Effect of Oat Bio-actives on Cardiometabolic Related Variables in Humans

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by

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Abstract

Consumers are becoming interested in oats as a healthy food. Several studies have investigated the impact of oats components on human metabolism. The purpose of this project is to study the influence of oat polar lipids on human cardiometabolic variables, which focused on four cardiometabolic variables, these were postprandial responses of glucose, insulin, cortisol, and subjective appetite variables. A randomized cross-over human study in 20 healthy subjects was conducted, in which subjects were assigned to consume test or reference breakfasts in random order. Test breakfasts consisted of white wheat bread (WWB) with spreads consisted of oat lipids containing 15 g polar lipids (OL), rapeseed oils (Raps), and a mixture of oat lipids (8.3 g) and rapeseeds oil (Mix). A plain WWB without lipids was included as a reference breakfast. All test breakfast contained similar amounts of lipids. Cardiometabolic test variables were determined at different times postprandially. The results showed that OL resulted in the lowest increase in concentrations of glucose and insulin after breakfast. The effect of oat polar lipids on cortisol wasn't statistically significant, however, this could be due to too few test subjects included in the study. Three appetite related variables: fullness, hunger, and desire to eat, were also measured. OL resulted in the lowest ratings of hunger and desire to eat. It is concluded that oat polar lipids are effective in decreasing postprandial blood glucose and insulin concentrations. At the same time, OL also has a beneficial effect on appetite variables, indicated by increasing satiety and decreasing hunger. The results provide information regarding oat polar lipids as possible new bioactive compounds with preventive potential against obesity and type 2 diabetes.

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List of Abbreviations

ACTH	Adrenocorticotropic hormone
ANOVA	Analysis of variance
AUC	Area under the curve
BMI	Body mass index
CCK	Cholecystokinin
CNS	Central nervous system
CRH	Crticotropin-releasing hormone
CRH	Corticotropin-releasing hormone
ELISA	Enzyme-linked immunosorbent assay
GIP	Gastric inhibitory polypeptide
GLP-1	Glucagon-like peptide-1
GLUT-4	Glucose transporter type 4
iAUC	Incremental area under the curve
MC4R	Melanocortin type 4 receptors
NPY/AgRP	Neuropeptide Y/ agouti-related peptide
POMC	Proopiomelanocortin
PPG	Postprandial glucose
PYY	Peptide YY
PYY3-36	Peptide YY3-36
TSH	Thyrotropin-releasing hormone
VAS	Visual analog scale

1. Introduction

The oat is a cereal grain that grows as an annual plant in temperate and subtropical regions with cold and dry climate (KJStaff, 2019). Oats are high in protein, fibre, vitamins, and minerals, among other nutrients, and compared with other cereals oats are high in lipids. Oats are a good source of beta-glucans, which can reduce cholesterol levels and improve postprandial glucose regulation (Behall et al., 2006). Oats will also increase satiety and reduce appetite because the beta-glucans have high viscosity and large hydration sphere (Rebello et al., 2013). Oats are now increasingly present in the diets of people. Many food companies have launched several oat products to keep up with the customer interest. Breakfast oat cereals are a popular product, which is eaten with milk or yoghurt. Additionally, oat flour, oat biscuits, and oat milk are also popular. However, oat lipids as innovation products have almost not been investigated before concerning metabolic health effects in humans.

In this project, a randomized cross-over meal study has been conducted, with the purpose to investigate metabolic effects of polar lipids from oats. The test products were consumed at breakfast and consisted of a white wheat bread (WWB) with 16.6 g oat lipids containing 90% polar lipids, or a WWB with similar amounts of rapeseed oil. A plain WWB without added lipids was used as a reference. 20 subjects were invited to participate in this human study, aged between 20 to 40 years old and with a body mass index (BMI) in the range of 19 kg/m^2 to 28 kg/m^2. Test variables measured in the blood were postprandial concentrations of glucose, insulin and cortisol. Subjective appetite-related sensations were also investigated.

2. Objectives

The objective of this project is to investigate acute postprandial and second meal effects of oat polar lipids, on four metabolic variables: postprandial blood glucose, insulin and cortisol responses, as well as subjective appetite variables (fullness, hunger and desire to eat). The project aims to address some questions pending in oat polar lipids research and provide some new information in this field. Several parts are including in the project. The first part is literature research on the health effects of oats, with a specific focus on the effects of oat polar lipids on postprandial blood glucose responses, insulin, cortisol and subjective appetite variables. The second part is to design and executes a meal study in healthy subjects. The third part is an analysis of postprandial blood glucose, insulin, cortisol concentrations and evaluation of subjective appetite-related variables. The last part is a statistical comparison, evaluation and discussions of the results on postprandial blood glucose, insulin, cortisol and subjective appetite variables.

3. Theoretical Background

3.1 Oats

Oats are a type of cereal grain from the Poaceae grass family of plants, formally called *Avena Sativa*. Oats are generally grown in temperate regions because they can grow in summer with lower temperatures and tolerate more rain (KJStaff, 2019). In Sweden, oats are the third-largest crop, and the production yield is

increasing. From 1987 to 2020, yield increased from 3500 kg/hectare to 4700 kg/hectare (SCB, 2020; SLU, 2020).

Oats have high nutritional value. It is rich in many minerals and vitamins, such as manganese, phosphorus, folate, vitamin B1 and vitamin B5 (Palsdottir, 2016). According to research, oats contain more protein and fat than most grains (Klose & Arendt, 2012). It is also known that oats are a good source of dietary fibre, especially β-glucan (Whitehead et al., 2014).

These nutrients make oats bring many benefits to people's health. Oats are high in dietary fibre, which is not digested and absorbed by the human body. The general route of dietary fibre in the human body is to move along the stomach, small intestine, large intestine and then be excreted from the body. According to the solubility of the dietary fibre components, it can be divided into insoluble dietary fibre and soluble dietary fibre. Insoluble dietary fibre is a type of fibre that cannot be dissolved in water and cannot be easily fermented by microorganisms in the large intestine. So it helps increase the weight of faeces and improve constipation (Oatmeal, 2020).

Soluble dietary fibre is the fraction of fibre that can be dissolved in water and swelled by water and can more easily, which compare with insoluble dietary fibre, be fermented by microorganisms in the large intestine. The speed of food movement from the stomach into the intestines can be slowed down by soluble fibre, which has a water absorption effect. After absorbing water, the volume of the material in the lumen of the gastrointestinal tract increases, resulting in a sensation of fullness which potentially may reduce energy intake. Therefore, the soluble dietary fibre can help in weight control and weight loss, also lowering blood glucose and blood lipids and preventing metabolic diseases. The viscous dietary fibre may also improve postprandial glucose regulation tough lower gastric emptying rate and gastrointestinal motility (El Khoury et al., 2012).

Oats contain β-glucan, which is a soluble dietary fibre. It can form a gel-like solution at a relatively low concentration and it has a high molecular weight, high viscosity and a wide range of hydration. This all helps oats decrease appetite and increase satiety (Rebello et al., 2013). At the same time, β-glucan intake also can result in increased bile acid production by the liver and lower cholesterol levels (Behall et al., 2006).

In addition to dietary fibre, there is starch in oats. From the nutritional point of view, starch in foods may be classified as rapidly digested starch, slowly digested starch and indigestible (resistant) starch, which is considered part of the dietary fibre. Rapidly digested starch will be rapidly broken down in the small intestine and absorbed as glucose. Slowly digested starch will be slowly broken down and absorbed (Zhang & Hamaker, 2009). Resistant starch, as other components of dietary fibre, is not digested enzymatically in the small intestine but is fermented in the large intestine to produce fermentation products, such as carbon dioxide, hydrogen (Nugent, 2005).

Oats also contain many antioxidants, especially avenanthramides, which is phenolic alkaloids and are almost exclusively found in oats (Meydani, 2009). Studies have shown that they may help to lower blood pressure by promoting the production of nitric oxide in the blood. Avenanthramides help dilates blood vessels and makes blood flow more smoothly (Nie et al., 2006). At the same time, avenanthramides are also beneficial for their anti-inflammatory properties (Sang & Chu, 2017; Sur et al., 2008).

3.1.1 Oat polar lipids

Oat polar lipids are commonly used in cosmetics to help moisturize and protect the skin since it is rich in polar lipids and antioxidants (vitamin E) (Cosmetics, 2021). However, there is almost no application of oat polar lipids in food, even if it has a strong nutritional potential. Oats contain about 13% lipids, of which 34% are polar lipids (glycolipids and phospholipids) (Hossain et al., 2021). This is suggested to be beneficial for appetite related variables, such as increase satiety (Ohlsson et al., 2014). Oat polar lipids has great potential to become an emerging healthy food, but research on the effect of oat lipids on the human body is scarce and it needs further research.

3.2 Blood glucose regulation

Blood glucose regulation is mainly controlled by two hormones: insulin and glucagon. ***Figure 1*** shows the blood regulation process.

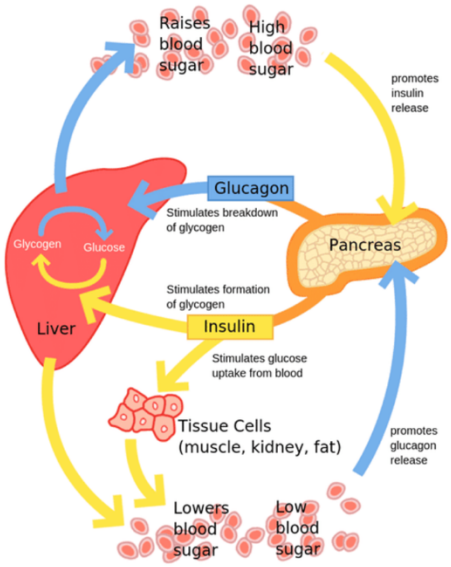

Figure 1. Blood glucose regulation in the human body (Babar et al., 2019)

After meals, blood glucose concentration will increase. Increase in glucose results in the release of the hormone insulin by β-cells in the pancreas. Insulin activates the conversion of glucose to glycogen in the liver (the process is called glycogenesis). At the same time, glucose is absorbed from the blood by muscle and adipose tissue cells through the glucose type 4 (GLUT-4) transporter, reducing blood glucose concentrations. When insulin binds to cell surface receptors, vesicles in the cells containing the GLUT4 transporter join the cytoplasmic membrane and fuse together via endocytosis, allowing glucose to diffuse easily into the cell. To retain the concentration gradient, glucose is phosphorylated to 6-phosphate glucose as it enters the cell (Lanham-New et al., 2019; Soman, 2009). As blood glucose levels decrease, glucagon is released from the α-cells of the pancreas. In the liver, glucagon binds to receptors on the surface of liver cells and stimulates them to break down glycogen in the cells and convert it to glucose (the process is called glycogenolysis). Liver cells release glucose into the bloodstream, causing blood glucose levels to rise (Lanham-New et al., 2019). Insulin and glucagon function together in a loop to keep blood glucose levels within adequate limits.

In addition, some other hormones can also affect the content of blood glucose, such as adrenaline, adrenal glucocorticoid, thyroid hormone, growth hormone, etc., all of which have the function of increasing blood glucose. In addition hormones (GLP-1 and GIP) are released from cells in the intestine after a meal and enhance the release of insulin.

3.2.1 Postprandial glucose regulation

Postprandial glucose (PPG) concentration means the concentration of plasma glucose after a meal. PPG is controlled by the absorption of carbohydrates, insulin and glucagon secretion, and their synergistic effects on the metabolism of glucose in the liver and peripheral tissues. Usually, the fasting blood glucose concentration is about 3.9 mmol/L to 6.1 mmol/L for individuals without diabetes, after 8 to 10 hours of fasting overnight. After eating, the glucose concentration begins to rise around 10 minutes later due to the absorption of carbohydrates. The glucose concentration reaches its peak in around 60 minutes and then returns to pre-meal levels within 2 hours after a meal (American Diabetes Association, 2001).

3.3 Cortisol regulation

Cortisol is a steroid hormone that belongs to the glucocorticoid class of hormones and is primarily produced by the adrenal glands of the adrenal cortex. Since cortisol receptors are present in almost every cell, cortisol can have various effects depending on the cell type. These functions include the control of blood glucose levels, the regulation of metabolism, the anti-inflammatory effect, the control of salt and water balance, the effect on blood pressure and the growth of the fetus (Endocrinology, 2013).

At various times of the day, cortisol levels naturally fluctuate. Cortisol levels are usually highest in the morning and lowest in the evening (Scott, 2021). Three interconnected regions of the body are primarily responsible for cortisol regulation: the brain, pituitary gland and hypothalamus. The hypothalamic-pituitary-adrenal axis is the name for this system. When cortisol levels are low, cells in the hypothalamic region of the brain release corticotropin-releasing hormone (CRH), which allows the pituitary gland to secrete another hormone into the bloodstream called adrenocorticotropic hormone (ACTH). High levels of ACTH are found in the adrenal glands, which promote cortisol production, resulting in elevated cortisol levels in the blood. They begin to prevent the hypothalamus from releasing CRH and the pituitary gland from releasing ACTH as cortisol levels rise. As a result, the level of ACTH drops, causing the level of cortisol to drop as well. This is called a negative feedback loop (Endocrinology, 2013).

Usually, the cortisol concentration will increase after meals, which is mediated by the secretion of ACTH and the regeneration of cortisol outside the adrenal glands (Stimson et al., 2014). Another study has compared the effects of different diets on cortisol. Compared with the high-fat diet and the high-carbohydrate diet, the high-protein diet is more likely to stimulate the secretion of cortisol (Slag et al., 1981).

Cortisol has an effect on human metabolism. The secretion of cortisol helps to convert some amino acids in muscles into glucose and glycogen into glucose (glycogenolysis). Maintaining high levels of cortisol for a long time has a negative impact on health, such as obesity. Cortisol can increase plasma cholesterol, activate the esterase under the skin of the limbs and promote the decomposition of subcutaneous fat, which is redistributed on the face, upper chest, back of the neck, abdomen and buttocks, forming central obesity. And cortisol makes the brain less sensitive to leptin, blocking the signal for satiety, so may make

people feel hungrier than usual. In addition, cortisol stimulates the appetite, because the production of cortisol can cause anxiety, people can alleviate anxiety by eating high-sugar and high-fat foods and results in weight gain (Hormone, 2019).

3.4 Appetite regulation

Pleasure and enjoyment during food intake are caused by body fluid substances, such as dopamine, endorphins and endocannabinoids. The regulation of energy and appetite is carried out through the interaction of peripheral signals with the central nervous system (CNS), of which the hypothalamus is the most critical. Two bunches specialized neurons coupled to appetite regulation, one release orexigenic neuropeptides and the other release anorexigenic neuropeptides. The orexigenic mediators stimulate the hunger centre, which is located in the lateral hypothalamus, to release orexins and melanin-stimulating hormones. The anorexigenic effectors stimulate the satiety centre, which in the ventromedial hypothalamus, to release corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone (TSH) and oxytocin. The orexigenic pathway contributes to increased appetite and decreased energy expenditure as well as the anorexigenic pathway leads to decreased appetite and increased energy expenditure (Perry & Wang, 2012).

An increase in ghrelin will induce hunger. In the stomach, ghrelin is secreted and contributes to increased neuropeptide Y (NPY) andagouti-related peptide (AgRP) expression in the hunger centre in the brain and mesolimbic reward centre activation. When people feel hunger and see the delicious food, pre-meal events occur, the pancreatic juice is secreted and hunger signals are released. The mesolimbic region that mediates food pleasure is simultaneously triggered. About 30 minutes after food intake, the intestinal tract, adipose tissue and liver release both short-term and long-term hormones of satiety (Perry & Wang, 2012).

The short-term hormones including CCK, peptide YY3-36 (PYY3-36), and glucagon-like peptide-1 (GLP-1), are released after a meal and affect the appetite centre of the hypothalamus. The upper intestine secretes CCK, the L cells of the small and large intestine release PYY3-36 and GLP-1, which has a high affinity for NPY/AgRP expressing Y2 receptors of the neurons. GLP-1 cooperates with PYY3-36 to respond to nutrients in the gut. After the food is ingested, GLP-1 can enhance insulin secretion and inhibit glucagon secretion (Nauck et al., 1997).

The long-term hormones controlling appetite also including insulin and in addition leptin. Through receptor-mediated transport, insulin enters CNS, and under normal condition its satiety potential increase in proportion to fat mass. Leptin crosses the blood-brain barrier and its receptor is activated. This activity contributes to proopiomelanocortin (POMC) or cocaine-amphetamine-related transcript activation and NPY/AgRP inhibition (Perry & Wang, 2012).

3.5 Metabolic syndrome

Metabolic syndrome is a cluster of factors that occurring simultaneously, increases the risk for developing illnesses (Cornier et al., 2008; Eckel et al., 2005). The factors including high blood pressure and blood glucose, overweight, abnormal cholesterol or triglyceride levels among others. People with metabolic syndrome are nearly twice as likely as those without metabolic syndrome to develop cardiovascular

disease, and the risk of type 2 diabetes is around five times greater. Obesity and a sedentary lifestyle are the main causes of metabolic syndrome. (Grundy, 2008).

Some studies have shown that in most countries, 20% and 30% of the adult population suffer from metabolic syndrome (Grundy, 2008). However, metabolic syndrome can be reversed and effectively prevented. Losing weight is the most useful method, which can be achieved through healthy eating habits. Therefore, this project is trying to find a new product that may help in lower postprandial glycemia and increase satiety.

4. Methodology

4.1 Study design and procedure

A randomized cross-over human study was designed, including 8 males and 12 females. The study was conducted in the laboratory at the Department of Food Technology, Engineering and Nutrition at Kemicentrum, LTH. The test participants were between 20 to 40 years old with the BMI around 19 kg/m^2 to 28 kg/m^2. Subjects were asked to participate on 4 experimental days and treated with 4 different breakfast meals, with around 7 days wash-out period in-between. Blood glucose was measured at the test days, and additional blood was collected for later measurements of the rest of the test parameters in blood. At each visit participants filled out an appetite form. Test breakfasts and the reference breakfast were given to subjects in random order. Before the study, all participants signed a written consent form. The study has been approved by the Regional Ethical Review Board in Lund, Sweden (Dnr. 2018/658).

An overview of the study design, including the test points for determinations of the test markers, are shown in ***Figure 2***. The human study lasted 330 minutes. At 8:00 am, the subjects came to the laboratory, after 8 to 10 hours of fasting overnight. Firstly, fasting blood glucose was measured directly through finger-prick blood test, and extra capillary blood samples were collected for insulin and cortisol analysis (time = 0 min). After that, the subjects got one of the test breakfasts or the reference, which was consumed in around 12 min. Then their blood glucose was tested repeatedly (at 15, 30, 45 60, 90, 120, 150, 180 and 210 min) and blood samples were collected for insulin analysis (at 30, 45, 60, 90, 120 and 210 min) and cortisol analysis (at 60 and 210 min). After 210 minutes, the subjects ate a standardized lunch. Then the measurement of blood glucose (at 225, 240, 255, 270, 300 and 330 min) and collection of blood samples for insulin (at 240, 255, 270, 300 and 330 min) and cortisol analysis (at 330min) were continued until the end of the test day. The collected blood samples for insulin and cortisol determinations were centrifuged and the plasma was kept at -40°C until analysis. At the same time, the subjects needed to fill out the appetite survey (at 0, 15, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300 and 330 min). Three appetite related variables were evaluated, which were fullness, hunger and desire to eat.

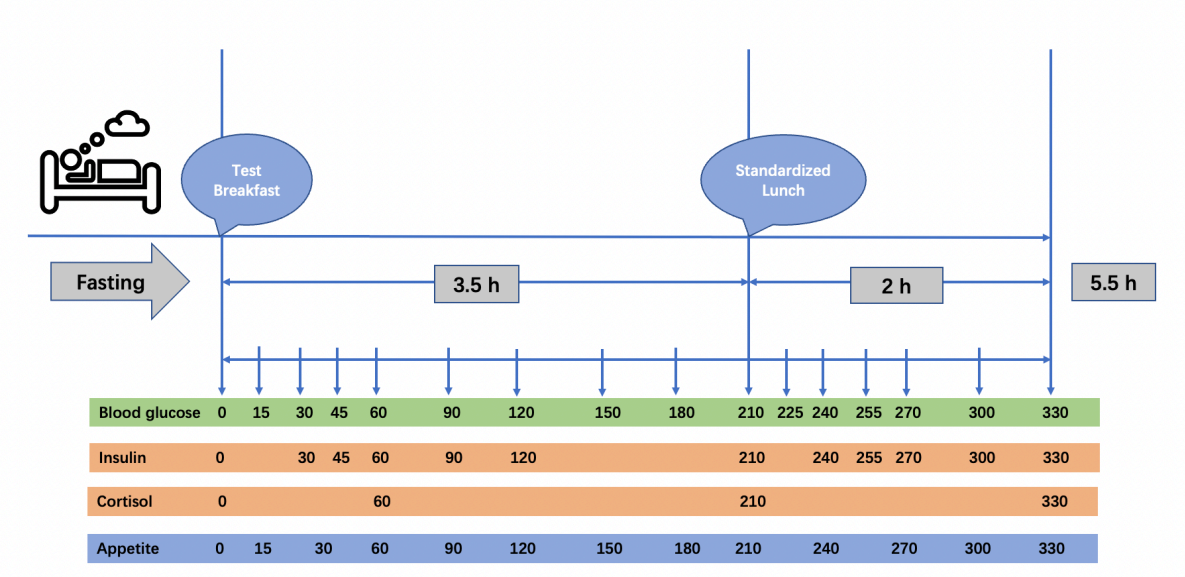

Figure 2. Schematic overview of the human study

4.2 Test breakfasts and standardized lunch

Table 1 shows the recipe for breakfasts and standardized lunch. Three test breakfasts and one reference breakfast were included. The test breakfasts contained 120 g of a WWB and 16.6 g of different lipids, or mixtures of lipids. The lipids included in the different test meals were oat lipids containing 90% polar lipids (OL), rapeseed oils (Raps), and a mixture (50/50) of OL and Raps (Mix). A plain WWB without spread with lipids were included as a reference breakfast product. Water, 250 ml, was consumed with the meals. The reason for choosing rapeseed oil for comparison to OL is that it is considered a healthy oil, which has a low saturated fat content but a high content of unsaturated fat. The standardized lunch contained 120 g of WWB (50 g available starch), 100 g of meatballs and 250 ml of water. The meatball sandwich was used as standardized lunch since meatballs is a common Swedish lunch. The image of the test breakfast can be found in ***Appendix A***.

Table 1. Formulation of test meals and references

Test breakfast	OL[1] + WWB[2]	120 g white wheat bread + 16.6 g oat polar lipids + 250 ml water
	Raps + WWB	120 g white wheat bread + 16.6 g rapeseed oils + 250 ml water
	Mix (OL &Raps) + WWB	120 g white wheat bread + 8.3 g OL + 8.3 g Raps + 250 ml water
Reference breakfast	WWB	120 g white wheat bread + no oils + 250 ml water
Standardized lunch	120 g white wheat bread + 100 g meatball + 250 ml water	

[1] *OL: Oat lipids contain 90% of polar lipids, so 16.6 g of oat lipids means that 15 g of polar lipids in the product;*
[2] *WWB: white wheat bread; Raps: Rapeseed oils.*

Table 2 shows the compositions of test breakfasts and standardized lunch. The available carbohydrates content is the same in all the test breakfasts and standardized lunch (50 g available carbohydrates). In addition, test breakfasts have 16.6 g of fat and 8.5 g of protein. However, the WWB reference product has

no added fat because it does not have any oils. Apart from the available carbohydrates, the standardized lunch also includes 18.5 g of fat and 21.5 g of protein.

Table 2. Macronutrient compositions of test breakfast and standardized lunch meals (per serving)

	Test breakfast and reference	**Standardized lunch (Meatball sandwich)**
Carbohydrate (g)	50	50
Fat(g)	16.6*	18.5
Protein(g)	8.5	21.5

** Fat in the spreads, not in the reference WWB*

4.3 Physiological test parameters

Blood glucose measurement

The blood glucose concentrations were measured on capillary blood using HemoCue equipment. Blood from a fingerpick test was collected in a HemoCue glucose cuvette and immediately placed in the HemoCue blood glucose meter for blood glucose concentration analysis.

Appetite variables

On three separate 10-cm visual analogue scales (VAS), subjects were asked to rate their feelings of fullness, hunger, and desire to eat at different intervals. For example, the level of fullness was expressed as score on a 10-cm scale, with 100 mm indicating absolute fullness and 0 mm indicating no fullness at all.

The appetite variable questionnaire can be found in ***Appendix B***.

Insulin & cortisol

After the blood samples were collected in capillary blood collection micro tubes, they were centrifuged to separate serum from blood cells and stored at -40°C. Insulin and cortisol concentrations were measured with the enzyme-linked immunosorbent assay (ELISA) method.

ELISA is a molecular experiment based on the characteristic of the specific bond between antigen and antibody for the identification and quantification of soluble substances such as peptides, proteins, antibodies, and hormones. The antigen or antibody bound to the solid surface (usually 96-well plates microplate) still maintains the intact natural structure of the protein. Using the bonding mechanism and the colour reaction catalysed by enzymes, the presence of antigen or antibody in a test sample can be detected and can be quantified according to the colour depth (Aydin, 2015).

There are several ELISA methods, such as direct ELISA, competitive ELISA, sandwich ELISA. In this project, sandwich ELISA was used to determine the concentration of insulin and cortisol. Sandwich ELISA is the most commonly used method since it has high specificity and high sensitivity. It can be used to detect two antibodies with different epitopes on the same antigen (Aydin, 2015).

The working principle of sandwich ELISA has serval steps. 96-well plates microplate is first coated with a capture antibody. Second, any antigen present in the sample binds to the capture antibody when it is added to the plate. Thirdly, a detecting antibody is added, which binds to the antigen. Following that, an

enzyme-linked secondary antibody is added, which binds to the detecting antibody. Finally, the substrate is added and converted to a detectable form by the enzyme (Schmidt et al., 2012).

4.5 Statistics

There are several statistical methods used in this project.

The statistical analyses of blood glucose and insulin concentrations are performed using delta values, which is the difference between the fasting value and the following values during the test day, showing the variation in concentrations from fasting. Cortisol and appetite variables were analyzed on the absolute values, that is the actual test data as a measure. At the same time, the standard error of the mean (SEM) was calculated, which measures how much the sample means may differ from the population mean.

After that, significant differences in results depending on the product were investigated. Based on the delta value, the incremental areas under the curves (iAUC) of blood glucose and insulin concentrations after the meals were calculated to judge whether they are significantly different. In the calculations of iAUC, the areas beneath the fasting concentration were ignored. According to the absolute value, the area under the curve (AUC) of cortisol concentrations and subjective appetite variables after the meals were calculated to determine the significant differences.

To examine the significant difference, the analysis of variance (ANOVA, general linear model) was used. When a significant difference between products was discovered, Tukey simultaneous test was used to investigate which products differed from each other. Since the 95% confidence interval (α= 0.05) is used, a P-value of less than 0.05 indicates significant differences in results. All statistical analyses and calculations of results were performed with GraphPad Prism and Minitab software.

5. Results

5.1 Postprandial glucose response

The incremental changes in blood glucose concentrations can be found in ***Figure 3***. There are two highest points: after test breakfasts and after standardized lunch, because blood glucose concentration rises after consuming test breakfast, then drops until it rises again after consuming standardized lunch. In the overall trend, WWB resulted in the highest blood glucose concentrations. OL resulted in the lowest rise in postprandial blood glucose concentrations along the whole length of the experiment.

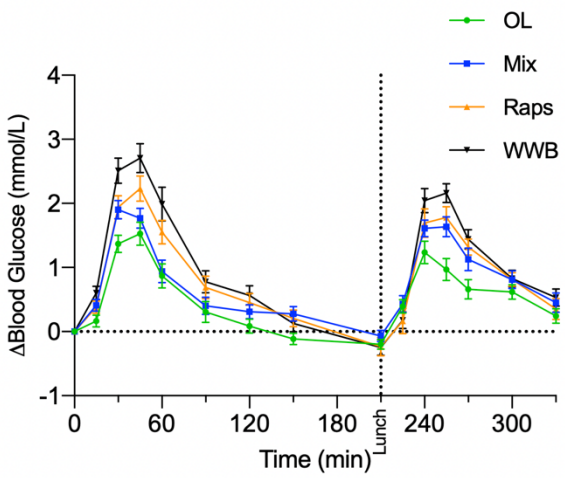

Figure 3. Incremental changes in blood glucose concentrations. Values are means ± SEM, n = 20 subjects. OL, white wheat bread with oat polar lipids; Mix, white wheat bread with the mixture of oat polar lipids and rapeseed oils; Raps, white wheat bread with rapeseed oils; WWB, white wheat bread.

The results regarding fasting and postprandial blood glucose concentrations (iAUC) are shown in ***Table 3***. There was no significant difference in the fasting value (p-value > 0.05). From 0 min to 120 min (i.e. after the test breakfasts), WWB and Mix, WWB and OL, Raps and OL are significantly different, since the p-value < 0.05. From 210 min to 330 min (i.e. after the standardized lunch), OL is significantly different from other products (p-value < 0.05).

Table 3. Blood glucose concentrations at fasting state and after consumption of test breakfasts and a standardized lunch [1]

Test Variables	WWB	Raps	Mix	OL
Fasting blood glucose (mmol/L)	4.92 ± 0.08 [a]	5.02 ± 0.07 [a]	4.93 ± 0.08 [a]	4.98 ± 0.07 [a]
Blood glucose iAUC = 0 - 120 min (mmol*min/L)	165.57 ± 14.90[a]	133.73 ± 12.70 [ab]	101.96 ± 10.40 [bc]	83.80 ± 11.10 [c]
Blood glucose iAUC = 210 - 330 min (mmol*min/L)	133.51 ± 10.80 [a]	119.33 ± 8.82 [a]	114.55 ± 9.97 [a]	79.13 ± 10.20 [b]

[1] *Data are presented as means ± SEM, n = 20 subjects. Values in the same row with different superscript letters are significantly different, p-value < 0.05. OL, white wheat bread with oat polar lipids; Mix, white wheat bread with the mixture of oat polar lipids and rapeseed oils; Raps, white wheat bread with rapeseed oils; WWB, white wheat bread.*

5.2 Insulin

The incremental changes in insulin are depicted in ***Figure 4.*** There are also two peaks, comparable with results of blood glucose concentrations, after test breakfast and after standardized lunch. After consuming test breakfast, the highest insulin concentration is after the WWB and Raps, which have similar results, followed by after MIX. OL resulted in the lowest postprandial insulin concentrations. After consuming standardized lunch, OL resulted in the lowest insulin concentrations and Raps in the highest concentrations.

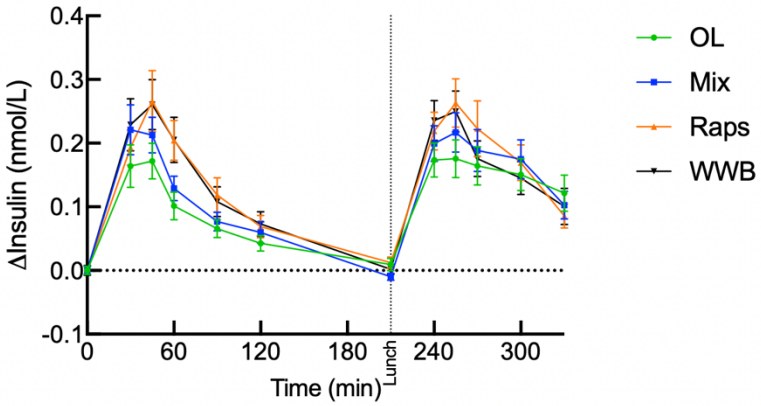

Figure 4. Incremental changes in insulin concentrations. Values are means ± SEM, n = 20 subjects. OL, white wheat bread with oat polar lipids; Mix, white wheat bread with the mixture of oat polar lipids and rapeseed oils; Raps, white wheat bread with rapeseed oils; WWB, white wheat bread.

Table 4 shows the differences in insulin concentration (iAUC). There was no significant difference in fasting blood insulin. From 0 min to 120 min, a significant difference can be found in WWB and OL, Raps and OL (p-value < 0.05). From 210 min to 330 min, only one set of comparison has a significant difference, Raps and OL (p-value < 0.05).

Table 4. Insulin concentrations at fasting state and after consumption of test breakfasts and a standardized lunch [1]

Test Variables	WWB	Raps	Mix	OL
Fasting blood insulin (nmol/L)	0.05 ± 0.01 [a]	0.04 ± 0.01 [a]	0.05 ± 0.01 [a]	0.05 ± 0.01 [a]
Insulin iAUC = 0 - 120 min (nmol*min/L)	18.05 ± 2.31 [a]	17.54 ± 2.19 [a]	14.31 ± 1.71 [ab]	11.19 ± 1.46 [b]
Insulin iAUC = 210 - 330 min (mmol*min/L)	18.93 ± 2.24 [ab]	20.46 ± 2.61 [a]	18.65 ± 2.17 [ab]	16.73 ± 1.83 [b]

[1] *Data are presented as means ± SEM, n = 20 subjects. Values in the same row with different superscript letters are significantly different, p-value < 0.05. OL, white wheat bread with oat polar lipids; Mix, white wheat bread with the mixture of oat polar lipids and rapeseed oils; Raps, white wheat bread with rapeseed oils; WWB, white wheat bread.*

5.3 Cortisol

The results regarding cortisol concentration can be found in ***Figure 5***. The reason for choosing three products (OL, Raps and WWB) for analysis is that, to the best of our knowledge, this is the first time effects of oat polar lipids on cortisol concentrations are investigated, and the main reason is to investigate effects, not possible dose response effects.

The cortisol concentration was measured at four different time points: 0 min, 60 min, 210 min and 330 min. This is the cortisol concentration at fasting, one hour after the test breakfast, prior to lunch, and two hours after the standardized lunch. No differences in concentrations after the different breakfast meals were observed up to 210 min. At 330 min, the cortisol concentrations after the WWB become the one with the highest concentrations and Raps resulted in the lowest cortisol concentration at this specific time point.

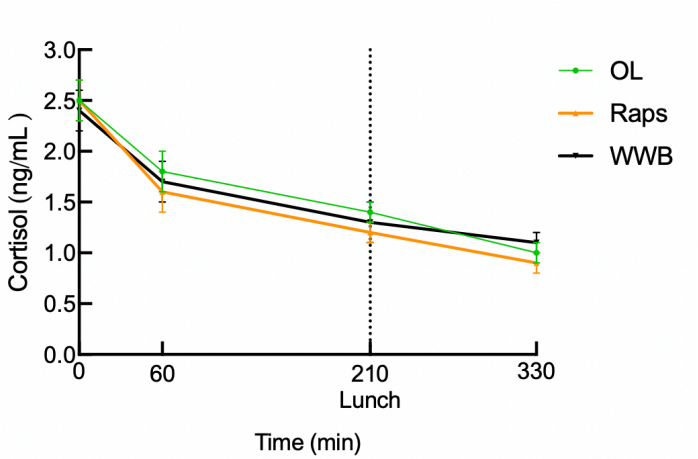

Figure 5. Concentrations of plasma insulin. Values are means ± SEM, n = 20 subjects. OL, white wheat bread with oat polar lipids; Raps, white wheat bread with rapeseed oils; WWB, white wheat bread.

Table 5 shows the results regarding cortisol concentration. There is no significant difference in the fasting cortisol as well as from 0 min to 210 min. Consumption of the Raps at breakfast resulted in significantly lower cortisol concentrations at 330 min compared with WWB breakfast (p-value < 0.05).

Table 5. Plasma Cortisol concentrations at fasting state and after consumption of test breakfasts and a standardized lunch [1]

Test Variables	WWB	Raps	OL
Fasting cortisol (ng/mL)	2.4 ± 0.2 [a]	2.4 ± 0.2 [a]	2.5 ± 0.2 [a]
Cortisol AUC = 0 - 210 min (ng*min/mL)	351 ± 38 [a]	329 ± 26 [a]	365 ± 4 [a]
Cortisol at 330 min (ng/mL)	1.1 ± 0.1 [a]	0.9 ± 0.1 [b]	1.0 ± 0.1 [ab]

[1] *Data are presented as means ± SEM, n = 20 subjects. Values in the same row with different superscript letters are significantly different, p-value < 0.05. OL, white wheat bread with oat polar lipids;; Raps, white wheat bread with rapeseed oils; WWB, white wheat bread.*

5.4 Subjective appetite variables

The mean absolute values of fullness can be found in ***Figure 6***. Fullness is lowest at 0 min because of the subjects in the fasting state. After consuming the test breakfast, fullness increases to a peak then decreases until it climbs again after consuming the standardized lunch, then falls again. From 0 min to 210 min, OL breakfast resulted in the highest sensation of fullness, after that is Raps. Mix and WWB are third and fourth respectively. After the standardized lunch, Raps over OL become the highest fullness. The values of Mix and WWB after lunch are very close.

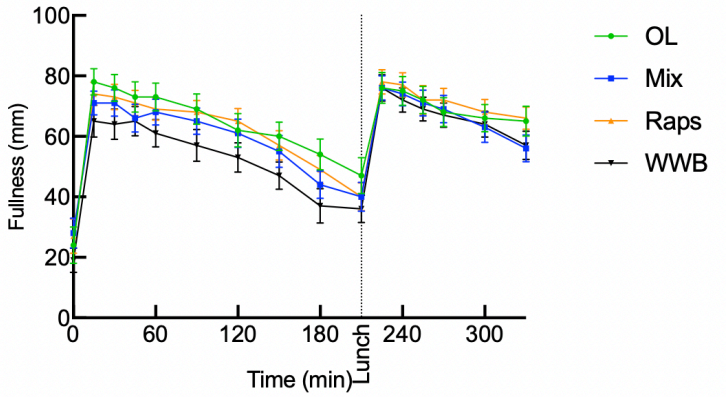

Figure 6. The mean value of fullness. Values are means ± SEM, n = 20 subjects. OL, white wheat bread with oat polar lipids; Mix, white wheat bread with the mixture of oat polar lipids and rapeseed oils; Raps, white wheat bread with rapeseed oils; WWB, white wheat bread.

The pattern of mean values of hunger is the opposite of that of the mean values of fullness (***Figure 7***). The value of hunger is highest at 0 min, then it decreases to the lowest point after consuming the test breakfast and then increases until it drops again after consuming standardized lunch and rises again. From 0 min to 210 min, the hunger of WWB is significantly higher than the other three products. Mix, Raps and OL are close, but OL is a little bit lower than Mix and Raps. After consuming the standardized lunch, the values of the four products are very close and the difference is very small.

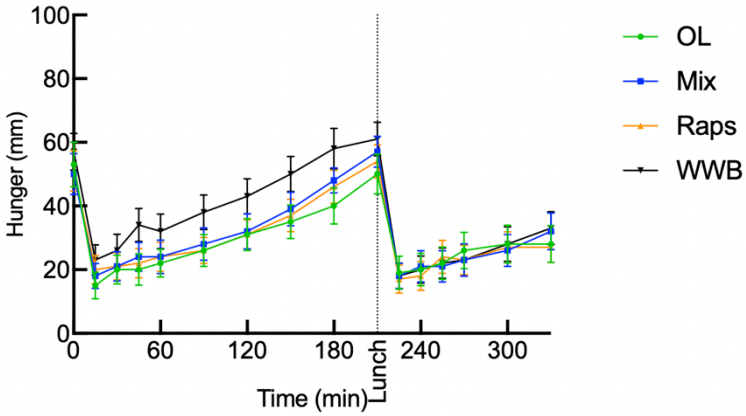

Figure 7. The mean value of hunger. Values are means ± SEM, n = 20 subjects. OL, white wheat bread with oat polar lipids; Mix, white wheat bread with the mixture of oat polar lipids and rapeseed oils; Raps, white wheat bread with rapeseed oils; WWB, white wheat bread.

The pattern of mean values of desire to eat is similar to that of hunger since hunger promotes the desire to eat (***Figure 8***). The value of desire to eat is highest at 0 min, then the value decreases to the lowest point after consuming the test breakfast and then increases until it decreases again after consuming standardized lunch and increases again. After consuming the test breakfast, WWB resulted in a significantly higher desire to eat than other products. OL, Mix and Raps are close to each other from the beginning, but Mix and Raps are higher than OL at 210 min. After eating the standardized lunch, the values

are very close to each other and do not have obvious differences. At 330 min, the four products are close to each other.

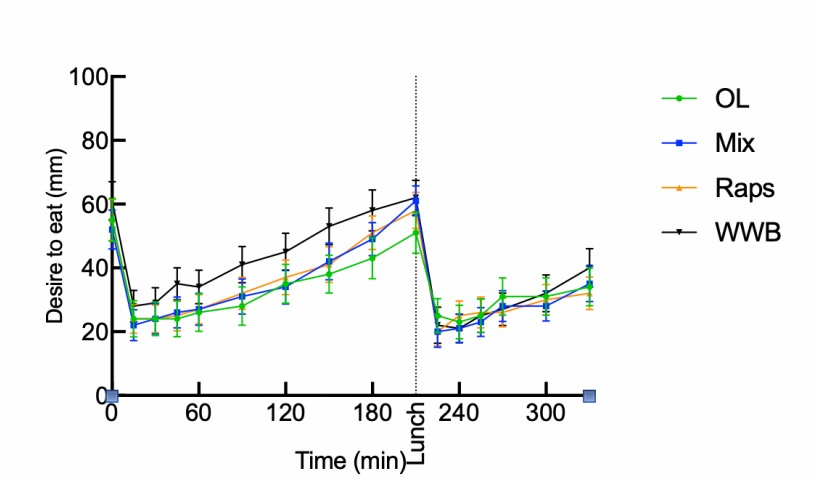

Figure 8. The mean value of desire to eat. Values are means ± SEM, n = 20 subjects. OL, white wheat bread with oat polar lipids; Mix, white wheat bread with the mixture of oat polar lipids and rapeseed oils; Raps, white wheat bread with rapeseed oils; WWB, white wheat bread.

Table 6 shows the statistical comparison between appetite variables. None of these three variables is significantly different from 210 min to 330 min. From 0 min to 210 min, WWB is significantly different from other products in fullness and hunger (p-value < 0.05). OL and WWB have a significant difference in desire to eat (p-value < 0.05).

Table 6. Appetite variables after consumption of test breakfasts and a standardized lunch [1]

Test Variables	WWB	Raps	Mix	OL
Fullness AUC = 0 - 210min (mm*min)	10741 ± 882 [b]	12242 ± 855 [a]	12828 ± 796 [a]	13268 ± 910 [a]
Fullness AUC = 210 - 330min (mm*min)	7808 ± 452 [a]	7904 ± 497[a]	8355 ± 457 [a]	8150 ± 544 [a]
Hunger AUC = 0 - 210min (mm*min)	8981 ± 962 [a]	7053 ± 902 [b]	6848 ± 852 [b]	6437 ± 944 [b]
Hunger AUC = 210 - 330min (mm*min)	3211 ± 535 [a]	3120 ± 551 [a]	3038 ± 525 [a]	3154 ± 643 [a]
Desire to eat AUC = 0 - 210min (mm*min)	9437 ± 1014 [a]	7765 ± 967 [ab]	7826 ± 941 [ab]	7113 ± 1156 [b]
Desire to eat AUC = 210 - 330min (mm*min)	3662 ± 589 [a]	3427 ± 528 [a]	3421 ± 521 [a]	3596 ± 655 [a]

[1] Data are presented as means ± SEM, n = 20 subjects. Values in the same row with different superscript letters are significantly different, p-value < 0.05. OL, white wheat bread with oat polar lipids; Mix, white wheat bread with the mixture of oat polar lipids and rapeseed oils; Raps, white wheat bread with rapeseed oils; WWB, white wheat bread.

6. Discussions & Conclusion

This project investigated the acute effect of oat polar lipids in humans after consuming them in a breakfast, followed by a second meal standardized lunch. Due to excessive intake of energy dense and processed foods, and sedentary lifestyles, people are at increased risk of cardiovascular-related diseases caused by

the metabolic syndromes. Dietary intervention with health promoting foods is one of the most economical and most convenient methods to reduce the risk of cardiometabolic illness. Oils are a common and essential part of the daily diet, so it is feasible to maintain health by eating healthier oils. For people with obesity and type 2 diabetes, it is important to consider dietary interventions to lose weight or lower blood glucose to improve health. This project has shown that consuming oat polar lipids can improve cardiometabolic risk elated variables and increase satiety, thus play a part in a preventive strategy against cardiometabolic disorders.

The results showed that the effect of OL in lowering blood glucose and insulin is significantly better than the other test products, both acutely postprandial after the test breakfast and after the standardized lunch. The second best product in lowering the postprandial blood glucose concentrations is Mix, followed by Raps. This demonstrates that oat polar lipids are effective in lowering blood glucose concentrations, the effects are dose dependent, and the effect can last a long time because the effect is not only after consuming test breakfasts but also after consuming the standardized lunch. In comparison to rapeseed oils, oat polar lipids have a more obvious effect on lowering blood glucose. Several hypotheses could be the possible reasons. One possibility is that the digestion of oat lipids is slow, partial digested or some parts of oat lipids may not be digested. One consequence of such mechanisms could be an increased release of gut hormones (GLP-1 and PYY) which are involved in blood glucose and appetite regulation. Another possibility is that polar lipids of oats may be responsible for reducing the activity of digestive enzymes (amylase or lipase).

Ohlsson et al. (2014) investigated the effect of consuming a liposome preparation of oat lipidson postprandial blood lipids and satiety hormones, with dairy lipids as a placebo. Subjects were tested for blood glucose and insulin from the fasting state up to 7 hours after test meals. Their results show that oat polar lipids are better than dairy lipids in lowering postprandial blood glucose concentrations (Ohlsson et al., 2014). Thus, the results observed in the current study were in accordance with previous studies indicating the beneficial effects of oat polar lipids on acute postprandial glucose regulation. Another research compared the effects of olive oil and corn oil on postprandial blood glucose. The results show that olive oil is more beneficial to lower postprandial blood glucose compared with corn oil (Violi et al., 2015). One suggestion for the future is to perform a human study on oat lipids and olive oil and compare which of the two oils has a more powerful effect on human metabolism.

There was no significant difference among products in effects on cortisol concentrations 0 min to 210 min after the breakfast. Two hours after consuming the standardized lunch, WWB resulted in the highest cortisol concentration and Raps in the lowest one. Cortisol increases with stress, indicating that rapeseed oils induced less stress in the body compared with oat polar lipids, these should be investigated in more details. Because the cortisol analysis has limitations since the small number of time points analyzed, and the small number of subjects. It is possible that if there are more samples, the findings could be different. Another possibility is there are no differences between the products in affecting cortisol concentrations.

The results of the three parts of the subjective appetite variables all indicate that oat polar lipids are useful in increasing satiety and decrease hunger because OL and WWB are significantly different after consuming test breakfasts. However, there are no significant differences between the lipid containing products. It could be due to the subjects failing to follow the instructions for filling out the scales. One suggestion for future studies is to explain to the subjects in more detail how to evaluate the appetite questionnaire

before starting the study and to check the evaluation of the subjects frequently during the period. Another possible reason is that there are too few test subjects, so the data is limited.

Ohlsson et. al. (2014) investigated subjective appetite variables on 15 female subjects consuming liposome as test meals and dairy lipids as a placebo. The same parameters were investigated: fullness, hunger and desire to eat. The results indicated that after consuming a test breakfast containing oat polar lipids, the subjective satiety of female subjects increased but there was no difference in *ad libitum* food intake compared to placebo (Ohlsson et al., 2014). Although the preparations used in that study and the present one was not identical, their outcomes were similar and this indicate that oat polar lipids have a positive effect on increasing satiety, perhaps stomach emptying is slower by prolonging lipid digestion and absorption.

This project has some limitations. For example, only 20 test subjects participated in the human study, so possibly there are not enough samples for cortisol analysis and subjective appetite variables to provide adequate statistical power to detect significant differences. The gender of the test subjects is not balanced, with more females than males. In addition, there are large differences in BMI between different subjects in the study, maybe it can evaluate results by multivariate analysis in further research.

To summarize, this project indicated that oat polar lipids have a positive effect on reducing postprandial glucose and insulin response in healthy subjects and it also works to increased satiety and reduced hunger. The project provides a good reference for long term study. People with metabolic syndrome can consider using oat polar lipids instead of other oils, which will help them relieve symptoms. Healthy people can also be recommended to consume oat polar lipids to reduce the risk of obesity and cardiovascular related diseases. However, people have to be aware of that irrespective of the type of oil, as a high energy density product, any oil that is consumed in excess may result in weight gain.

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Appendix A – Test breakfast

Appendix B – Appetite survey

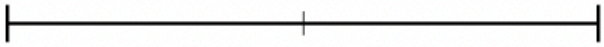
Initialer:
Datum:

Tid: 0 min
(Precis före brödfrukost)

Markera med ett streck den position på skalorna som bäst överensstämmer med dina aptitförnimmelser

Hur MÄTT (eng. FULL) känner du dig just nu?

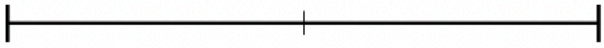
Inte mätt alls (Not FULL att all) **Väldigt mätt (Very FULL)**



Hur HUNGRIG (eng. HUNGRY) känner du dig just nu?

Inte hungrig alls
(Not hungry at all)

Extremt hungrig
(Extremely hungry)



Hur gärna vill du ÄTA (eng. DESIRE TO EAT)?

Vill inte äta
(Do not want to eat)

Vill väldigt gärna äta
(Strong desire to eat)