, diabetes causes end-organ damage, dysfunction and failures ensuing in kidney failure, blindness, heart diseases and eventually can lead to early death [2, 3]. In addition, diabetes is predisposing to atherosclerotic cardiovascular diseases (CVDs) and diabetic cardiomyopathy, which accelerates heart failure, and increases the risk of cognitive impairment and dementia [4, 5]. World Health Organization [6], CVDs are the leading cause of death for an estimated 17,9 million people, yearly.

T2DM and CVD share many pathophysiology features due to common genetic and environmental risk factors. Common known pathophysiologies for T2DM and CVD are: obesity, insulin resistance, subclinical inflammation, oxidative stress, hypercoagulability, hypertension, hyperlipidemia and dyslipidemia [5]. T2DM and to a major part also CVDs can be counteracted or prevented by lifestyle modifications according to the Nurses' Health Studies and the World Health Organization [3, 7]. The World Health Organisation suggests "following a healthy diet, enough physical activity, maintaining normal body weight and avoiding tobacco and harmful use of alcohol" [3]. In addition, postprandial changes in glycemic and blood lipid profiles strengthen the atherosclerosis process and thus aggravate CVDs, meaning that controlling postprandial blood glucose concentrations is a pivotal way to counteract or prevent both T2DM and CVD [8].

Several clinical studies suggest that altered lipid metabolism is the primary cause of imbalanced glucose homeostasis in T2DM patients [9]. Phosphatidylcholine (PC) is an example of a plant PL studied to affect lipid metabolisms [10, 11]. Studies suggest that supplementation of PC significantly reduces atherogenic lipoprotein and increases high density lipoprotein (HDL)

concentrations [12]. Besides, dietary lipids have the potential to regulate glucose responses and induce satiety levels as a result of the release of gut hormones and delaying gastric emptying [13].

1.2 Issue and aim

A previous cross-over study conducted at Lund University [13], demonstrated that intake of 30 g (g) of oat oil containing high concentrations of PL (40%, mainly glycolipids) lowered the glucose and insulin response after breakfast (contained the PLs) and a standardised second meal (without added PLs, 3.5 hours after breakfast), compared to 30 g of oat oil containing a low dose of PL (4%). Likewise, after lunch, the intake of high concentration oat PLs at breakfast resulted in an increase in the hormones glucagon-like peptide 1 (GLP-1) and pancreatic peptide YY (PYY). These results suggested that oat PLs have the potential to affect acute and second meal postprandial metabolic responses.

However, a study investigating the postprandial effects of different PLs have shown contradictory results [14]. Consequently, 5, 10 and 15 g of a plant PLs did not result in significantly beneficial effects on acute postprandial glucose and insulin responses compared to an oil-based reference product without plant PL.

To draw conclusions regarding effects on postprandial glucose regulation of plant PL, this degree project was established. This study aimed to investigate both the acute and second meal postprandial response on the blood glucose concentrations in healthy young adults on different concentrations of plant-based PL X, further referred as PL X and X is confidential information. In addition, the aim of this study was to investigate effects of PLs on the subjective appetite levels of satiety, hunger and desire to eat. PL X product consisted of a highly concentrated PLs preparation of plant origin, which allowed the preparation of test meals without resulting in excessive fat contents. A plant oil without PL was used as a reference meal. All test meals consisted of a similar amount of total fat. An additional test meal without added lipids was included as a negative control meal. All test and reference meals contained similar amount of available carbohydrates (43 g). The study was performed as a randomized cross-over single blind study.

1.3 Structure

The report starts with a literature review that addresses glucose regulation, PL and appetite hormones. Afterwards, the implementation of the study is explained in detail in the material and methods section. Results of the blood glucose responses and appetite parameters are presented as graphs and tables in the subsequent chapter. The following chapter discusses the results and presents a conclusion. Lastly, the report ends with a reference list and additional data are presented in the appendices.

2. Theoretical background

2.1 Glycemic regulation and cardiometabolic risks

2.1.1. Digestion and metabolism of carbohydrates

The body takes fuel from meals during the day and converts the macronutrient (carbohydrates, fat and protein) into helpful energy. These components can be oxidised entirely or stored in the body's adipocytes and glycogen in the muscles. [15, 16]

After digestion in the small intestine, glucose is absorbed and enters the circulation and in a first step travels to the liver. The liver's role in glucose metabolism is to release and take up compounds to maintain homeostasis of glucose concentrations [16]. In the absorptive state, the body takes up the nutrients through the intestine, and the absorption of glucose in the circulation results in an increase in blood glucose concentrations. This rise stimulates the liver to take up the glucose and phosphorylates it into glucose-6-phosphate. At this point, peptide hormones, insulin and glucagon, regulate the glycogen synthesis or degradation. Insulin stimulates glucose uptake into cells and converts it into glycogen in the liver or muscles and to fat in the adipose tissue. [15, 16]

2.1.2. Glycemic regulation

Glycemic regulation is defined as the homeostasis in the body to regulate an average glucose concentration in the blood. Before a meal, in the postabsorptive state, the blood glucose concentration is around 5 mmol/L, which can rise to 10 mmol/L after a meal in healthy subjects [16]. The peptide hormones insulin and glucagon regulate this increase.

The pancreas secretes insulin into the bloodstream via beta-cells. When a high glucose concentration in the blood circulation occurs, the beta-cells are stimulated to secrete insulin.. Insulin stimulates the uptake of glucose into cells and the liver converts glucose into glycogen for storage in the liver, muscles. The liver can store about 100 g of glycogen per day [15, 16]. During the fed state, insulin release is stimulated since glucose concentrations are increasing. When the glucose concentrations decrease, the release of insulin decreases as well. [15, 16]

In the postabsorptive state, when the glucose concentrations are low, the body uses the stored glycogen for gluconeogenesis to release glucose into the bloodstream to enhance the balance of glucose concentrations. Glucagon is a polypeptide hormone secreted by a-cells from the pancreas and is responsible for stimulating the liver for gluconeogenesis when the blood glucose concentrations decrease. [16]

The insulin concentration is evaluated in the absorptive state since the body takes the nutrients. The postabsorptive state starts about four hours after a meal when the intestine lacks nutrients. The blood glucose concentrations are decreasing, and the predominant insulin is decreasing while glucagon is starting to dominate to regulate the blood glucose concentrations. [16]

2.1.3. Fasting value

As described above, the glycemic regulation assures the glucose concentration in the blood. A low blood glucose concentration is related to overnight fasting, where the body has entered the postabsorptive state. Therefore, insulin concentrations are low, glucagon concentrations are relatively high, and glucose is produced from glycogen in the liver or via gluconeogenesis. The glucose concentration will be around 5 mmol/L for healthy individuals after overnight fasting. If a fasting value is measured higher than 6.1 mmol/L, this can indicate dysregulation in the glycemic regulation and might cause prediabetes or Impaired Fasting Glucose. [17, 18] After a meal, the glucose concentrations should not exceed 11 mmol/L and, after two hours, should be reduced to 7.8 mmol/L. If the two-hour postprandial is higher than 7.8 mmol/L, it can be defined as postprandial hyperglycemia [19].

2.1.4. Cardiometabolic risks

As mentioned before, T2DM is an increasing problem. The cause of this metabolic disorder is that the insulin generally produced by the pancreas is no longer controlled adequately in the body, which is called insulin resistance. The muscles in the body can gain some resistance to the insulin they produce. Therefore, the pancreas needs more effort to secrete insulin to regulate blood glucose effectively. This non-communicable disease, T2DM, differs from type 1 diabetes mellitus since the latter defines the inability of the pancreas to produce insulin, whereas T2DM is caused by insulin insensitivity. [16, 18]

T2DM causes by hyperglycemia, which increases the risk of CVDs. Hyperglycemia is defined by high blood glucose concentrations, comprising 6.9 mmol/L while fasting [20]. Hypoglycemia is opposed to hyperglycemia since it refers to a low blood glucose concentration comprising <3.3 mmol/L [21]. Acute or postprandial hyperglycemia is also related to the increased risk of CVDs in healthy and diabetic individuals. Hyperglycemia is caused by highly increased glucose concentrations, which can lead to a postprandial hypoglycemia, where the blood glucose concentrations decrease lower than usual after the initial postprandial blood glucose peak, below base levels. Hypoglycemia is a risk indicator of CVD and all-cause mortality in T2DM subjects and is more common after consumption of food with a high glycemic index.

These risks show the importance of keeping a balanced glycemic regulation. A controlled glycemic regulation can be achieved by reducing the amount of carbohydrates in the body or consuming food with low glycemic index. Likewise, increased dietary fibre and protein intake can help reduce the risk of a high blood glucose concentration. [22]

2.2 Dietary lipids and regulation

Lipids are hydrophobic molecules, such as oils, phospholipids, cholesterol, and other oily substances, which vary in structure and function according to their origin. Lipids are essential components of the body but carry a negative stigma today because of overconsumption and causes of chronic diseases [15]. Lipids mainly found in foods and the body are triglycerides (TAG), phospholipids, and sterols. TAGs are composed of three fatty acids, in the body its stored in the adipocytes. Pohospholipids are soluble in water and fat or oily substances due to their chemical structure. Fatty acids are the building blocks for the characteristics of fats and oils. Those which are not attached are called free fatty acids. They are chains with a carboxyl group (-COOH) and a methyl group (CH3) at the side of the chain. [15, 23]

2.2.1. Types of lipids and dietary intake

Within lipids, there are different types which all have various influences on a nutritional level. The Nordic Nutrition Recommendation (NNR) states that the total daily fat intake should be 25 - 40 E%. It is recommended not to reduce the intake of total fats below 25% since this can influence

the solubility, thus bioavailability, of fat-soluble vitamins. Low-fat diets also reduce HDL cholesterol) and impair glucose tolerance, which is unfavourable. [24]

The recommendation for fat intake is divided into saturated and unsaturated fatty acids. The intake of saturated fatty acids (SFA) should be <10% of the daily intake [24]. Unsaturated fatty acids (USFA) have double bonds in their carbon chain. Thus, it is less saturated with hydrogen. In case the molecule has only one double bond, it is defined as a mono-unsaturated fatty acid (MUFA), and the presence of several double bonds is defined as polyunsaturated fatty acids (PUFA). The NNR recommended intake is 10-20 E% for MUFAs and PUFAs 5-10 E%. NNR also states that the dietary sources of fatty acids have a significant role in the correlations to health, i.e. fatty fish, nuts and seeds Dietary intake is associated with CVD, T2DM, and certain cancers. [15, 24]

Espically omega-3 has heart healthy effects such as dialating blood vessels, reducing subclinical inflammation and discouraging blood clotting [15, 16]. Omega fatty acids are defined as essential fatty acids (EFA), as the body does not synthesise them themselves, as opposed to omega-9 fatty acids, which the body can synthesise and therefore is a non-essential fatty acid [15]. The recommended intake for omega-3, according to the NNR, is > 1 % of the daily intake [24]. Excess consumption of fats leads to the storage of triglycerides in the adipose tissue. Trans fatty acids (TFA) can be formed due to the hydrogenation of USFA, making it more saturated [15]. The intake of TFA increases the risk of CVD since it stimulates a rise in low density lipoprotein (LDL) cholesterol and a decrease in HDL cholesterol [15, 25]. Concerning these health risks and substantial evidence, the NNR recommends a TFA intake of <1 E% [24].

2.2.2. Plant lipids

In the past decades, researchers found numerous results on the beneficial effects of PUFA on CVD [26]. Dietary plant lipids are commonly high in PUFA and MUFA. Therefore, as mentioned above, they benefit individuals suffering from CVD and T2DM. Foods enriched in plant lipids are oilseeds, soy, oil-rich vegetables and nuts. Major part of plant lipids consist of PLs and plant sterols. Research shows the beneficial effects of plant sterols on reducing LDL and increasing HDL, thereby decreasing the risk of CVD [27]. Plant lipids can be polar, such as phospholipids, or non-polar, such as TAG. PLs are phospholipids and glycolipids, which are both amphipathic.

Due to their amphiphitic nature they help emulsify TAG in the GIT by forming micelles. PLs have a delayed digestion. Non-PLs do not have a water-loving head and therefore do not have the amphipathic characteristic [15].

Sterols and PLs can be synthesised in the body, meaning they are not EFA. Sterols are a group of lipids differ significantly in their structure compared with other lipdis. Most sterols do not contain any TAG but exist as hydrocarbons with a multiple-ring structure [15]. Most commontly known sterol is cholesterol which is found only in animal origin food, but plant sterols is the one also found in plant food, which has a role in CVD. Excess cholesterol in the body can lead to the accumulation of cholesterol in the blood and adipose tissue. In order to reduce the body's cholesterol, studies proved that the intake of plant lipids high in PUFA and MUFA could reduce LDL and increase HDL. [15, 16]

2.2.3. Plant lipids in diet

Plant-based diets are known to be healthy diets and have beneficial effects when it comes to preventing or treating diseases such as T2DM. For example, the largest follow-up study evaluated dietary choices and T2DM incidences of 4.1 million persons' who participated in the Nurses Health Study, Nurses' Health Study 2 and the Health Professionals Follow-up Study and revealed that the risk of developing diabetes decreased by 34% when adherent to the plant-based dietary index compared to those being less adherent to the plant-based dietary index [28]. Body mass index and other diabetes risk factors were not associated with the results. Plant lipids, particularly, consist of mainly USFA known to improve blood cholesterol and overall heart health. Likewise, a study that performed a systematic review and meta-analysis on randomised controlled feeding trials of the effect of SFA, PUFA, MUFA and carbohydrates on glucose homeostasis revealed a significant improvement of insulin secretion capacity, glycemic response and insulin resistance after replacing carbohydrates, SFA and even MUFA with PUFA [29]. Showing a significant beneficial effect of PUFA on blood glucose response. However, contradictory results can be drawn from a study investigating the influences of degree of fat saturation on glycmic, insulinemic or satiety responses in ten healthy men [30]. The conclusion was that inclusion of fat does reduce glycemic response however, no influence by the degree of fat saturation on glycemic, insulinemic or satiety response compared to a carbohydrate meal was found. Meaning that replacement of SFA

with USFA did not have a significant acute postprandial effect. Overall, these results show that fat in general, has an beneficial influence on the blood glucose response, although further studies must investigate the impact of the different types of fat both acute and second meal. Also, the World Health Organization recommends preferring the intake of USFA over SFA [31]. However, a higher total fat intake still increases the development of T2DM, disturbance in glucose metabolism or impaired glucose tolerance. Hence why both the quality as quantity are important for the fat intake.

2.2.4. Phospholipids

The chemical structure of phospholipids is similar to triglycerides, composed of three fatty acids, since they contain glycerol and fatty acids. There are two primary sorts of phospholipids, namely: glycerophospholipids and sphingolipids. Glycerolphospholipids can be defined as diglycerides. One of the glycerides is replaced by a phosphate group with a nitrogen component, polar head and two fatty acids, non-polar tail, with glycerol at the end to define the different types of glycerophospholipids [12, 15]. The phosphate-nitrogen component is hydrophilic, making phospholipids amphipathic, and excellent emulsifiers due to their compatibility with water and oil. [12, 27]

2.2.5. Digestion of lipids

Digestion of lipids starts in the mouth, where chewing and digestive enzyme lingual lipase creates tiny fat droplets, which are easier to digest [15]. Gastric lipase in the stomach breaks down 30% TAGs to diglycerides and free fatty acids. The primary digestion of TAGs takes place in the small intestine, which stimulates the secretion of cholecystokinin (CCK) and secretin from duodenal cells. Chyme from the stomach is mixed with secretions from the pancreas (pancreatic juice) and thus pancreatic lipase, which digest TAG into single fatty acids. CCK signals the gallbladder to release bile, which helps emulsify fat droplets and break globules into tiny pieces so lipase can interact with the surfaces. Bile salts form micelles, which transport the monoglycerides and free fatty acids in the intestine till they are absorbed by the intestinal mucosal cells. [15, 16]

The digestion of PLs has a similar pathway [15]. The PLs digestion starts later in gastrointestinal tract, namely in the intestinal lumen. They are not hydrolysed by gastric lipases but by phospholipases, which produce also PLs and free fatty acids. Dietary PLs are absorbed for nearly

>90% and synthesised into the surface layer of chylomicrons. Some studies have indicated the passive absorption of PLs and incorporation in HDL fractions; this way, PLs affect the composition of lipid lipoprotein [32].

Dietary PLs are functional during the digestion and absorption of food as they help break off fats in the stomach into tiny pieces for easier digestion [15]. They continue emulsifying in the intestine, where they are secreted from bile. PLs, are also present on the surface of lipoproteins to carry lipid particles through the body. PLs originated from plant and animal sources and are most common in egg yolks, liver, soybeans, sunflower and peanuts. Since their emulsifying characteristics, they are often used as additives in food matrices.

2.3 Plant PL X

2.3.1. Composition and structure

PL X is a mixture of various phospholipids of which mainly are glycerophospholipids [32]. PL X compositions may vary depending on the genetic, environmental and agronomical factors where they originate from. Meaning there is a variation within a source group, leading to different fatty acid and phospholipid compositions. Table 1 shows the average fatty acid composition of PL X, and Table 2 shows the average bioactive phospholipid composition of PL X according to van Nieuwenhuyzen and Thomas [33].

Table 1: Average fatty acid composition plant based PL X [33]

Fatty acid	**%**
16:0	11
18:0	4
18:1	18
18:2	63
18:3	0
Others	4

Table 2: Average phospholipid composition of liquid PL X analysed by 32P-NMR method [33]

	Phosphatidylcholine (PC)	**Phosphatidylethanolamine**	**Phosphatidylinositol**	**Phosphatidic acid**	**Other phospholipids**
PL X in liquid form[1]	16	8	14	3	6

[1] commercially produced liquid PL X from kernels

PL X is specifically abundant in the phospholipid phosphatidylcholine (PC). PC is a glycerophospholipid occurring in and building up cell membranes of all living organisms [15]. PC consists of unsaturated and saturated fatty acids, glycerol, choline and a phosphatidic acid group. The chemical structure of PC is similar to triglycerides, except it consists of a glycerol backbone with two fatty acids instead of three, making it a diglyceride. A nitrogenous component occupies one attachment of the backbone, choline, also called a phosphocholine molecule. The phosphocholine molecule makes a hydrophilic head, whereas the fatty acids component creates a hydrophobic tail. These surface-active properties make the molecule a great natural emulsifier and homogenising agent. PC increases the synthesis, availability and release of acetylcholine as it is a precursor for choline [34]. Thus PC helps to improve brain functions and memory. A more important effect of PC is the role on cholesterol transportation by increasing the uptake of HDL cholesterol and improving bile secretion [35, 36]. [15]

2.3.2. Functions of PL X

PL X has a broad range of functions and is used in the food industry as a food additive or by the human body in metabolic processes, for example lipid transportation.

PL X is present in all human cellular and sub-cellular membranes and is a constituent of bile juice. The phospholipids form a selectively permeable lipid bilayer. This specific structure forms boundaries, thereby protecting body cells and creating a suitable environment for bioactive compounds and proteins. Likewise, they play a role in crucial cellular processes such as metabolism, lipid transportation, stabilisation of lipid droplets and solubilisation of cholesterol

[37]. The phospholipid composition determines the emulsifying capacity of PL X, and is also involved in cell signalling, creating an essential role for communication and interaction between the body cells. In this way, phospholipids are involved in DNA replication, cell development or secretion processes. [32, 34]

2.3.3. Legislation

PL X is an authorised food additive according to EFSA Regulation (EC) No 1333/2008 Annex I and II on food additives [38]. Where food additives are defined as: "Food additives are substances that are not normally consumed as food itself but are added to food intentionally for a technological purpose described in this Regulation, such as the preservation of food" by the EFSA [38]. Also, it was concluded by the Panel of the Commission Regulation (EU) No 257/2010 that there is no need for an acceptable daily intake (ADI) or safety concerns for the general population (>1 year old) on PL X as a food additive [39].

2.3.4 Effects of PL X on metabolic health

Since 1990 there have been reported studies investigating the effect of PL on human health and the prevention or treatment of metabolic disorders. As said before, the PL X has an amphiphilic nature, which has been shown to modulate lipoprotein metabolism and lower cholesterol concentrations in the body. These mechanisms can benefit metabolic disorders since T2DM is associated with altered lipid metabolism, and an elevated cholesterol level is associated with higher risk of CVD *[32]*. However, studies are still lacking investigating PL X and its effects on metabolic disorders. Also, no systematic review is available concluding the potential role of PL X on metabolic disorders*, according to Robert* et al. *[32].*

2.4 Appetite regulation and hormones

Besides the digestion of nutrients, hormones and neural communication pathways are also involved in our body's appetite regulation. Those three together are responsible for regulating energy homeostasis. The hormones are secreted from endocrine cells in the gastrointestinal tract, initiating hunger, satiety and satiation feelings [40]. This way, the body controls feeding behaviour and body weight changes. The hormones that will be discussed are ghrelin, leptin, PYY and GLP-1.

Ghrelin, also called the hunger hormone, is a peptide secreted by the stomach when empty, signalling the brain to stimulate food intake. Subsequently, the food intake leads to a rapid reduction of the ghrelin hormone, implicating that these hormone concentrations vary throughout the day depending on the eating behaviour. It is reported that patients with obesity have low ghrelin levels. [40]

Leptin, a protein produced by white adipose cells and the stomach, regulates homeostasis between food intake and energy expenditure in the long run. Together with insulin, it controls the body weight by inhibiting hunger and creating a feeling of satiation. [40]

PYY and GLP-1 are hormones released by the small and large intestines, inhibiting food intake and promoting satiety. The response for PYY starts after eating and is at its highest concentration in the blood 1-2 hours after ingestion, depending on the type and amount of nutrients consumed. GLP-1 hormones give a rapid response after 10-15 min and a prolonged response at least after 30-60 min. Likewise, blood glucose concentrations are regulated by GLP-1 via reduced gastric emptying rate, inhibition of glucagon secretion and promotion of glucose-dependent insulin secretion. Knowledge about these hormones, which foods that may be specifically efficient to stimulate the release, and their signalling pathways can help prevent or manage health diseases like obesity and improve our dietary choices. [40]

As said, nutrients affect the appetite regulation in our body as well. It is reported that lipids affect satiety levels through several mechanisms, of which the release of the hormones mentioned above and the inhibition of gastric emptying are some [36]. Nonetheless, not all lipids result in the same appetite response. Likewise, not all bodies respond the same.

2.5 Previous studies

Randomised controlled clinical trials demonstrated a relationship between glycemic control and microvascular and neurological diabetes complications, and showed improvements when glycemic concentrations were controlled, respectively [41]. According to the Chinese Da Qing Study, the

US Diabetes Prevention Program and the Finnish Diabetes Prevention Study, glycemia concentrations diagnosed for diabetes can be postponed by a healthy diet and exercise [41].

Clinical studies have shown that atypical lipid metabolism is the leading cause of glucose metabolism disturbance in T2DM [42]. A disorder in lipid metabolism can activate insulin resistance and cellular dysfunction, thus is related to the progression of diabetes [43]. In Chengdu, China, a study on serum lipids suggested that participants with impaired glucose regulation have high concentrations of LDL and TG, simultaneously with decreased concentrations of HDL [44].

The quality of the dietary fats may affect the management of hyperglycemia. Numerous clinical studies identified the impact of fat composition on hyperglycemia, resulting in data indicating that SFA worsens insulin sensitivity and counteracts the effects of MUFAs in the diet [45]. The Mediterranean diet, rich in MUFA, is associated with a lower risk of CVD and T2DM. PLs in eggs benefit the increase of HDL cholesterol, decrease in LDL cholesterol, and regulate lipid metabolism and inflammation [46]. Poreba *et al.* [26] examined the intake of linolenic acid PLs on the glycemic response in T2DM patients showing positive results, indicating that, in particular, PL linoleic acid is linked to a lower risk of T2DM. To the researcher's knowledge, they are the first to show that poor glycemic control in diabetic subjects is associated with lower concentrations of Eicosapentaenoic Acid/Arachidonic Acid (EPA/AA) ratio in the serum PL fraction.

Controlled human studies on milk PLs resulted in positive effects on reducing inflammation, promoting gut health and managing dyslipidemia [47, 48]. The amphiphilic characteristics of phospholipids have essential roles in the coating of lipid droplets and lipoproteins in the body, therefore, lipid transportation and metabolism. This way, phospholipids modulate lipoprotein metabolism by increasing HDL concentrations. PL X, high in phospholipids, can potentially prevent and treat metabolic disorders [32].

A previous study in oat PL investigated the blood glucose response, appetite regulation and changes in gastrointestinal hormones compared to a commonly used oil, such as rapeseed oil [13]. The latter is low in glycolipids, while the oat PL are high. The consumption of oatt PL during a meal decreased the peak in blood glucose mmol/L and insulin concentrations both in the acute

postprandial period, but also at the next standardised meal consumed after 3.5 hours. Significant results were obtained via a crossover study (*n=20*) showing the difference between the control and reference. This study also showed increased values for GLP-1 and PYY after the standardised lunch. Based on previous observations it is suggested that plant lipids can influence glycemic control positively, and possibly have beneficial implications in appetite control..

3. Methodology

3.1 Study subjects

Twenty healthy subjects participated in the study, ten men and ten women, aged (Mean +/- SEM) 24.3 +/- 0.7 years and average weight (Mean +/- SEM) 23.1 +/- 0.6 kg/m^2. The study was carried out in the spring 2023. The requirements for the healthy subjects were being non-smokers, BMI 19-28 kg/m^2, age between 20 - 40 years and no metabolic disorder or food allergies. Participants in the study could follow both an omnivorous or vegetarian diet; no participants follow a vegan diet. The Regional Ethics Review Board in Lund, Sweden, approves the study. Subjects were comprehensively informed about the protocol and purpose (Dnr. 2018/658) of the study, and all subjects gave written consent for their participation.

3.2 Study Design and Procedure

The randomised cross-over study was performed at the Food Technology, Engineering and Nutrition Department of Lund University. For every test day, a different test breakfast was served. Every subject consumed in total four test breakfast in a randomised order. The subjects were also served a standardised lunch, composed of mashed potatoes and meatballs. Trial days for each subject were separated with approximately one-week wash-out. Test parameters investigated were postprandial glucose and subjective appetite response. Test variables were determined in the acute and second meal postprandial period, i.e. after the test breakfast and after the standardised lunch.

Subjects were asked to avoid the intake of antibiotics and probiotics four weeks in advance and during the trial days. The day before each experiment, the subjects were informed to avoid fibre-rich foods, high-intensity exercise and alcohol intake. Subjects were informed about standardising their meal the previous day and consuming the evening meal at 18:00 h. A standardised evening meal was consumed at 21:00 h, consisting of commercial white bread with a spread of choice. Each subject selected their amount of bread themselves and repeated it on every occasion. Keeping a similar breakfast and lunch on the previous day was recommended. Small quantities of water were allowed, on trial days before arriving at the experimental site. The same routine was kept throughout the experiment. Meal records were obtained from the day prior to the trial days to facilitate the standardisation of meal patterns.

At 07:30 h of the trial day, the subjects were expected at the research unit. After they rested and filled in the information sheet about their weight, the fasting value was taken via the first finger-prick blood sample. Ten min later, the second fasting value was recorded (at 0 min, baseline). Later the two fasting values were used to calculate the average fasting value for that experiment day. At the same time point, before breakfast, the subjective appetite scores (satiety, hunger and desire to eat) were obtained. The subjects were then instructed to consume the breakfast within 12 min. After breakfast, time points 15, 30, 45, 60, 90, 120, 150, 180, and 210 min were used to determine the blood glucose concentrations and subjective appetite score. At 120 min, a small glass of water was served. The standardised lunch was served and instructed to be consumed within 12 min after the finger-prick blood sample at 210 min. After lunch, blood glucose concentrations and subjective appetite scores were obtained at 225, 240, 255, 270, 300 and 330 min. Participants were not allowed to eat or drink anything but the breakfast and lunch meals including the water. Participants were required to stay seated in the experiment room. The entire trial day lasted six hours.

Figure 1 presents a schematic overview of the experimental design.

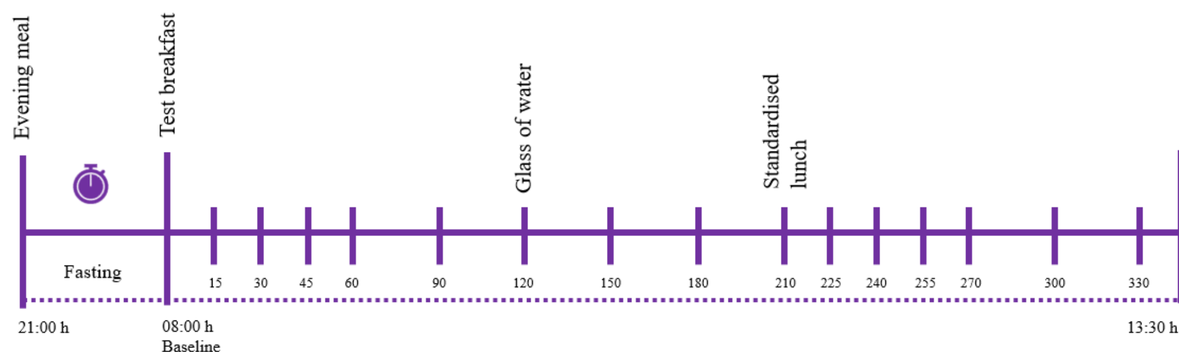

Figure 1: Schematic overview of the trial day.

3.3 Test meal and standardised lunch

3.3.1 Composition and nutritional values of test meal, including PL X

All breakfast test meals consisted of a standardised amount of available carbohydrates (43 g) from commercially bought white bread. The slices of bread were served with a spread on top, developed specifically for the study. Glucose and cacao powder was added to all spread in equivalent amounts to create a pleasant flavour for the test meals. Other ingredients in the spread were water, lipid,

and the active ingredient PL X. Test meal i. contained a high concentration of PL X (PLH), test meal ii. contained a low concentration of PL X supplemented by a reference oil (PLL) and test meal iii. contained a reference oil (RSO). A negative sample containing no lipids (NL) was added as a control meal. The active ingredient, PL X, was commercially purchased from Helhetshälsas, Borghamn, Sweden. In addition to the slices of bread, a glass of 200 ml water was served during the breakfast. Table 3 shows recipes for all four test meals, and Table 4 the nutritional composition of the test meals per serving.

Table 3: Recipes of all four test meals per serving

	i. PLH	**ii. PLL**	**iii. RSO**	**NL**
Bread (g)	105.6	105.6	105.6	105.6
Glucose (g)	6	6	6	6
Cacao powder (g)	2	2	2	2
PL X (g)	17.6	8.8	-	-
Reference oil (g)	-	8.7	17.6	-
Water (g)	51.1	25.5	6.4	20

Table 4: Nutritional composition of all four test meals per serving

	i. PLH	**ii. PLL**	**iii. RSO**	**NL**
Available carbohydrates	43	43	43	43
Total fat	17.4	16.7	16.1	-
PL X	17.4	8.6	-	-

Test meal i, ii and iii. were served as slices of bread smeared with the corresponding spread (see Appendix A, figure A6). The consistency of the test meal NL was too thin and therefore was

presented in a bowl in which the bread was to be dipped by the subjects (see Appendix A, figure A7).

3.3.2 Composition and nutritional value of the standardised lunch meal

Every trial day, the standardised lunch consisted of 65 g of vegan mashed potato powder (Eldorado, Stockholm, Sweden) mixed with 300 ml water and 1 gram of salt, served with 56 g of vegetarian meatballs (Felix, Eslöv, Sweden) and a glass of 250 g of water. The lunch comprised 54 g of carbohydrates, according to the nutritional declaration of the producers. Table 5 shows the nutritional composition of the standardised lunch per serving of 422 g.

Table 5: Nutritional composition of the standardised lunch per serving (422 g)

	Mashed potato	**Vegetarian meatballs**	**Standardised lunch meal**
Total Energy in Kcal	227	137	364
Carbohydrates (g)	48	6	54
Fat (g)	>1	10	10
Protein (g)	5	7	12
Fiber (g)	-	2	2

3.4 Blood glucose and subjective appetite parameters

A HemoCue® Glucose 201+ System was used to determine blood glucose concentrations. On the morning of an experiment day, the right amount of glucose Microcuvettes had been removed from the fridge to reach room temperature before use. Then, the HemoCue® Glucose 201+ System was verified for accuracy. As the experiment started, capillary blood finger-prick tests were performed on the subjects with a Hemocue safety lancet. After that, blood was drawn into a Microcuvette in one flow and without air bubbles and was to be inserted into the HemoCue® Glucose 201+ System. Within one minute, the system showed the corresponding blood glucose concentration. The system

was cleaned at the end of every experiment day. All equipment and materials were used as prescribed.

In addition, subjective appetite parameters were registered. Subjects were given a questionnaire to answer every time after a capillary blood finger-prick test was taken. At the start of the experiment, instructions were given to the subjects on how to fill in the form. They were asked to read the questions, think about it briefly and mark a vertical line on the position that best corresponds to their appetite sensation at that moment, excluding the utmost positions. The three questions were about fullness, hunger and desire to eat with a 0-100 mm Visual Analogue scale (VAS) and were the same every time (see Appendix B, table B8 and B9). After the experiment, both of the two performing students measured the results per question (in mm) using the same ruler.

3.5 Statistical analysis

Data for blood glucose and subjective appetite results were expressed as mean +/- SEM. All data graphs were plotted in GraphPad Prism (version 9.5.1) and used for further analysis. Incremental areas under the curve (iAUC) and areas under the curve (AUC) for postprandial blood glucose and subjective appetite results, respectively, were calculated using a trapezoid model in GraphPad Prism (version 9.5.1). Calculations were made for each test subject and meal, and all areas were considered. iAUC data were used for statistical evaluation of postprandial blood glucose concentrations. The fasting value (at 0 min) was used as the first baseline, and the blood glucose concentration prior to lunch (at 210 min) was considered second baseline. AUC data is used for the statistical evaluation of postprandial subjective appetite parameters. The fasting value (at 0 min) was considered as the baseline.

An ANOVA test was performed on the iAUC and AUC results to detect possible significant differences between test meals. Statistical Software MiniTab (version 21.4) was used. First, the Descriptive Statistics test was performed to inspect data and identify outliers. Data were normally distributed, and no outliers were found. After that, a General Linear Model followed by a Tukey Pairwise Comparisons method was performed. A significant difference between groups was detected if $p<0.05$ (95% confidence).

4. Results

4.1 Postprandial acute and second meal blood glucose response to PL X

Figure 2 presents incremental glucose responses acutely after the test meals and after the standardized lunch. The dotted line indicates the start of the second meal at 210 min. Table 6 shows the significant differences between test variables. The results showed significant differences in postprandial glucose responses between test products during the whole test day (at 0-330 min, $p < 0.05$), indicating significantly lower glucose response after RSO and PLL breakfasts compared to NL. However, PLH resulted in the lowest glucose response, significantly lower than NL, RSO and PLL. PLH decreases blood glucose response with 42% compared to NL, while RSO and PLL reduces blood glucose with 20% and 26%, respectively.

After breakfast, (at 0-120 min) significantly lower postprandial glucose response appears after the breakfasts containing RSO, PLL and PLH, i.e. all products high in fat, compared to the negative NL ($p < 0.05$). Simultaneously, the individual incremental highest peak values (iPeak) after these products were lower than after consuming the negative, NL. Postprandial glucose response after lunch showed no differences between PLL and PLH (at 210-330 min, $p > 0.05$). However, a significantly lower response for PLH compared to RSO and NL was observed ($p < 0.05$). iPeak after the standardised lunch is significantly lower after PLH breakfast compared to NL, RSO and PLL (at 210-330 min, $p < 0.05$).

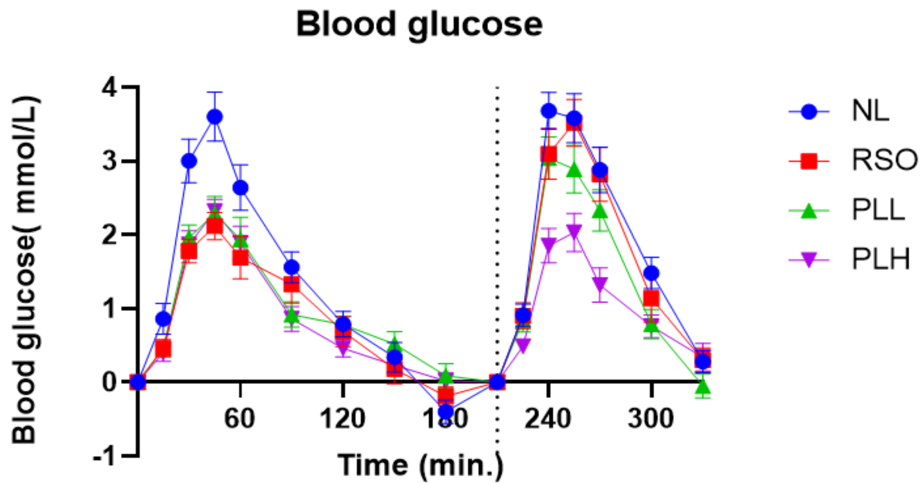

Figure 2: Graphical representation of incremental blood glucose response after test breakfast and standardised lunch meals. Values are presented as mean values (+/- SEM), *n=20* healthy subjects. NL = white wheat bread negative sample without lipids, RSO = white wheat bread with spread and commercially reference oil, PLL = white wheat bread with spread and low concentration PL X, PLH = white wheat bread with spread and high concentration PL X.

Table 6: Blood glucose concentrations at fasting and after consumption of test meals at breakfast, followed by a standardised lunch meal.[1]

Test variables	*NL*	*RSO*		*PLL*		*PLH*	
Glucose			Delta % [2]		Delta % [2]		Delta % [2]
Fasting blood glucose (mmol/L)	5.3 ± 0.1^{B}	5.5 ± 0.1^{A}	3.8	5.3 ± 0.1^{AB}	0.0	5.3 ± 0.1^{AB}	0.0
Blood glucose prior to std. lunch (at 210) (mmol/L)	4.6 ± 0.1^{B}	5.0 ± 0.1^{A}	8.7	5.3 ± 0.1^{A}	15.2	5.2 ± 0.1^{A}	13.0
Blood glucose iAUC = 0-120 (mmol*min/L)	227.3 ± 19.1^{A}	151.7 ± 16.2^{B}	-33.3	151.1 ± 13.7^{B}	-33.5	146.5 ± 11.8^{B}	-35.6
Blood glucose iAUC = 210-330 min (mmol*min/L)	240.1 ± 19.3^{A}	220.4 ± 21.2^{AB}	-8.2	186.5 ± 16.2^{BC}	-22.3	127.9 ± 11.6^{C}	-46.7
Bloog glucose iAUC = 0-330 min (mmol*min/L)	521.8 ± 35.6^{A}	417.7 ± 37.4^{B}	-20.0	387.3 ± 25.4^{BC}	-25.8	304.2 ± 18.4^{C}	-41.7
Blood glucose iPeak after breakfast	3.9 ± 0.3^{A}	2.5 ± 0.2^{B}	-35.9	2.7 ± 0.2^{B}	-30.8	2.5 ± 0.2^{B}	-35.9
Blood glucose iPeak after lunch	4.0 ± 0.3^{A}	4.0 ± 0.4^{A}	0.0	3.5 ± 0.3^{A}	-12.5	2.4 ± 0.2^{B}	-40.0

[1] Data is shown in mean values +/- SEM, *n=20* healthy subjects. Data in the same row, showing different superscript letters are significantly different (*p <0.05,* ANOVA, Tukey's Pairwise test). iAUC = incremental area under the curve.

[2] The difference in percentage is calculated from test meal NL.

4.2 Postprandial acute and second meal subjective appetite response to PL X

Figures 3, 4 and 5 present the acute and second meal subjective appetite ratings (satiety, hunger and desire to eat, respectively). The y-axis represents the VAS scale (0-100 mm.). Table 7, 8 and 9 shows the significant differences between test variables (satiety, hunger and desire to eat, respectively). The results did not show any significant differences depending on test breakfast for satiety, hunger or desire to eat ratings (at 0-330 min, *p >0.05*). Nor a significant difference was observed in subjective satiety, hunger or desire to eat ratings after breakfast (at 0-120 min, *p >0.05*) or after standardized lunch (at 210-330 min, *p >0.05*) when analysed separately. Before lunch, at 210 min, significantly higher subjective satiety ratings were observed when the test subjects had consumed PLH at breakfast compared to when they consumed NL (*p <0.05*). In addition, at 120 min, significantly lower subjective hunger and desire to eat ratings were found after the PLH breakfast compared to NL breakfast (*p <0.05*).

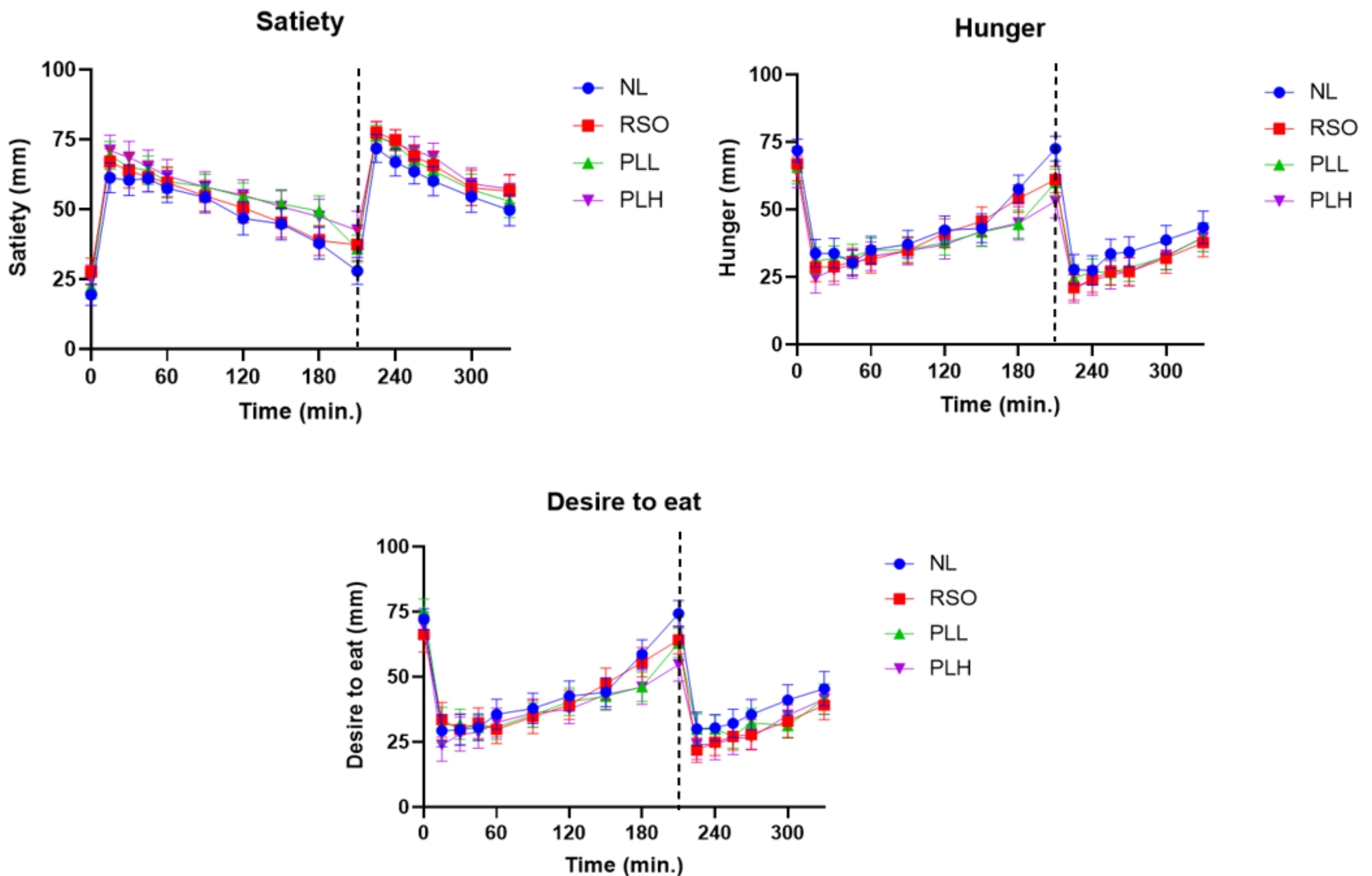

Figure 3, 4 and 5. Graphical presentation of subjective appetite response (satiety, hunger and desire to eat, respectively) after test breakfast and standardised lunch meals. Values are presented as mean values(+/- SEM), *n=20* healthy subjects. NL = white wheat bread negative sample without lipids, RSO = white wheat bread with spread and commercially bought oil, PLL = white wheat bread with spread and low concentration PL X, PLH = white wheat bread with spread and high concentration PL X.

Table 7: *Satiety* scores following the intake of different test meals during breakfast, followed by a standardised lunch meal.[3]

Test variables	***NL***	***RSO***		***PLL***		***PLH***	
Satiety			**Delta %[4]**		**Delta %[4]**		**Delta %[4]**
Fasting rating (mm)	19.5 ± 3.9 [A]	27.7 ± 4.8 [A]	+ 42.1	22.3 ± 4.3 [A]	+ 14.4	25 ± 5.2 [A]	+ 28.2
Rating prior to std. lunch (at 210) (mm)	27.9 ± 4.9 [A]	37.4 ± 5.8 [AB]	+ 34.1	35.9 ± 4.9 [AB]	+ 28.7	42.5 ± 6.8 [B]	+ 52.3
AUC = 0-330 min (mm*min)	17090 ± 1489 [A]	18322 ± 1467 [A]	+ 7.2	18911 ± 1391 [A]	+ 10.7	19449 ±1652 [A]	+ 13.8
AUC = 0-120 min (mm*min)	6512 ± 532 [A]	6839 ± 579 [A]	+ 5.0	7052 ± 514 [A]	+ 8.3	7220 ± 609 [A]	+ 10.9
AUC = 210 – 330 (mm*min)	6977 ± 566 [A]	7643 ± 544 [A]	+ 9.5	7463 ± 510 [A]	+ 6.9	7813 ± 584 [A]	+ 12.0

Table 8: *Hunger* scores following the intake of different test meals during breakfast, followed by a standardised lunch meal.[3]

Test variables	***NL***	***RSO***		***PLL***		***PLH***	
Hunger			**Delta %[4]**		**Delta %[4]**		**Delta %[4]**
Fasting rating (mm)	71.6 ± 4.3 [A]	66.9 ± 6.3 [A]	- 6.6	65.7 ± 6.3 [A]	- 8.2	64.0 ± 5.8 [A]	- 10.6
Rating prior to std. lunch (at 210) (mm)	72.5 ± 4.7 [A]	61.1 ± 4.9 [AB]	- 15.7	60.1 ± 5.1 [AB]	- 17.1	53.2 ± 6.3 [B]	- 26.6
AUC = 0-330 min (mm*min)	13750 ± 1443 [A]	13771 ± 1545 [A]	+ 0.2	11899 ± 1272 [A]	-13.5	11417 ± 1507 [A]	- 17
AUC = 0-120 min (mm*min)	4361 ± 488 [A]	4365 ± 530 [A]	+ 0.1	4063 ± 398 [A]	- 6.8	3613 ± 545 [A]	- 17.2
AUC = 210 – 330 (mm*min)	4360 ± 597 [A]	4506 ± 684 [A]	+ 1.0	3840 ± 559 [A]	- 13.9	3632 ± 583 [A]	- 18.6

Table 9: *Desire to eat* scores following the intake of different test meals during breakfast, followed by a standardised lunch meal.[3]

Test variables	*NL*	*RSO*		*PLL*		*PLH*	
Desire to eat			*Delta %*[4]		*Delta %*[4]		*Delta %*[4]
Fasting rating (mm)	72.2 ± 4.0 ^{A}	69.7 ± 5.2 ^{A}	- 3.5	75.3 ± 4.6 ^{A}	+ 4.3	66.2 ± 6.7 ^{A}	- 8.3
Rating prior to std. lunch (at 210) (mm)	74.3 ± 5 ^{A}	64.2 ± 5.4 ^{AB}	- 13.6	63.1 ± 5.6 ^{AB}	- 15.1	54.7 ± 6.4 ^{B}	- 26.4
AUC = 0-330 min (mm*min)	13965 ± 1620 ^{A}	12660 ± 1701 ^{A}	- 9.3	12617 ± 1426 ^{A}	-9.7	11981 ± 1717 ^{A}	- 14.2
AUC = 0-120 min (mm*min)	4464 ± 610 ^{A}	4228 ± 662 ^{A}	- 5.3	4332 ± 503 ^{A}	- 3.0	4106 ± 635 ^{A}	- 8.0
AUC = 210 – 330 (mm*min)	4561 ± 642 ^{A}	3819 ± 619 ^{A}	- 16.3	4013 ± 593 ^{A}	- 12.0	3809 ± 636 ^{A}	- 16.5

[3] Data is shown in mean values +/- SEM, *n=20* healthy subjects. Data in the same row, showing different superscript letters are significantly different ($p < 0.05$, ANOVA, Tukey's Pairwise test). AUC = area under the curve.
[4] The difference in percentage is calculated from test meal NL.

5. Discussion and conclusion

5.1 Discussion

This study investigated whether PL X has a beneficial effect on postprandial blood glucose concentrations and subjective appetite parameters in healthy adults, acutely after breakfasts containing the lipids and after a standardised lunch meal, without the test lipids. For the study, the effect of different doses of PL X were compared to commercially bought oil. The results suggest that supplementing a breakfast meal with PL X can reduce cardiometabolic risk factors associated with T2DM and CVD, reliant on the dose.

In line with the hypothesis and outcomes of previous studies [13, 46, 47, 48, 49], the results of our study indicate an improved postprandial acute and second-meal blood glucose tolerance, for a period of 5.5 hours, following a breakfast meal supplemented with PL X. More precisely, a meal containing 17 g of PL X (PLH) significantly decreases the postprandial blood glucose response compared to a carbohydrate-equivalent meal containing a commercial reference oil (RSO) or no added lipids (NL). In addition, PLH consumed at breakfast significantly decreases the blood glucose response following a subsequent standardised lunch meal (i.e., 3.5 hours after the PL containing breakfast) compared to NL and RSO. Test meal PLL with 9 g of PL X significantly decreased the blood glucose response compared to NL, acute and after the second meal.

Considering the individual highest peak responses, PLH, PLL and RSO result in lower peaks after breakfast than NL. Whereas the beneficial effects of PLH also reduce the peak after lunch compared to PLL, RSO and NL. The overall blood glucose response observed before lunch (at 210 min) was significantly improved after PLH, PLL and RSO compared to NL, indicating that these lipid-containing meals prevent postprandial hypoglycemia [21].

Likewise, subjective appetite responses prior to lunch (at 210 min) were improved by PLH compared to NL for both satiety, hunger and desire to eat markers. These results show prolonged satiety sensation after consumption of PLH, indicating better short-term control over food intake and potentially being useful to prevent obesity. It could be suggested that adding components known for their high satiety-producing effects, like soluble dietary fibres and proteins, to a meal with PLs, help improve these satiety feelings even more and thereby be a stronger tool in appetite regulation, help weight loss and prevent obesity.

PLs are only a small component of our diet, with an average intake of 3-5 g per day, even though they are essential lipid constituents in all cell membranes of our body [49]. Our study increases the ongoing investigation of potential beneficial health effects of PLs, and suggests that their intake should be increased to establish a healthier diet.

The results of this study build on existing evidence from previous studies investigating the postprandial effects of plant PLs. Ohlsson *et al.* [49] reported that liposomes with PLs from fractionated oat oil significantly improve the plasma glucose concentrations, appetite-regulating hormones PYY, GLP-1 and CCK and satiety levels with a dose of 35 g total lipids, 10 wt%, and 14 g total lipids in their follow up study. In addition, Hoissan *et al.* [13] results indicate that supplementing a breakfast meal with PLs from oat oil (40% glycolipids) reduces postprandial blood glucose and insulin concentrations acutely and at a second-meal consumed after 3.5 hours, and increases gut hormones concentrations of GLP-1 and PYY. These studies suggest that PLs from oats have the potential to improve postprandial blood glucose response and appetite, matching our results. While those studies focus on the influence of oat PL preparations with high concentrations of glycolipids [13, 49], our study demonstrates similar results on the postprandial glucose response with another PL preparation of plant origin (PL X), abundant in phospholipids (>50%), mainly PC. The structure of the PL X is different from those of oat lipids and undergo potentially different digestion, absorption, and transportation mechanisms in the body.

To our knowledge, only Yao *et al.* and Shen *et al.* have previously investigated the same health effects of PLs, rich in phospholipids, in healthy humans [14, 50]. However, their study outcomes differed from those of the previously mentioned studies, and indicated no significant improvement by 5, 10 and 15 g of PLs on blood glucose concentrations, insulin concentrations and subjective appetite sensations. Nonetheless, these studies did not include second-meal responses. Our results, however, show the beneficial effects of PL X on blood glucose and satiety responses using various doses and including second-meal effects.

A review by Robert *et al.* describes that plant PLs, with >50% abundance of phospholipids, have significant physiological effects [32]. They have an essential role in lipoprotein transport, functionality and metabolism. Due to their amphiphilic nature, they stabilise lipid droplets. In addition, plant PLs can potentially be used to treat dyslipidemia [32]. Robert *et al.* [32]state that

data about plant PLs as a food ingredient and supplement is lacking. Therefore, our research promotes interest in researching these beneficial effects.

Specifically addressing post-breakfast response, no significant difference in blood glucose response (at 0-120 min) between PLH, PLL and RSO was recorded, meaning the glucose responses were similar after the lipid-added breakfasts. These results match existing studies. A study performed by MacInthos *et al.* [30]investigated the influence of the fat content and its degree of saturation, they concluded that fat, in general, reduces glycemic response. However, no difference was found in the acute postprandial effect between SFA and USFA in ten healthy men. Also, the systematic review and meta-analysis of SFA, PUFA, MUFA and carbohydrates done by Imumara *et al. [29],* showed a significantly improved glycemic response by PUFA compared to SFA and even MUFA. Since all three lipid preparations in the presently described study contain equivalent amounts of total fats (+/- 16.5 g of which mainly MUFA and PUFA), no difference in blood glucose response between all three fat-included test meals may be expected post breakfast. PLH, PLL and RSO all show a significant difference with the negative breakfast sample NL, matching the expectations as well after reviewing the literature, and so it reinforces the results of this study.

After 5,5 hours (0-330 min), a significant improvement of 42% in blood glucose concentrations was observed for PLH compared to NL. In addition, RSO and PLL improved blood glucose concentrations with 20% and 26% respectively, indicating significant decrease compared to NL. However, PLH resulted in the significant lowest blood glucose response. These are interesting findings, showing a dose-response effect by supplementing a breakfast with PL X, and variation in between test meals prepared with lipids.

More exciting results were shown after lunch (at 210 – 330 min). A dose-response effect could be observed. Test meals with a high dose of PL X (PLH) significantly improve the blood glucose concentrations after lunch compared to samples RSO and NL, whereas a low dose of PL X (PLL) only resulted in improved blood glucose response compared to NL. No improvements were seen after the RSO compared with NL.

The consumption of plant and animal PLs positively affects subclinical inflammation, promoting gut health and managing dyslipidemia [47, 48]. Gastric emptying is a major determinant for the

(initial) rise of blood glucose concentrations[13]. The acute increase in blood glucose concentrations is directly related to the stomach emptying rate. Lipids reduce the gastric emptying rate, corresponding to our results, after the supplemented breakfast meals (0-120 min) with PL X showing no significant difference between meals prepared with lipids. Interestingly, our study implies that a dose of 17 g of PL X also efficiently improves blood glucose concentrations after standardised lunch meals. However, our study does not provide results on the mechanism behind this effect and therefore this deserves investigation.

A known mechanism for fat is that it reduces gastric emptying rate, promotes release of gastric and gut hormones and thereby induces satiety [49]. This mechanism may explain why only significant difference in subjective appetite response, between meals prepared with lipids, was found at 210 min. However, the appetite response after negative sample NL was similarly to meals prepared with lipids. The difficulty in observing subjective appetite parameters might explain this result. External factors such as tiredness, excitement, commitment or stress levels could have played a disturbing role when filling in the form. Since the VAS is a subjective form of assessing satiety, hunger and desire to eat, individuals may interpret these questions differently. After all, significance was found at the same time point for satiety, hunger and desire to eat (at 210 min), indicating that the perception of the VAS was valid.

A trend is observed for the sensation of hunger and the desire to eat., showing a reduced hunger sensation and desire to eat after PLH, but it is insignificant. As stated above, lipids are known to affect satiety levels through several mechanisms, like the release of specific gut hormones and inhibition of gastric emptying rate [49]. An animal study by Nishimukai *et al.* [51] supplemented soybean lecithin to the diet of rats, and recording decreased gastric emptying rate and food intake. A reason for this could be the secretion of CCK in the small intestine, which promotes satiety and reduces the risk for the development of obesity [32]. These effects show the potential in rats, but data from human studies are needed. Another mechanism was suggested by Ohlsson *et at.,* implying the increased release of gut hormones GLP-1 and PYY due to delayed absorption in the upper gastrointestinal tract, which could lead to a higher feeling of satiety [49]. However, the concentration of hormones was not examined during our study, so no conclusions can be drawn, and further research is needed.

Another interesting aspect of this study is that the distribution of men and female subjects is as desired. However, a limiting factor in the study is the low number of subjects, which may influence the statistical power of the determination of blood glucose concentrations and appetite variables. The generalisation of the sample group could be more homogeneous since 15 out of 20 subjects are migrants living in Sweden for about one-and-a-half years. It is known that immigration may lead to variations in gut bacteria and absorption of nutrients. It is also important to note that a dietary record helps participants to prepare for the trial day. However, we still rely on the subject's compliance, and results can depend on their sleep schedule, daily exercise, and dietary habits. The desired wash-out period of one week between trial days was not always reached since the subject's schedules not always allowed this. The actual minimum wash-out period for our study was four days. Considering that for food to travel from consumption to leave the body as faeces takes about 36 hours, depending on the individual, the relatively brief wash-out period might not have influenced the results majorly.

Another limitation of the study is the intra-individual difference in fasting values seen in glucose concentrations between the test days. Wishfully, blood glucose concentrations at fasting should be similar each trial day, but results show a significant difference between NL and RSO, which may influence the results. However, incremental areas under the curve are used for our calculations, and these differences in fasting concentrations are thus taken away.

The lipid spread used contains variable amounts of water depending on the sample; this was necessary to create appropriate consistency and taste. The amount of water per test breakfast (i.e. PLH 51 g, PLL 26 g, RSO 6 g and NL 20 g per serving) may influence satiety, since it results in a difference in the meal volume. The amount of lipids also differs among the ingested samples. For PL X, the total amount of lipids was calculated with the composition information provided in the previous batch from the same supplier, which had a lower purity. Therefore, the samples used here with the active ingredient have slightly higher total lipids (i.e. HPL 17.4 g, PLL 17.7 g, RSO 16.1 g per serving). This slight difference does not influence the significance of the results. In future studies, these minor errors should be removed so that all samples have equal amounts of total lipids. Also, the physical chemical structure of PL X might influence the effect on blood

glucose concentrations and satiety response. In this study, PL X is manually mixed into the spread. However, using for example a homogeniser can result in a different structure and thereby might give a different effect.

Another limitation with the study being ongoing when the clock was set to summertime because of Daylight Saving Time Changes. This hour difference may have influenced the satiety of the subjects, and possibly also the glucose regulation in the following days. Not all subjects were directly affected by the difference in time due to their trial days schedule, and no outliers were excluded, considering the randomisation of the order of test meals. We recommend conducting further experiments without the scope of Daylight Saving Time Changes.

It is beyond the scope of this study to address the postprandial effects of insulin concentrations and gut hormones, so this response was not determined. The amphiphilic nature of PL X can influence the absorption of fatty acids since it can emulsify the TAG and improve lipid digestion, absorption and further transportation. The insulin and plasma fatty acid concentrations were not examined. Further studies are required to establish whether PL X increases the release of gut hormones. Also, more research is needed to set the dose-response relationship by examining the effects on different concentrations to find out the minimal effective concentration. The evidence behind specific mechanisms of plant PLs lowering the blood glucose response needs to be revised; more research should be conducted to substantiate our findings.

5.2 Conclusion

This research verified that PL X significantly reduces acute and second-meal (after 3.5 h) postprandial blood glucose concentrations. The results indicate a dose-response effect. In addition, PL X significantly improved subjective appetite response before lunch. These results clearly present the beneficial effects of PL X on postprandial blood glucose response and appetite variables, thereby could have a role in the prevention and treatment of obesity, T2DM and CVD. However, it raises the question of which mechanisms are behind the beneficial effects. Future researchers should consider investigations of additional test markers to enhance comprehension of the underlying mechanisms, including gastrointestinal hormones involved in metabolic and appetite regulation.

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Appendices

Appendix A: Test meal presentations examples

Figure A6: Example test meal i., ii., and iii.

Figure A7: Example test meal iii.

Appendix B: Questionnaire subjective appetite parameters

Hunger, satiety, and desire to eat

Name:

Date:

Product code:

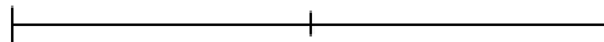
Figure B8: Front page subjective appetite questionnaire

Time: 0 min
(Just before breakfast)

Mark with a line the position on the scale that best corresponds to your appetite sensation

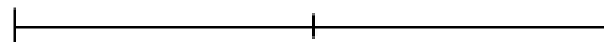
How FULL do you feel right now?

Not FULL at all **Very FULL**



How HUNGRY do you feel right now?

Not hungry at all **Extremely hungry**



How much do you want to EAT (DESIRE TO EAT)?

Do not want to eat **Strong desire to eat**

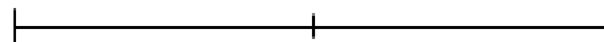


Figure B9: Example page of the subjective appetite questionnaire