A randomized, controlled, crossover trial investigating the effects of bread containing guar galactomannan on glucose response in healthy overweight adults

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ABSTRACT

The increased prevalence of lifestyle diseases, such as type 2 diabetes mellitus (T2DM) and obesity, and the implication of hyperglycaemia in the aetiology of these diseases have called for more research on dietary modification to address the specific metabolic disturbances involved in these conditions. Several studies have demonstrated the effectiveness of guar galactomannan, a dietary fibre, in reducing postprandial glucose and insulin levels, but more knowledge is needed on its effect as part of bread. Bread is a staple food in Norway, and can have a high glycaemic index, thus contributing to diabetes risk. The purpose of this study was therefore to investigate the effects of breads containing guar galactomannan varying in molecular weight and concentration on postprandial glucose response in healthy, overweight (mean BMI 27.6 ± 2.5 kg/m^2) adults (n=9). This randomized, controlled, crossover trial comprised five breads which were tested, one per study day. Diabetes mellitus was excluded by confirming HbA1c concentrations well below the diagnostic cutoff for DM. The primary outcome was postprandial glucose response, measured by samples of capillary and venous plasma glucose, and serum insulin 0, 15, 30, 45, 60, 90, 120, and 180 minutes post-consumption. The secondary outcome was whether the different effects of guar galactomannan on postprandial glucose response is dependent on the molecular weight or concentration of guar galactomannan added to the breads. The breads were consumed in a double-blind, randomized order, and contained 25 g available carbohydrate each. The area under the curve (AUC), the maximum concentration (C_{max}) and the time of C_{max} were calculated and compared by non-parametric statistical tests.

No significant differences in the AUC of glucose was observed after consumption of the guar-breads, relative to the control. All guar-breads produced significant ($p<0.05$) reductions in mean maximum insulin concentrations of 34% - 68% compared to the control. When comparing individual guar-breads to the control, significantly reduced insulin AUC 0-120 minutes were observed after consumption of two of the test breads. Our findings demonstrate that breads containing guar galactomannan reduces the postprandial insulin rise, without affecting blood glucose response, in healthy, overweight adults. The study was affected by the Covid-19 pandemic, and instead of 12 participants, only 9 were included. This may have influenced the results due to reduced power of the study. More research is required to determine the effects of guar added to carbohydrate staple foods.

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BMI	Body Mass Index
C_{max}	Maximum concentration
CVD	Cardiovascular disease(s)
DM	Diabetes Mellitus
EDTA	Ethylenediaminetetraacetic acid
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization
g	G-force
GI	Glycaemic Index
GL	Glycaemic Load
GR	Glycaemic Response
HbA1c	Haemoglobin A1c
iAUC	Incremental Area under the curve
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
MBF	Department of Medical Biochemistry and Pharmacology
MW	Molecular weight
NaF	Sodium Fluoride
NCD	Non-communicable disease(s)
NEFA	Non-esterified fatty acids
NHANES	The National Health and Nutrition Examination Surveys
NNR	Nordic Nutrition Recommendations
PHGG	Partially hydrolysed guar gum
REK	Regional Committee for Medical and Health Research Ethics

RUHS	Research Unit for Health Surveys
SAFE	Secure Access to Research Data and E-infrastructure
SAT	Subcutaneous adipose tissue
SCFA	Short-chain fatty acids
SD	Standard Deviation
SEM	Standard Error of the Mean
STOP-NIDDM	Study to Prevent Non-Insulin-Dependent Diabetes Mellitus
T=0	Time zero
T2DM	Type 2 Diabetes Mellitus
T_{max}	Time of maximum concentration
VAT	Visceral adipose tissue
WAT	White adipose tissue
WHO	World Health Organization

INTRODUCTION

Among the macronutrients, carbohydrates constitute a major proportion of dietary energy, accounting for between 40 and 80 percent of total energy intake (1). Dietary carbohydrates are defined as naturally occurring sugars, added sugars, starch, resistant starch and dietary fibre/non-starch polysaccharides (1). Research on the content of carbohydrates in foods is growing as some types of carbohydrates have properties that may help prevent the development of lifestyle diseases (2-5).

Worldwide, chronic diseases such as cardiovascular diseases, diabetes, respiratory diseases and cancer, collectively called noncommunicable diseases (NCDs), contribute to approximately 71% of all deaths (6). The development of NCDs is the result of a combination of genetic, physiological, environmental and behavioural factors (6). The view that refined carbohydrates such as sugar and flour could be a major cause of obesity, CVD and T2DM was promoted from the 1960s (7). As carbohydrate is the main dietary component affecting postprandial glycemia and insulin secretion (8) it is implicated in the aetiology of many chronic diseases. Postprandial hyperglycaemia is thought to play a direct pathogenic role in disease progression (9). Thus, the habitual diet is a major modifiable risk factor in the development of chronic diseases.

1.1 Diet and chronic disease risk

1.1.1 Diabetes mellitus

In 2019, approximately 463 million adults were living with the diagnosis diabetes mellitus (DM). This number is expected to rise to 700 million by 2045 (10). Among other factors, genetic predisposition and environmental factors are implicated in the aetiology of DM (11, 12). This is especially true for type 2 DM (T2DM, formerly known as non-insulin-dependent diabetes mellitus), the most prevalent variant, existing in over 90% of people with diabetes (13, 14). The increase in T2DM is thought to be associated with changes in diet and physical activity levels that have promoted overweight and obesity (15).

T2DM is characterized by increased glucose levels – hyperglycaemia – which is caused by diminished action of insulin and β-cell dysfunction (16). Insulin is continually produced in pancreatic β-cell. It is stored in vacuoles and released in response to elevated blood glucose levels. It is the primary hormone regulating the uptake of glucose from the blood into most cells, including the liver, adipose tissue and skeletal muscle tissue (16). People who develop

T2DM go through an early phase of impaired glucose tolerance, considered a prediabetic stage (17), where diminished action or secretion of insulin may eventually lead to the development of glucose intolerance (an umbrella term for metabolic conditions that result in hyperglycaemia) (18). This desensitization of the body's tissues to the action of insulin is generally known as insulin resistance. Insulin resistance in most cases is believed to be a post-receptor defect, relating to deficiencies in the cells that respond to insulin rather than insulin production (16, 19).

However, the pancreatic β-cells can adapt to changes in insulin action (20). Compensatory hyperinsulinemia occurs if the β-cells can produce enough insulin to compensate for insulin resistance, maintaining glucose homeostasis (19). During this certain period glucose tolerance is assumed to remain normal (18). In individuals who pass from impaired glucose tolerance to diabetes, insulin resistance progressively increases, while the secretion of insulin decreases (21). Kahn *et al,* provide a model for a hyperbolic relationship between insulin sensitivity and insulin secretion in normal subjects (22). To maintain a constant glucose tolerance the varying insulin sensitivity is balanced by altering the insulin secretion of the β-cells (23). With progressively increasing insulin resistance, pancreatic β-cell compensation is eventually followed by β cell failure, resulting in persistent hyperglycaemia and development of type 2 diabetes (24). Therefore, faults in both β-cells and insulin-sensitive tissues are required for the development of fasting hyperglycaemia and T2DM (23).

Blood glucose control is the main aim in management of diabetes mellitus, as normalized blood glucose is associated with reduction in development, disease progression, and progression of complications (25, 26). Improving the glycaemic control through diet modification could reduce the risk of disease-related complications, minimise or even avoid the need for medication, improving quality of life for people with diabetes and increase life expectancy (27).

Studies such as the Diabetes Prevention Program (28) provide evidence that intensive diet and exercise programs are highly effective in delaying or preventing diabetes. Many prospective studies have shown a strong association between daily physical activity and reduced T2DM risk (29). Additionally, an animal study found an increase in insulin-stimulated glucose uptake in obese Zucker rats following acute exercise (30). Furthermore, findings from the STOP-NIDDM Study (31), showed that an intervention with the α-glucosidase and α-amylase inhibitor acarbose (which acts on the enzymes involved in the digestion of carbohydrates) in

subjects with impaired glucose tolerance delayed progression to diabetes, irrespective of age, sex and body mass index (BMI). These data indicate that reducing the rate of carbohydrate absorption can delay, or even prevent the advancement of impaired glucose tolerance to diabetes.

A major proportion of people with T2DM are overweight or obese, and obese people are at a greater risk of developing T2DM (32). Meta-analyses have documented a relationship between BMI and T2DM risk (33, 34), establishing excess body weight as a leading risk factor for T2DM (35). Although there is a strong relationship between body adiposity and T2DM, not all overweight or obese develop T2DM, and conversely, not all persons with T2DM are overweight (36).

1.1.2 Overweight and obesity

Worldwide, in 2016, more than 1,9 billion adults (over 18 years of age) were overweight, and over 650 million people were obese (37). BMI is used as an indicator of nutritional status, defined as a person's weight in kilograms divided by the square of the person's height in meters (kg/m^2) (38). Obesity is caused by excessive accumulation of adipose tissue, which is influenced by diet, physical activity level, genetic predisposition, developmental stage and age (39). According to common standards (38), obesity is defined as a BMI $\geq$30 kg/m^2, overweight as a BMI of 25.0-29.9 kg/m^2, and normal weight as a BMI of 18.5-24.9 kg/m^2.

The risk of developing T2DM is associated with increasing BMI, especially in early adulthood (40). In a meta-analysis of prospective cohort studies quantifying the relationship between BMI and the risk of T2DM, overweight was associated with a 3-fold increased risk, and obesity with a 7-fold increased risk of diabetes when compared to subjects with normal weight (33). Currently, BMI is the cornerstone of the classification system for overweight and obesity and offers a proxy measure of body fatness that has been useful in international population surveillance, and clinical assessments performed on an individual level (41). Recently, there has been an increased interest in body composition analyses in obesity research (42-44), as BMI does neither discriminate between lean tissue and fat tissue, nor describe the distribution of body fat.

Obesity is often linked to insulin resistance (18, 45), and both obesity and T2DM are associated with insulin resistance (46). Prospective studies have promoted insulin resistance as a predictor of T2DM (47, 48). In a study conducted by Abbasi *et al,* results showed that

individuals expressed increased insulin resistance the greater their BMI (49). Furthermore, the presence of insulin resistance at any given BMI heightened the risk of T2DM (49). However, the authors also concluded that insulin sensitivity varies at any given level of BMI, displaying that overweight and obese subjects can be insulin sensitive, and normal weight subjects can be insulin resistant. These data correspond with results from the European Group for the Study of Insulin Resistance (50), where only 25 % of the obese individuals were insulin resistant.

Similarly, data from The National Health and Nutrition Examination Surveys (NHANES) 1999-2004, which linked BMI categories (normal weight, overweight, and obesity) with body-size phenotypes (metabolically healthy, and metabolically abnormal), showed a high prevalence of cardiometabolic components among adults with a normal BMI, while a large proportion of overweight and obese adults had a favourable metabolic profile (51). A review by Karelis *et al,* (52), provide further evidence for these observations. A 2012 study including 732 participants found independent associations between excess visceral fat and insulin resistance with T2DM, while no associations were found for BMI, total body fat or abdominal subcutaneous fat when compared with the same outcome (44). These data suggest that the measure of overall body fat is not the main determinant of insulin resistance.

Adipose tissue is traditionally divided into two categories, white adipose tissue (WAT), which serves as an energy storage, and brown adipose tissue, which mainly produces heat through lipid oxidation (53). WAT can be divided further into subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT), which surround the intra-abdominal organs (54). There is a large body of evidence suggesting that the distribution of excess body fat is predictive of insulin resistance, especially the abnormal accumulation of VAT, which stems from an inability of adipose tissue to store the excess energy (55-62). While some studies show that both SAT and VAT are associated with insulin resistance (58-60, 62, 63), most studies describe a distinct role of VAT as a correlate of insulin resistance (57, 64-66). Recently, McLaughlin *et al,* found that VAT increased the risk of insulin resistance, whereas SAT was associated with a decreased risk of insulin resistance, in overweight and moderately obese individuals (67).

Visceral obesity is commonly associated with a complex condition also known as the metabolic syndrome. The metabolic syndrome is defined as a multicomponent risk factor cluster in which hypertension, insulin resistance, dyslipidaemia and abdominal fat distribution

(often referred to as visceral/central obesity) are key features (13, 68). The increasing prevalence of the metabolic syndrome worldwide is associated with the global epidemic of obesity and diabetes (69). The National Cholesterol Education Program's adult Treatment Panel III report (70) considered the "obesity epidemic" as mainly responsible for the increasing prevalence of the metabolic syndrome. Still, some researchers put greater emphasis on insulin resistance as the underlying cause (71, 72). However, isolating the role of insulin resistance in pathogenesis is complicated as it is strongly linked to obesity.

There is now evidence establishing the role of adipose tissue as an endocrine organ (73, 74), participating in the regulation of several biological processes (75, 76). Adipose tissue can impact metabolism by releasing non-esterified free fatty acids (NEFAs), and various adipokines, including leptin and adiponectin, and cytokines that are involved in both autocrine/paracrine (local) and endocrine (systemic) signalling (74, 77). Some of the adipokines secreted from adipose tissue can affect the immunological response. In VAT, higher concentrations of plasminogen activator inhibitor 1 and interleukin-6 are observed (78). Proinflammatory cytokines and plasminogen activator inhibitor 1 promote a proinflammatory state (79), which coupled with adipocyte dysfunction (80), contribute to an obesity-induced state of chronic low-grade inflammation (81). This state of inflammation is recognized clinically as slightly elevated C-reactive protein levels (79). Several studies have discovered strong associations between chronic inflammation and reduced insulin sensitivity, linking obesity, inflammation and insulin resistance (82-84).

Different characteristics of SAT and VAT might explain the differences in metabolic effects of adipose tissues (85). Literature suggests that VAT possess a higher lipolytic activity than SAT (86) in addition to being more resistant to the anti-lipolytic effects of insulin (insulin decreases the activity of hormone-sensitive lipase, which releases fatty acids from lipid droplets (87, 88), leading to an increased release of NEFAs. Increased plasma NEFA levels have been shown to contribute to insulin resistance (79, 89).

1.2 Glycaemic Index and Glycaemic Load

Nutritionally, carbohydrates are commonly characterized as being glycaemic or non-glycaemic, the former referring to carbohydrates that are digested and absorbed in the small intestine causing the blood sugar levels to rise, and the latter referring to carbohydrates that enter the large intestine as dietary fibre. The postprandial glucose and insulin response depend on the structure, particle size and fibre contents of dietary carbohydrate, and the rate of which

they are absorbed and digested (2). Earlier, carbohydrates were usually classified as simple or complex, in the belief that rates of digestion and absorption is determined by saccharide chain length (90). However, differences in absorption and post-prandial effects are not explained by glucose chain length (91). This led researchers to propose a classification system for dietary carbohydrates based on their bioavailability, which describes the utilisation and biological effects of carbohydrates in the diet (92). Glycaemic carbohydrates in the diet mainly comprise the polysaccharide starch, which is a polymer of glucose units, free sugars such as the disaccharides sucrose and lactose, monosaccharides such as glucose and fructose, and maltodextrins, which is partially hydrolysed starch. The non-glycaemic carbohydrates mainly include non-starch polysaccharides and resistant starches (92).

Glycaemic response (GR) describes the postprandial rise in blood glucose levels after consuming a food or meal that contains available carbohydrate. Available carbohydrates refer to carbohydrates that are hydrolysed by digestive enzymes in the upper gastrointestinal tract and absorbed in the small intestine as monosaccharides (93). Findings from a study conducted by Crapo *et al.* showed that equal amounts of carbohydrate had different effects on blood glucose (94) indicating that the inherent properties of carbohydrates lead to different postprandial responses. Glycaemic index (GI) is a concept first introduced in the 1980's by Jenkins *et al.* (2), to quantify the GR to different carbohydrate-containing foods. GI is defined as the incremental area under the glucose response curve (iAUC) of a test food relative to the response elicited by a reference food in the same subject (2, 95). Both the test and reference food (usually glucose or white bread) must contain the same amount of available carbohydrate (5). The mathematical definition of GI is as follows (Figure 1):

$$GI = \frac{\sum_{n}^{x=1} 100 \text{ X } F_x / \bar{G}_x}{n}$$

Figure 1. The mathematical definition of GI, as defined in "Glycemic index of foods: a physiological basis for carbohydrate exchange", by Jenkins *et al,* (2).

F_x is the iAUC in subject *x* elicited by 50 g of available carbohydrate from the test food and $\bar{G}_x$ is the mean AUC in subject *x* elicited by 50 g glucose, tested on two or three separate occasions. The GI is the mean of these values in *n* subjects, where n≥10 according to the

current internationally accepted method (96). High-GI foods are characterized by their rapid absorption, inducing high postprandial glucose and insulin responses.

The utility of the concept of GI as a useful nutritional tool has been criticized, since the glycaemic effect of carbohydrates is also influenced by biological variation between individuals, meal and nutrient composition, and the rate and extent of digestion and absorption. Still, this does not take away the value of measuring the characteristics of carbohydrates, since the purpose of the GI ranking is to provide a guiding tool for the consumer, in food selection (92). When considering the clinical use of the GI, the most common way is to classify foods as either low- (GI≤55), medium- (GI 56-69) or high- (GI≥70) GI (97, 98).

However, the level of postprandial glycaemia and insulin demand depends on not only the type of carbohydrate consumed, but also the amount of carbohydrate per serving of food (9). Thus, the concept glycaemic load (GL) was introduced (99, 100), and proposed as a global estimate of postprandial glycaemia and insulin demand. GL is the product of the GI and the total available carbohydrate content in a test food (101). Therefore, a food can have a high GI, but low GL, depending on its carbohydrate content.

An increasing body of evidence indicate that a low-GI diet may be associated with health benefits, including reduced risk of diabetes (99, 100, 102), cardiovascular diseases (103-106) and metabolic syndrome (68, 107). A systematic review including 11 randomized controlled trials found indications that glycaemic control improved significantly in people with diabetes with a low-GI-diet compared to a high-GI-diet (27). A recent meta-analysis including 37 prospective observational studies found that a high-GI/GL-diet increased the risk of diabetes, heart disease, gallbladder disease and breast cancer (9), which indicates that postprandial hyperglycaemia may potentially act as a universal mechanism for disease progression (8, 9).

The results of studies examining the relationship between dietary GI, GL, and disease risk have been inconsistent (9, 108). A Swedish study including 36246 men without diabetes or prior CVD did not find a positive association between increasing GI/GL or CVD risk or mortality in a six-year follow-up (109). Similarly, studies investigating the relationship between dietary GI/GL and risk of developing T2DM: Krishnan *et al,* (110), Sahyoun *et al,* (111) and Mosdøl *et al,* (112) showed an inverse or no relationship between GI/GL and T2DM risk. Because of inconclusive data from scientific literature, the European Food Safety

Authority (EFSA) has not established dietary reference values for GI/GL based on type 2 diabetes or cardiovascular disease risk (113).

A recent systematic review found that a high consumption of dietary fibre and whole grains have a stronger association with beneficial health outcomes than GI and GL (108). Although dietary measures of GI and GL have been promoted as indicators of the carbohydrate quality of foods, some authors have argued that GI does not apply in mixed meals (114-116). Foods are usually consumed in combination and thus the GR of the meal would be affected by the GI of its individual components. Thomas Wolever (95) comments that there is a distinction between GR and GI that needs to be accounted for when considering GI values of different foods. One researcher (115) stated that "Eating a food as part of a mixed dish or meal changes the GI. For example, the addition of nuts can drop the GI of a food by as much as half". GI and GR are not necessarily interchangeable terms, meaning that the addition of nuts may reduce the GR to the meal, but not the GI of the food item (95). It has been demonstrated that the GR of mixed meals with similar macronutrient composition, but different carbohydrate sources, is proportional to the GI of the individual components of the meal (117-119). Furthermore, it has been shown that combining GI values of individual foods can accurately predict the GR to mixed meals containing fat and protein (120). In other words, GI is measured in individual foods, and calculated for mixed meals.

1.3 Blood sampling methods

Measurement of blood glucose values is a common clinical diagnostic test. In the diagnosis and management of T2DM, the accuracy and precision of results is important as it affects clinical outcomes and patient safety (121). Measurements of fasting plasma glucose, 2-hour plasma glucose during an oral glucose tolerance test, and HbA1c (122), corresponding to the average, long-term blood glucose levels, are equally appropriate for diagnostic screening (123). From 2011, HbA1c is usually measured in whole blood with EDTA as anticoagulant. HbA1c measurements were suggested to be reported as mmol glycated Haemoglobin (Hb) per mol of Hb (diagnostic criterion ≥ 48 mmol/mol) instead of per cent (diagnostic criterion ≥ 6.5%), in response to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference method (124). In Norway, the IFCC reference method was implemented in September 2018 (125).

Glucose measurements can be performed in whole blood, serum or plasma, but for the diagnosis of T2DM, venous plasma is recommended (126). The glucose concentration in

plasma is ~11% higher than in whole blood, if the haematocrit (vol% of red blood cells) is normal (126). Once the blood sample is drawn the glucose concentration will decrease due to glycolysis by erythrocytes, white blood cells and blood platelets (127, 128). Therefore, blood samples should be drawn into tubes containing a glycolysis inhibitor in addition to an anticoagulant (129). Common stabilizers and anticoagulants include citrate buffer (sodium citrate-citric acid), sodium fluoride (NaF), ethylenediaminetetraacetic acid (EDTA), heparin, and oxalate (129, 130). Blood samples should be drawn in the morning after an overnight fast of at least 8 hours, due to diurnal variation (higher in the morning than in the afternoon) of fasting plasma glucose levels (131). An advantage of plasma samples compared to serum is the added anticoagulants, to avoid clotting. In contrast, serum samples require 30-60 minutes in room temperature to clot (132). Thus, plasma samples can be centrifuged with no waiting time. However, although red and white blood cells are removed from plasma samples, platelets can still be present. Platelets can metabolise glucose and affect the glucose measurements over time. Therefore, serum has been suggested as a better sample when there is an expected time delay before glucose measurements (133).

Self-monitoring of blood glucose by capillary blood measurements is the traditional point-of-care practice for glycaemic control in patients with T2DM (134, 135). The most common site of capillary blood tests is the fingertip because of its easy access and high vascularization (136). In some cases, blood sampling at the fingertip is contraindicated (137, 138), as repeated pricking can lead to tissue damage and damage sensory nerves (139-141). Therefore, the accuracy and reliability of alternative sites, such as the earlobe, thigh, palm and forearm have been studied (136, 141-145).

Immediately after a meal, changes in blood glucose may be detected earlier at the fingertip than sites such as the thigh or forearm as there is a suggested lag time in glucose equilibration in periods where glucose changes rapidly (141, 144, 145). This has clinical relevance as the detection of hypoglycaemia by self-monitoring of blood glucose may be delayed (146), and may be an issue in research settings that investigate the glycaemic response to carbohydrate intake.

1.4 Bread – a staple food in Norway

Bread is one of the most important sources of carbohydrates in the Norwegian diet with an average intake of approximately 200 g per day and capita (147). Many common carbohydrate-rich staple foods such as potatoes, breakfast cereals and bread products have a high GI (148).

Bread is consumed in various forms, with differing nutrient compositions. For example, white wheat bread (which according to Norkost 3 (147) contributes to approximately 19 % of total bread intake in women and men, respectively) is commonly made from refined flour, producing a high glycaemic response (149). Grain is composed of endosperm (~80%), germ and bran. The refining process removes the germ and bran from the starch-containing endosperm. The endosperm is then ground to flour. In removing the bran, important micronutrients, phytochemicals (plant chemicals) and antinutrients are removed, leading to a lower nutrient density flour (150).

As bread is a main staple food in Norway, and given the variation in GI values of breads, there is a great potential for dietary improvement by introducing breads with lower GI. In a study exchanging common high-GI bread containing low amounts of dietary fibre with low-GI bread high in dietary fibre for three weeks, the insulin sensitivity of patients with impaired glucose tolerance was improved (151). An increasing body of evidence indicate the potential health benefits of a low-GI diet. While there are many alternatives for lunch and dinner meals, there is not an abundance of breakfast products, such as bread and cereals (148). Many breakfast cereals have high GI and high added sugar content. Several studies have proposed that a low-GI breakfast may improve glucose tolerance in subsequent lunch meals – a so-called second meal effect (152-154). The mechanism of the 2nd meal effect is thought to relate to an extended absorptive phase after consuming a low-GI food, promoting a more efficient suppression of free fatty acids, which in turn improves insulin sensitivity in the following meal (153). This effect has also been seen from the evening meal to breakfast (155).

Bread is mostly available in medium- to high-GI forms (156). It has been assumed that producing breads with wholemeal flour or whole grains make for a low-GI bread (157, 158). Interestingly, comparing wholegrain wheat bread and white wheat bread, the glycaemic responses are similar even though the wholegrain bread contains a higher amount of dietary fibre (149, 159). Fardet *et al,* suggests that the minor differences in GR may be caused by a similar digestive process, despite a higher fibre content. Furthermore, they suggest that the glycaemic response is determined by the physical property of the food rather than the raw materials (149).

Bread has a porous texture which is generated through the kneading of the bread and the action of the yeasts. This creates a network in which the protein (gluten) surrounds the starch. This interaction between gluten and starch has been shown to impact the glycaemic response

to white bread, as gluten-free bread produced a significantly higher glycaemic response than normal bread (160). Other means of lowering the GI of bread products include changing the ratio of amylose to amylopectin by mixing different types of flours to complement each other. Amylopectin makes up approximately 70-80% of starches and is characterized by containing both linear and branched glucose polymers (161). In normal wheat flour, amylose, a linear chain of glucose polymers, comprises 30% of total starch (149). Amylose is digested more slowly than amylopectin, and is prone to form resistant starch, an oligosaccharide that is nondigestible (161). In the presence of water and heat, swelling and dissolution of starch occurs, known as gelatinisation (113). This process makes the starch more accessible to digestive enzymes. By increasing the content of amylose, which is more compact, and thus more slowly degraded it is possible to significantly reduce the GI of the bread (162, 163).

Intact grains have also been used to create breads that reduce glycaemic response, likely because the insoluble fibre surrounding the starch forms a physical barrier that reduces access to the digestive enzymes (149). An additional benefit of consuming intact grains is a delayed gastric emptying. In addition, organic acids such as acetic, propionic and lactic acid (or their corresponding salts) can reduce metabolic responses when added to bread as ingredients (164), or when present in the bread as a result of bacterial fermentation as seen in sourdough breads (165). The likely mechanism for the reduced glycaemic responses seen in these studies is a delayed gastric emptying due to a lowered pH. Organic acids are also thought to affect the rate of starch breakdown (164, 166).

Even though bread constitutes a major source of dietary fibre in the Norwegian diet, and many European countries, the average intake of dietary fibre of many people (147) is lower than the recommended amount (113). The selection of low-GI staple foods needs to be broadened to increase the consumption and appeal of foods with low-GI, especially among breakfast products. Among the variety of bread products, there are few low-GI alternatives. Typical low-GI breads often contain large amounts of whole grains or high levels of organic acids, with textures and tastes that some may not like (5).

1.5 Dietary fibre

Dietary fibre has attracted increasing interest due to the potential health benefits, which can be mainly explained due to its impact on absorption and gut function, and its impact on carbohydrate and lipid metabolism (167). Most countries adhere to the definition of the Codex Alimentarius from 2009 (168), which defines dietary fibre as edible carbohydrate polymers

consisting of 10 or more monomeric units (some countries use a lower degree of polymerization of 3-9), that are not hydrolysed by endogenous digestive enzymes in humans (169). The EFSA Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre (113) considers the main types of dietary fibre to be: non-starch polysaccharides, resistant oligosaccharides, resistant starch, and lignin. The Nordic Nutrition Recommendations (NNR) 2012 (170), have adopted the EFSA definition of dietary fibre.

The main constituents of dietary fibre are called non-starch polysaccharides, which comprise several different polymers that vary vastly in terms of molecular size, structure, and monomeric composition (171). From a physiological standpoint, dietary fibre can be divided in two categories: insoluble and soluble fibres - characterized by their behaviour in aqueous solutions (172). Insoluble fibres include cellulose, lignin, some resistant starches and many hemicelluloses. Most of these fibres are structural components of the plant cell wall. They are not digested in the upper gastrointestinal tract of humans, and act as bulking agents and increase intestinal transit time (172). Soluble dietary fibres include polysaccharides with a high water-holding capacity, such as pectins, gums, mucilages and some hemicelluloses (173). Pectic substances and hydrocolloids, such as gums and mucilages, may form highly viscous solutions in water (113). It is important to note that the distinction between soluble and insoluble fibre is method-dependent and does not always predict physiological effects. Therefore, FAO/WHO suggested that this differentiation should be gradually phased out (171).

Some dietary fibre components are subject to fermentation by colonic microflora. The level of fermentation is dependent on a combination of factors, including the physical form and molecular structure of the substrate, transit time, and the composition of the bacterial flora (113). Some fermentable dietary fibres are known as “prebiotics” because of their ability to stimulate the growth of beneficial gut bacteria, such as Bifidobacteria and Lactobacilli, which produce lactate and short-chain fatty acids (SCFA, including acetate, propionate and butyrate) (174). Acetate and propionate provide energy for the host, while butyrate is a major source of energy for the colonic mucosa (175).

1.5.1 Functional dietary fibre

Previous studies have found that adding certain types of soluble fibre as a functional ingredient to food products may slow down the rate of digestion and absorption (167, 172, 176). Soluble dietary fibre absorbs water and swells in the intestine, mixing with the food to

form an intertwined network (177). The presumed mechanisms of action of soluble dietary fibres on blood glucose control include delayed gastric emptying, impact on the release of gut and digestive hormones, reduced starch degradation, and delayed sugar absorption (93).

Different types of dietary fibre have been tested in this regard. A recent study by Rieder *et al,* examined the effects of cereal beta-glucan on postprandial glucose response when added to breads in different amounts (5). The health claims of cereal beta-glucan have been approved by EFSA on reduction of postprandial glycaemic responses (178) and reduction of blood cholesterol levels (179, 180). The study measured the glycaemic effects of four different test breads and one control bread in 14 healthy adult subjects (excluding people with diabetes). The authors reported that the breads with the highest beta-glucan contents significantly decreased peak blood glucose rise, iAUC and GI when compared to a wheat control. However, to reach the EFSA claim on reduction of postprandial glycaemic responses a high dose of 4 g beta-glucan per 30 g available carbohydrate is required, which is difficult to achieve in bread, thus limiting the utility of the claim (180). The researchers showed that by optimizing the processing conditions to maximize beta-glucan solubility and molecular weight, approximately the same effect can be achieved with half the amount of beta-glucan (5).

1.5.2 Guar galactomannan

Another dietary fibre with wide applications in the food industry is guar gum. Guar is a galactomannan derived from the endosperm portion of the seeds of *Cyamopsis tetragonoloba*, a member of the Leguminosae family (181). Food-grade guar gum for commercial use has a high content of total dietary fibre with a reported content of approximately 80% guaran (182) which is the polysaccharide fraction of the endosperm. The guar plant has been grown for centuries in India and Pakistan, where it is used in both human food and animal feed (181). Guar gum is approved as food additive E412 in the European Union (183).

Different variants of galactomannan are found in the four primary plant sources: Guar (*Cyamopsis tetragonoloba*), Locust bean gum (*Ceratonia siliqua L.*), Tara plant (*Caesalpionia spinosa*) and Fenugreek (*Trigonella foenum-graecum*) (184), of which guar is the most important economically (185). They vary mainly by the proportion of mannose to galactose in the backbone (186) and fine structure (187). Guar has been reported to have a mannose:galactose ratio of 2:1 (188). The viscous property of galactomannans is thought to be influenced by the proportion of sugars in the polysaccharide backbone, where a higher

galactose content generally leads to decreased viscosity due to steric hindrance (decrease in the rate or occurrence of chemical reactions due to the molecular structure) posed by galactose residues (189).

Galactomannans consist of linear chains of (1-4)-β-D-mannopyranosyl units with α-D-galactopyranosyl units attached by (1-6)-linkages (190). The major functionality of galactomannans is related to their ability to impart high viscosity in cold water, even at low concentrations (191, 192). This ability to hydrate without heating makes it appealing for the food industry. The guar gum industry developed from around the 1940s (193) with common applications in dairy, baked goods, confectionary, meat and beverage industries as a thickener, stabilizer and emulsifier (189, 194). Until recently, the use of guar gum has been an efficient and cost-effective food additive. However, due to increased demand from non-food industry such as oil and gas drilling wells, and production of explosives and paper, the price is increasing (194).

The galactomannan molecules are not hydrolysed by human digestive enzymes in the small intestine and thus function as dietary fibre (195). As a dietary fibre, galactomannan has been used to create low-GI food products such as breakfast cereals (196), pasta (197), biscuits (198), and bread (199). However, because guar is very viscous it is difficult to integrate in food products in high enough amounts to achieve a physiological effect. Newer studies have examined the utility of partially hydrolysed guar gum (PHGG) as a prebiotic fibre, however mostly aimed at improving symptoms of the irritable bowel syndrome (200, 201). PHGG is water-soluble but does not exhibit the same gel-forming properties as guar gum, hence it may be used to increase the dietary fibre content without negatively affecting the nutritional or sensory properties of foods and beverages (194).

The effectiveness of guar in reducing postprandial glucose and insulin levels, and low-density lipoprotein-cholesterol has been documented both in healthy subjects and in patients with diabetes (176, 191, 196, 202-204). Hydrocolloids such as guar gum form a continuous network which traps the starch granules in a coherent gel that acts as a barrier, reducing the accessibility of the starch to digestive enzymes (205). This effect may in part explain the mechanism of reduced postprandial glycaemia as seen in previous studies. The viscous properties of guar gum vary depending on the molecular weight of the galactomannan (194). There is a huge potential for bread products featuring functional fibre such as guar galactomannan for their blood glucose lowering effect. However, there is a gap in our

knowledge on the impact of guar of different molecular weights on glucose absorption. Therefore, the present study investigated the postprandial effects of bread containing different amounts and molecular weights of guar galactomannan in healthy, overweight adults.

2 OBJECTIVES

The goal of this study was to investigate whether bread with added guar galactomannan can reduce the postprandial glucose response in healthy overweight adults. The effects of different breads on glucose levels will be tested using both capillary blood samples and venous plasma samples, and the differences will be investigated. The five test breads comprised one wheat control and four breads with galactomannan added in different amounts and viscosities. More specifically we wanted to investigate whether:

1. The postprandial glucose response is changed after ingestion of bread containing guar galactomannan compared to similar amounts of bread without guar galactomannan
2. The effect of guar galactomannan on postprandial glucose response is dependent on the dose and molecular weight of guar galactomannan.
3. The insulinemic response is changed after ingestion of bread containing guar galactomannan compared to similar amounts of bread without guar galactomannan
4. The effect of guar galactomannan on the insulinemic response is dependent on the dose and molecular weight of guar galactomannan.

3 SUBJECTS AND METHODS

3.1 Study design

This single-centre randomized clinical crossover study aimed to assess the postprandial effect of guar galactomannan in healthy, overweight adults. The participants will complete one screening visit and five separate test day visits with a wash-out period of minimum 3 days in between. Each participant served as their own control.

3.2 Study population

This trial was planned during the spring of 2019 and is currently being conducted at the Research Unit for Health Surveys (RUHS) in Bergen, Norway. The study is a collaboration between Centre for Nutrition, University of Bergen, and the food research institute Nofima AS, Norwegian Institute of Food, Fisheries and Aquaculture Research, Ås. Due to the Covid-19 pandemic, the study is on hold after the inclusion of 9 participants. Thus, this thesis will include data from these 9 participants. This study is registered at ClinicalTrials.gov with identifier: NCT04289545.

Inclusion and exclusion criteria

Healthy adults (aged 18-60 years) with a BMI between 25-30 kg/m^2 were eligible to participate in the study. The rationale for including persons with a BMI between 25-30 kg/m^2 was the expected higher blood glucose response in overweight people compared to normal weight people. Participants with a BMI >30 kg/m^2 were not included in the study as they have an increased risk of diabetes (69). Participants under the age of 18 years were not included as this study aims to assess the effects of guar galactomannan in the adult population. This study did not include persons over the age of 60 years as this age group has a higher risk of developing chronic disease (206).

The aim was to include 15 participants in the study with an expected dropout rate of 20 % which would result in 12 subjects completing the entire study. 12 participants provide 80 % power to detect a 30 % difference in AUC glucose after 120 min. Thus, the power of the study will be affected due to the Covid-19 lockdown.

Table 1. Inclusion and exclusion criteria for participation in the study.

Inclusion criteria	Exclusion criteria
Subjects between 18 – 60 years of age	Known diagnosis of diabetes mellitus type 2
Subjects with a BMI between 25 – 30 kg/m^2	HbA1c at screening ≥ 48 mmol/mol
	Other chronic diseases (heart disease, cancer) within the last 3 years
	Pregnancy or lactation
	Known drug or alcohol abuse
	Use of medication that interferes with glucose metabolism
	Unwilling to follow the study requirements

3.3 Recruitment process

Healthy, overweight volunteers were recruited to the study via posters that were placed on the different campuses around Bergen city centre, including cafeterias, informational boards and training centres. In addition, we advertised the study on social media and recruited among colleagues, mostly at Centre for Nutrition, Bergen. People who were interested in participating in the study were contacted via e-mail or telephone. At first contact they received information about the study requirements and were invited to come for a screening visit. This is an ongoing intervention study with continuous recruitment of participants fulfilling inclusion criteria. By March 2020, 9 participants had completed all 5 visits of the study.

Screening visit

On the baseline visit participants were provided with a written briefing of the study. Upon obtaining informed written consent (Appendix A), the volunteers were asked to fill out a screening questionnaire on self-reported health (Appendix B). In addition, anthropometric measurements, including height, weight and waist circumference were obtained. The level of physical activity was assessed, and eligible subjects were asked not to change the level of physical activity during the study period. In addition, subjects were instructed not to change

their diet composition or intake. One non-fasted blood sample was drawn to measure HbA1c, in order to identify undiagnosed DM.

Screening blood sample

On the screening visit blood was drawn for analysis of HbA1c, reflecting the mean blood sugar the last 6-8 weeks. One 3 ml tube containing EDTA (VACUETTE® (ref# 454246), Greiner Bio-one). was drawn from an antecubital vein. The tube was stored in room temperature and delivered to the Department of Medical Biochemistry and Pharmacology (MBF), Haukeland University Hospital, Bergen, Norway for analyses (Bio-Rad D100 (total error <6%) by Roche Diagnostics AS).

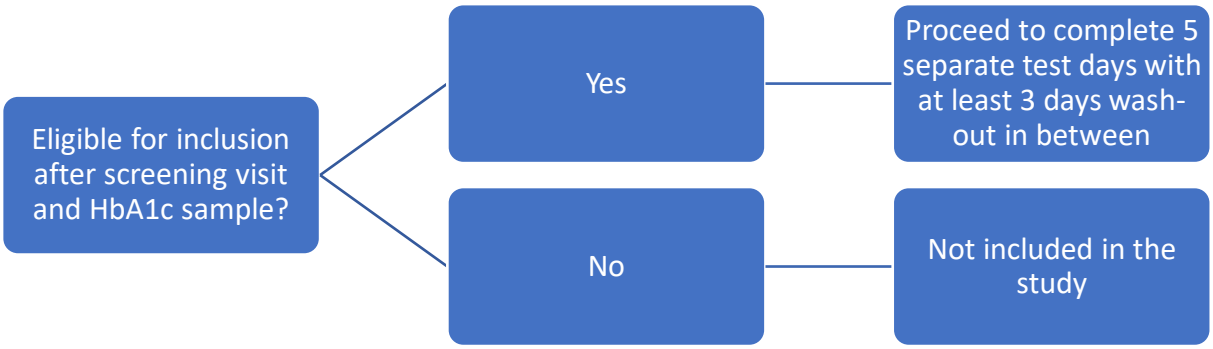

Figure 2. Process chart of the inclusion of subjects to the study.

Visits 1 through 5

If the HbA1c sample was within the reference range (<48mmol/mol), (providing very low likelihood of a diagnosis of diabetes mellitus type 2) and the BMI was within the desired range, and after signing informed consent, the participant was eligible for inclusion in the study. On study days, the participants came to the research unit at approximately 08.00 hours, after an at least 12 h overnight fast, meaning they were instructed to eat their last evening meal before 20.00 hours the day before. Subjects were asked to eat a carbohydrate-based meal as an evening meal before each test day, and to eat approximately the same meal before each test day. The participants were instructed to avoid smoking, alcohol consumption and any strenuous exercise the day before a study visit. There was an at least 72 h wash out period between the test days.

The rationale behind choosing serving sizes containing 25 g available carbohydrate instead of the more common 50 g was to ensure that all subjects could consume the entire portion of test bread within the set time frame of 15 minutes. In a previous study conducted by Rieder et al. (5) they similarly had to reduce the serving size.

Upon arriving on the test days, the subjects were asked to fill out a form (Appendix C) asking for any changes in disease, supplements or medications. Weight and waist circumference were measured at each visit. Lastly, the participants were to fill out at what time they had consumed their last meal, and if they had followed the eating and drinking restrictions.

Then, either a nurse or a bioengineer for inserted a cannula in the medial cubital vein in the upper arm. After resting for 10 minutes the fasted (baseline) blood sample was drawn. The subjects consumed the test bread at a comfortable pace within 15 minutes along with 250 ml of water. The researcher noted the time (time zero, t=0) at which the participant had fully consumed the test bread. Time zero represented the baseline for the remaining blood samples. This means the first blood sample was drawn 15 minutes after the bread was fully consumed. During the test day, the study participants were instructed to remain seated and were not permitted to eat.

3.4 Description of the different test breads

Five different breads were included in the study. The breads were based on a standard wheat dough (wheat flour, 1 % dry yeast, 1,5 % salt and water). Powdered guar gum (galactomannan) of four different molecular weights (M_W) were added to the doughs. Two doughs contained 10 % guar gum (2 different M_W) and two doughs contained 15 % guar gum (2 different M_W). See Table 2 for an overview of the concentrations and amounts of guar in the different breads. One dough with no added guar gum served as a control. After fermentation, the doughs were divided into pieces containing 25 g available carbohydrate. The pieces were moulded by hand, proved and baked. The finished products were blast frozen and kept frozen until the experiment. On the day before the study visit, the bread was thawed on a plate, covered with plastic foil and marked with participant ID number and bread number.

Table 2. Detailed overview of the concentration and molecular weights of guar galactomannan added to the different breads.

Bread number	**Molecular weight (M_W)**	**Guar flour type**[a]	**MW in kDA and g/mol (in parenthesis)**	**Guar concentration**	**Combination**
Bread 1 (B1)	-	-	No added guar (0.13 ± 0.01)[b]	0%	Control
Bread 2 (B2)	High	M100	511.1 ± 4.6 (5.10 ± 0.07)	15%	High M_W /high concentration
Bread 3 (B3)	Low	M30	194.5 ± 1.6 (3.56 ± 0.06)	10%	Low M_W /low concentration
Bread 4 (B4)	Low	M5	37.1 ± 0.6 (5.52 ± 0.08)	15%	Low M_W /high concentration
Bread 5 (B5)	High	M400	720.1 ± 13.4 (3.43 ± 0.04)	10%	High M_W /low concentration

[a] Higher number behind the "M" indicates higher M_W guar

[b] Sugar analysis detected small amounts of galactose and mannose present in the control bread

3.5 Randomized order of consumption of the test breads at each study day

The five different breads were numbered from 1-5 and provided in a double-blinded and randomized order, to exclude any unknown factor that might affect blood glucose. Since this is an experiment involving food, difficulties in the blinding cannot be excluded, but will be minimized as far as possible. The breads were quite similar in appearance (Figure 3). The key disclosing the content of each test bread was locked until completion of the study. Due to the Covid-19 pandemic, the key was revealed to the investigator after 9 participants had completed the study.

Figure 3. Comparison of the five test breads: 1 and 2 on top, 3 to 5 on bottom, from the left.

Five dice were colour coded (red, blue, yellow, green, white) with each colour representing one type of bread. Since were marked with numbers upon arrival, the order of colours listed above was transformed to the numerical order 1-5, keeping the contents of the different breads de-identified. All five dice were thrown simultaneously, and the highest rolls removed until only one remained. This was then logged as the bread to be consumed at the first test day. The dice throwing process then continued with four dice, then three, and finally two dice, until the order was set. This process was repeated 20 times, allowing for 20 participants to be included (Table 3).

Table 3. The order of test bread consumed at each visit was randomized for up to 20 participants.

Participant ID number	Visit 1 (bread #)	Visit 2 (bread #)	Visit 3 (bread #)	Visit 4 (bread #)	Visit 5 (bread #)
1	1	3	4	2	5
2	1	4	5	3	2
3	5	2	4	3	1
4	2	1	3	4	5
5	3	5	2	4	1
6	2	5	3	4	1
7	4	5	1	3	2
8	2	5	3	1	4
9	4	5	3	2	1
10	5	4	3	2	1
11	3	4	1	2	5
12	3	4	1	5	2
13	3	2	5	4	1
14	4	1	3	5	2
15	5	4	1	2	3
16	1	5	3	2	4
17	3	4	1	5	2
18	5	4	1	3	2
19	3	5	1	4	2
20	2	3	5	4	1

3.6 Blood samples

Over the course of the study the subjects underwent blood sampling performed by qualified health personnel. Both venous blood samples from the venous catheter, and capillary blood samples were taken.

Venous blood samples

Venous blood samples were collected into one serum tube (BD Vacutainer® Serum Separation Tube™ II Advance (ref#367957) from BD Diagnostics) containing an acryl-based gel with silica particles as clot activator, and one plasma tube (VACUETTE® FC Mix (ref#454513) from Greiner Bio-One) containing FC Mix (Na2-EDTA, NaF, and sodium citrate-citric acid). The baseline sample was drawn first, and the remaining samples at 15, 30,

45, 60, 90, 120 and 180 min after minute 0. Serum samples were used for insulin analysis, and plasma samples for in vivo glucose analysis. After blood draw serum tubes were left still in room temperature for 30-60 min. Plasma blood samples were centrifuged immediately. Blood samples were centrifuged (serum samples: Hettich 320 centrifuge at 2200 g for 10 minutes at 20°C, plasma samples: Eppendorf Centrifuge 5702 R at 1800 g for 10 min at 4°C) on-site. The samples were then pipetted (serum and plasma layer respectively) into two aliquots each, containing ≥ 0,5 ml. Both serum aliquots and one plasma aliquot were immediately placed in a temporary -20°C freezer and moved to a -80°C freezer for storage at the end of the test day. Plasma samples were delivered to the MBF laboratory for analyses (Cobas 8000 series c702 module by Roche Diagnostics AS).

Finger-stick capillary blood samples

Capillary blood samples were collected by finger-stick, simultaneous as the venous blood samples (the baseline sample was collected first, then the remaining samples were collected after minute 0) at 15, 30, 45, 60, 90, 120 and 180 min after minute 0, and analysed on-site (Accu-chek blood glucose meter by Roche Diagnostics Norge AS). Subjects performed hand wash before the first and second test. The finger was gently massaged near the site of sampling for a few seconds. The top cap of the blood glucose meter was twisted to select the desired depth of the lancet needle. A new lancet was inserted into the tip of the device before each finger-stick sample. The tip of the blood glucose meter was pressed against the side of the fingertip and the release button was pressed to penetrate the skin of the fingertip. The fingertip was gently squeezed to get a small sample of blood. The blood drop was applied to the test strip which was immediately analysed in the device. The used test strip and lancet was then removed and discarded.

Serum samples for insulin measurement

The two aliquots containing serum were intended for insulin analyses. One aliquot served as a backup. The serum samples were sent to the hormone laboratory at MBF for analyses (method: chemiluminescence immunoassay, Siemens Immulite 2000 XPi (6 % analytical variation at 45 mIE/L)). Remaining samples are destroyed after obtaining the respective results.

3.7 Anthropometric measurements

Height measurements

Height was only measured during the screening visit. The same stadiometer (SECA217) was used for all height measurements. The participant was asked to remove their shoes and place their heels against the back plate of the stadiometer and look straight forward in the Frankfurt plane. A sliding horizontal headpiece was then adjusted to rest on the top of the head.

Weight measurements

Weight was measured on the screening visit and all five study visits. The subject was asked to remove their shoes and outer clothing such as coats and jackets. The scale was tared before performing each weight measurement.

Waist circumference

Measurements of waist circumference were performed on the screening visit and all five study visits. Measurements were performed while standing, using a standard flexible measuring tape, on bare skin. Measurements were obtained from the slimmest part between the navel and the top of the hipbone just after an exhale. The measuring band was tightened snug around the waist without compressing the skin.

3.8 Ethical considerations

The study was conducted in agreement with the ethical principles of the Declaration of Helsinki and the study design was approved by the Regional Committees for Medical and Health Research Ethics (REK Vest) in August 2019 (Reference number: 2019/912-1). The study protocol was subject to minor changes which were approved January 2020 (ID 11367). Informed, written consent was collected from all subjects prior to the start of the study. Participation was entirely voluntary, and withdrawal from the study was possible at any time without further justification.

All personal information was de-identified and handled in a confidential manner. Link key containing subject information was kept behind two locked doors. The obtained blood samples are stored specifically for this study only and are destroyed at the end of the study. No individual personal information will be published or available for persons who are not in charge of the study.

3.9 Data analysis

Data were plotted in an excel spreadsheet on a secure desktop: "Secure access to research data and e-infrastructure" (SAFE), created by University of Bergen for secure processing of sensitive personal data in research. SAFE is based on the Norwegian Code of Conduct for information security in the health and care sector. GraphPad Prism version 6 for Windows, (GraphPad Software, La Jolla California, USA) was used for graphic representation. Statistical testing was performed in IBM SPSS Statistics for Windows, version 25 (IBM Corp. Released 2017, Armonk, NY).

Area under the curve (AUC)

AUC values for fluoride venous plasma glucose, capillary glucose and serum insulin were computed using the trapezoid rule (207), ignoring the area beneath the fasting concentrations, using GraphPad Prism version 6. The trapezoid rule can be used to estimate AUC when the values on the X-axis are not evenly spaced. A straight line is drawn between each time point on the glucose response curve, creating several AUC-segments (Figure 4.). The total AUC represents the sum of all these AUC-segments.

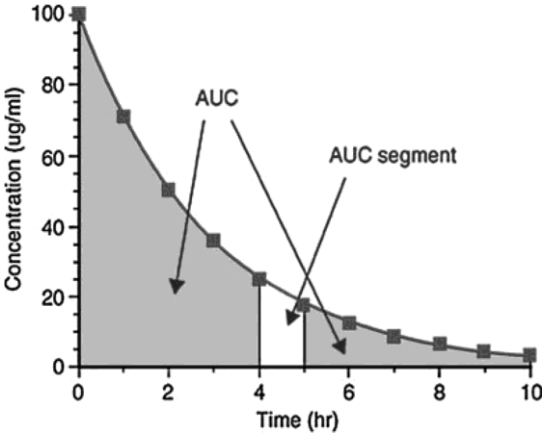

Figure 4. Illustration of the trapezoid rule for estimating AUC. Reprinted from Wolff *et al.* 2017 (208).

AUC calculations were performed according to the formula:

$AUC = \frac{1}{2}(C_1 + C_2)(t_2 - t_1)$, where C represents the glucose concentration at each timepoint (t).

Calculations of incremental AUC (iAUC) were performed using the fasting blood glucose concentration as the baseline. iAUCs were calculated for capillary and plasma glucose, and AUCs for serum insulin from 0-120 minutes ($iAUC_{120}$/AUC_{120}).

Statistical analysis

Statistical tests comparing plasma and capillary glucose, and insulin values were performed using tests for paired samples (related-samples Wilcoxon signed rank test), and independent-samples Kruskal-Wallis test in SPSS. The criterion for statistical significance was determined at the level of $p<0.05$.

Related-Samples Wilcoxon Signed Rank Test:

We performed a related-samples Wilcoxon signed rank test as a non-parametric alternative to a dependent samples t-test, because it does not assume normality of the data. The test was applied to capillary C_{max}, T_{max} and $iAUC_{120}$, plasma C_{max}, T_{max} and $iAUC_{120}$, and insulin AUC_{120}. We compared differences between the mean values of each guar-bread to the control.

Independent-Samples Kruskal-Wallis test

An independent-samples Kruskal-Wallis test was applied to the individual C_{max}, T_{max}, $iAUC_{120}$ and AUC_{120} values from each participant, using the results from each different bread.

4 RESULTS

Among the recruited volunteers, 19 subjects agreed to come for a screening visit to assess if they were eligible to participate in the study. Among these, five people did not fulfil the inclusion criteria. Three subjects who were included in the study gave notice that they wanted to drop out before their first test day. Additionally, two subjects dropped out during study visits due to challenges with blood sampling. Due to incomplete sample sets, the blood samples from these subjects were not analysed and thus not included in the dataset. In total, nine subjects completed all five visits and were included in the dataset.

4.1 Demographics

Baseline demographics for the study population showed a male predominance, a mean age of 30 years, and a mean BMI of almost 28 kg/m^2 at inclusion in the study. All the included volunteers were of Caucasian ethnicity and had HbA1c levels well within reference range (Table 4).

Table 4. Demographics and subject characteristics for the study population at baseline. Mean ± SD

Subject characteristics	**Subjects completing all five visits (n=9)**
Age[a] (years)	30 ± 8
Weight (kg)	87.8 ± 8.5
BMI (kg/m^2)	27.6 ± 2.5
Waist circumference[a] (cm)	91 ± 9
Sex (n)	
Female	2/9
Male	7/9
Ethnicity (n)	
Caucasian	9/9
HbA1c (mmol/mol)	32.4 ± 3.0

[a] Rounded to the nearest whole number.

4.2 Missing data

Capillary blood glucose measurements were not performed on *B1* in two subjects because of lack of equipment on the first study day. All graphical and statistical analyses calculated for *B1* on capillary blood samples were performed on this smaller sample size (n=7).

4.3 Postprandial glucose response

In capillary blood samples, there were no significant differences neither in $C_{max,}$ nor in T_{max} between the test breads and the control (Wilcoxon Signed Rank test, all p-values > 0.05). Two p-values were below 0.1, C_{max} B3 (p = 0.07) and T_{max} B5 (p = 0.08). The time of the mean maximum glucose concentration (T_{max}) occurred earliest at 32 (B3) and latest at 47 minutes (B5) (Figure 5). All guar-breads had higher maximum glucose concentrations (C_{max}) than the control. Among the test breads, B4 and B5 had a delayed T_{max} compared to the control (T_{max} 40 ± 12 min), without achieving significance.

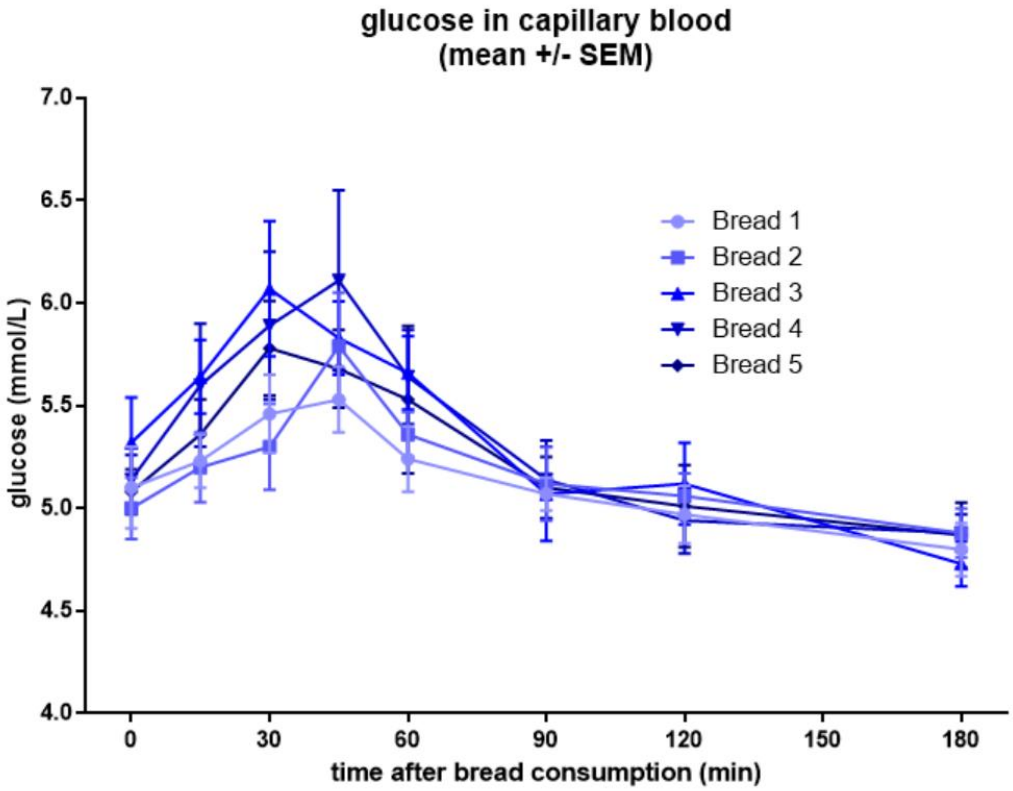

Figure 5. Postprandial glucose concentration in capillary blood (n=9) after consumption of five different test breads. SEM: standard error of the mean.

In plasma samples, there were no significant differences neither in C_{max} nor in T_{max} between the test breads and the control (Wilcoxon signed rank test, all p-values > 0.05). The earliest

T_{max} was observed at 32 (B3) and the latest at 45 (B5) minutes (Figure 6). Some breads achieved mean glucose concentrations below the baseline (fasting) values during the later phase of the study (>60 minutes). In 3 of the test breads we observed an increase in plasma glucose after 120 minutes.

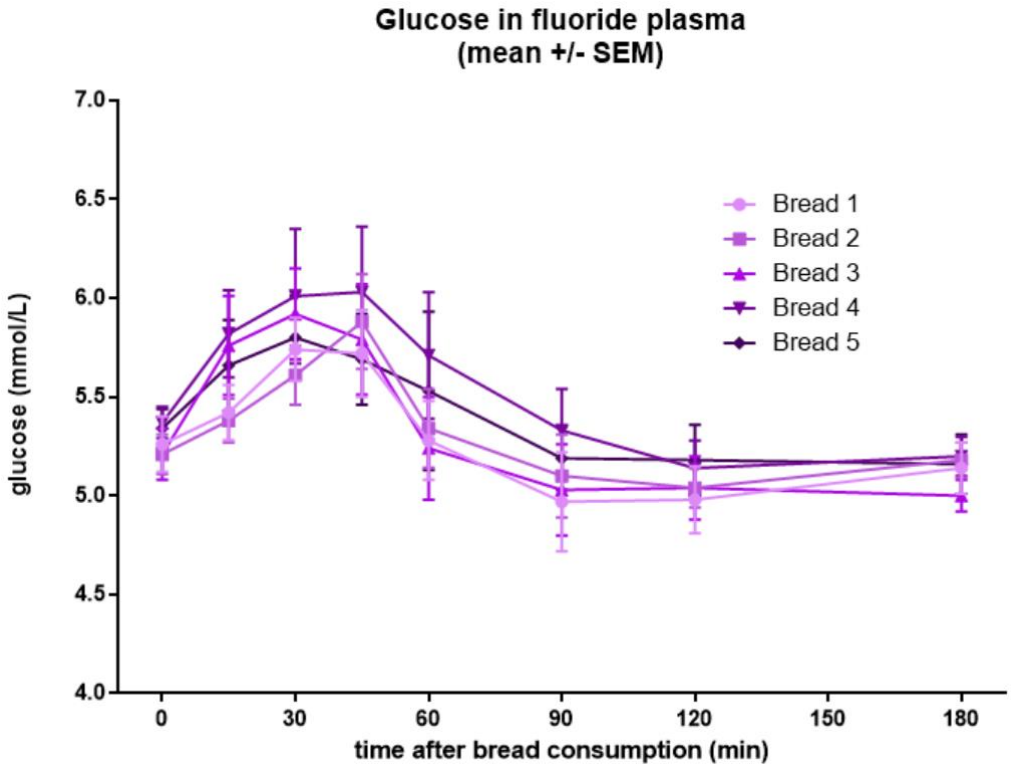

Figure 6. Postprandial glucose concentration in venous plasma (n=9) after consumption of five different test breads.

When evaluating the differences in glucose concentrations measured by the two different methods of blood sampling, we observed variability in glucose concentrations measured until 120 minutes (Figure 7). From 120 – 180 minutes, plasma glucose values were higher than capillary glucose.

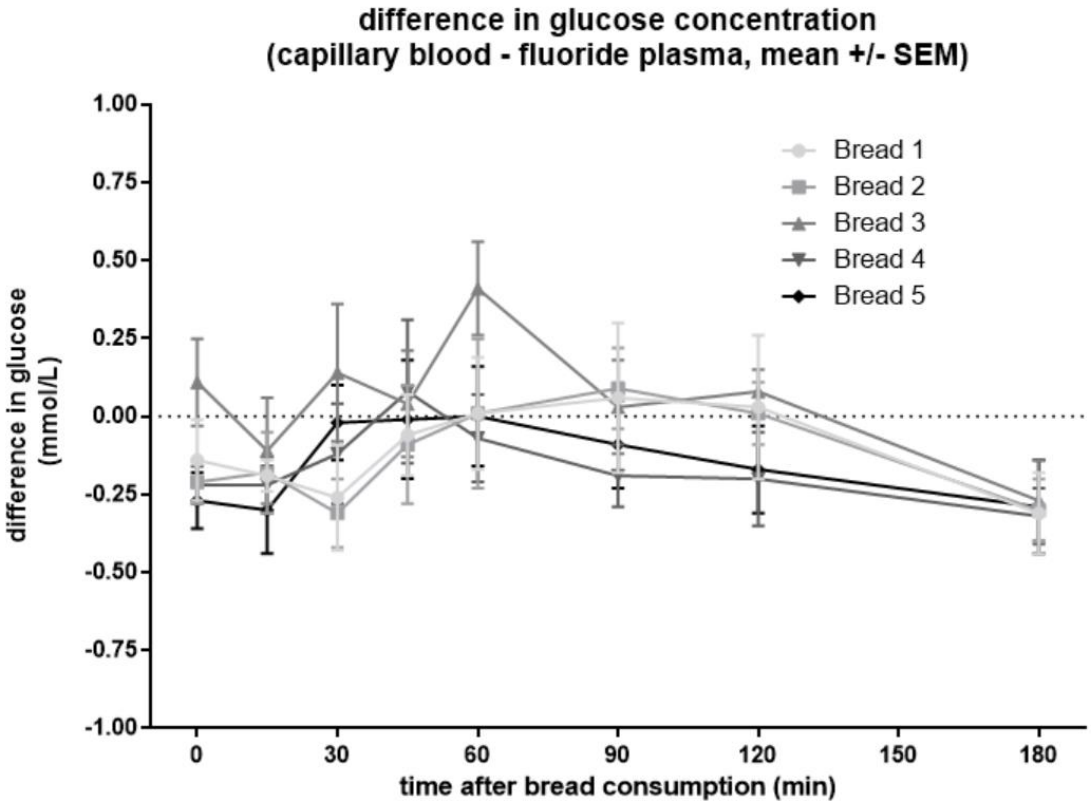

Figure 7. Differences in capillary and venous plasma glucose concentrations after consumption of five different test breads.

Incremental area under the curve 0-120 minutes ($iAUC_{120}$)

There were no significant differences in either capillary or plasma $iAUC_{120}$ (Wilcoxon signed rank test, all p-values > 0.05) when comparing each bread to the control. There were also no significant differences when comparing the same bread between capillary and plasma $iAUC_{120}$ (related-samples Wilcoxon signed rank test, lowest p-value in B1 = 0.128).

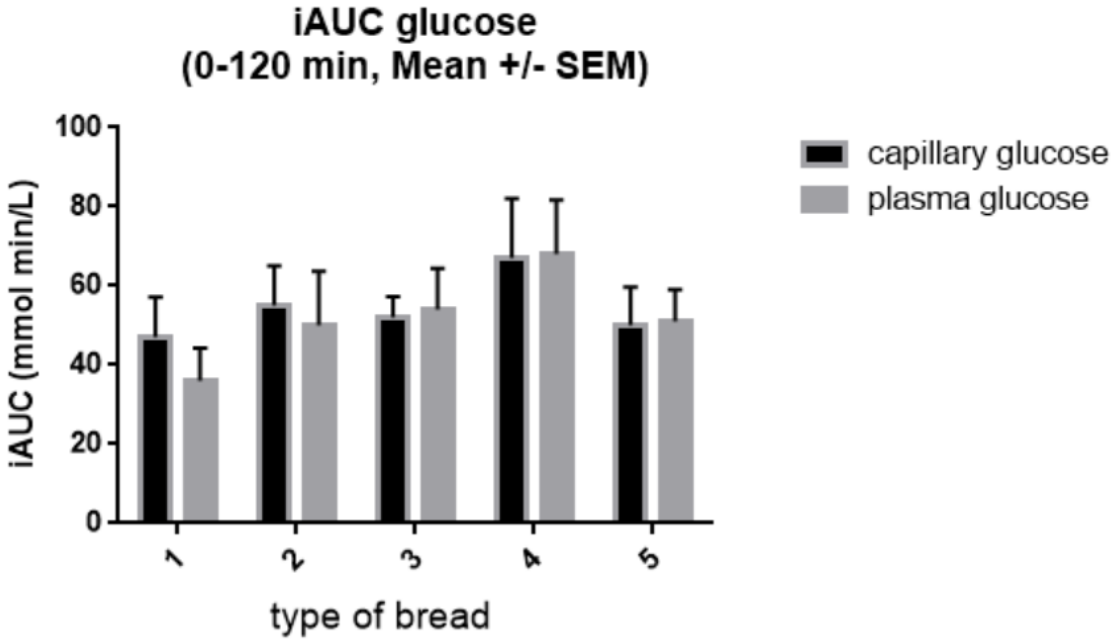

Figure 8. Capillary and plasma glucose $iAUC_{120}$ (n=9) after consumption of five different test breads. SEM: standard error of the mean.

Independent-samples Kruskal-Wallis test

We applied an independent-samples Kruskal-Wallis test to the individual participant's (n=9) capillary C_{max}, T_{max}, and $iAUC_{120}$, and plasma C_{max}, T_{max}, and $iAUC_{120}$ to assess for any differences across the breads, comprised of (B1 – B5). There were no significant differences (all p-values >0.05).

Table 5. Postprandial blood glucose response (n=9) after consumption of five different test breads.

Test food	C_{max} [a] capillary glucose (mmol/L)	Capillary iAUC[c] 120 (mmol*min/L)	T_{max} [d] capillary glucose (min)	C_{max} [a] plasma glucose (mmol/L)	Plasma iAUC[c] 120 (mmol*min/L)	T_{max} [d] plasma glucose (min)	Capillary PBGR[e] (mmol/L)	Plasma PBGR[e] (mmol/L)
Control	5.7 ± 0.4[f]	47 ± 27[f]	40.0 ± 12.2[f]	5.9 ± 0.6	36 ± 25	37.5 ± 8.0	0.7 ± 0,4	0.7 ± 0.5
Bread 2	6.0 ± 0.7	55 ± 30	39.4 ± 25.3	6.0 ± 0.6	50 ± 29	40.0 ± 13.0	1.0 ± 0,8	0.8 ± 0.6
Bread 3	6.3 ± 0.9	52 ± 14	32.1 ± 10.4	6.1 ± 0.7	36 ± 25	31.9 ± 14.9	1.2 ± 0,3	1.0 ± 0.6
Bread 4	6.3 ± 1.1	67 ± 45	43.3 ± 11.7	6.3 ± 1.0	50 ± 41	43.1 ± 18.7	1.2 ± 0,8	1.1 ± 0.7
Bread 5	6.3 ± 0.9	50 ± 29	46.7 ± 29.5	6.2 ± 1.0	54 ± 31	45.0 ± 34.0	1.2 ± 0,8	1.0 ± 0.9

Values are reported as means ± SD.

[a] C_{max}: maximum glucose concentration

[b] Area under the curve. Rounded to the nearest whole number

[c] Incremental area under the curve: difference between peak and baseline AUC. Rounded to the nearest whole number

[d] T_{max}: time of maximum glucose concentration

[e] PBGR: peak blood glucose rise: difference between maximum and fasting (baseline) glucose concentration

[f] Sample size (n=7)

4.4 Postprandial insulin response

Among the five test breads, the highest mean maximum insulin concentration (C_{max}) was seen in B1 (Figure 9). All breads containing guar elicited a significantly (Wilcoxon signed rank test, all p-values <0.05) lower mean insulin C_{max} (12.0 - 23.9 mIE/L) than the control. The lowest insulin C_{max} was seen in B2, followed by B5. Consumption of the guar-containing breads led to a reduction in mean (n=9) peak insulin concentration of 34 % (B4), 41% (B3), 61% (B5), and 68% (B2) compared to B1. There were no significant differences in insulin T_{max}.

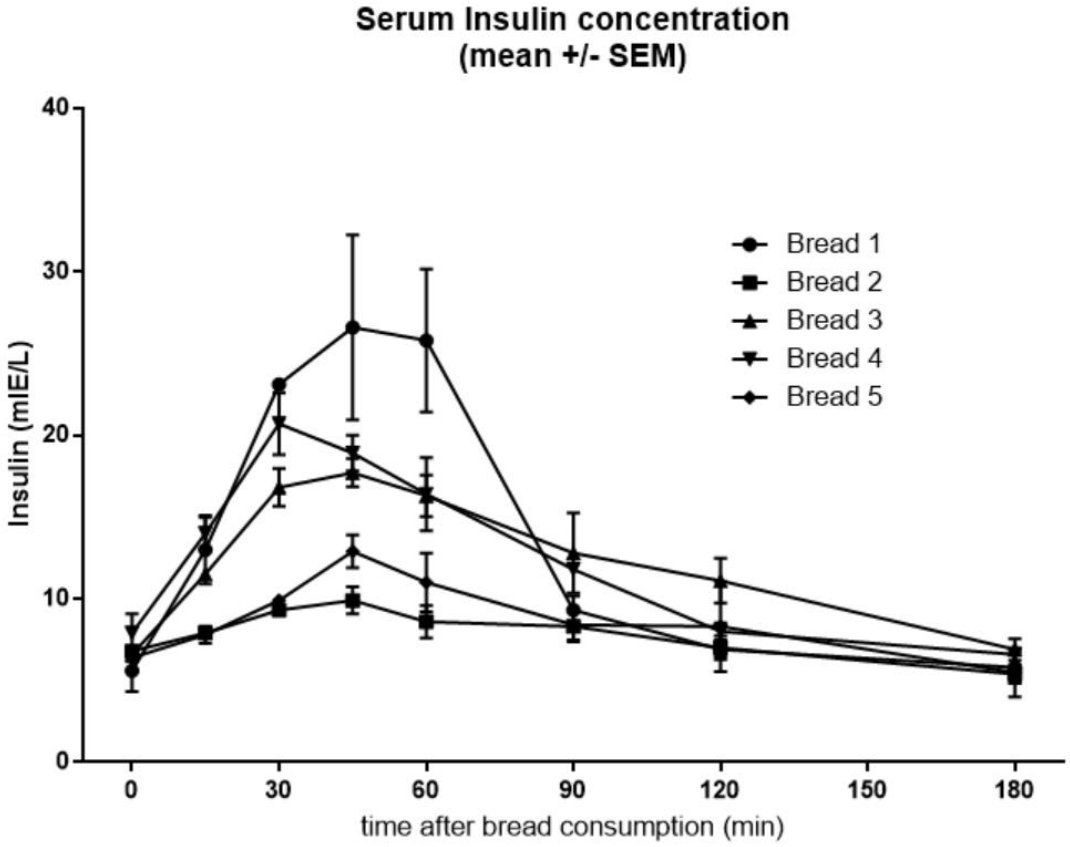

Figure 9. Postprandial serum insulin concentration (n=9) after consumption of five different test breads.

Area under the curve 0-120 (AUC_{120})

For serum insulin analysis, AUC_{120} (Figure 10) values were calculated instead of $iAUC_{120}$, as insulin concentrations may be close to zero (area of detection >2.0 mIE/L) in the fasted state. Wilcoxon signed rank test revealed significant differences in insulin AUC_{120} B5 ($p = 0.008$) and B2 ($p = 0.008$), compared to the control. Insulin AUC_{120} was reduced by 45% and 48% in B5 and B2 respectively.

There were no significant differences in individual (n=9) insulin AUC_{120} values when compared across all five breads (Independent-samples Kruskal-Wallis test, $p = 0.171$).

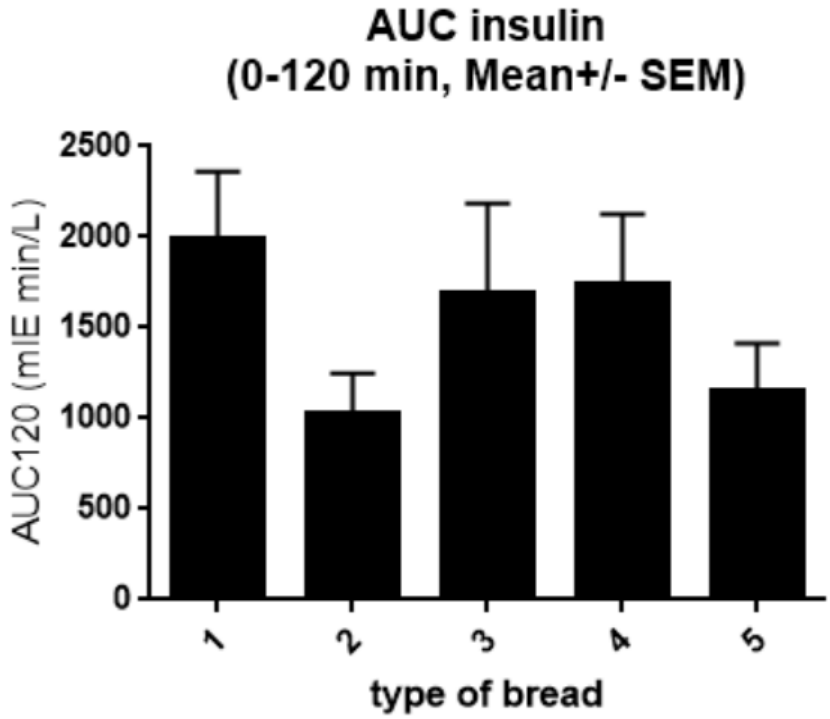

Figure 10. Insulin AUC_{120} (n=9) after consumption of five different test breads.

Table 6. Postprandial insulin response (n=9) after consumption of five different test breads.

Test food	C_{max}[a] insulin (mIE/L)	AUC_{120}[b] insulin (mIE*min/L)	T_{max}[c] insulin (min)
Bread 1 (control)	34.8 ± 20.8	1983 ± 1130	43.3 ± 11.7
Bread 2	10.9 ± 6.6	1026 ± 660	43.3 ± 30.4
Bread 3	20.5 ± 18.5	1687 ± 1490	56.7 ± 30.7
Bread 4	22.8 ± 19.1	1742 ± 1150	41.7 ± 12.5
Bread 5	13.7 ± 10.9	1151 ± 789	40.0 ± 35.2

Values are reported as means ± SD.

[a] C_{max}: maximum insulin concentration

[b] Area under the curve. Rounded to the nearest whole number

[c] T_{max}: time of maximum insulin concentration

5 DISCUSSION

Globally, the obesity epidemic has been associated with an increased risk of NCDs such as T2DM (33, 209), characterized by deficient production or response to the action of insulin, as manifested by hyperglycaemia (16). Dietary carbohydrates, closely linked to the postprandial glucose and insulin response (8), are thus implicated in the aetiology of such lifestyle diseases. The vast research on the relationship between carbohydrates and disease risk has given rise to terms such as GI (2), and GL (99, 100), reflecting the glycaemic response to both individual foods and mixed meals.

Dietary carbohydrates are digested and absorbed at different rates, and to various extents (210). In search of alternative food products to lower the postprandial GR, different low-GI bread products have been developed (5, 151, 158, 165). The metabolic effects of bread can vary to a great extent, depending on the whole grain content and the amount of dietary fibre (149), but also on the particle size of the flour and the use of sourdough (164, 165). At present, it is acknowledged that some dietary fibres may have an important role in modulating postprandial glycaemia (93). Soluble dietary fibres, characterized by their high water-binding capacity (173), have the potential to delay or reduce the rate of digestion and absorption (93, 172).

There is evidence of reductions in the postprandial glucose and insulin response after consumption of foods enriched with guar (176, 196, 202-204), however, these studies did not test bread with added guar. Until recently, the practical application of guar galactomannan in food products was faced with challenges in making beneficial doses palatable (176). The addition of guar into food products such as breads and biscuits seem to be the most effective mode of administration (196). Still, not much is known about the postprandial response to guar galactomannan of different viscosities and doses. Thus, the current study aimed to investigate the postprandial effects of bread with added guar of different molecular weights and concentrations.

5.1 Main findings

We performed this randomized clinical crossover study to assess the postprandial effects of bread containing guar galactomannan in healthy, overweight adults. In this study sample of nine overweight persons, all participants consumed five different test breads in a randomized

order on five separate test days with a wash out period of at least 3 days in a fully blinded manner, and neither the subject nor the investigator knew the contents of the different breads.

The present study demonstrated that in healthy, overweight adults, the consumption of four breads containing different concentrations and molecular weights of guar galactomannan did not lead to significant changes in postprandial capillary or venous glucose concentration, when compared to a wheat control bread with no added guar. Indeed, maximum concentrations and iAUC were higher than that of the control, without achieving significance. The insulin response, however, was significantly affected. Insulin C_{max} was significantly reduced by 34% - 68%, compared to the control, after consumption of the breads containing guar. Furthermore, the insulin response, reflected by insulin area under the curve, AUC_{120}, was significantly reduced in both test breads containing high-M_W guar, compared to the control.

To the best of our knowledge, no previous study has examined the effects of guar galactomannan of different M_W's and concentrations on postprandial glucose and insulin response in healthy, overweight humans. There is evidence suggesting that guar as an ingredient in foods is effective in decreasing postprandial glucose and insulin concentrations in human subjects (176, 191, 196, 211-213). In the current study, diagnosis of T2DM was an exclusion criterion, since the glycaemic response is expected to be higher in persons with T2DM. Therefore, we have mostly focused in this discussion on the results from studies investigating the postprandial effects of guar consumption in people without T2DM.

5.2 Suggested mechanisms

In the present study, postprandial insulin concentrations (Figure 10) were significantly reduced after consumption of all breads containing guar galactomannan (Table 2), contradicting the results of Groop *et al,* in which guar gum was suggested to stimulate rather than suppress insulin secretion (214). However, as opposed to what has been previously reported, we observed that both capillary (Figure 5), and venous (Figure 6) glucose concentrations were not different from those of the control (Table 5), after consumption of guar-containing breads.

The effectiveness of guar in reducing the postprandial glycaemic response seems to be determined mainly by its ability to increase the viscosity of the intestinal content (215), due to the expansion of hydrated galactomannan chains, which may delay gastric emptying and

reduce the propulsive and mixing effects of peristaltic movements (93, 215) and in turn lead to a reduced postprandial glycaemic response (216). However, whether guar will change the gastric emptying time is a matter of debate, as studies have reported conflicting results (215, 217).

Other mechanism of action assumes that guar acts as a physical barrier between enzymes and substrate in the intestine (218). The entanglement of guar chains with other polymers in the digesta (192) such as starch molecules, may impact gut functions through an effect on both the rate and degree of diffusion of substrates, enzymes or product, or on enzymatic reactions (177). In an animal model, Brennan *et al,* inspected chyme from the small intestine of pigs fed wheat bread containing guar, and discovered that galactomannan had formed a layer covering the starch granules (218), suggesting that guar may exert its effects at the level of the food matrix. An in vitro study suggested that guar can reduce or delay starch digestibility (177). Pancreatic α-amylase is crucial enzyme in the first stage of starch digestion. Slaughter *et al,* demonstrated that the effects of guar gum in lowering the postprandial glycaemic response include inhibition of the initial stage of starch degradation, as galactomannan acts as a non-competitive inhibitor of α-amylase (219).

The effects of guar galactomannan on reducing postprandial glucose and insulin response is mainly attributed to its capacity to increase the viscosity of gastrointestinal contents and the width of the unstirred water layer covering the intestinal mucosa, by readily absorbing water, which limits the movement and release of nutrients in the stomach or small intestine (191, 199, 215, 216). In a study investigating the effects of different dietary fibres and fibre analogues, guar, the most viscous substance among the ones tested, led to the greatest reductions in postprandial glucose and insulin concentrations (191). The mechanism of action is assumed to involve association with the carbohydrates in the food matrix, which limits the rate of starch digestion (176, 191, 218), by competing for available water, thus inhibiting starch gelatinization, or by creating a barrier around starch granules, or between glucose and small intestinal mucosal cells (204, 220).

Viscous, soluble fibres form thick gels when they are mixed with water. While the viscous properties of guar gum are dependent on factors affecting the extent and rate of hydration (199), they are also dependent on the M_W and concentration of the galactomannan, two factors that were modified in the present study. A trial performed in healthy male subjects suggested that increasing amounts of guar gum led to greater decreases in postprandial insulin

concentrations (221). Regarding the impact of altering guar M_W, a galactomannan of high M_W would, in theory, produce a higher viscosity solution than a galactomannan of low M_W. Typical guar-flours used in research are M60 (low), M90 (medium) and M150 (high), which have been tested in both humans (199) and animals (216).

In a study with similarities to the present one, 17 normal weight (mean BMI 22.8 kg/m^2) subjects without T2DM consumed breads containing equivalent concentrations of standard food grades of guar (199). However, the breads (50 g available carbohydrate) were served with butter and jam (25 g available carbohydrate). All guar-breads significantly reduced the postprandial insulin response, but they did not find any significant differences between low-M_W and high-M_W galactomannan flours, although their results suggested that low-M_W guar was less effective than guar of higher M_W. Furthermore, they observed no significant changes in postprandial glucose, with reported mean PBGR (maximum concentration relative to the fasting levels) of 1.2 mmol/l at approximately 30 minutes, and close to or somewhat below the baseline after the peak. This is similar to the results (Figure 5 and 6, Table 5) reported in the present study.

In the current study, all four test breads, containing both low- and high-M_W guar galactomannan (Table 2) significantly reduced insulin C_{max} compared to B1. Comparisons of the AUC, however (Figure 10), after bread consumption (Figure 9) suggested that the higher-M_W guar was more effective than the lower-M_W guar. Interestingly, B3 and B4 had lower C_{max}, but similar AUC as the control, probably due to higher late insulin concentrations (>90 minutes). There was a sharp decrease in insulin concentration after consumption of the control after 60 minutes. Interestingly, there was also a rise in glucose concentrations after consumption of B3 (Figure 5 and 6) in the same time interval. Other studies reported that much of the effect of the guar-containing breads on postprandial insulin response can likely be explained by a decreased rate of small intestinal glucose absorption (199, 204, 220). However, since the measured insulin concentration represents a combination of insulin secretion and insulin degradation we cannot, at present, explain the underlying mechanisms of the observed effect.

Another mechanism that has been suggested in the literature is that guar may affect the postprandial insulin response by modulation of the secretion of gastric inhibitory polypeptide (GIP), an insulin-stimulating hormone (214, 216, 222). In a trial performed in healthy adults, Blackburn *et al*, demonstrated that the rate of intestinal glucose absorption was significantly

reduced in the presence of guar, but returned to control-values when there was no guar present (215). Additionally, the secretion of GIP has been demonstrated to be directly related to the rate and site of glucose absorption (223). In the current study we did not measure or quantify the extent of glucose absorption in the intestine. However, the potential hormone modulating effects of guar may serve as a possible explanation why the present, and other studies (198, 199, 222) did not observe any significant effect of guar galactomannan on postprandial glucose concentrations, whilst the insulinemic response was indeed significantly affected.

5.3 Discussion of methods

As briefly mentioned in Section 1.3, measurements of fasting and postprandial glucose concentrations vary depending on the site of sampling (224), which is important to consider when interpreting an individual's glycaemic response to a meal. Measurements on peripheral blood, such as venous or capillary blood is representative of the net effect of several post-absorptive processes, since nutrient-rich blood is transported from the small intestine, via the liver, to peripheral tissues (216).

Considering the medium of choice for blood glucose measurements, studies have found that there is less random error associated with capillary glucose measurement compared to venous plasma (225). Thus, differences in glycaemic response are potentially detected more readily with capillary rather than venous glucose measurements (226). There is also evidence of increased within-subject variation in plasma glucose concentrations compared to capillary blood (227). In the current study, we decided to measure both capillary and venous blood, since capillary blood sampling is incorporated in the recommended glycaemic index methodology used for calculating AUC (228), which is reflective of the glycaemic response.

However, several postprandial studies determine the glycaemic response based on measurements of venous plasma, or even serum (examples presented in (229, 230)). Ideally, measurements of glucose concentrations in portal blood would provide more precise results, as it was observed in an animal model, where the investigators detected a more pronounced glucose-lowering effect of guar in portal blood than in peripheral blood (216). Nevertheless, for obvious ethical and practical reasons, such blood samples are impossible to acquire in human studies. Also, with capillary blood, the glucose concentration differs by body site, and it may be speculated in how great the difference is between fingertip and antecubital vein, as opposed to the differences in capillary blood obtained from different body sites (141, 145).

In the present study we performed blood sampling up to 180 minutes. Still, we determined $iAUC_{120}$ values to be more reflective of the postprandial glucose response to the different breads, rather than $iAUC_{180}$. This decision was based on the discrepancies in glucose concentrations between the two methods of blood sampling between 120 and 180 minutes (Figure 7). When calculating iAUC, GraphPad Prism does not include the area beneath the fasting glucose concentrations (Figure 11) (207). Thus, $iAUC_{120}$ avoids the potential inclusion of negative peaks (glucose concentrations below the fasting value, which is illustrated in Figure 11). In addition, iAUC from 0 - 120 minutes seems to be the standard practice, as it is used in other postprandial studies investigating postprandial glycaemic response (2, 120, 199, 228). To achieve congruence in our results, insulin AUC_{120} was reported rather than AUC_{180}.

We chose iAUC rather than AUC to reflect the glucose response to the test foods, as we were interested in measuring the relative change from fasting concentrations over time, as opposed to the average concentration over time, where a large part of the area is determined by the fasting glucose concentration (228). iAUC is calculated based on measurements of glucose concentrations at various time points, usually in intervals of 15 - 30 minutes (231). The most precise measurements of changes in glucose concentrations over time, however, would be obtained from continuous glucose monitoring, which according to an expert on glycaemic index, is almost impossible to achieve (231), even though there are now wearable devices that can measure glucose concentration almost continuously (232).

There is, however, variation in the methods used to calculate and report AUC, which could make it difficult to compare results across studies. An interlaboratory study revealed that errors in calculating AUC are common (233). AUC values reported by approximately 50% (14 of 26 laboratories) to a central laboratory were considered incorrectly calculated or reported (233). Using the simplest method, calculations of iAUC are based on the trapezoid rule (207), which connects straight lines between each point of glucose measurement. However, we acknowledge that this is not the most accurate physiological representation of the glycaemic response (231). One consideration is whether the time intervals are appropriate for detecting changes in glucose concentrations, and another is what is happening between these time points.

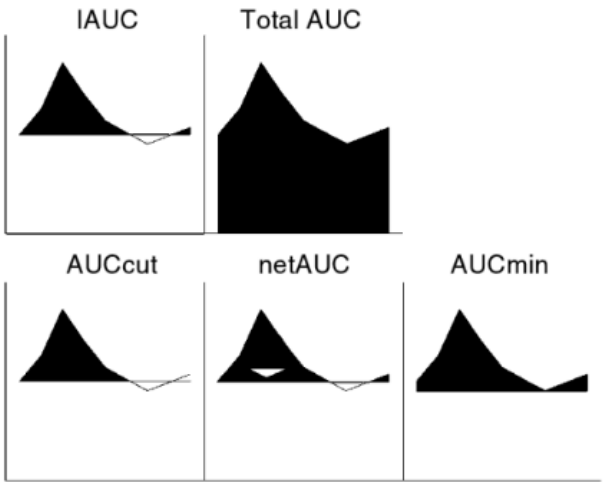

Fig. 2.4. Area subtended by different methods used to calculate area under the curve.

Figure 11. Areas defined by different methods used to calculate area under the curve (AUC). Reprinted from "The Glycaemic Index, A Physiological Classification of Dietary Carbohydrate" (231).

When performing analyses of $iAUC_{120}$ (Figure 8) no significant differences were detected between capillary and plasma samples, or between the test breads and the control, in either capillary or plasma samples. When comparing the iAUC's of the two methods of blood sampling, the most pronounced difference was found in B1 (the control). However, this can be due to the lower number of comparisons (n=7) in capillary samples of B1.

Since iAUC is a measure of the glycaemic response, the magnitude of the response depends on the amount of available carbohydrate in the food serving. The definition of GI is based on the relative glycaemic response to 50 g available carbohydrates, compared to 50 g available carbohydrate of a reference food, which is usually glucose or white bread (2). The intent of the term GI is thus to quantify the ability of a carbohydrate-food to raise blood glucose, relative to the reference food. Therefore, the reported amount of available carbohydrates in a certain food serving is dependent on how the amount of "available" and "unavailable" carbohydrate is defined and calculated. For instance, the mere definition of unavailable carbohydrates as dietary fibre, when GI was a novel concept, was relatively simple to apply in practice. Now it is known that certain groups of carbohydrates may exhibit properties that define them as "partially available", including resistant starches, modified starches and sugar

alcohols (231). Additionally, it is almost impossible to precisely quantify the amount of carbohydrate from foods that is actually absorbed in the gut (231).

With regard to the addition of functional fibre as ingredients to food one should consider that there is a maximum threshold to the amount of which guar gum can be added before affecting the sensory properties of the food product, as was also experienced in the present study. In our study, the breads were blinded for both the investigator and the study subjects. Some breads, however, were described as "drier" and "tougher to chew", although we did not systematically investigate this. Ellis *et al*, suggests a guar concentration of 5 - 10% to achieve a balance between physiological efficacy and palatability (234).

Compared to the results from a recent bread study conducted by Rieder *et al,* (5), the postprandial glucose concentrations measured in the present study are markedly lower, even in the control bread, which is surprising, as both studies used portion sizes reported to contain 25 g available carbohydrate. At present we do not have an explanation for these differences. However, reported AUCs to different breads are highly variable and our results are within the variation of reported values (as illustrated on p. 21 in (231)). Still, direct comparison of results is limited by the fact that we did not determine the exact distribution between carbohydrate, fats and protein of the breads in the present study. It is still important to consider the impact of nutrient composition, as it has been previously shown that high amounts of fat may lead to a reduced glucose response (198). Furthermore, the addition of a glucose reference as a sixth "test food" would prove beneficial as a benchmark value when evaluating the reliability of the glucose measurements after consumption of the breads.

One aspect of the methodology of the current study which is important to consider when interpreting and/or comparing results, is the definition of what is the baseline (time zero, t=0) for postprandial blood samples. In this study, t=0 was defined as the timepoint when the subject had fully consumed the bread, similarly to a recent postprandial study (230). This is in contrast to blood samples based on the timepoint when the subject began consuming the test food, as is more commonly used (199, 222, 227-229). Unclear definitions of t=0 also exists in the literature (215). Standardizing t=0 as the time of test meal commencement would be easier to keep track of, if multiple persons are involved in conducting the experiment. However, the pace at which an individual consumes the serving of test food is susceptible to variability, both between subjects, and within-subjects (from one test day to another), which is difficult to control for in postprandial studies. By defining t=0 at the start of the meal, the variability in

pace of consumption is not accounted for. Conversely, defining t=0 at the end of the meal potentially impacts the detection of very early changes in blood glucose.

One possible explanation for the low PBGR observed in the current study (Table 5) may be the low amount of carbohydrates in the servings of bread. The glycaemic response in relation to different amounts of available carbohydrate has previously been demonstrated (231). When comparing the glycaemic response to three different (25, 50 and 100 g) levels of available carbohydrate relative to white bread with the same content of available carbohydrate in five different foods, the mean relative response varied by less than 5% across all levels of carbohydrate, suggesting that the relative responses of foods are proportionate to the amount of available carbohydrate (231). Thus, we believe it is reasonable to assume that the servings containing 25 g available carbohydrate tested in the present study would accurately reflect the relative changes in postprandial glucose concentrations after consuming the test breads. However, the observed fall in glucose concentrations beneath the fasting values (Figure 5 and 6) would eventually be avoided by increasing the amount of available carbohydrate in the food servings. Additionally, we observed a tendency of raised plasma glucose concentrations at 180 minutes compared to the prior measurement at 120 minutes (Figure 6). While the same effect was not observed in capillary blood samples, the increased glucose concentrations at 180 minutes may perhaps be attributed to the stimulation of gluconeogenesis and/or glycogenolysis, due to the low amount of food, the timeframe of blood sampling, and also the prior overnight fast.

Potential degradation of the breads owing to either the transport of storing conditions may serve as another possible explanation for the low PBGR values observed (Table 5). The breads used in the present study were produced at in-house facilities at Nofima, Ås. Although the breads were immediately blast frozen following production, they had to be transported to the study site in Bergen. When arriving at RUHS, the breads were immediately transferred to a dedicated on-site freezer. The temperature of the breads at arrival was not controlled, however. Additionally, there is no guarantee that the freezer where the breads were stored kept a constant temperature. The freezer was only open for short amounts of time when collecting breads to thaw, in preparation for the test days. Still, we acknowledge the possibility that the integrity of the breads may have been affected by transport and storage. Additionally, upon writing this thesis, nearly one year has passed since the breads were produced. Starch retrogradation may potentially have occurred in the breads, influencing the

glycaemic response when performing the tests. However, a recent review examining the effect of hydrocolloids, such as guar, added to bakery products to improve shelf life, reports that guar decreased the retrogradation of starch (235). Still, whether this would have occurred in the test breads used in this study, and to what extent, is of interest and will be solved by later analysis of the remaining breads.

Few studies have observed the long-term effects of guar supplementation on body composition. Administration of guar gum before meals (twice daily) for eight weeks significantly reduced body weight in obese patients (236). In addition, patients reported increased satiety even though they were instructed to maintain their regular dietary habits. This effect was assumed to be related to the effect of guar gum on gastric emptying time (236). These results agree with findings from a previous study in which adding guar gum to a meal increased satiety, and where gastric emptying time was found to significantly correlate with a measure of satiety (237). This potential effect of guar gum as a regulator of appetite is important when considering the link between obesity and disturbances of metabolism, as weight loss is acknowledged as a first-line therapy to reduce risk factors (79). However, a meta-analysis of 20 randomized trials suggests that guar is not efficacious for body weight reduction (238). In the present study, participants were weight stable with a mean weight change of -0.2 ± 2.3 kg between visit 5 and visit 1. The mean change in WC (2.5 ± 2.8 cm) from the first to the final visit is likely related to measurement errors. The short time span of the study along with the infrequent consumption of guar makes it difficult to evaluate the long-term effects of guar galactomannan on body composition.

Subject compliance to the study requirements may also affect the reliability of our results. The time of day tests are done affects the results of glycaemic response studies, as blood glucose and insulin responses have diurnal variation (131, 225). It is acknowledged that a phenomenon of improved glucose tolerance may occur post-consumption of a low-GI food (152, 155). This 2nd meal effect should perhaps be accounted for when considering the variability in glycaemic responses. Our tests were performed in the morning after an overnight fast, since it is not reasonable to expect participants to fast for several hours during the day.

The researcher should consider what is economically feasible and applicable in practice, as expensive and demanding restrictions may have little or no effect (233). The summarized recommendations from the interlaboratory GI-study (233), favours moderate, rather than

extensive subject restrictions, which is in agreement with the degree of restrictions presently used. In this study we did not provide participants with a standardized evening meal. Instead, they were instructed to eat a similar carbohydrate-based evening meal before each test day. In addition we aimed to eliminate confounding factors of lifestyle that may influence glucose metabolism, by urging subjects to avoid alcohol, nicotine and any strenuous physical activity before the test day. Indeed, the variation coefficient in baseline glucose concentrations was rather low and did not exceed 6% in any of the participants (data not shown).

5.4 Strengths and limitations

The study design is a strength of this study. The order of breads was double-blinded and randomized to remove potential confounding and placebo effects related to the order in which they were consumed. In addition, because of the crossover design, each participant served as their own control, which enabled comparisons between the different groups (breads). However, the crossover design can also be regarded as a weakness of the study. Since each participant contributes a major part of the total data, it is sensitive to dropouts. The group of subjects studied was relatively homogenous. However, the study is limited by the number of participants (n=9) which affects the statistical power to detect any significant differences. Recruitment of participants had to be stopped due to the Covid-19 outbreak. The study is now on hold, awaiting recruitment of remaining subjects (n=3, for a total of n=12).

The servings of bread were calculated to contain 25 g available carbohydrate each. Subjects were instructed to consume the breads within the timeframe of 15 minutes, which did not present any challenges. We did not, however, estimate the GI or analyse the nutrient composition of the different breads provided for this study, which may provide useful insight into the glycaemic properties of the different breads. To remove potential carry-over effects after consumption of the test breads, we included a wash out period of at least 3 days between visits. All test days were scheduled in the morning. In addition, we standardized the duration of the overnight fast before each test day, to remove potential effects of the last meal on glycaemia. The duration of the overnight fast was self-reported, displaying some deviation in hours fasted (min: 10 h, max: 14.5 h) from the 12 hours stated in the study protocol.

The study is strengthened by performing double blood samples, both venous and capillary, at each timepoint (fasting and postprandial), which enables comparisons between and within the two methods, which was also blinded to all personnel involved in blood sampling and handling. Drawing of blood samples and handling of blood samples was in accordance with

accepted laboratory guidelines and methods (Analyseoversikten.no). Capillary glucose samples were not obtained (B1) from two participants on one test day due to lack of equipment. During the first study days, handwash was not performed in all subjects before capillary blood sampling. Higher capillary glucose concentrations may have been reported due to the potential presence of for instance sugar residues on the fingers. Thus, some of the measured glucose concentrations from capillary blood samples may have been inflated. The near-significant ($p<0.1$) differences found in capillary B3 C_{max} and B5 T_{max}, and plasma B3 T_{max} may have occurred by chance, since we conducted many tests.

In some of the blood samples drawn from the venous catheter (one serum tube and one plasma tube), we observed haemolysis, which may have impacted the analyses performed at MBF. All blood samples were drawn within a range of ± 4 minutes of the time intervals.

Anthropometric measurements were performed before consumption of the breads at each test day. Measurements were obtained by different personnel, which increases the likelihood of measurement errors. Two of the included participants had BMI values that were below (BMI: 24.8 kg/m^2) or above (BMI: 33.4 kg/m^2) the inclusion criteria.

Conflicts of interest

Nofima AS funded the study, but had no influence on the design, analysis or reporting of the study.

5.5 Future perspectives and conclusion

There is a huge potential for functional dietary fibres added to carbohydrate staple foods, such as bread, since the intake of many people is lower than the recommended amount (147). Guar galactomannan is a soluble fibre capable of increasing the viscosity of solutions, even at low concentrations (215), a mechanism that is thought to be key for its effect on reducing postprandial glucose and insulin concentrations (176, 191, 196, 211-213). The beneficial effects of this soluble fibre make it interesting as a functional ingredient added to foods, for instance to make low-GI food alternatives for improved control of postprandial glycaemia. More research is needed on the optimal mode and medium of administration of guar galactomannan, since the rate and extent of hydration seems to be important for its physiological effects (199). Further studies are warranted on the effects of guar galactomannan on glucose response in normal weight samples. The viscous properties of guar gum are dependent on the M_W and concentration of the galactomannan, but also on the extent

and rate of hydration, which along with factors such as temperature and access to water, depend on the particle size of the guar gum preparation. For future projects it would be interesting to add a marker of glucose absorption as an ingredient to breads, to assess the extent of small intestinal absorption of glucose.

In the present study we investigated the postprandial response to breads containing guar galactomannan of four different molecular weights and two different concentrations, in 9 healthy, overweight adults. We found that consumption of breads containing guar did not lead to any significant change in postprandial glycaemia compared to a control. However, all guar-breads elicited significantly lower postprandial maximum insulin concentrations, and our results suggest that increased guar molecular weight, rather than concentration, affects the insulinemic response (reflected by insulin AUC), as the two breads containing high-M_W guar significantly decreased insulin AUC, compared to the control. In conclusion, bread with added guar galactomannan significantly reduces the postprandial rise in insulin, and high-M_W guar significantly reduces insulin area under the curve compared to a wheat-control, in healthy, overweight adults.

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7 APPENDICES

APPENDIX A: Informed consent form

Forespørsel om deltakelse i forskningsprosjektet

postprandiale effekter av funksjonell brød

Dette er et spørsmål til deg om å delta i et forskningsprosjekt for å utprøve metabolske effekter av et nytt type brød som har tilsatt kostfiber (galaktomannan). Den undersøkelsen utføres av Universitetet i Bergen i samarbeid med Nofima, Ås. Prosjektleder er professor i klinisk ernæring, Jutta Dierkes, og studien gjennomføres ved 'Forskningsenhet for Helseundersøkelser (FHU)'.

Hva innebærer PROSJEKTET?

I den undersøkelsen skal brød med eller uten tilsatt galaktomannan spises, og effekten på blodsukker og insulinutskillelse registreres. Galactomanan er en type kostfiber som er godkjent som tilsetningsstoff til matvarer.

Prosjektet innebærer at du kommer fem ganger til FHU i Årstadveien, og hver undersøkelse vil ta inntil 4 timer. Du skal komme fastende, det vil si at du ikke skal ha spist eller drukket noe siden kl 20 kvelden før studiedagen. Du skal spise brød uten noe annet, og blir fastende etterpå. Det vil bli tatt både venøse og kapillære blodprøver ved innkomst, og etter 15, 30, og 45 minutter, og videre etter en, en et halv, to og tre timer. Du få en perifert venekateter (venflon) lagt inn for venøse prøver, og kapilløre prøver tas vedstick fra fingertuppene.

I prosjektet vil vi innhente og registrere opplysninger om deg. Vi henter inn informasjon om din helsetilstand med et spørreskjemaet som en av de medarbeider i studien tar kontakt med deg på forhånd, og dere går gjennom spørreskjemaet sammen, og blodprøveresultater vil inngår i et forskningsprosjekt. Opplysninger som er relevante inkluderer om du har fått påvist diabetes mellitus, høyt blodtrykk, har hjerte-kar sykdommer eller hadde kreft. Vi skal også måle din vekt, høyde og midjemål (omkrets) og måle langtidsblodsukker (HbA1c) ved screeningsundersøkelse, og glucose og ulike hormoner under studien.

Mulige fordeler og ulemper

Før studien begynner, vil du delta i en screeningundersøkelse. Her skal vi utelukke at du har diabetes mellitus ved å bruke spørsmål om kliniske symptomer, og måling av blodsukker og langtidsblodsukker HbA1c. Du får altså bedre opplysning om din helse.

Som ulempe må tidsbruk beregnes (fem ganger ca 4 timer), og at det blir tatt blodprøver. Det er veldig lite risiko ved blodprøvetaking. Blodprøvene blir tatt ut av autorisert erfarende helsepersonell ved FHU. Likevel kan det, i sjeldne anledninger, oppstår blåmerker. Kapillære blodprøver fra fingertupper er vanlig til kontroll av blodsukker hos diabetikere. Det er også ingen kjente negative effekter ved å spise det berikede brødet. Videre skal deltagerne møte fastende til undersøkelsene. Kvelden før skal det inntas et bestemt måltid mat, og deltakerne skal ikke røyke eller drikke alkohol eller spise noe etter kl 20.

Frivillig deltakelse og mulighet for å trekke sitt samtykke

Det er frivillig å delta i prosjektet. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke. Dersom du trekker deg fra prosjektet, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner. Dersom du senere ønsker å trekke deg eller har spørsmål til prosjektet, kan du kontakte prof. Jutta Dierkes, telefon 55977344, epost jutta.dierkes@uib.no.

Etter avsluttet studie vil du få en kompensasjon av 1500 NOK. Den betales kun ved fulført studie.

Hva skjer med OPPLYSNINGENE om deg?

Opplysningene som registreres om deg skal kun brukes slik som beskrevet i hensikten med prosjektet. Du har rett til innsyn i hvilke opplysninger som er registrert om deg og rett til å få korrigert eventuelle feil i de opplysningene som er registrert. Du har også rett til å få innsyn i sikkerhetstiltakene ved behandling av opplysningene.

Alle opplysningene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger gjennom en navneliste. Det er kun prosjektleder Jutta Dierkes som har tilgang til denne listen.

Opplysningene om deg vil bli anonymisert eller slettet fem år etter prosjektslutt.

Hva skjer med prøver som blir tatt av deg?

Prøvene som tas av deg skal oppbevares for analyser som er beskrevet i prosjektet.

Forsikring

Under studien er du forsikret med pasientskadeloven.

Godkjenning

Regional komité for medisinsk og helsefaglig forskningsetikk har vurdert prosjektet, og har gitt forhåndsgodkjenning (saksnr. REK (2019/912). Etter ny personopplysningslov har behandlingsansvarlig institusjon, (Universitetet i Bergen), og prosjektleder Jutta Dierkes et selvstendig ansvar for å sikre at behandlingen av dine opplysninger har et lovlig grunnlag. Dette prosjektet har rettslig grunnlag i EUs personvernforordning artikkel 6 nr. 1a og artikkel 9 nr. 2a og ditt samtykke.

Du har rett til å klage på behandlingen av dine opplysninger til Datatilsynet.

kONTAKTOPPLYSNINGER

Dersom du har spørsmål til prosjektet kan du ta kontakt med prof. Jutta Dierkes, Telefon 55977344, jutta.dierkes@uib.no

Personvernombud ved institusjonen er Janecke Helene Veim (janecke.veim@uib.no).

Jeg samtykker til å delta i prosjektet og til at mine personopplysninger og mitt biologiske materiale brukes slik det er beskrevet

Sted og dato	Deltakers signatur
	Deltakers navn med trykte bokstaver

Appendix B: Form that was filled out during the screening visit

	Kvinne: Mann:	
Målinger	Vekt:_____kg Høyde:____cm Midjemål_____cm Alder:_____	
Tidligere sykdom og år av diagnose		
Kosttilskudd	1. 2. 3.	
Medikamenter	1. 4. 2. 5. 3. 6. Annet:	
Alkoholbruk (enheter pr uke)	Daglig: 1-2 x pr uke: < 1x pr uke: < 1x pr mnd aldri: Hva drikker du til hver anledning (antall enheter): Øl (33 cl): Vin (15 cl): Brennevin (4 cl):	
Røyking /Snus	Røyker sigaretter: Ja/Nei Hvis ja, _____antall pr dag Bruker snus: ja/nei hvis ja, _____antall pr dag Hvis nei, har du røykt tidligere: ja/nei hvis ja, når har du sluttet?____	
Aktivitetsnivå	Ved jobb: Stillesittende stående Fysisk arbeid	

	Ved fritiden: Treningsøkter pr uke / turer pr uke / hagearbeid pr uke Intensitet (lav/middels/høy):

APPENDIX C: Form that was filled out on each study day

Studiedag nr.		Dato:
Målinger	Vekt:_____kg Høyde:____cm Midjemål_____cm	
Endringer i kosttilskudd eller medisinering	1. 2. 3.	
Siste måltid i går	Kl	
spist eller drukket noe siden siste måltid	Spist ja / nei Drukket ja / nei Røykt ja / nei	

Studiedag nr.		Dato:
Målinger	Vekt:_____kg Høyde:____cm Midjemål_____cm	
Endringer i kosttilskudd eller medisinering	1. 2. 3.	
Siste måltid i går	Kl	
spist eller drukket noe siden siste måltid	Spist ja / nei Drukket ja / nei Røykt ja / nei	

Studiedag nr.		Dato:
Målinger	Vekt:_____kg Høyde:____cm Midjemål_____cm	
Endringer i kosttilskudd eller medisinering	1. 2. 3.	
Siste måltid i går	Kl	
spist eller drukket noe siden siste måltid	Spist ja / nei Drukket ja / nei Røykt ja / nei	

Studiedag nr.		Dato:
Målinger	Vekt:_____kg Høyde:____cm Midjemål_____cm	
Endringer i kosttilskudd eller medisinering	1. 2. 3.	
Siste måltid i går	Kl	
spist eller drukket noe siden siste måltid	Spist ja / nei Drukket ja / nei Røykt ja / nei	

Studiedag nr.		Dato:
Målinger	Vekt:_____kg Høyde:____cm Midjemål_____cm	
Endringer i kosttilskudd eller medisinering	1. 2. 3.	
Siste måltid i går	Kl	
spist eller drukket noe siden siste måltid	Spist ja / nei Drukket ja / nei Røykt ja / nei	

APPENDIX D: Study synopsis

Study synopsis: Postprandial effects of bread containing galactomannan, a soluble fiber

Jutta Dierkes, Gülen Arslan Lied (Center of Nutrition, UiB)

Simon Ballance, Anne Rieder (Nofima, Ås)

Title	Postprandial effects of bread containing galactomannan, a soluble dietary fiber
Objectives	Investigate whether bread with added galactomannan can reduce the postprandial glucose response in healthy overweight adults. Four types of bread have added galactomannan in different amounts and viscosities, and one bread is the control bread.
Interventions	The test persons will consume bread (quantity 25g available carbohydrates) at 5 different days (at least 3 days in between), in random order, starting at around 8:00 h. Blood samples will be taken at baseline, at 15, 30, 45 and 60 minutes, 90 and 120 minutes and at 180 minutes. Blood sampling starts at 15 min after complete consumption of the test bread. The test bread has to be consumed within 15 minutes with 250 ml of water It is recommended to take arterialized venous blood into test tubes with anticoagulant (heparin or EDTA). Blood samples will be immediately centrifuged and divided into 3 aliquots. One aliquot will be sent to the central laboratory at Haukeland University hospital after all samples have been taken, the other aliquots will be frozen at -80 C degrees.
Key inclusion and exclusion criteria	Inclusion: Healthy adults with overweight (BMI 25 – 30 kg/m2) Exclusion: Known diagnosis of diabetes mellitus type 2 HbA1c at screening $\geq$ 48 mmol/mol Other chronic diseases (heart disease, cancer) within the last 3 years Pregnancy and lactation Known drug or alcohol abuse Unwilling to follow the study requirements
Outcomes	Primary endpoint: AUC glucose, difference of the different intervention breads to the control bread Cmax glucose, differences of the different intervention breads Secondary endpoint: AUC insulin, difference of the different intervention breads to the control bread
Study type	Single center randomized clinical study
Statistical analysis	Comparison of AUC glucose after the different bread types
Sample size	To be screened n=30 (expected) To be allocated to the study n=15

	To be analysed n=12
Study duration	October 2019 - January 2020

Timeline per visit:

Day before study: no strenuous exercise

Evening before study day: last meal at 20:00 h, thereafter only water

The meal should be the same before all test days, and should be carbohydrate rich (include pasta, potatoes or rice)

Test day: no coffee, tea, smoking or sniff, water is allowed

8:00 h arrival at FHU, placement of canule,

baseline blood sample (arterialized venous blood)

Ca. 8:15 h consumption of test bread within 15 min, and 250 ml water

15 min upon consumption: blood sampling at 15 min

Blood sampling at 15, 30, 45, 60, 90, 120 and 180 minutes

Ca. 11:30 h test day finished, canule drawn