THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Efficacy of a low glycemic index diet and effectiveness of oat β-glucans on cardiometabolic risk factors

Results from randomized controlled trials

THERESE HJORTH

Department of Life Sciences

CHALMERS UNIVERSITY OF TECHNOLOGY

Gothenburg, Sweden 2024

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THERESE HJORTH
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Department of Life Sciences
Chalmers University of Technology
SE-412 96 Gothenburg
Sweden
Telephone + 46 (0)31-772 17 25

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ABSTRACT

Background: Cardiometabolic diseases (CMD) are major causes of death and morbidity world-wide. Adhering to a healthy diet, such as the Mediterranean diet (MED), has proven effective for prevention of these conditions. Aspects of carbohydrate quality, including glycemic index (GI) and dietary fiber also play an important role for prevention of CMD. Low-GI foods improve glycemic response and have potential beneficial effects on glycemic control. Dietary fiber, gut microbiota, and short chain fatty acids (SCFAs) have been linked to cardiometabolic risk. The oat-derived β-glucan fiber has shown benefits for short-term blood glucose response.

Aim: This thesis aimed to evaluate the effects of GI and dietary fiber on metabolic health among individuals at elevated cardiometabolic risk. The aim was further to evaluate long-term effects of low GI on cardiometabolic risk factors in non-diabetic individuals; identify individual postprandial glucose responses associated with metabolic effects; investigate relationships between SCFAs, type 2 diabetes (T2D) risk factors, and gut microbiota composition; and to assess the impact of β-glucan-enriched bread on long-term glucose control in individuals at T2D risk.

Results: The low-GI diet improved postprandial glucose control compared to the high-GI diet. The MED lowered daily blood glucose in both groups, but only the low-GI diet reduced daily glycemic variability. Two distinct postprandial glucose response clusters were identified and associated differently with T2D risk markers, glycemic control, and gut microbiota. No association was found between SCFAs and T2D risk factors or glycemic control. However, significant positive association was found between acetate concentrations and blood pressure. In a pragmatic study, β-glucan-enriched bread did not improve long-term glycemic control in high-risk individuals.

Conclusion: Low-GI foods within a Mediterranean diet improved postprandial glucose control and reduced glycemic variability, emphasizing GI as a key dietary component for glycemic regulation. The association between acetate and blood pressure suggests a possible role for SCFAs in CMD risk. Although β-glucans have shown benefits for acute glycemic responses, their effectiveness for long-term glycemic control was limited in real-world settings. Findings on individual differences in glucose responses and gut microbiota profiles highlight the need for dietary strategies considering individual variability, i.e., precision nutrition, for cardiometabolic health.

Keywords: Cardiometabolic disease; Cardiovascular disease; Type 2 diabetes; Carbohydrate quality; Dietary fiber; Whole grains; Glycemic Index; Mediterranean diet; Short chain fatty acids; Gut microbiota metabolites; β-glucans

LIST OF PUBLICATIONS

The doctoral thesis is based on the work presented in the following papers

I. Robert E. Bergia*, Rosalba Giacco*, **Therese Hjorth***, Izabela Biskup, Wenbin Zhu, Giuseppina Costabile, Marilena Vitale, Wayne W. Campbell, Rikard Landberg, and Gabriele Riccardi. *Differential Glycemic Effects of Low- versus High-Glycemic Index Mediterranean-Style Eating Patterns in Adults at Risk for Type 2 Diabetes: The MEDGI-Carb Randomized Controlled Trial*. Nutrients, 2022 Feb 8;14(3):706

II. Viktor Skantze*, **Therese Hjorth***, Carl Brunius, Johan Dicksved, Anders Esberg, Marilena Vitale, Rosalba Giacco, Robert Bergia, Mikael Wallman, Mats Jirstrand, Wayne Campbell, Gabriele Riccardi, Rikard Landberg. *Differential Responders of a Mixed Meal Tolerance Test Associated with Type 2 Diabetes Risk Factors and Gut Microbiota: Data from the MEDGI-Carb Study*. Nutrients, 2023 Oct 15;15(20):4369

III. **Therese Hjorth**, Viktor Skantze, Wayne W. Campbell, Rosalba Giacco, Gabriele Riccardi, Rikard Landberg. *Plasma concentrations of gut fermentation products in relation to risk factors of type 2 diabetes and gut microbiota: Data from the MEDGI-Carb study*. Manuscript in preparation.

IV. **Therese Hjorth**, Alena Schadow, Ingrid Revheim, Ulrike Spielau, Klara Meyer, Anne Rieder, Paula Varela, Simon Ballance, Antje Koerner, Rikard Landberg, Anette Buyken, Jutta Dierkes, Hanne Rosendahl-Riise. *Effectiveness of Regular Oat β-Glucan Enriched Bread Consumption on Long-Term Glycemic Control in Adults at Risk of Type 2 Diabetes: Results from the CarbHealth Randomized Controlled Trial*. Submitted.

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- Marilena Vitale, Giuseppina Costabile, Robert E. Bergia, **Therese Hjorth**, Wayne W. Campbell, Rikard Landberg, Gabriele Riccardi, Rosalba Giacco. *The role of sex on plasma glucose and insulin profiles after Mediterranean diets with low or high glycemic index: data from MEDGI-Carb randomized clinical trial.* Clinical Nutrition 2023 Oct;42(10):2022-2028.

- Giuseppina Costabile, Robert E. Bergia, Marilena Vitale, **Therese Hjorth**, Wayne Campbell, Rikard Landberg, Gabriele Riccardi, and Rosalba Giacco. *Effects on cardiovascular risk factors of a low- vs high-glycemic index Mediterranean diet in high cardiometabolic risk individuals: the MEDGI-Carb study.* European Journal of Clinical Nutrition 2024 May;78(5):384-390.

- Sebastian Åberg, Marie Palmnäs-Bédard, Therese Karlsson, **Therese Hjorth**, Kia Nøehr Iversen, Rikard Landberg. *Evaluation of subjective appetite assessment under free-living vs-controlled conditions: A randomized crossover trial comparing whole-grain rye and refined wheat diets (VASA-home).* Nutrients 2023 May 25;15(11):2456.

Contribution Report

Paper I: Therese Hjorth (TH) was the shared first author and was responsible for data management, including the provision of sociodemographic, anthropometric, and metabolic data, as well as the calculation of all dietary data from the Swedish site as part of the multicenter trial. TH contributed to the writing and participated in revising the manuscript in collaboration with the co-authors during the editing process.

Paper II: TH was the shared first author and co-interpreted the data and results; wrote part of the manuscript related to nutritional aspects as well as type 2 diabetes and blood glucose responses. TH also co-wrote the computational aspects with the other shared first author.

Paper III: TH was the first author and carried out analyses of short-chain fatty acids, data processing and handling, analyzed and interpreted the data, wrote the first manuscript, and revised the manuscript together with co-authors.

Paper IV: TH wrote the protocol and ethical application for the Swedish site as part of the multicenter trial, conducted the trial at the Swedish site, collected and processed the data from the other sites, analyzed and interpreted the data, wrote the first draft of the manuscript and revised the manuscript together with co-authors.

ABBREVIATIONS

ALT	Alanine Aminotransferase
ANOVA	Analysis of variance
AST	Aspartate Aminotransferase
BMI	Body mass index
CONGA	Continuous overall net glycemic action
CC	Complete case analysis
CGM	Continuous glucose monitoring device
CMD	Cardiometabolic diseases
CRP	C-reactive protein
CVD	Cardiovascular disease
E%	Percent of energy intake
EFSA	European Food Safety Authority
FLI	Fatty liver-index
GI	Glycemic index
GL	Glycemic load
GLM	Generalized linear model
GLP-1	Glucagon-like-peptide-1
HbA1c	Glycated hemoglobin
HDL-C	High-density lipoprotein cholesterol
HOMA-IR	homeostasis model assessment insulin resistance
HSI	Hepatic steatosis index
KNN	K-nearest neighbor
LC	Liquid chromatography
MAG	Mean absolute glucose
MAGE	Mean amplitude of glucose excursions
MED	Mediterranean diet
MS	Mass spectrometry
NOFIMA	Norwegian Institute for Food, Fisheries, and Aquaculture Research
ITT	Intention-to-treat
IPA	Indole-3-propionic acid
LDL-C	Low-density lipoprotein cholesterol
MMTT	Mixed meal tolerance test
MetS	Metabolic syndrome
OGTT	Oral glucose tolerance test
QUICKI	Quantitative insulin sensitivity check index
RCT	Randomized controlled trial
SCFAs	Short chain fatty acids
SD	Standard deviation

SEM	Standard error of the mean
T2D	Type 2 diabetes
tAUC	Total area under the curve

TABLE OF CONTENTS

Introduction 1
2 Aim and Objectives 3
3 Background 5
3.2 Healthy dietary patterns and cardiometabolic disease 5
3.3 Carbohydrate quality 7
3.4 Whole grains, dietary fiber and cardiometabolic health 8
3.5 Oat β-glucans: postprandial- and long-term metabolic effects 9
3.6 Glycemic Index 9
3.7 Gut microbiota, carbohydrates and cardiometabolic health 11
3.8 Microbial metabolites and cardiometabolic health 11
3.9 Precision nutrition and cardiometabolic health 14
3.10 The postprandial phase and personalized guidelines 16
3.11 Efficacy vs Effectiveness 16
4 METHODS AND DATA 19
4.1 Hypotheses and research strategies 19
4.2 Study designs 22
4.2 Experimental design 22
4.3 Study populations 24
4.4 Intervention diets 25
4.5 Dietary assessments and adherence 26
4.6 Clinical examinations 27
4. 7 Continuous glucose monitoring 27
4.8 Postprandial assessments in the MEDGI-Carb trial 28
4.9 Gut microbiota composition and gut microbiota derived metabolites 28
4.10 Clinical chemistry 30
4.11 Differential metabolic responders in the MEDGI-Carb trial 30
4.12 Data analysis and sample size estimates 31
5 Results and discussion 33
5.1 Study population 33
5.2 Dietary intake 37
5.3 The MEDGI-Carb study: Effect of GI on glycemic control (Paper I) 38
5.3.1 Postprandial MMTT glucose and insulin responses 38
5.3.2 Twenty-four-hour glycemic variability 40

5.3.3 Fasting and OGTT and insulin responses 42
5.4 The MEDGI-Carb study: Identification of differential responders to a mixed meal tolerance test (Paper II)....43
5.4.1 Identification of clusters based on a mechanistic model 43
5.4.2 Clusters linked to type 2 diabetes risk markers and glucose control....44
5.4.3 Clusters and gut microbiota....45
5.5 The MEDGI-Carb study: Plasma concentration of gut microbiota metabolites in relation to risk factors of type 2 diabetes 47
5.5.1 Intervention effects on plasma SCFA and IPA concentrations 47
5.5.2 Associations between baseline tAUC tertiles of SCFAs, IPA and glycemic control .47
5.5.3 Plasma SCFA and IPA tAUC and changes in risk factors of type 2 diabetes 48
5.5.4 Differences in microbial abundance and diversity between healthy and impaired glycemic control groups....48
5.6 CarbHealth, β-glucans and metabolic control 50
5.6.1 Bread consumption, acceptance and adherence 52
6 General discussion 55
7 Strengths and Limitations 59
8 Conclusions 61
9 Future perspectives....63
10 Acknowledgements....64
11 References....67

Introduction

Cardiometabolic diseases including cardiovascular disease (CVD) and type 2 diabetes are the leading causes of morbidity worldwide (1) and the global prevalence are increasing (2, 3). This can partly be attributed to increased age in the population and an increase in the prevalence of obesity and overweight (2). Metabolic syndrome (MetS) is a recognized cluster of cardiometabolic dysfunctions that increase the risk of developing type 2 diabetes and CVD. The key components of MetS include insulin resistance, impaired glucose tolerance, obesity, hypertension, and dyslipidemia (4).

Many of these conditions can be significantly modified through lifestyle changes, particularly diet. Growing evidence suggests that healthy diets such as the Mediterranean diet which is rich in fruits, vegetables, whole grains, and unsaturated fats diet is effective in improving body weight, hypertension, dyslipidemia, and type 2 diabetes whereas a diet high in refined grains, added sugar and saturated fat increases the risk of these conditions (5, 6). A healthy dietary pattern has also been shown to influence gut microbiota composition and derived metabolites, with evidence suggesting a favorable effect on the microbiota composition (7). Growing research indicates that the interaction between diet, gut microbiota, and their metabolites may have a significant impact on host health (8).

Among dietary components, carbohydrate quality rather than carbohydrate quantity has emerged as an important factor for prevention of cardiometabolic diseases (9). Carbohydrate quality can be assessed in various ways and is often described across four dimensions: whole grains, dietary fiber, glycemic index (GI) and glycemic load (GL), and sugar (10). In the Nordic- and Northern European countries, carbohydrate-rich cereal foods are staple foods and they comprise the primary energy source as well as the main source of carbohydrates, dietary fibre and plant protein in the diet (11). Therefore, improving carbohydrate quality in the diet, via good quality cereal foods, could generate substantial health benefits for the population.

High whole grain intake has consistently been associated with reduced risk of developing type 2 diabetes and CVD. The health benefits are partly attributed to the dietary fiber content (12). Dietary fibers vary widely in terms of physiochemical properties that result in functionalities such as solubility, viscosity, fermentability and bulking capacity (13). These diverse properties are closely linked to the different health benefits of dietary fibers. β-glucans are a viscosity forming dietary fiber found in high amounts in oat and barley (14). β-glucans have proven beneficial effects on acute postprandial glucose responses and blood lipid concentrations in studies under ideal and controlled conditions (efficacy trials) (15). However, less is known about the long-term effects of β-glucans on blood glucose control such as glycated hemoglobin (HbA1c) in a real-world

context (effectiveness).

Another key aspect of carbohydrate quality is the concept of glycemic index (GI). Glycemic index is a feature of a food that describes the impact of a carbohydrate rich food on blood glucose response under standardized conditions, where high GI foods results in a high postprandial peak and low GI foods results in a lower peak (16). Postprandial glycemia has been suggested to be as important as fasting glucose levels for the development of impaired insulin sensitivity and insulin secretion observed in the progression toward type 2 diabetes and CVD (17, 18). Immediate effects of GI have been demonstrated, but data on long-term consumption of low GI foods among non-diabetic individuals are lacking, particularly regarding the effect on glycemic control and cardiometabolic risk factors. Furthermore, the role of GI in the context of a healthy diet pattern has yet to be established.

A diet with high content of dietary fiber has been associated with increased concentration of microbial fermentation products such as short chain fatty acids (SCFA) and the tryptophan metabolite Indole-3-propionic acid (IPA) (19, 20). Shorth chain fatty acids and IPA have been suggested to play important roles in cardiometabolic health, such as improved glucose control, improved blood pressure and blood lipid concentration (21, 22). However, most research on the role of SCFA and IPA in glycemic health has been conducted in animal models or observational studies, highlighting the need for randomized controlled trials to further explore the potential causality of observed associations with host health.

Dietary recommendations are often given at a population level and the adherence to general guidelines are often poor (23). Furthermore, general guidelines do not take metabolic responses to foods into account, nor the large individual variation in such response to diet. A Landmark study have shown a high degree of inter-individual variability in postprandial metabolic responses (24). The postprandial phase has been shown to be important in identifying metabolic dysfunctions that may be of importance to long-term health. Static fasting measurement fails to capture the metabolic process that occurs after a meal (25, 26). Precision nutrition, defined as providing the right diet for the right person at the right time, has the potential to complement current dietary recommendations by better taking differences in metabolic responses into account, and thereby providing more efficient promotion of long-term health (27). Understanding individual variations in metabolic postprandial responses is therefore an important element in providing more personalized advice for improved health.

2 Aim and Objectives

The overall aim of this thesis was to evaluate two dimensions of carbohydrate quality, glycemic index (GI) and dietary fibre, on metabolic health and cardiometabolic risk in individuals at elevated risk, using data from two dietary intervention trials; one with a controlled design i.e., an efficacy study (**Paper I**, **II**, **III**) and one designed to be more pragmatic and reflective of real-world conditions i.e., an effectiveness study (**Paper IV**).

The specific objectives were to:

A. Evaluate the long-term effect of glycemic index on glucose homeostasis in the context of a Mediterranean dietary pattern among non-diabetic individuals (**Paper I**)

B. Identify differential postprandial glucose responders after a single mixed meal and investigate their associated metabolic effects (**Paper II**)

C. Investigate the association of postprandial plasma concentrations of gut fermentation products with risk factors of cardiometabolic health and gut microbiota composition (**Paper III**)

D. Evaluate the effectiveness of β-glucan enriched bread as a strategy to improve long-term glucose control among adults at elevated risk of developing type 2 diabetes (**Paper IV**)

3 Background

3.1 Cardiometabolic diseases

Cardiometabolic diseases comprise a group of common and to some extent preventable conditions including type 2 diabetes, cardiovascular diseases (CVD), non-alcoholic fatty liver disease, and metabolic syndrome. The prevalence of cardiometabolic diseases has reached epidemic proportions globally (3, 28-30). Metabolic syndrome is a recognized cluster of cardiometabolic abnormalities that significantly elevates the risk of the individual developing type 2 diabetes, and cardiovascular disease (CVD). The key components of the metabolic syndrome include impaired glucose tolerance, or insulin resistance, obesity, hypertension, and dyslipidemia, characterized by elevated triglycerides and low levels of high-density lipoprotein cholesterol (HDL-C) (31). Risk factors for cardiometabolic diseases can be classified into modifiable and non-modifiable categories. Non-modifiable risk factors include age, sex, and genetics (32, 33), while modifiable risk factors include smoking, physical inactivity, overweight and obesity, and dietary habits (1, 34, 35). Given that modifiable risk factors, such as poor dietary habits, play a significant role in the development of cardiometabolic diseases, adopting healthier dietary patterns may have a large impact on reducing cardiometabolic disease risk (36).

3.2 Healthy dietary patterns and cardiometabolic disease

Evidence suggests diets rich in fruits, vegetables, whole grains, legumes, and unsaturated fat, are highly effective in preventing and managing conditions such as type 2 diabetes, obesity, hypertension, and dyslipidemia (37). In contrast, diets high in refined grains, salt, added sugars, and saturated and trans- fats increase cardiometabolic disease risk (38).

The Dietary Approach to Stop Hypertension (DASH) study was a landmark trial designed to assess the effects of different dietary patterns on blood pressure (39). The study included 459 adults, both with and without hypertension, and compared three diets: a control diet that was low in fruits and vegetables but included full-fat dairy products; a diet rich in fruits and vegetables; and the DASH diet, which emphasized fruits, vegetables, and low-fat dairy products while reducing saturated and total fat. Sodium intake and body weight were maintained at constant levels throughout the trial. The DASH diet significantly lowered systolic- and diastolic blood pressure compared to control diet, and it was particularly effective in participants with hypertension, producing effects comparable to those achieved with medications (39). Systematic reviews and meta-analyses have verified these results among different populations i.e., adults with hypertension and pre-hypertension (39, 40).

The healthy Nordic diet has been investigated in slightly different versions in several intervention trials for the effects on cardiometabolic health. The SYSDIET-study, involving 200 individuals with metabolic syndrome, assessed the impact of a healthy, isocaloric Nordic diet on insulin sensitivity, lipid profiles, and inflammation markers. The healthy Nordic diet included foods such as whole grains, fatty fish, berries, root vegetables, rapeseed oil and low-fat dairy products while the control diet was an average Nordic diet. No significant changes were observed in insulin sensitivity or blood pressure, but significant changes were observed in lipid profile and low-grade inflammation (41). The SYSDIMET study involving 131 individuals with impaired glucose control and features of the metabolic syndrome in a 12-week dietary intervention trial aimed to investigate the effect of a healthy diet on inflammation and endothelial dysfunction. Participants were randomized into one of three diet groups: a healthy diet consisting of whole grains, fatty fish, and 300 grams of bilberries per day; a whole-grain-enriched diet; or a control diet consisting of refined grains and low-fiber products. Results from the trial showed that the healthy diet had significant beneficial effects on inflammation and endothelial function, compared to the control group (42). Similarly, the NORDIET aimed to investigate the effect of a healthy Nordic diet on cardiovascular risk factors among individuals with slightly elevated cholesterol concentrations. Eighty-eight subjects were randomized to either an ad libitum healthy Nordic diet, consisting of fruit, berries, vegetables, low-fat dairy products, and fatty fish, or a control diet, which was the subjects' habitual Western diet, for six weeks. After the intervention, the healthy Nordic diet was found to improve the blood lipid profile, systolic blood pressure, and insulin sensitivity compared to the control diet (43). A systematic review and meta-analysis comprising of five randomized controlled trials (RCT) consisting of 513 participants investigated the effect of the Nordic diet on cardiovascular risk, and concluded that the Nordic diet improves systolic and diastolic blood pressure and markers of blood lipid profile (44).

The traditional Mediterranean diet is characterized by a high intake of plant-based foods, including fruits, vegetables, legumes, breads, and other cereals, minimally processed. It also emphasizes a high consumption of olive oil and nuts, moderate intake of eggs, dairy products, fish, and poultry, and a low intake of red meat and added sugars (45). The Mediterranean diet has consistently been linked to health benefits such as lower cardiovascular risk factors including blood lipids and blood pressure, reduced risk of type 2 diabetes, and metabolic syndrome (45, 46). Both the Mediterranean diet as a whole and its components have cardiometabolic health benefits. The monounsaturated fatty acids in the olive oil may improve postprandial glycemia via an improved postprandial insulin sensitivity (47), and improve LDL-C levels (48) as well as beneficial effect on endothelial function (49). The Mediterranean diet also includes high intake of whole grain foods rich in dietary fiber which has been related to reduced inflammation, improved lipid profiles and blood pressure (50), and improved glucose metabolism (51). These health effects

may partly be due to improved gut microbiota composition including enhanced production of gut microbiota metabolites such as SCFA and the tryptophan metabolite IPA from the diet (52, 53).

The PREDIMED study aimed to investigate the impact of a Mediterranean diet on CVD prevention in individuals at high risk but without history of CVD. The trial included 7447 participants who were assigned to one of three diets: a Mediterranean diet supplemented with extra-virgin olive oil, a Mediterranean diet supplemented with mixed nuts, or a low-fat control diet. The study showed a significant reduction of 30% in the incidence of major cardiovascular events such as heart attack, stroke, or death from cardiovascular cause, in participants following either of the Mediterranean diet groups compared to the control diet group (54).

While these dietary patterns emphasize the inclusion of whole foods, a key factor underlying their benefits is the quality of the carbohydrates.

3.3 Carbohydrate quality

Carbohydrate quality can be measured in various ways and can be described by four dimensions including: the amount and type of whole grain vs refined carbohydrates, dietary fiber and their sources, glycemic index (GI) and glycemic load (GL), and sugar content of a diet or food (55, 56). Carbohydrates comprise simple sugars, oligosaccharides, starch, and non-starchy polysaccharides and represent the backbone in the diet worldwide. National dietary guidelines suggest that 45-65% of total caloric intake should be derived from carbohydrates (57, 58) and that the dietary fibre intake should be in the range of ≥ 25 - 35 grams per day (58). While total sugar intake should not exceed 10 E% (percent of energy intake) (58). Cereals and grains represent a significant portion of carbohydrate intake worldwide, and the form in which they are consumed, whether as whole grains or refined grains, has substantial implications for health (59). This distinction is important, as whole grain consumption has been consistently linked to reduced risk of type 2 diabetes, cardiovascular disease, and metabolic syndrome, while diets high in refined grains have been associated with increased risk of these conditions (60).

Diets rich in whole grains, legumes and high fiber foods seen in both the Mediterranean and Nordic diets are associated with improvements in glucose metabolism, insulin sensitivity and reduced inflammation (41, 61, 62). In contrast, diets high in refined carbohydrates, sugars and low in fiber may increase the risk of type 2 diabetes and metabolic syndrome (63, 64). Meta-analysis of randomized controlled trials have shown that improving the quality of carbohydrates by focusing on whole grains and reducing sugar intake can help regulate postprandial blood glucose levels, improve lipid profiles,

and reduce insulin resistance, key factors in preventing cardiometabolic diseases (65-67).

3.4 Whole grains, dietary fiber and cardiometabolic health

Although an official global definition of whole grains is lacking, there is broad consensus that it includes all three parts of the grain kernel: the bran, germ, and starchy endosperm, in proportions similar to those found in the intact grain (68). The bran and germ are rich in dietary fiber, vitamins, and bioactive compounds such as polyphenols, while the starchy endosperm mainly contains starch and some proteins (69). Extensive research has found that a high whole grain intake has been consistently associated with lower risk of developing type 2 diabetes, cardiovascular disease, and certain cancers (70). These health benefits are attributed to various components of whole grains, with cereal fiber suggested as one of the most important (71).

Dietary fiber comprise complex, non-starch polysaccharides and lignin that are not digestible within the upper gastrointestinal tract and pass undigested into the colon, where they to a variable degree are fermented by gut bacteria (72, 73). There is great heterogeneity among the components that are classified as dietary fibers, resulting in major differences in water solubility, viscosity, water binding and bulking capacity, and fermentability. These differences contribute to varied effects on host metabolism and cardiometabolic health (74). An important functionality of dietary fibre is whether it is soluble or not and to what degree it is gel-forming (soluble), fermentable (soluble and insoluble), and bulking (insoluble) (74). The different types of fibers produce a wide range of physiological effects, from slowing gastric emptying to serving as substrates for gut microbiota in the colon (75, 76). The diverse functionalities of fiber result in varying health benefits depending on the type and source of fiber. An overview of different fiber types and their associated health benefits is provided in **Table 1**.

Table 1. Overview of fibers and associated health benefits

Type of fiber	Example of fibers	Health benefits
Fermentable (soluble and insoluble)	Inulin, resistant starch	Stimulates SCFA production, supports gut health, regulates blood glucose, improves insulin sensitivity
Gel-forming (primary soluble)	β-glucans, pectin	Regulates blood glucose, lowers cholesterol, increases satiety, regulates gastrointestinal transit time
Bulk (insoluble)	Cellulose, lignin	Adds bulk to stool, speeds up transit time, prevents constipation, regulates blood glucose

3.5 Oat β-glucans: postprandial- and long-term metabolic effects

β-glucans are viscosity-forming dietary fiber found in high amounts in oats and barley, and comprise a group of fiber molecules that vary in molecular weight and water solubility (77, 78). β-glucans have been shown to reduce acute postprandial glucose responses and lowering blood cholesterol levels in controlled intervention studies (79, 80). Such effects may aid long-term health.

The well-established beneficial effects on short-term blood glucose levels and long-term blood lipids have led to authorized health claims. According to European Food Safety Authority (EFSA), consuming 4 grams of oat or barley β-glucan per 30 grams of available carbohydrates can reduce postprandial glucose responses, while 3 grams of β-glucan is sufficient to lower blood cholesterol in individuals with slightly elevated cholesterol levels (15). However, the evidence of the beneficial postprandial glucose concentrations is primarily based on acute, short-term intervention studies performed under controlled conditions (81, 82). Large scale intervention studies investigating the effectiveness of β-glucans in a real-world situation are lacking. Most of the existing studies have been performed in individuals with manifest type 2 diabetes and not among those with high risk of developing type 2 diabetes, and studies on the effect on long-term glucose control such as glycated hemoglobin (HbA1c) are inconclusive (81-84).

The beneficial effects of β-glucan are not uniform but depends on molecular weight and water solubility, and food processing can affect these properties (78, 85, 86). Research has explored various food matrices to determine the most effective delivery vector of β-glucans, with bread (solid form) and porridge (semi-solid form) recognized as particularly promising. These forms have been found to enhance the functional benefits of β-glucans, making them ideal vectors for maximizing the health benefits, such as improved cholesterol levels and glycemic control (85). A recent systematic review and meta-analysis concluded that bread enriched with fibers such as β-glucan or whole grains, has the potential to improve glycemic control primarily among individuals with type 2 diabetes (87).

3.6 Glycemic Index

Another key aspect of carbohydrate quality related to cardiometabolic risk is the Glycemic Index (GI) and the related Glycemic Load (GL). The GI concept was developed in 1981 as a tool to estimate the impact of a food or meal on glucose regulation (88). GI takes into consideration factors such as dietary fiber content, added sugar, the ratio of starch to sugar, and the liquid to solid ratio to present the glycemic potential per gram of carbohydrate (67).

The GI of a food item is determined by measuring postprandial plasma glucose excursions following the consumption of a standardized amount (usually 50 gram) of the

food, compared to the glucose excursions from consuming 50 gram of pure glucose, measured over the first 120 minutes post-consumption (16, 89) (**Equation 1**).

$$GI_{food\ item} = \frac{(area\ under\ the\ curve, plasma\ glcose\ concentration_{50\ g\ food\ item)}}{(area\ under\ the\ curve, plasma\ glucose\ concentration_{50\ g\ glucose})} \times 100$$

Equation 1. Calculation of GI of a food item

Low GI foods such as dairy products, legumes, pasta, and lentils result in lower postprandial increases in plasma glucose concentrations while high GI foods such as potatoes produce larger postprandial increases in plasma glucose concentrations (90). Low GI foods are often categorized as ≤ 55, medium 56-69, while high GI foods are categorized as ≥ 70 (91). To date approximately 4000 food items have been categorized according to GI (90). Several factors determine the GI of a food including type of carbohydrate, as well as content of protein, fat and quantity and type of fiber, food particle size, and pH (92). Glycemic load was introduced as a means of reflecting total glycemic burden of a food with a certain GI. GL considers the GI and the amount of available carbohydrates in proportion of the food eaten (GL = GI × available carbohydrate in a given amount of food).

Postprandial glycemia may contribute as much as fasting blood glucose levels to the pathogenesis of impaired insulin sensitivity and insulin secretion seen in the progression towards type 2 diabetes (93, 94). Studies have linked high GI diets, due to the high postprandial spikes, to adverse health outcomes, including increased risk of type 2 diabetes, CVD, and obesity (95). The rapid postprandial glucose spikes associated with high GI foods contribute to insulin resistance, dyslipidemia, and chronic inflammation, key factors in the pathogenesis of cardiometabolic diseases (96). On the contrary, low GI foods, that result in smaller postprandial glucose excursions, are associated with improved blood glucose control, improved insulin sensitivity, and reduced risk of cardiometabolic conditions (95). However, not all studies have reported such benefits. For example, a review examining the relationship between GI and disease risk found no significant association between the GI of foods and disease outcomes (97). While acute effects of low GI foods have generally been demonstrated, data on long-term consumption of low GI diets in non-diabetic populations are limited, particularly regarding their impact on glycemic control and other cardiometabolic risk factors (98).

As mentioned above, over 4,000 foods have been categorized according to their GI values, but it still remains unclear how GI interacts with healthy lifestyle patterns, such as the Mediterranean diet on cardiometabolic disease risk. Understanding the role of GI in the context of a healthy diet patterns could help refine dietary advice to individuals at elevated risk.

3.7 Gut microbiota, carbohydrates and cardiometabolic health

The gut microbiota has emerged as an important factor influencing cardiometabolic diseases and appears to play a role in metabolic disorders, immune function, and overall cardiometabolic health (99-101). The gut microbiota includes bacteria, archaea, fungi and viruses with large impact on our health (102). Studies have established that gut microbiota composition in healthy individuals differs significantly from that in individuals with obesity (103) and also differs between individuals with type 2 diabetes and healthy individuals (104). Microbial diversity, often assessed by indices such as the Shannon, Simpson and Chao1 has been reported to be lower among individuals with type 2 diabetes (105). The Shannon index accounts for both richness i.e., number of species and evenness i.e., abundance distribution (106), while the Simpson index emphasizes the dominance of particular species within the microbial community (107). The Chao1 index is an estimator of species richness, providing an estimate of total number of species, including those present in low abundance (108). Microbial diversity has been reported to be lower among those with type 2 diabetes, with specific bacterial genera such as *Akkermansia* and *Faecalibacterium* consistently being reported less abundant in type 2 diabetes patients. Conversely, potentially harmful bacteria like *Ruminococcus* and *Escherichia* have been found to be more prevalent in people with type 2 diabetes (109, 110). Such alterations in the gut microbiota have been linked to metabolic dysfunctions, including insulin resistance and chronic inflammation, which are central to type 2 diabetes pathogenesis (109, 110).

Many studies investigating the role of gut microbiota on metabolic health are observational and often of cross-sectional design, which may not infer causality (111-113). This is a major limitation that makes it difficult to determine whether alterations in gut microbiota represent a cause or consequence of metabolic dysfunction (114). Prospective, longitudinal studies and randomized controlled trials are needed to establish clearer causal relationships.

3.8 Microbial metabolites and cardiometabolic health

Gut microbiota may mediate effects on human health in different ways. One way is via production of metabolites with adverse or beneficial effects on health and disease that are influenced by dietary intake (113, 115). Several metabolite classes have been identified as important mediators of gut microbial activities on health outcomes (22).

Trimethylamine N-oxide (TMAO) is one such gut derived metabolite that has been linked to increased CVD risk, and all-cause mortality (116, 117). Dietary precursors to TMAO are animal-derived foods such as red meat, egg yolk and full fat dairy products. TMAO is also found naturally in fish and certain types of seafood (118). Studies suggest that circulating TMAO is an important link to vascular damage and adverse cardiovascular outcomes (119).

Bile acids represent another group of compounds that provide a link between gut microbiota, diet and health. When a high fat meal is consumed, bile acids are released into the digestive system to aid in the digestion of lipids as well as absorption of cholesterol and fat-soluble vitamins (120). Most bile acids are reabsorbed in the ileum and transported back to the liver via portal blood circulation to inhibit bile acid synthesis (121). However, bile acids can also be transformed into secondary bile acids by different gut microbiota, thereby modifying the composition of the total bile acid pool (122). Some of these secondary bile acids are not reabsorbed but excreted in stool, subsequently this will enhance bile acid neo-synthesis in the liver and result in lowering low-density lipoprotein concentration (123).

Fermentable dietary fibers, including resistant starch, play an important role in modulating gut health by influencing microbial composition and promoting the production of beneficial metabolites such as short-chain fatty acids, which are modulators of metabolic health (124). Short-chain fatty acids are the main end products of the fermentation of non-digestible carbohydrates by gut bacteria (124). The three main SCFAs are acetate, propionate and butyrate and they are typically found in molar ratio of 3:1:1, respectively (125, 126). Approximately 5-10 % of the gut microbiota metabolites are excreted in feces (127, 128). Acetate is the most prevalent SCFA in peripheral circulation, with venous concentrations ranging from 98 to 143 μmol/l, while venous concentrations of propionate and butyrate are significantly lower. Their typical concentrations range 3.8 to 5.4 μmol/l and 0.5 to 3.3 μmol/l, for propionate and butyrate respectively (129, 130).

Although each of the three SCFAs has different functions and pathways in the host metabolic system, they all help lower colonic pH, which inhibits the growth of potentially pathogenic bacteria (73). The potential health effects of SCFA on the host are various. Butyrate serves as the main energy source for colonocytes and is primarily metabolized by the colonic epithelium and are thereby an important substrate for the intestinal epithelium (113, 130). Propionate can serve as a precursor for intestinal gluconeogenesis while both propionate and butyrate induce gluconeogenic enzymes (22, 127). Acetate has been suggested to suppress appetite through central mechanisms (22). Furthermore, SCFA have been suggested to play a role in regulation of glucose homeostasis by enhancing insulin secretion from the pancreas and gluconeogenesis in the liver and skeletal muscles, as well as reducing plasma glucose and cholesterol concentrations (131).

After the non-digestible carbohydrates have been depleted, gut microbiota use protein for fermentation, and it is estimated that approximately 12-18 g of protein reach the large intestine daily (132, 133). Excess protein are fermented into several different metabolites such as SCFA, branched-chain fatty acids, amines and indoles (134). However, these

metabolites have heterogeneous effects on host health. Some are beneficial to human health and others contribute to pathophysiology of diseases (135). Conversely, Indole-3-propionic acid (IPA), is produced by intestinal bacteria from tryptophan and has been associated with lower risk of developing type 2 diabetes (136). The possible role of IPA in the development of type 2 diabetes is suggested to be its protective effect by preservation of β-cell function (137). After being produced in the gut by specific bacteria through the reductive metabolism of tryptophan, IPA enters the bloodstream directly from the gut and IPA serum concentrations typically ranges from 1 to 10 µmol/L (138).

SCFAs and IPA play important roles in cardiometabolic health, with diet being one of the strongest modulators of their production (22, 137). These metabolites improve gut barrier integrity, regulate glucose and lipid metabolism, modulate the immune system, control inflammatory responses, and influence blood pressure (139) (**Figure 1**). However, most of the research investigating the role of SCFA and IPA on glycemic health has been performed on animal model or observational studies and there is a need for randomized controlled trial to further investigate the associations with host health.

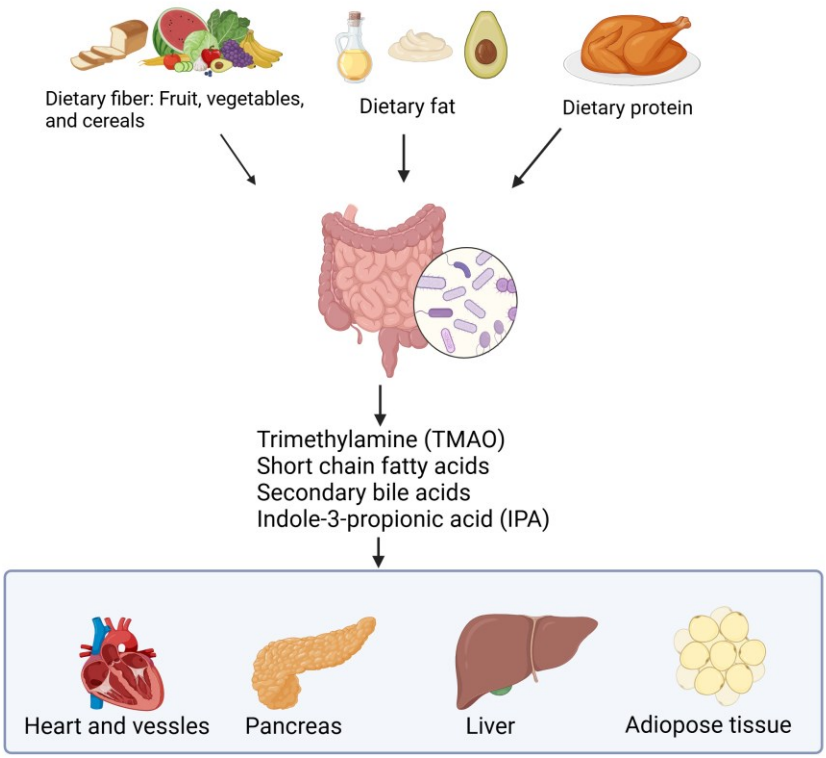

Figure 1. The influence of diet and gut microbiota-derived metabolites and their influence on host health. Modified from Schroeder and Bäckhed (22). Created in BioRender. Hjorth, T. (2024) https://BioRender.com/b83b286

3.9 Precision nutrition and cardiometabolic health

Current dietary recommendations are primarily designed to promote health, prevent nutrition deficiencies, and reduce chronic disease risk at a population level (58, 140, 141). Policies and dietary guidelines are aimed at the general population with specific guidelines to groups that are of risk of deficiencies, including children, pregnant women, elderly, and individuals with chronic illnesses, to ensure nutritional needs are met, with country specific variations (58). The population-based approach of dietary guidelines comes with some challenges. This includes the fact that adherence to dietary recommendations among the public is generally low (23, 142), Furthermore, generalized guidelines do not account for acute metabolic responses or the large individual variability in response to diet due to including genetic, metabolic, and gut microbiota differences (143).

Personalized nutrition, or precision nutrition, may offer a more effective approach to reach both improved adherence and to take inter-individual differences into account to improve effectiveness of advice. Some studies have, for example, shown increased adherence and more effective change in diet followed upon personalized approaches (144, 145). The concepts of personalized- and precision nutrition are often used interchangeably and there is no consensus on terminology although attempts have been made (146). Precision nutrition aims to create individualized dietary recommendations based on biological principles. It incorporates genomic data, microbiome analysis, and metabolomics to address molecular-level differences. Beyond biological factors, it also considers personal preferences, lifestyle, environmental influences, and general health data, such as gender, age, and body weight, to recommend the right diet for the right person at the right time (147, 148). In this thesis, the term precision nutrition is used, as it implies a broader approach that includes improved dietary assessment and tailoring diets for both individuals and groups of individuals.

Personalized diet plans as part of precision nutrition have often been based on individual's postprandial blood glucose, blood lipid, and gut microbiome responses. Such personalized diet plans have been shown to significantly improve triglycerides, body weight, waist circumference and HbA1c compared to standard dietary advise (149). An increased number of studies indicate that postprandial metabolic responses are of major importance for long-term health and thus, may be important targets to be taken into account for dietary guidelines (150, 151).

Individuals exhibit a wide variation in their responses to different foods, with significant inter-individual variation in postprandial plasma glucose (24, 151), triglycerides (152) and insulin responses (153). Determinants of responses can be attributed to metabolic profile such as body weight, sex, and genetics (143). Additionally, circadian rhythms, which refers the body's internal clock, may influence how dietary intake impacts health

outcomes, a field known as chrono-nutrition that has emerged as part of precision nutrition (154). Lifestyle choices such as smoking, physical activity, stress, and the composition of the gut microbiome also play significant roles in modulating individual dietary responses (146, 147, 155) (**Figure 2)**. Given the considerable inter-individual variability, tailoring dietary advice to individual needs could potentially enhance the effectiveness of public health strategies (156).

Precision nutrition has not yet been implemented in the prevention of diseases at larger scale or included in official dietary guidelines, as the field is still in its infancy, despite a surge of research over the past 10 years (147, 157). More research is needed to establish effectiveness and efficacy of precision nutrition as well as efficient strategies for implementation of personalized guidelines. Recent studies indicate that the currently recommended healthy dietary patterns provide benefits across the general population, although the magnitude of these benefits may vary based on individual susceptibility (158). Although precision nutrition is still in its early stages, it has potential to complement current dietary recommendations. Understanding individual variations in metabolic responses to meals with particular emphasis on the postprandial phase might play an important role in determining personalized guidelines for optimal health.

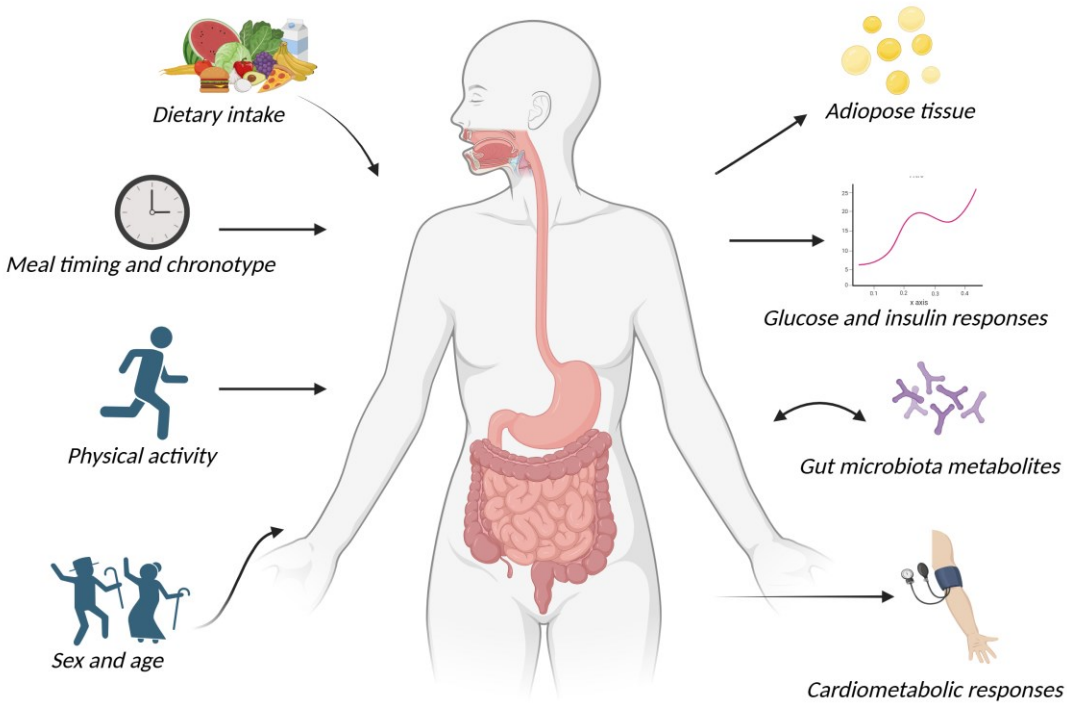

Figure 2. Key factors that may affect individual variability in responses and diet-health outcomes. Created in BioRender. Hjorth, T. (2024) https://BioRender.com/b12j061

3.10 The postprandial phase and personalized guidelines

Clinical studies have traditionally focused on assessment of hormonal- and metabolic responses during fasting. However, the postprandial state, i.e. the period after a meal, has gained attention, as most individuals spend most of their day in that state (159). The typical duration of the postprandial phase depends on the composition of the meal: 2-3 hours for carbohydrate-rich meals, 3-5 hours for mixed meals, and up to 8-10 hours for fat-rich meals. As a result, most people remain in a postprandial state for nearly all waking hours (159). The postprandial response has been shown to be crucial in identifying metabolic dysfunctions, as static fasting measurements fail to capture the dynamic processes that occur after consuming a meal.

The postprandial state has been linked to an increased risk of both cardiovascular disease and type 2 diabetes (25, 26, 93). Zeevi et al conducted the first large hallmark study in the field of precision nutrition were postprandial glucose concentrations where predicted in response to different foods (24). They used an algorithm where they included parameters such as anthropometrics, dietary habits, physical activity, and gut microbiota to predict differential responses. It was concluded that there was high interpersonal variability in glucose response to the same meals and that this response was associated with the different parameters and that it was predictable based on some basic key information provided by the individual (24). They also used the algorithm to categorize food as ‘good’ or ‘bad’ based on individual responses, and foods classified as ‘bad’ for some individuals were classified as ‘good’ for others, demonstrating the significant inter-individual variation.

The personalized responses to dietary components (PREDICT 1) (160) clinical trial were designed to quantify and predict individual variations in postprandial triglyceride, glucose and insulin responses to standardized meals. In total, 1002 individuals were enrolled in the PREDICT 1 trial, data on genetics, metabolic, microbiome-composition, meal-composition and meal-context data was collected to distinguish predictors of individual responses to meals. It was concluded that significant differences were observed in how individuals responded to identical meals in terms of triglycerides, glucose and insulin levels. The study concluded that even identical twins exhibit different responses to the same meals. These findings support the potential for personalized nutrition strategies to optimize health outcomes (160).

3.11 Efficacy vs Effectiveness

Assessing outcomes from dietary interventions may consider efficacy or effectiveness. Efficacy refers to how well an intervention works under ideal, controlled conditions within a specific, well-defined population. In contrast, effectiveness measures how the same intervention performs in more diverse populations under every day, real-world contexts

(161, 162). Although efficacy and effectiveness studies are both important for evaluation of interventions, they serve distinct purposes and have different study designs. While assessment of efficacy maximizes the likelihood of observing an intervention effect under controlled conditions, effectiveness research considers external factors such as individual dietary habits, environmental influences, and broader lifestyle factors that may moderate the intervention's effect in real-world settings (163). As a result, effectiveness research may be more relevant to policymakers (164). Studies have consistently demonstrated a gap between the outcomes observed in intervention studies under controlled settings and those in real-world environments (161). This gap has been documented across multiple domains, including dietary change, tobacco cessation, and physical activity interventions (165, 166).

For instance, while RCTs have shown that whole grains can improve glycemic control and reduce the risk of type 2 diabetes under ideal conditions, the real-world effectiveness can be influenced by variables such as the type of whole grain consumed, the overall diet, and adherence to dietary guidelines. Although whole grains offer benefits, their impact may be less significant in diverse populations with varying dietary habits (66). Similarly, β-glucans have been demonstrated to have clear physiological effects of relevance to health in controlled settings (167). However, in everyday conditions, factors such as food processing, cooking methods, and individual dietary practices including spreading the dose over the day may diminish their effectiveness, but such conditions have rarely been studied (78).

In summary, while efficacy studies are critical for evaluating interventions under ideal conditions, their limitations in terms of external validity should be more widely acknowledged. Effectiveness studies, which assess interventions in real-world settings, provide a necessary complement but have surprisingly rarely been reported for dietary interventions. Both designs offer unique insights that contribute to a fuller understanding of an intervention’s potential impact across different populations and contexts (163).

4 METHODS AND DATA

4.1 Hypotheses and research strategies

In this thesis it is hypothesized that different dimensions of carbohydrate quality can influence cardiometabolic outcomes in individuals at elevated risk of cardiometabolic disease. The two dimensions of carbohydrate quality at focus in this thesis are glycemic index, and dietary fiber. This thesis not only investigates the overall impact of carbohydrate quality on cardiometabolic risk markers but also examines health effects associated with carbohydrate quality in relation to gut microbiota, gut microbiota metabolites, and their relevance within the context of precision nutrition.

Specific hypotheses

I. It was hypothesized that long-term consumption of low glycemic index foods would reduce postprandial insulin and glucose responses more effectively than high glycemic index foods and improve markers of glycemic variability and insulin sensitivity.

II. It was hypothesized that a mechanistic model can effectively identify differential glucose responders following a mixed meal tolerance test and that these responses are associated with type 2 diabetes risk factors and gut microbiota composition.

III. While gut microbiota and their associated metabolites have been extensively studied, the association between postprandial gut microbiota fermentation products and long-term metabolic outcomes remains unexplored. Therefore, it was hypothesized that postprandial gut fermentation metabolite concentrations, in response to standardized meals, are associated with changes in type 2 diabetes risk factors. Additionally, it was hypothesized that long-term consumption of a Mediterranean diet increases these postprandial metabolite concentrations, positively influencing metabolic health outcomes.

IV. Whole grains and dietary fiber have consistently been associated with a lower risk of type 2 diabetes. Dietary fiber β-glucan have been shown to improve postprandial glucose responses and blood lipid concentrations in acute settings. Therefore, it was hypothesized that long-term consumption of β-glucan-enriched bread significantly improves key risk markers of type 2 diabetes, such as HbA1c, under real-life conditions.

To investigate these hypotheses, data from two large multicenter, international, parallel group dietary intervention trials were used. In the first study, the effect of the relative contribution of glycemic index in a healthy dietary pattern in individuals with the metabolic syndrome was investigated. This trial resulted in **Paper I**, **II**, and **III**. In the second study, a large multicenter, international, parallel group, pragmatic, dietary intervention trial investigating the effectiveness of β-glucan among individuals at risk of developing type 2 diabetes was conducted. This trial resulted in **Paper IV**. An overview of the studies as well as their links to the specific objectives are presented in **Table 2**.

Table 2. Overview of the two studies included in the thesis

	The MEDGI-Carb study (Papers I, II and III)	**The CarbHealth study (Paper IV)**
Design	12 weeks, 2-armed, single-blind, parallel, randomized, international multicenter, n = 213	16 week, 2-armed, double-blind, parallel, randomized, international multicenter, n = 194
Inclusion criteria	Males and females, 30-69 years BMI 25-37 kg/m^2, Waist circumference > 102 cm (males), > 88 cm (females) + trait from the metabolic syndrome; high blood pressure, elevated fasting plasma glucose, or dyslipidemia	Males and females, 40-70 years, BMI ≥ 27 kg/m^2, HbA1c 35-50 mmol/mol
Intervention	High vs low glycemic index carbohydrates in a context of a healthy Mediterranean diet	β-glucan enriched bread vs a whole grain wheat bread
Primary outcome	Postprandial insulin and glucose response	HbA1c
Secondary outcomes	Indices of glycemic variability. Fasting glucose and insulin, HbA1c.	Fasting glucose and insulin, blood lipids, fatty liver index, hepatic steatosis index, indices of insulin sensitivity.
Thesis objective(s) and hypotheses responded to.	It was hypothesized that low GI foods could improve postprandial glucose and insulin response as well as risk markers of cardiometabolic diseases. Furthermore, it was hypothesized that differential responders could be identified from a MMTT using a mechanistic model. Postprandial gut microbiota metabolites were hypothesized to be associated with glycemic control and risk factors of type 2 diabetes. The study responded to thesis objective A, B and C	It was hypothesized that the dietary fiber β-glucan could improve long-term glycemic control. The study responded to the thesis objective A, B and D.

BMI, body mass index; HbA1c, glycated hemoglobin; MMTT, mixed meal tolerance test

4.2 Study designs

Data from the MEDGI-Carb trial (168) was used to investigate the relative contribution of GI on postprandial glycemia and glycemic variability in a context of a healthy Mediterranean diet over a twelve week dietary intervention period (**Paper I**). The MEDGI-Carb trial was also used to identify differential responders of postprandial glucose in response to standardized meals served during test days at baseline and post-intervention (**Paper II**). Furthermore, the MEDGI-Carb trial was used to examine how plasma concentrations of short chain fatty acids and indol-3-propionic acid were influenced by a high-fiber Mediterranean diet, their association with changes in cardiometabolic risk factors and glycemic control, and the relationship with different gut microbiota genera (**Paper III**).

Data from the CarbHealth study (169) was used to investigate the effectiveness of regular consumption of an oat-derived β-glucan-enriched bread as part of a habitual diet on glycated haemoglobin (HbA1c), measures of glycaemic control and blood lipids in comparison to a wholegrain wheat bread in adults at risk of type 2 diabetes (**Paper IV**).

4.2 Experimental design

MEDGI-Carb

The MEDGI-Carb trial was an international, multi-center, randomized, parallel-group study conducted over 15 weeks in adults with components of metabolic syndrome i.e., at risk for development of diabetes. During the intervention period, participants followed a Mediterranean-style healthy eating pattern, weight-maintenance diet, consuming either a low-GI or high-GI diet with specific foods. During baseline and post-trial, diet, general health sleep quality, fecal microbiota profile and blood pressure were assessed. Outcome measurements were obtained during testing days to determine glucose homeostasis by completion of an 8-hour mixed meal tolerance test (MMTT) including breakfast and lunch with intervention specific foods, an oral glucose tolerance test (OGTT), and continuous glucose monitoring at baseline, mid-point (only in USA) and post-intervention (**Figure 3.** Study schematics of the MEDGI-Carb trial). All participants were instructed to maintain levels and types of physical activity during the intervention. The primary outcome was postprandial insulin concentration; secondary outcomes include fasting plasma glucose and insulin, HbA1c, and indices of postprandial glycemia and glycemic variability.

The study was conducted at three centers: (i) Federico II University, Naples, Italy, (ii) Chalmers University of Technology, Gothenburg, Sweden, and (iii) Purdue University, West Lafayette, IN, USA. The study was initiated in January 2018 and the last participant finished the trial in December 2019.

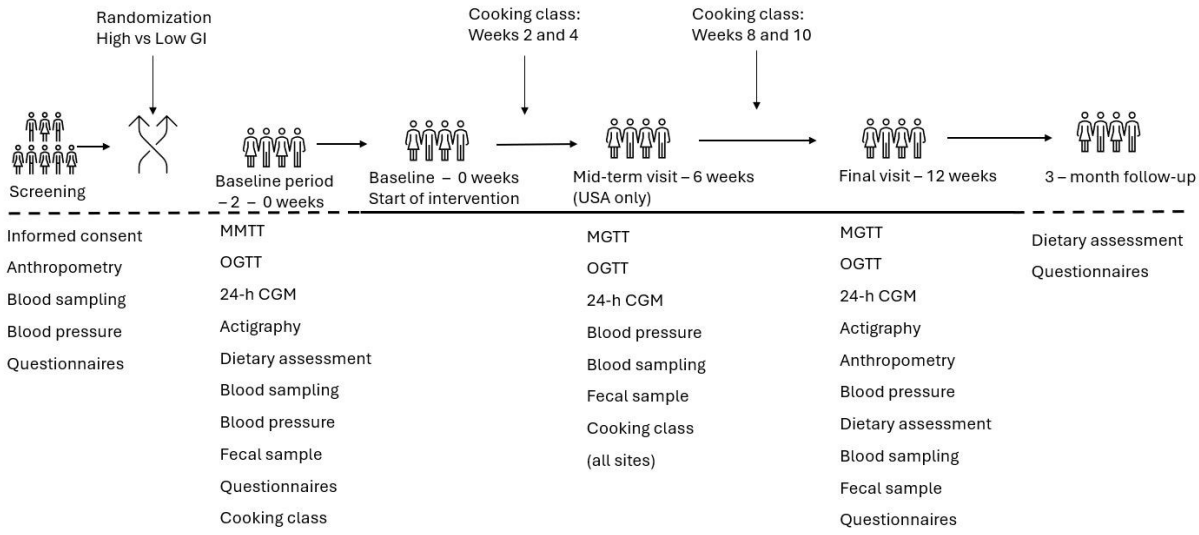

Figure 3. Study schematics of the MEDGI-Carb trial, a randomized, single-blinded, controlled trial assessing the effects of low- vs high glycemic index in the context of a Mediterranean-style healthy eating pattern. CGM, continuous glucose monitoring; MMTT, meal glucose tolerance test; OGTT, oral glucose tolerance test. Blood samples include complete metabolic panels and lipids. lipoprotein profile: questionnaires include the International Physical Activity Questionnaire, Health and Well-being (SF-36v2) Questionnaire, Pittsburgh Sleep Quality Questionnaire, Epworth Sleepiness Scale, and the Mediterranean Diet Assessment.

CarbHealth

The CarbHealth trial was a multicenter, double-blind, randomized, controlled 16-week dietary intervention conducted in participants with HbA1c concentrations ranging from 35 to 50 mmol/mol at screening. The CarbHealth study aimed to investigate the effectiveness of an oat- β-glucan enriched bread vs a whole-grain wheat control bread as part of a habitual diet in glycemic control in adults at high risk of developing type 2 diabetes. Throughout the 16-week intervention period, participants in each group were instructed to consume three to six slices of pre-sliced intervention or control bread daily, for at least six days per week, replacing their habitual bread. The study breads were specially developed and produced for the trial by the Norwegian Institute for Food, Fisheries, and Aquaculture Research (NOFIMA).

Outcome measurements were obtained at baseline, 8 weeks, and 16 weeks. The primary outcome was the difference in HbA1c between the diets after 16 weeks. Secondary outcomes included changes in fasting capillary glucose, serum insulin, blood lipids, anthropometric measures, fatty liver index, hepatic steatosis index, indices of insulin sensitivity, and consumer acceptance (**Figure 4**. Study schematics of the CarbHealth trial).

The study was conducted at four universities across three countries: (i) University of Bergen, Bergen, Norway; (ii) Chalmers University of Technology, Gothenburg, Sweden; (iii) Paderborn University, Paderborn, Germany; and (iv) Leipzig University, Leipzig, Germany. Recruitment for the study started in July 2021, and the last participant completed the trial in September 2023.

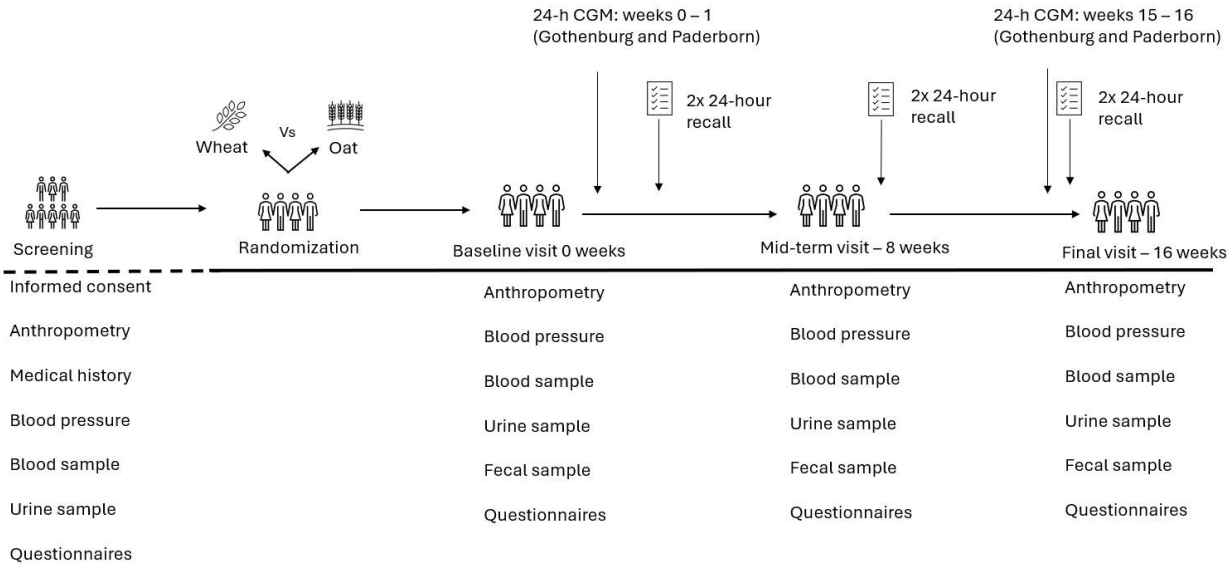

Figure 4. Study schematics for the CarbHealth study, a randomized, double-blinded, controlled trial assessing the effectiveness of a β-glucans enriched bread vs a whole-grain wheat control bread as part of a habitual diet in glycemic control. CGM, continuous glucose monitoring. Blood panels include glycemic measurements and blood lipids: questionnaires include the International Physical Activity Questionnaire, Health and Well-being (SF-36v2) Questionnaire. Figure adapted from Hjorth et al 2022 (169) under CC BY-NC 4.0 license.

4.3 Study populations

MEDGI-Carb

The eligibility criteria were designed to select middle-aged and older adults at risk of developing CVD or type 2 diabetes. Adults with a waist circumference >102 cm (males) or >88 cm (females), along with one additional feature of Metabolic Syndrome were considered eligible. These features included blood pressure >130/85 mmHg or use of antihypertensive medication, fasting plasma glucose levels between 100–126 mg/dL, fasting triglycerides between 150–400 mg/dL, HDL cholesterol <40 mg/dL (males) or <50 mg/dL (females). Other inclusion criteria were age 30–69 years, BMI 25–37 kg/m^2, stable weight (±3 kg in the last three months), no acute illness, no cardiovascular events (myocardial infarction or stroke) in the six months preceding the study, haemoglobin >12 g/dL, and no renal or liver failure (creatinine <1.7 mg/dL and ALT/AST <2 times the normal values). Exclusion criteria were as follows; diabetic, pregnant, lactating, vegetarian, on a diet incompatible with the intervention, smoking more than 20 cigarettes per day, or

engaging in a stable intensive physical activity regimen (>3 hours/week of moderate or high-intensity resistance or aerobic exercise). Participants using antihypertensive or statin medications were instructed to maintain their current type and dosage throughout the study and to notify the research investigators of any changes that occurred.

CarbHealth

The eligibility criteria were designed to select adults at an elevated risk of developing type 2 diabetes. Individuals aged 40–70 years with a body mass index of ≥ 27 kg/m^2 and HbA1c levels between 35 and 50 mmol/mol were eligible to participate. The exclusion criteria included: a diagnosis of type 1 diabetes or pharmacologically treated type 2 diabetes, fasting blood glucose levels above 7.0 mmol/l, non-fasting blood glucose levels above 11.1 mmol/l, urine glucose exceeding 180 mg/dl, or protein excretion in urine. Additional exclusions applied to those with food allergies preventing the consumption of the study breads, pregnancy, lactation, or plans to become pregnant during the intervention period. Individuals with systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 100 mmHg, a history of gastrointestinal issues, myocardial infarction, heart failure, stroke, or cancer within three years prior to screening, as well as a history of alcohol abuse, were also excluded. Furthermore, the use of antidiabetic medications at the time of screening or initiation of such medication during the trial was reason for exclusion.

4.4 Intervention diets

MEDGI-Carb

The trial started with a 3-week baseline period where participants consumed their habitual diet, followed by a 12-week controlled dietary intervention. During the intervention period, participants were instructed to follow an individualized, iso-caloric, Mediterranean-style diet using prescribed menus. Both groups consumed the same amount of metabolizable carbohydrates (270 g/day) and dietary fiber (35 g/day), but half of the carbohydrate intake was group-specific, based on GI values (High GI ≥70, Low GI ≤55). Modulation of daily energy intake was achieved by adjusting intakes of protein and fat. Participants were given selected food items to use for their meals (high-GI jasmine rice, potato, mashed potatoes, couscous, whole grain bread, and rusks; low-GI pasta, brown rice, flatbread, all bran, and wheat plus rye bread and seeds) to improved adherence to the diets.

CarbHealth

Participants were instructed to consume 3-6 slices of intervention bread per day for six days a week while maintaining their habitual diet and physical activity levels. No strict dietary guidance was given, allowing participants flexibility in how they incorporated the bread into their day, whether as part of a meal or a snack. The intervention bread provided 286 kcal, 16.6 g dietary fiber, and 6.0 g of β-glucan per three slices, while the control

bread provided 244 kcal and 5.0 g dietary fiber with only 0.02 g β-glucan. The intervention bread was matched in starch and fat content to the control bread, with the β-glucan having a molecular weight of approximately 1000 kDa. Dietary intake was measured through six 24-hour recalls spread across the study period.

4.5 Dietary assessments and adherence

MEDGI-Carb

Assessment of dietary intake during the intervention differed slightly between the study sites and has previously been described in detail of the MEDGI-Carb trial (168). In brief, dietary intakes were assessed by four-day food records (three weekdays, and on weekend day) at baseline, at week 4, week 8 (Italy only), week 12 and post intervention in Sweden and Italy. In the United States center, intake was assessed via three-day dietary recalls on non-consecutive days during baseline weeks and 3 months after completing the intervention. There were also center specific procedures on measuring compliance and adherence to the intervention diets. At the Italian center, diet adherence was tracked through four-day dietary records at weeks 4, 8, and 12, with support from dietitians via bi-weekly counseling and weekly phone calls. Compliance was calculated as a percentage of starchy foods consumed relative to the prescribed amount, with partial credit for incomplete consumption. Swedish center: Adherence was monitored using four-day dietary records (at weeks 4 and 12) and daily menu compliance checklists. Participants checked off consumed items and noted deviations. Compliance was calculated as a percentage of all food items consumed, with partial credit for incomplete consumption. At the US-center, compliance was assessed through daily menu checklists and weekly reviews of online grocery orders. Participants weighed portions, checked off consumed items, and noted percentages for incomplete consumption. Menu booklets were reviewed mid-point and post-study, and compliance was calculated as the percentage of items consumed, with partial credit for incomplete ingestion.

CarbHealth

For the CarbHealth study (169), dietary assessments were conducted using 24-hour dietary recalls with country-specific food composition data. The method varied slightly between centers: Bergen, Paderborn, and Leipzig: Six unannounced 24-hour recalls (weeks 0–2, 7–9, and 15–16) were conducted using *myfood24*, which is based on the German and Norwegian food composition databases for respective countries. Gothenburg: Since *myfood24* is unavailable for Swedish data, six 24-hour recalls were done at the study site and by phone. Portion sizes were estimated using images or kitchen measures. Nutrient composition was determined using *DietistNet Pro*, based on the Swedish Food Composition Database. During the intervention period, participants recorded bread consumption by ticking the numbers of slices consumed each day in a pre-coded journal.

4.6 Clinical examinations

MEDGI-Carb

Participants underwent clinical examinations and measurements of anthropometrics, including blood pressure, at baseline, week 6 (in the USA only), and week 12. Blood samples were collected for the analysis of HbA1c, insulin, glucose, HDL-C, LDL-C, and triglycerides. Markers of glucose homeostasis were assessed through an eight-hour mixed meal tolerance test (MMTT), an oral glucose tolerance test (OGTT), and six days of 24-hour continuous glucose monitoring (CGM) at both baseline and post-intervention. Insulin sensitivity indices, including QUICKI, Stumvoll, and Matsuda indices, were calculated using OGTT data. Follow-up data on eating patterns, sleep quality, general health, and well-being were collected via email or regular mail three months after the completion of the 12-week intervention period.

CarbHealth

Clinical study visits involved collection of blood, fecal and urine samples, measurements of blood pressure, and anthropometry at baseline, mid-point (8-weeks), and post-intervention. Blood samples were collected for the analysis of HbA1c, insulin, glucose, ALT, AST, creatinine, Gamma-Glutamyl Transferase, HDL-C, LDL-C, and triglycerides.

4. 7 Continuous glucose monitoring

MEDGI-Carb

Medtronic iPRO2 Professional continuous monitoring devices (CGM) (Northridge, CA, USA) were used to measure 24-hour interstitial glucose concentrations at 5-minute intervals for seven days during baseline and post-testing weeks. The data was then used for the calculation of glycemic variability, including metrics such as mean amplitude of glucose excursions (MAGE), continuous overall net glycemic action (CONGA), mean absolute glucose (MAG), and the lability index.

CarbHealth

CGM data from the CarbHealth trial was not used in this thesis and will only be described briefly here but are described in detail elsewhere (169). In the CarbHealth trial CGM data was measured for seven days a baseline and study week 1 and study week 15 to study week 16 at the sites in Gothenburg and Paderborn. CGM data was collected to analyze glucose peaks, mean glucose levels, the coefficient of variation, total area under the curve, and differences in postprandial responses to morning and evening meals between the two breads, as well as to examine whether individual chronotype influences bread consumption and metabolic health

4.8 Postprandial assessments in the MEDGI-Carb trial

Visits for clinical assessments at baseline and post-intervention included an 8-h MMTT (breakfast and lunch) and 2-h OGTT. For the OGTT and MMTT, fasting blood samples were collected at -15 minutes and -5 minutes timepoint (TP). The 75 g glucose drink (OGTT) or the breakfast test meal (MMTT) was consumed at TP 0. During the OGTT, blood samples were collected 60 minutes and 120 minutes after consumption of the glucose beverage. Subjects were not allowed additional fluid during the test. The breakfast test meal was consumed at TP 0, and consumed in two parts, the participants had 7.5 minutes to consume the first part and 7.5 minutes to consume the last part, to control the pace of the meal consumption. The participants were allowed to drink eight ounces of water (approx. 2.4 dL) during the meal. Blood samples were collected at TP 15 after the breakfast test meal and then at TP 30, TP 45, TP 60, TP 90, TP 120, TP 180, and TP 240. A standardized lunch meal was served at TP 240, again with 7.5 minutes to consume the first half and 7.5 minutes to consume the last part. The blood sampling followed the same pattern as after breakfast (**Figure 5**). The test meals were standardized over the three centers. Due to practical reasons, all participants were served the same portion size i.e., kilocalories, regardless of energy requirement for practical reasons.

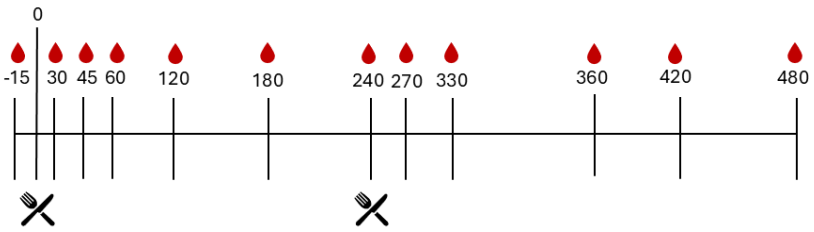

Figure 5. Schematics of blood sampling during the MMTT. Standardized meals were served at 0 and 240 minutes.

4.9 Gut microbiota composition and gut microbiota derived metabolites

MEDGI-Carb

During pre- and post-intervention days, participants collected fecal samples at home using a EasySampler Stool Collector. After the sample was collected at home it was stored at -20°C and transported to the clinic in a cooling box with an icepack within 72 hours. At the clinic the sample was transferred to -80°C within 24 hours and thereafter stored in the study biobank until the study was finalized. The samples were analyzed using 16S rRNA gene amplicon sequencing at the Swedish University of Agricultural Sciences, Uppsala. A comprehensive description of the gut microbiota analysis and subsequent data processing is provided by Iversen and Dicksved (170). Briefly, DNA was extracted from fecal samples, and the V3-V4 region of the 16S rRNA genes was amplified using specific primers to generate sequencing libraries. Amplicon sequence variants were then compared against reference databases, and the microbiota data was aggregated to the genus level prior to analysis

In addition to gut microbiota analysis, four microbial compounds were measured: three short-chain fatty acids (acetate, propionate, and butyrate) and one tryptophan metabolite, IPA. These compounds were analyzed in heparin plasma samples collected during the MMTT, with twelve blood samples collected per individual on each occasion, totaling twenty-four samples. Full details of the SCFA and IPA analysis methods are described by Fristedt et al (171). In brief, for each batch, plasma samples, blanks, and quality control samples were prepared and mixed with derivatizing reagents, followed by shaking and centrifugation. The reaction was then quenched with quinic acid, followed by additional shaking and centrifugation. Finally, an internal standard was added to each sample. The samples were analyzed using liquid chromatography-mass spectrometry (LC-MS QTRAP 6500+). The mobile phase consisted of water and acetonitrile, delivered in a gradient. Samples were randomized such that those from the same individual were placed within the same batch to minimize between-batch variability. This approach helped prevent batch-related differences from influencing the analyses, ensuring that observed effects could be attributed to the actual variables under study rather than technical inconsistencies. Data from the Swedish site was exclusively used for this analysis, as SCFA and IPA measurements were only available from this part of the study.

For the analysis of SCFA and IPA, participants from both the High and Low GI groups were combined into a single group, as the primary interest was not to compare the effects of the GI diets. Instead, the aim was to assess overall changes in plasma SCFA and IPA concentrations before and after the intervention. To achieve the highest precision in calculating the total area under the curve (tAUC), a pilot study was conducted to determine the number of data points required for reliable tAUC calculation of plasma SCFA time profiles. Seventeen blood samples were collected from eight randomly selected participants, and tAUCs were calculated using 8, 10, and 12 samples, applying the trapezoid rule across all time points for each compound. Results showed the largest reduction in tAUC errors between 8 and 10 samples; however, precision remained inadequate with fewer than 12 samples. Thus, tAUCs for acetate, propionate, butyrate, and IPA were calculated using 12 time points for each occasion
For the analysis of SCFA and IPA, participants were divided into tertiles based on their baseline tAUC concentrations

CarbHealth
Similarly, in the CarbHealth trial, participants were asked to provide fecal samples at baseline, week 8, and week 16, but gut microbiota analysis has not yet been performed and is not included in this thesis. Fecal samples were collected to investigate mechanistic explanations for differential responses.

4.10 Clinical chemistry

MEDGI-Carb

Blood samples were obtained from an antecubital vein and placed in tubes containing a cloth activator to obtain serum or sodium/lithium heparin to obtain plasma. Serum tubes were held at room temperature for at least 15 minutes and then centrifuged at 4000 × g at 4°C for 15 minutes (3000 × g at 4°C for 10 minutes in Sweden). EDTA plasma, serum, and heparinized plasma samples were aliquoted into microtubes. Plasma and serum aliquots were frozen at -20°C within 2 h of sample collection, stored at this temperature for a maximum of one week, and then stored at -80°C until thawed for analysis. EDTA plasma samples were used to assess insulin, glucose, SCFA and IPA concentrations. All samples were analyzed at the end of the study to minimize batch effects.

CarbHealth

Fasting blood samples were drawn from the antecubital vein into tubes with lithium heparin and EDTA for plasma and a clot activator for serum. EDTA-plasma, serum, and Li-heparin plasma were refrigerated or kept on ice, processed, aliquoted into microtubes, frozen at -20°C within 2 hours, and transferred to -80°C within 24 hours. Samples were analyzed in batches at the Department of Medical Biochemistry and Pharmacology at Haukeland University Hospital, Bergen, Norway, with maximum storage time for HbA1c samples of 8 weeks.

4.11 Differential metabolic responders in the MEDGI-Carb trial

Differential responders from the mixed meal tolerance tests at baseline and post-trial in the MEDGI-Carb trial were examined using a modified version of the minimal glucose model (172). This model, originally developed for OGTT data, uses two differential equations to describe the feedback loop between glucose and insulin (173, 174). This analysis has been described in detail in Paper II (175). Briefly, the model simplifies the glucose-insulin feedback system and assumes a delay in glucose entering the bloodstream, where insulin facilitates its absorption into tissues or conversion into glycogen. It also accounts for glucose removal via the kidneys and insulin secretion in response to plasma glucose. However, the model assumes a linear relationship between insulin and glucose, omitting the influence of other hormones like GLP-1. Key glucose dynamics are described by four parameters: baseline glucose level (Gb), amplitude of concentration changes (A), frequency of oscillations (ω), and the damping coefficient (α). These parameters determine the response pattern, with faster or slower glucose removal rates impacting the shape of the postprandial glucose curve. **Figure 6** illustrates these dynamics, showing variations in glucose response depending on the body's regulatory efficiency. Insulin was excluded from the model during the derivation process, making it useful in settings where insulin cannot be directly measured. We chose to include only the breakfast meal in the analyses of differential responders to avoid any second-meal effect.

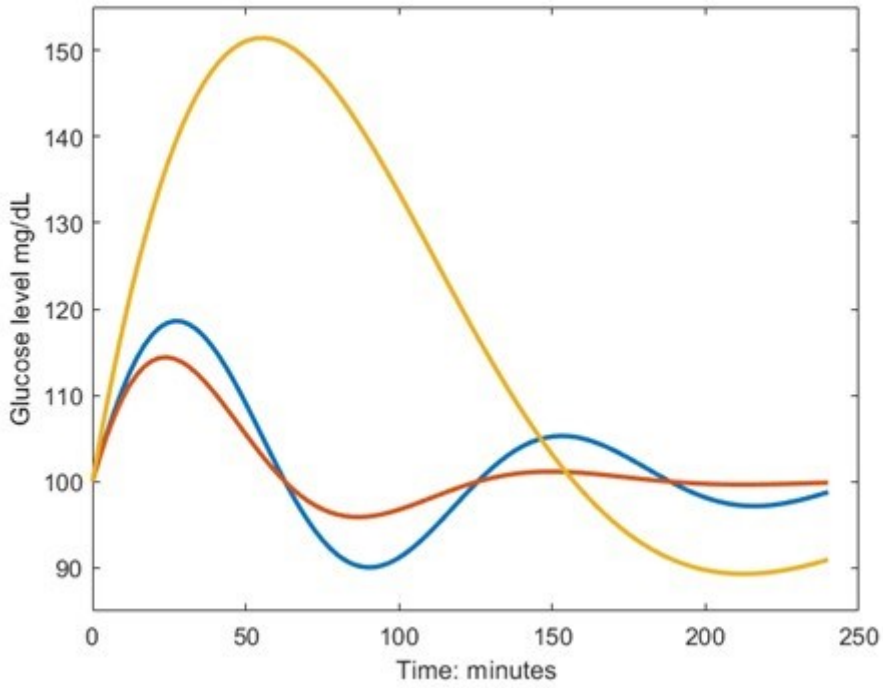

Figure 6. Example dynamics generated from the model. The blue curve displays a fast biphasic response to the MMTT, characterized by a high frequency (ω) and a low amplitude (A). The red curve, with a higher damping coefficient (α), results in a quicker monophasic return to baseline. In contrast, the yellow curve represents a slow response to the MMTT, indicative of poor glucose regulation. It follows an inverse parameter relationship to the blue curve but shares the same damping coefficient. Figure taken from Skantze et al 2023 (175) under CC BY-NC 4.0 license.

4.12 Data analysis and sample size estimates

The different statistical models used in the different research objectives are summarized in **Table 3**. Across the studies, covariates such as age, BMI, and study site were consistently adjusted. The outcome variables varied, with Paper I focus was on postprandial glucose and insulin, in Paper II it was on plasma glucose response using MMTT, in Paper III it was on SCFAs and microbiota diversity, and in Paper IV the focus was on HbA1c and glycemic control. Data imputation methods were applied in Papers I, III, and IV, using approaches like multiple imputation and K-nearest neighbor (KNN). Power calculations differed across studies, with Papers II and III being exploratory, while Papers I and IV targeted specific power thresholds to detect meaningful changes, such as a reduction in HbA1c. Testing methods included intention-to-treat (ITT) or complete case analyses (CC), and statistical tests, depending on the study design

Table 3. Overview of statistical models, covariates and outcome variables across the papers

Aspect	Paper I	Paper II	Paper III	Paper IV
Software	SAS (Version 9.4)	Monolix (2021R2, Lixoft SAS)	R Studio (Version 4.3.0)	R Studio (version 4.3.0), Minitab (v21 for consumer analysis)
Statistical model	Repeated measures, mixed model to assess group, time and interaction effects	Nonlinear mixed-effects model	Linear models for metabolic outcomes, p-trend test	Generalized Linear Model (GLM)
Covariates	BMI, waist circumference, age, smoking status, study site	Age, study site	Age, sex	Baseline value, study site
Outcome variables	Postprandial effects on glucose and insulin response.	Plasma glucose response using MMTT, clustering based on glucose-time profiles	SCFA (acetate, propionate, butyrate) and indole-3-propionic acid (IPA), microbiota diversity	HbA1c, glycemic control, consumer acceptance, satiety, satiation
Gut microbiota analysis	*NA*	16S rRNA	16S rRNA	*NA*
Data imputation	Multiple imputation using chained equations	Not applicable	Values below the limit of detection were imputed with pre-decided values. KNN method	Multiple imputation method. Age, BMI, study site
Testing methods	ITT. Covariates included in all models	CC. ANOVA, Chi-squared for continuous and categorical data	Kruskal-Wallis, Wilcoxon signed-rank test	ITT. Generalized Linear Model (GLM)
Power calculation	30% difference in postprandial insulin expected; 180 individuals needed for > 80% power	No prior power calculation due to exploratory nature. 155 participants included	No prior power calculation due to exploratory nature. 53 participants included	HbA1c reduction by 3 mmol/mol expected. 250 participants needed for 90% power, accounting for 45% dropout rate

BMI, body mass index; CC, complete case analysis; GLM, generalized linear model, HbA1c, glycated hemoglobin; ITT, intention-to-treat analyses.

5 Results and discussion

5.1 Study population

Detailed background characteristics and information regarding diet and compliance of the two trials have been presented in the papers and will be only briefly described here. The two studies had similar samples sizes with 213 individuals in MEDGI-Carb trial and 194 individuals in the CarbHealth trial. **Table 4** shows the BMI, age, sex and metabolic characteristics of the participants in the two studies, and respective subgroups.

Table 4. Summary of participant characteristics across the two clinical trials and corresponding subgroups							
	MEDGI-CARB Paper I[1]		**MEDGI-Carb Paper II**[2]		**MEDGI-Carb Paper III**	**CarbHealth Paper IV**[4]	
	low-GI	**high-GI**	**low-GI**	**high-GI**	***NA***	**Oat**	**Control**
n (f %)	102 (54)	111 (58)	83 (54)	72 (50)	53 (58)	102 (60)	92 (59)
Age (years)	55 ± 10	55 ± 11	56.0 ± 10.5	55.8 ± 9.9	60.1 ± 7.9	57 ± 8	59 ± 9
BMI (kg/m^2)	31.1 ± 3.1	30.3 ± 3.0	31.1 ± 3.2	30.8 ± 3.0	30.0 ± 2.8	32.3 ± 4.8	31.9 ± 4.4
Waist circumference (cm)	106 ± 8	105 ± 9	105 ± 9	107 ± 9	107 ± 8	109 ± 11	109 ±11
Glucose mmol/L	5.4 ± 0.6	5.4 ± 0.7	5.7 ± 0.6	5.9 ± 0.6	5.8 ± 0.5	5.6 ± 0.8	5.5 ± 0.6
Insulin (µU/mL)	14.4 ± 1.0*	15.0 ± 1.0*	14.6 ± 8.9	15.8 ± 8.6	11.7 ± 5.9	13.5 ± 11.6	12.3 ± 8.3
HbA1c %	5.4 ± 0.0*	5.5 ± 0.0*	5.4 ± 0.4	5.5 ± 0.3	5.4 ± 0.3	5.6 ± 0.3	5.6 ± 0.3
HbA1c (mmol/mol)	35.9 ± 0.4*	36.3 ± 0.4*	35.8 ± 4.0	36.7 ± 3.7	35.1 ± 3.7	37.8 ± 3.0	38.0 ± 3.0
Total Cholesterol (mmol/L)	4.9 ± 1.0	5.0 ± 0.9	5.0 ± 0.9	4.9 ± 0.8	5.0 ± 0.8	5.5 ± 1.0	5.5 ± 1.0
Triglycerides (mmol/L)	1.3 ± 0.6	1.3 ± 0.6	1.4 ± 0.8	1.3 ± 0.5	1.2 ± 0.5	1.5 ± 0.6	1.4 ± 0.6
HDL-C (mmol/L)	1.4 ± 0.4	1.3 ± 0.4	1.3 ± 0.3	1.3 ± 0.3	1.4 ± 0.3	1.5 ± 0.4	1.5 ± 0.4
LDL-C (mmol/L)	3.2 ± 0.8	3.3 ± 0.7	3.0 ± 0.7	3.0 ± 0.7	3.1 ± 0.7	3.6 ± 1.0	3.6 ± 1.0
Systolic blood pressure (mm Hg)	126 ± 14	126 ± 14	129 ± 14	125 ± 12	129 ± 13	134 ± 17	129 ± 15
Diastolic blood pressure (mm Hg)	82 ± 9	83 ± 8	82 ± 9	81 ± 9	84 ± 8	85 ± 9	83 ± 10

Means ± SD. *Mean ± SEM. BMI, Body Mass Index; HbA1c, glycated hemoglobin, HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol. [1] Intention-to-treat analyses, [2] Complete case analyses, [3] Only the Swedish site, [4] Intention-to-treat analyses. Table modified from Paper I, II, III, and IV.

MEDGI-Carb

The CONSORT participant flow diagram for the MEDGI-Carb trial is presented in **Figure. 7**. In total, 584 participants were screened for eligibility, and 213 participants were subsequently enrolled in the trial. Of the initially enrolled participants, 27 (low-GI; n = 12, high-GI; n = 15) dropped out prior to starting the dietary intervention, and 26 individuals (low-GI; n = 8, high-GI; n = 18) dropped out during the intervention. Approximately half of the participants (47 %) dropped out due to considering the protocol/diet to demanding while the other half dropped out due to "other". In total, 160 individuals (low-GI: n = 86; high-GI: n = 74) completed the trial, comprising the complete-case (CC) analysis group. The intention-to-treat (ITT) analysis group included 213 individuals (low-GI: n = 106; high-GI: n = 107). There were 56 % females in the MEDGI-Carb study, average BMI was 31.1 kg/m^2 in the low-GI group and 30.3 kg/m^2 in the high-GI group (**Table 4**). Forty-four percent of the participants had two traits of the metabolic syndrome, 37 % had three traits, 16 % had four traits and 3 % had five traits. All participants in the MEDGI-Carb study had elevated waist circumference (by design), elevated blood pressure was the most common secondary trait (60 %), followed by elevated fasting glucose (46 %), low HDL-C (38 %), and elevated triglycerides (33 %).

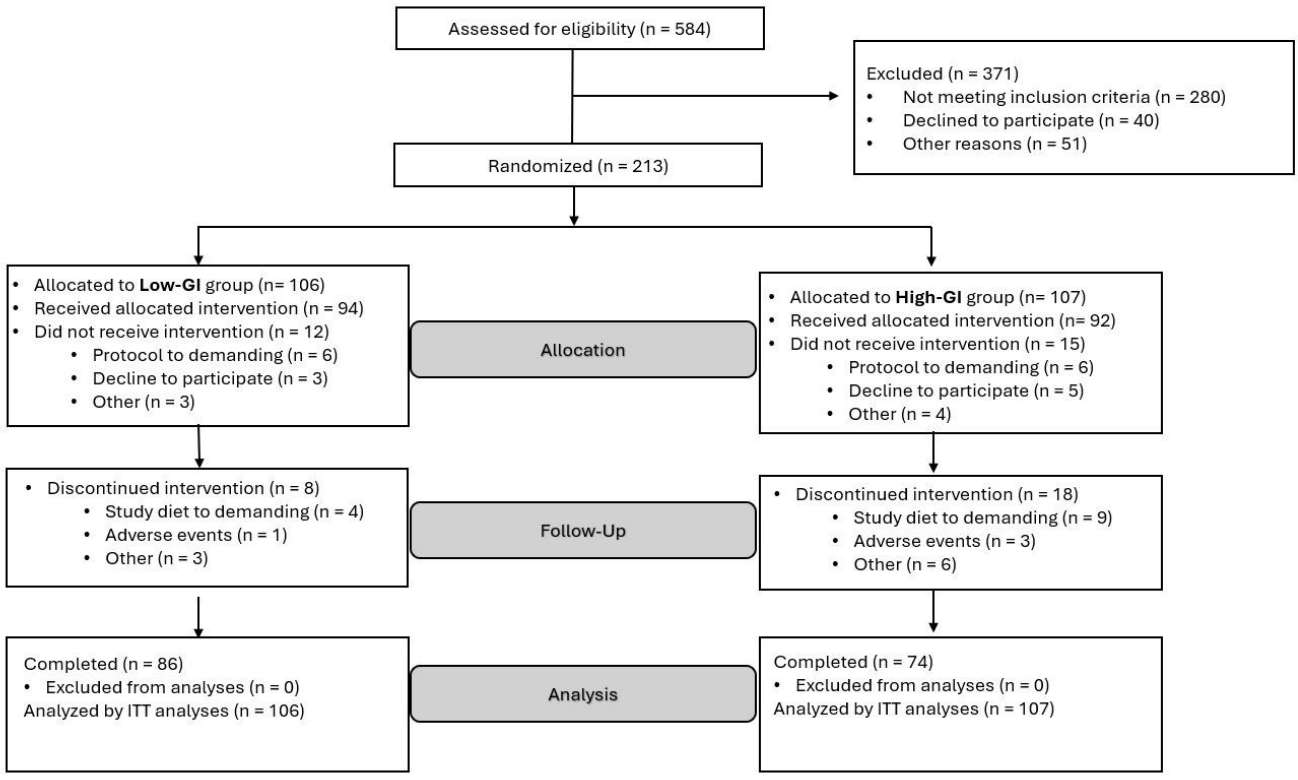

Figure 7. CONSORT flow diagram for all sites of the MEDGI-Carb trial. Figure modified from Bergia et al. 2022 (176) under CC BY-NC 4.0 license.

CarbHealth.

During the recruitment phase, 352 individuals were screened for eligibility and 202 were enrolled in the trial (**Figure 8**). Of the initially 202 recruited participants, seven individuals (oat; n = 2, wheat; n = 5) dropped out prior to starting the intervention. Thirty-eight individuals (oat; n = 23, control; n = 15) dropped out during the intervention. Most (62 %) stated "other" as the reason for drop out. In total, 156 individuals (oat: n = 78; control: n = 78) completed the intervention, comprising the complete-case (CC) analysis group. The intention-to-treat (ITT) analysis group included all randomized individuals who attended the baseline visit (oat: n = 102; control: n = 93). One individual (control) was excluded from analyses due to developing manifest type 2 diabetes during the intervention. Approximately 61% of the included participants were females, all participants had elevated BMI and waist circumference. HbA1c levels were slightly increased 37.8 mmol/mol in the oat group and 38.0 mmol/mol in the control group, while fasting blood glucose levels were normal. Both groups were considered normocholesterolemic, with normal cholesterol and triglyceride levels (**Table 4**).

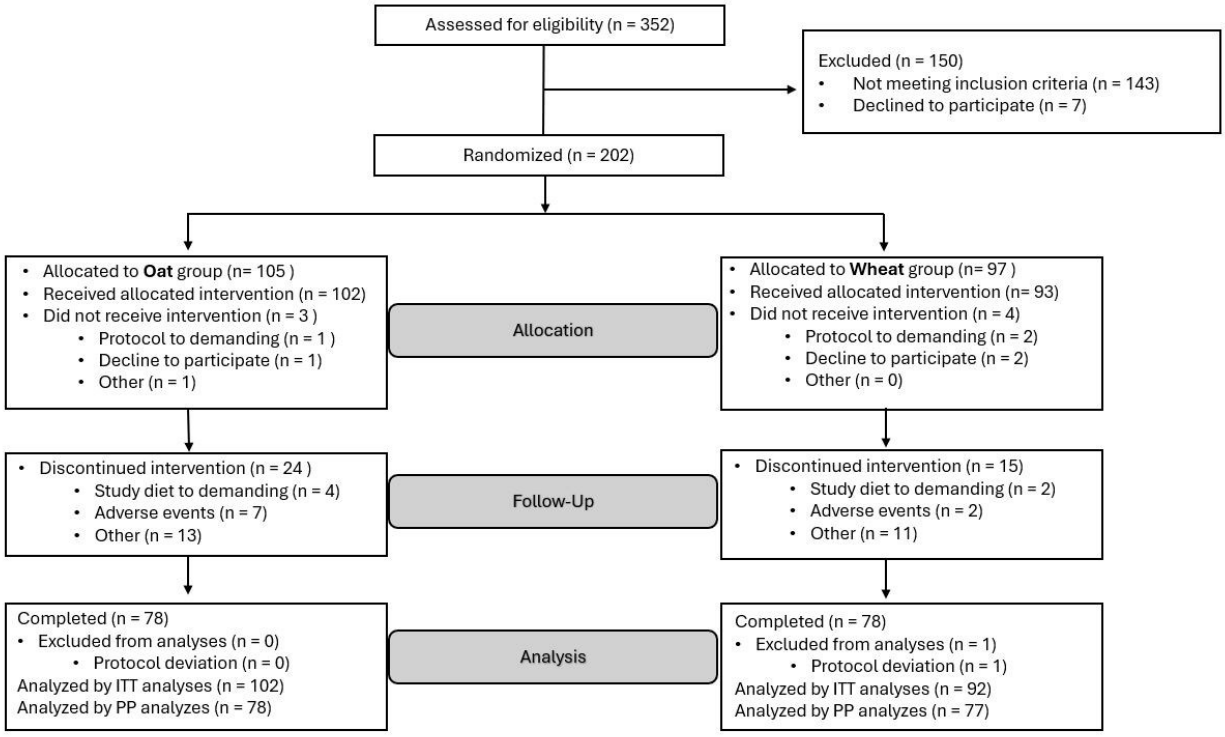

Figure 8. CONSORT flow diagram for the CarbHealth trial

5.2 Dietary intake

MEDGI-Carb

None of the groups in the MEDGI-Carb study showed a difference in energy or macronutrient intake at baseline or post-intervention. However, after the trial, energy intake increased by around 300 kcal in both groups. Moreover, dietary fiber, monounsaturated fats, and carbohydrates increased while alcohol consumption, along with total and polyunsaturated fat intake, decreased in both groups. There was no significant difference in average GI at baseline, with 59.0 ± 6.3 in the low-GI group and 59.4 ± 5.6 in the high-GI group ($p = 0.299$). The targeted differences in GI were achieved, with a significant difference between groups. The low-GI group had an average GI of 46.8 ± 3.1, while the high-GI group averaged 66.2 ± 4.7 ($p < 0.001$).

CarbHealth

In the CarbHealth trial, both groups reported similar energy, and macronutrient intake at both baseline and post-trial both measured as E% and gram per day. There was a slight increase in energy of approximately 100 kcal per day post-intervention compared to baseline. Intake of dietary fiber remained the same post-trial compared to baseline in both groups, intake of dietary fiber was approximately 20 gram per day in both groups. As dietary intake was not part of the planned analysis, no formal comparison of dietary intake between the groups has been performed.

5.3 The MEDGI-Carb study: Effect of GI on glycemic control (Paper I)

5.3.1 Postprandial MMTT glucose and insulin responses

A total of 213 (119 female, 94 male) participants were included in the analyses of the effect of GI on glycemic control. At baseline there was a significant difference in average postprandial insulin responses after the high-GI test meal compared to the low-GI test meal ($p = 0.004$), but this difference between the groups was no longer present after the intervention ($p = 0.17$). From baseline to post-trial, postprandial insulin response decreased in the high-GI group, but not in the low-GI group ($p = 0.046$). Postprandial glucose levels were about 17 % higher after the high-GI meal compared to the low-GI meal at baseline ($p = 0.02$). This difference increased to approximately 35 % after the intervention ($p < 0.001$). The difference between the groups was mainly due to an increase in average postprandial glucose over 12 weeks in the high-GI group (Δ 0.2 ± 0.1 mmol/L; $p = 0.03$) (**Figure 9**). In contrast, glucose responses in the low-GI group remained unchanged post-intervention, similar to the postprandial insulin responses. Overall, the differential effects of GI were more pronounced during lunch meals, while glucose and insulin responses showed less variation between the groups after breakfast meals.

Contrary to expectations, while postprandial insulin was lower in the low-GI group compared to the high-GI group at baseline, this difference was no longer observed post-intervention. Interestingly, insulin levels decreased more in the high-GI group than in the low-GI group, but this reduction occurred only after breakfast. In contrast, post-lunch insulin levels remained significantly higher in the high-GI group by more than 50 %. Given that elevated postprandial insulin is associated with increased cardiovascular risk, this persistent elevation after lunch may represent a negative consequence of the high-GI diet (177). Moreover, this finding aligns with the second meal effect, where the glycemic index of one meal influences the insulin and glucose responses to a subsequent meal. Multiple studies have shown that consuming a low-GI meal at breakfast can improve the glycemic and insulin response to lunch, potentially offering a protective effect by reducing postprandial glucose spikes and improving insulin (178-181).

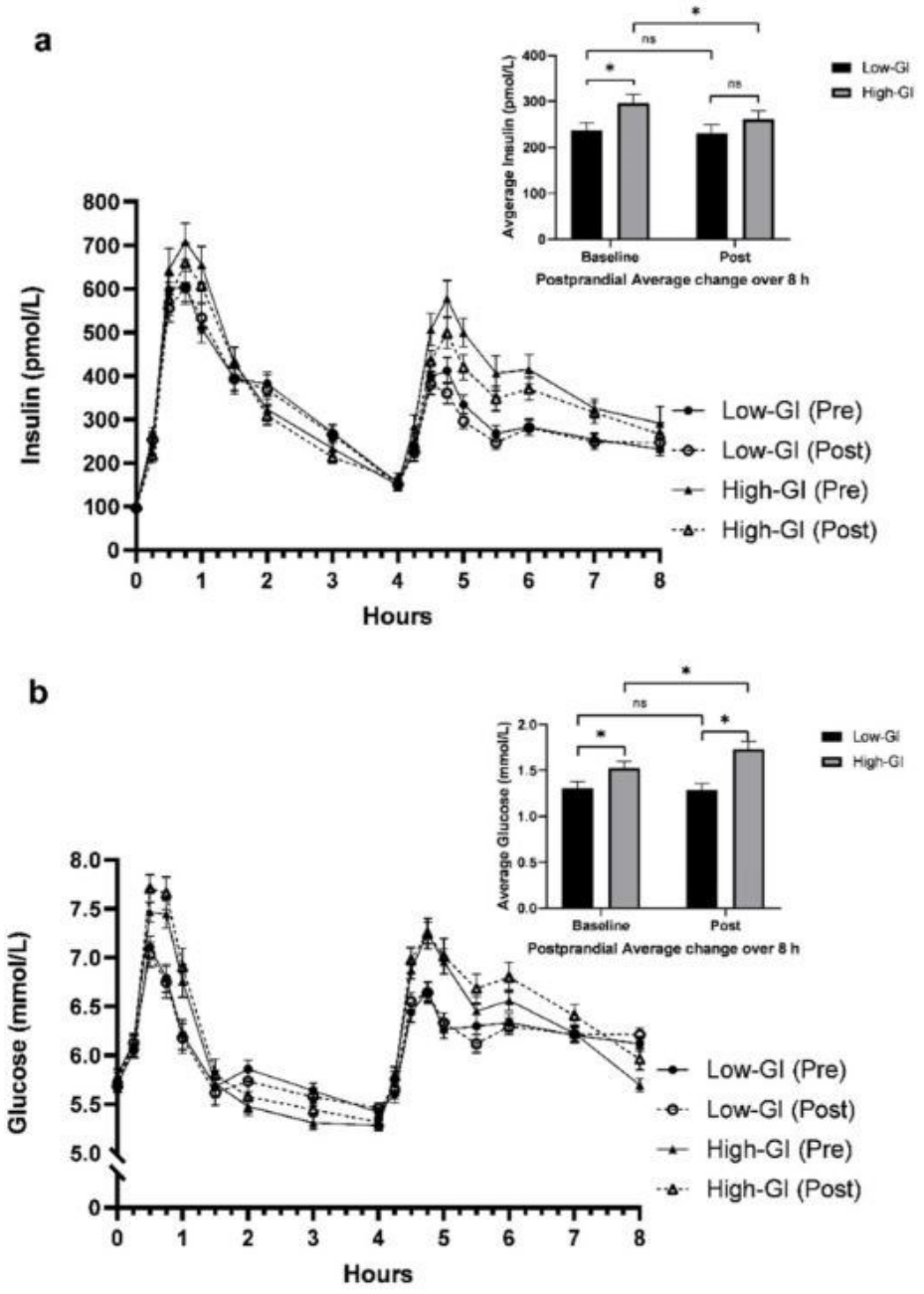

Figure 9. Insulin (**a**) and glucose (**b**) responses to a low-GI and high-GI 8-h meal glucose tolerance test at baseline and after a 12-week dietary intervention. Inset bar graphs display average postprandial insulin and glucose elevations above fasting concentrations over the 8-h period. Data are means ± SEM. * Statistically significant, $p < 0.05$. ns, no significance. Figure taken from Bergia et al. 2022 (176) under CC BY-NC 4.0 license.

5.3.2 Twenty-four-hour glycemic variability

At baseline, measurements from the 24-hour continuous glucose monitoring (CGM) variables, i.e., glycemic variability; including average 24-hour glucose concentrations, 24-hour standard deviation, mean amplitude of glucose excursions (MAGE), mean absolute glucose (MAG), lability index, or continuous overall net glycemic action (CONGA) did not differ between the groups. During the intervention, average 24-hour glucose concentrations and CONGA improved in both groups. However, the low-GI group showed greater reductions in several glycemic variability indices compared to the high-GI group by the end of the intervention, including MAGE ($p < 0.01$), MAG ($p = 0.02$), and lability index ($p = 0.04$) (**Figure 10**). The result of the 24-h CGM measurements add important context to the findings from the postprandial measurements. While the 24-h glucose concentrations decreased in both groups, only the low-GI group showed improvement in glycemic variability indices. Glycemic variability, referring to oscillations in blood glucose levels and includes both hyperglycemia and hypoglycemia, is linked to an elevated risk of CVD and can contribute to the progression from prediabetes to type 2 diabetes (182, 183). Based on the improvement in glycemic variability indices observed in the low-GI group, the results suggest more stable blood glucose concentrations following a low-GI diet, which may lead to several health benefits. The mechanistic support on the relevance of glycemic variability indices suggest that temporary hyperglycemia has been shown to induce even more vascular damage than sustained hyperglycemia, mainly mediated by oxidative stress (183). However, there has not been a consensus of the most important glycemic variability indices to use in clinical settings and the relative contribution of each index (183, 184). Although there is still no consensus, accumulating evidence suggests that glycemic variability is associated with both microvascular and macrovascular complications, particularly in individuals with type 2 diabetes (185). Assessing glycemic variability in individuals at risk for type 2 diabetes may be crucial, as disruptions in glucose homeostasis can be an early sign of glucose dysregulation (26).

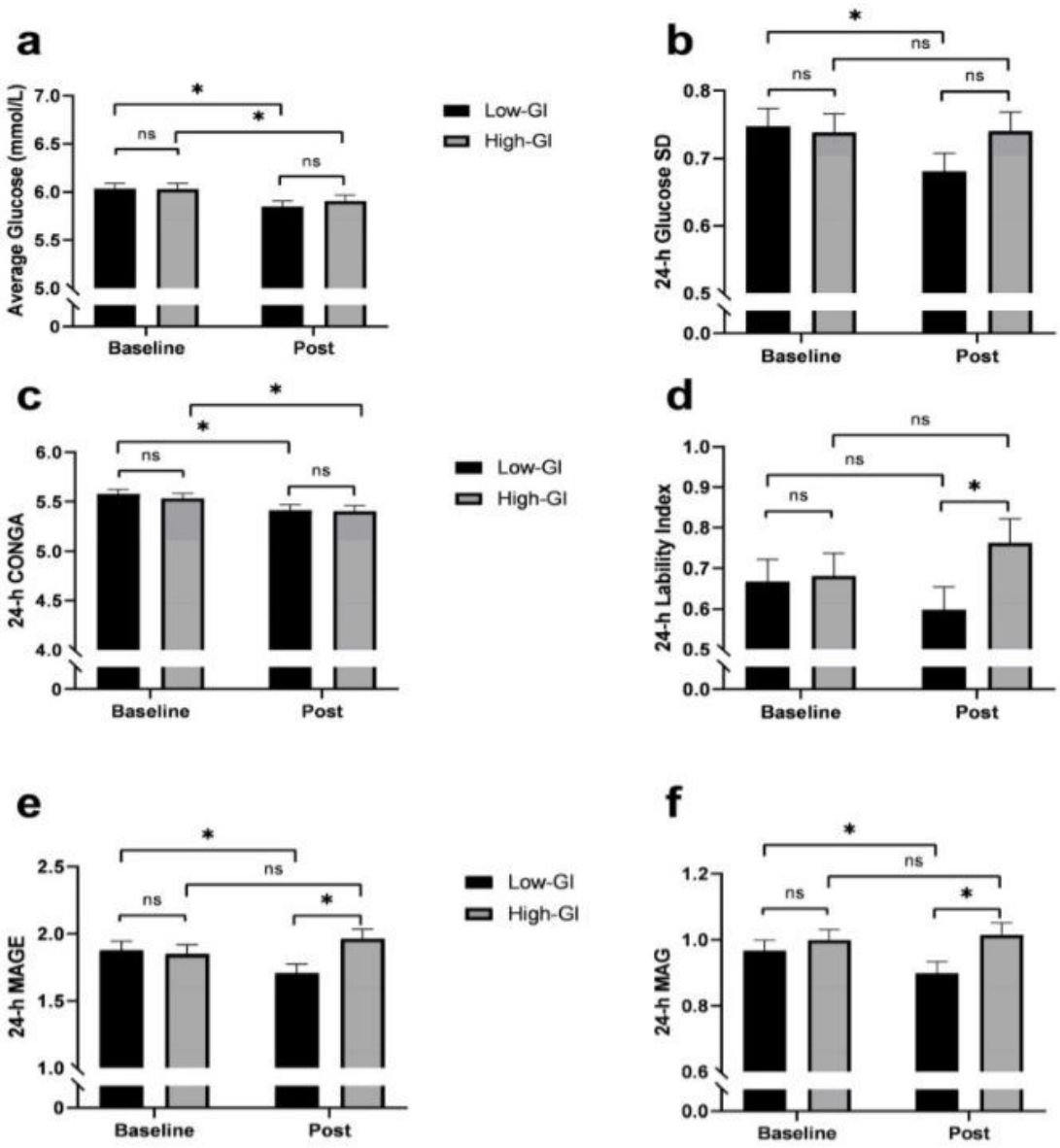

Figure 10. 24-h continuous glucose monitor-derived measures of glycemic variability at baseline and after a 12-week dietary intervention. (**a**) Average 24-h glucose concentration, (**b**) standard deviation (SD), (**c**) continuous overall net glycemic action (CONGA), (**d**) lability index, (**e**) mean amplitude of glucose excursions (MAGE), and (**f**) mean absolute glucose (MAG). The presented data are means ± SEM. * Statistically significant, $p < 0.05$. ns, no significance. Figure taken from Bergia et al. 2022 (176) under CC BY-NC 4.0 license.

5.3.3 Fasting and OGTT and insulin responses

No significant changes were observed in fasting glucose or insulin concentrations, HOMA- IR, or HbA1c levels between the groups at the end of the intervention. However, we saw that the glucose response to the OGTT, but not insulin, decreased over the course of the intervention ($p = 0.02$) irrespective of GI. The lack of result in fasting glucose was expected, as fasting plasma glucose tends to remain stable during weight-maintaining dietary interventions, particularly in individuals who are normoglycemic or have slightly elevated glucose levels (186, 187). This stability likely reflects that fasting glucose is less responsive to short-term dietary changes compared to postprandial glucose, which is more directly influenced by meal composition and timing (188). Consequently, interventions that do not target weight loss or calorie reduction often fail to impact these measures. A systematic review and meta-analysis reported that low-GI diets had a positive effect on fasting glucose levels but not on fasting insulin, but the reduction was inversely correlated to body weight (189). Additionally, HbA1c is a less precise marker of glucose control for values below 5.5 % (37 mmol/mol), which included most participants in this trial (190-192). Improvements in OGTT outcomes are typically seen only in long-term weight-loss interventions, particularly in individuals with diagnosed impaired glucose tolerance (193, 194).

In summary, participants who followed a low-GI diet showed better post-meal glucose control compared to those on a high-GI diet, and this difference grew over time, largely due to worsening glucose control in the high-GI group. The Mediterranean diet alone helped lower daily blood glucose levels, but only the low-GI diet reduced daily glycemic variability. These findings underscore the importance of incorporating low-GI foods, which are typical of the Mediterranean diet, to enhance health benefits in non-diabetic individuals.

5.4 The MEDGI-Carb study: Identification of differential responders to a mixed meal tolerance test (Paper II)

A mechanistic glucose model was applied to glucose responses from standardized mixed meal tolerance tests (MMTT) conducted at baseline and post-testing on 155 participants (81 females, 74 males) that completed the two measurements in the MEDGI-carb trial.

5.4.1 Identification of clusters based on a mechanistic model

Results showed two distinct clusters (A and B) that were estimated from the model using the baseline MMTT. These clusters were well separated based on the parameters of amplitude and frequency (**Figure 11**). The clusters formed from baseline data did not show any association with site or intervention group (High vs Low GI). The clusters consisted of approximately 46 % of the individuals in cluster A and 54 % of cluster B. The same analysis was applied to the post-intervention data, and similar cluster membership was observed. However, using the Euclidean silhouette measure, we found that the distance between clusters decreased after the trial, indicating that the clusters were more distinct when using baseline data. However, this result was expected, as participants in both groups improved their daily glucose control.

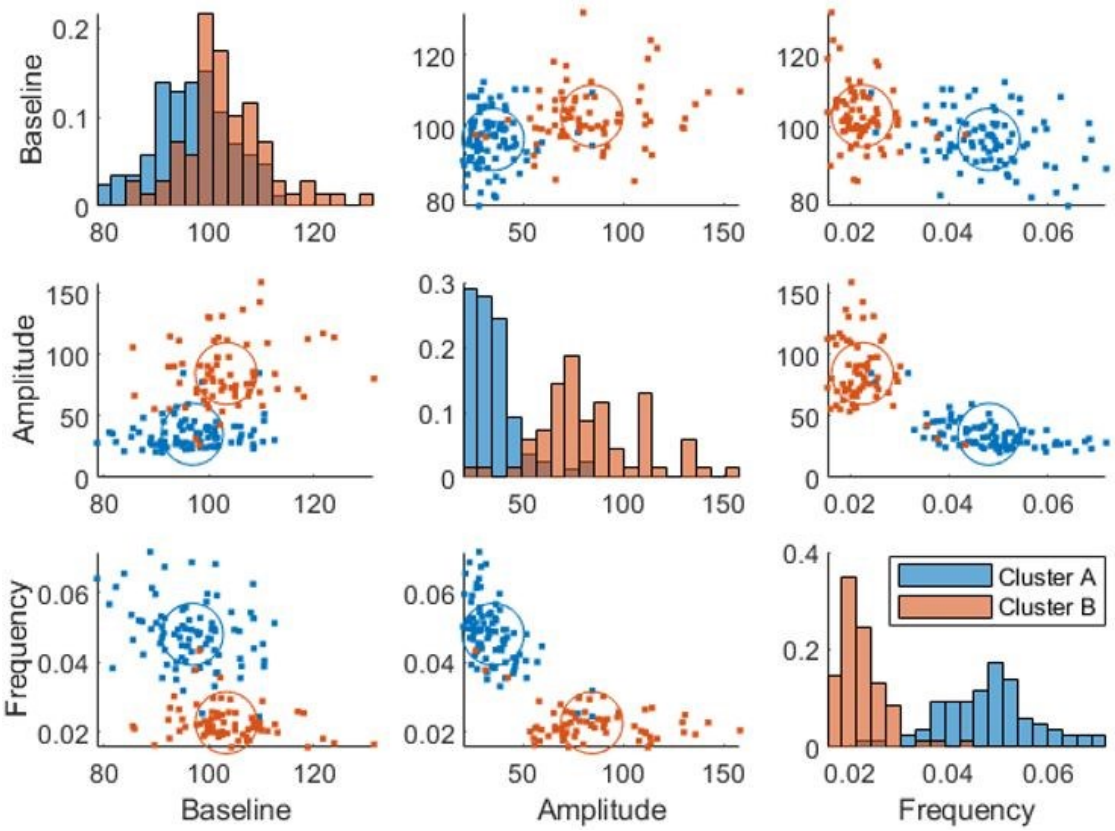

Figure 11: Joint parameter distribution from fitting the model to postprandial breakfast MMTT data. Blue represents Cluster A, and red represents Cluster B. The diagonal shows histograms of the parameter distributions, while the off-diagonal plots show pairwise joint distributions of the parameters. Figure taken from Skantze et al 2023 (175) under CC BY-NC 4.0 license.

5.4.2 Clusters linked to type 2 diabetes risk markers and glucose control

The clusters were significantly associated with various risk markers for type 2 diabetes, including HbA1c ($p < 0.001$), insulin sensitivity indices (QUICKI, Stumvoll, and Matsuda, all $p < 0.001$), and waist circumference ($p < 0.001$) using one-way ANOVA (**Figure 12**). The clusters were also differently associated with clinical cut-offs for glucose control, such as prediabetes defined by HbA1c (≥ 39 mmol/mol or ≥ 5.7 %) and fasting blood glucose > 5.6 mmol/L (100 mg/dL) ($p = 0.01$), insulin resistance ($p < 0.01$) as measured by the Matsuda index (≤ 2.5), and overall glucose control (normoglycemic), impaired glucose control, or diabetic) ($p < 0.01$) (195, 196), using the Chi-squared test. These results suggest that using a standardized breakfast meal could predict meaningful data to predict risk factors of type 2 diabetes from glycemic glucose response measurements.

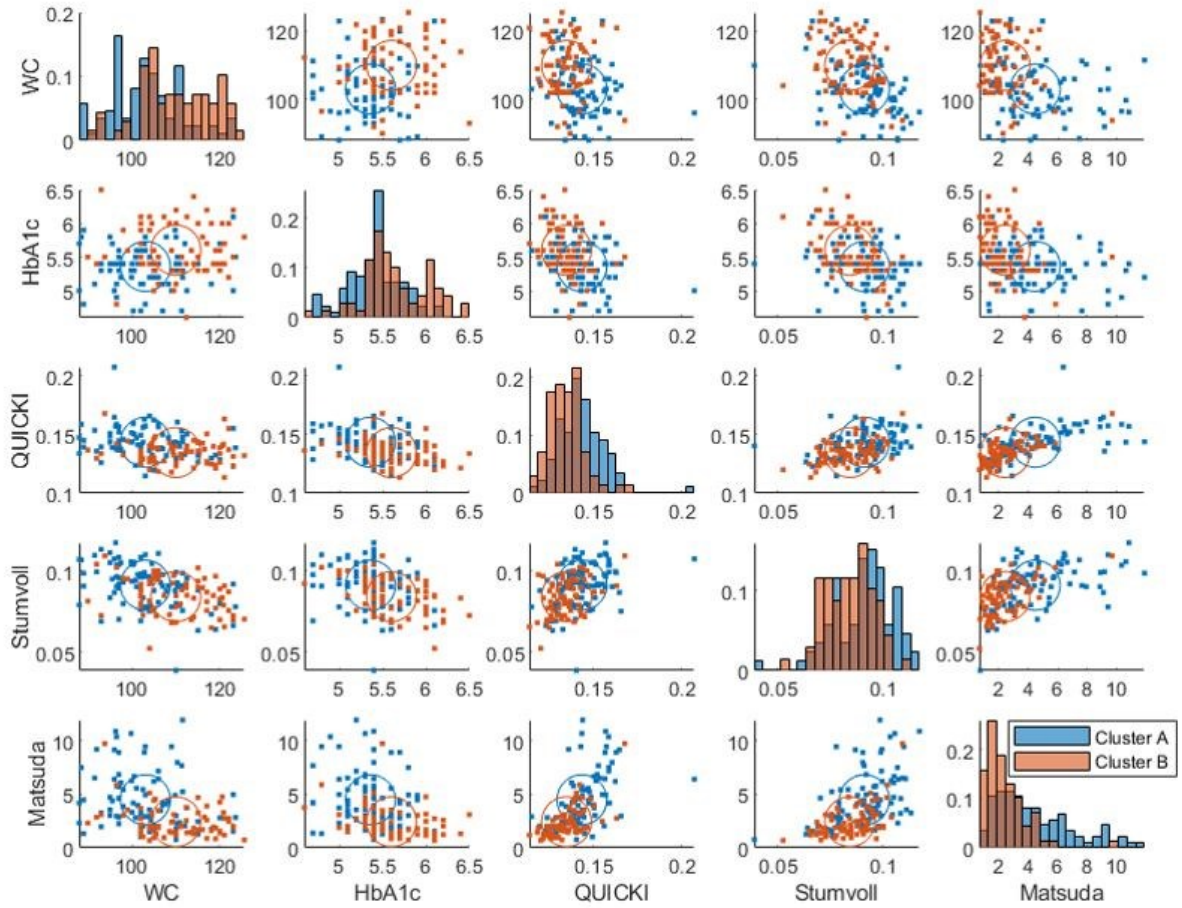

Figure 12: Joint distribution of baseline diabetes risk markers that showed significant associations with the clusters. Figure taken from Skantze et al 2023 (175) under CC BY-NC 4.0 license.

5.4.3 Clusters and gut microbiota

The clusters also differed in associations with gut microbiota genera, such as *Clostridium sensu stricto 1* (ANOVA p = 0.007) and *Blautia* (ANOVA p = 0.024), both linked to glucose metabolism. Cluster A had a higher proportion of *Clostridium sensu stricto 1* compared to Cluster B, while the opposite was true for *Blautia*. Furthermore, individuals in Cluster A showed a lower and earlier peak in plasma glucose response, suggesting more efficient glucose regulation (**Figure 13**). These findings align with previous reports linking these genera to glucose control (197, 198), although the causal relationship with postprandial glucose response clusters remains unclear.

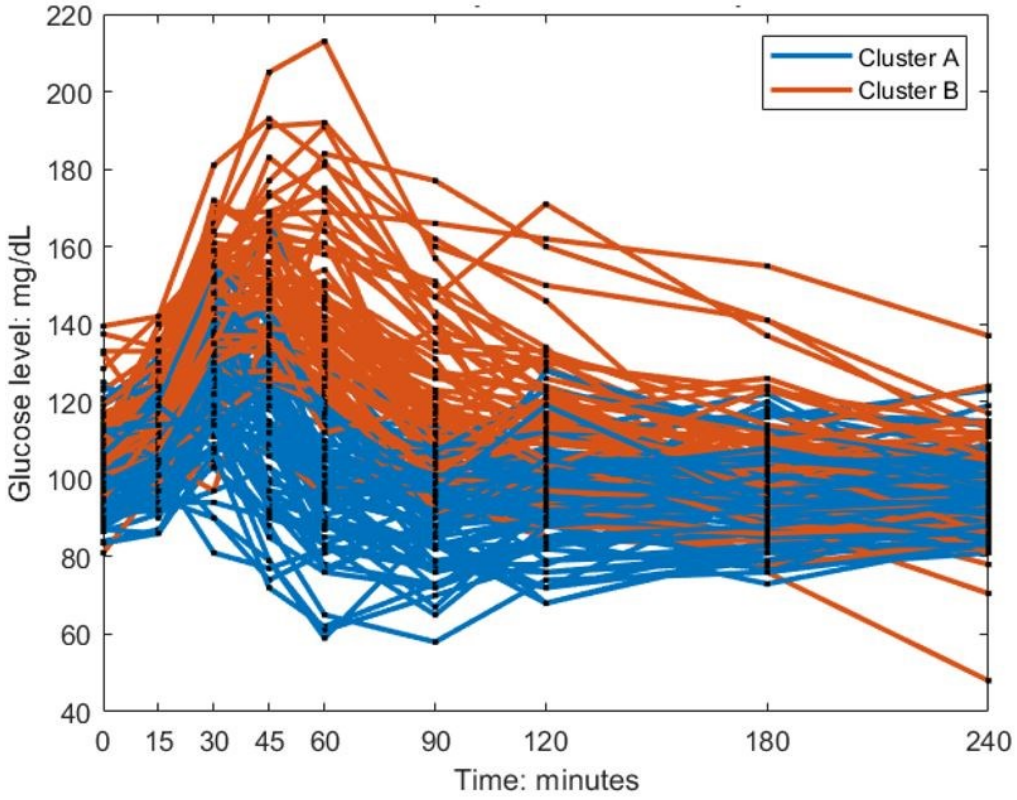

Figure 13: Baseline postprandial breakfast MMTT response, color-coded by clusters. Figure taken from Skantze et al 2023 (175) under CC BY-NC 4.0 license.

In summary, two distinct clusters were identified using a mechanistic model. The two clusters were differently associated with clinical risk markers of type 2 diabetes, glycemic control, and gut microbiota.

5.5 The MEDGI-Carb study: Plasma concentration of gut microbiota metabolites in relation to risk factors of type 2 diabetes

Four gut microbiota metabolites, acetate, propionate, butyrate and IPA were analyzed in plasma from 53 individuals (31 females, 22 males) from the Swedish site in the MEDGI-Carb trial.

5.5.1 Intervention effects on plasma SCFA and IPA concentrations

Results showed a significant increase in acetate total area under the curve (tAUC) concentrations post-intervention compared to baseline ($p < 0.001$). In contrast, propionate and butyrate did not exhibit significant changes in median tAUC concentrations ($p = 0.43$ and $p = 0.21$, respectively). Similarly, IPA tAUC concentrations showed no statistically significant change ($p = 0.057$), although the result approached significance (**Table 5)**.

Table 5. Total tAUC concentrations of acetate, propionate, butyrate, and IPA at baseline and 12 weeks

Compound (µM·min)	Baseline	Week 12	Median Δ (95% CI)[1]	P-value[2]
Acetate	11204 (6452)	13831 (8067)	2815 (782; 5799)	<0.001
Propionate	527 (349)	578 (325)	52 (-38; 142)	0.43
Butyrate	194 (115)	220 (117)	26 (-18; 70)	0.21
Indol-3-propionic	738 (657)	811 (594)	102 (-131; 308)	0.057

Data presented as Median + IQR. [1] Median difference between baseline and 12 weeks. 95% CI calculated with boot strapping: [2] Wilcoxon rank-sum test comparing changes from baseline to week 12.

5.5.2 Associations between baseline tAUC tertiles of SCFAs, IPA and glycemic control

No associations were observed between baseline tertiles tAUC concentrations of SCFAs or IPA and the probability of being classified as healthy (normal glucose control, n = 35) vs impaired glucose control (n = 18) (all $p > 0.05$) (**Table 6)**. This suggests that baseline levels of these metabolites may not be strong predictors of glucose regulation status in our cohort. Plasma SCFAs have been associated with improved glucose homeostasis and insulin sensitivity, though these effects appear modest and vary based on factors like sex, gut microbiota composition, and genetics (21). This suggests that while SCFA production is linked to dietary patterns, its direct impact on glycemic control may be less clear or influenced by other variables. Likewise, IPA has been associated with improved insulin sensitivity and a lower risk of type 2 diabetes, although these effects also vary based on individual metabolic status and gut microbiota composition (199, 200).

Table 6. Baseline tAUC concentrations of SCFAs and IPA and glycemic control (healthy (n = 35) vs impaired glucose control n = 18)

Compound	Tertiles (μM·min)[1]	Odds Ratio (OR)[2]	95% CI[3]	p-value[4]
Acetate	Low (8267)	0.9999	0.9999, 1.000	0.82
	Medium (11205)			
	High (17200)			
Propionate	Low (382)	1.0010	0.9989, 1.003	0.34
	Medium (527)			
	High (904)			
Butyrate	Low (127)	0.9998	0.9952, 1.004	0.91
	Medium (194)			
	High (320)			
IPA	Low (407)	0.9989	0.9975, 0.9999	0.11
	Medium (738)			
	High (1501)			

IPA: Indole-3-propionic acid. [1] Data presented as median, [2] Odds ratios (ORs) represent the odds of being classified as healthy (normal glucose control) for each tertile, compared to the reference category of impaired glucose control. An OR > 1 indicates increased odds of being classified as healthy, while an OR < 1 indicates decreased odds. [3] 95% confidence interval (CI); [4] p-value from the logistic regression model

5.5.3 Plasma SCFA and IPA tAUC and changes in risk factors of type 2 diabetes

A significant linear trend was observed between baseline tertiles of TAUC acetate and intervention-mediated changes in systolic blood pressure (p = 0.036) across plasma acetate TAUC tertiles. However, no significant linear trends were found for baseline tertiles of propionate, butyrate, or IPA tAUCs in relation to blood pressure or other risk factors, including diastolic blood pressure, HbA1c, QUICKI, Matsuda index, HOMA-IR, insulin, LDL, HDL, total cholesterol, or triglycerides (p > 0.05 for all comparisons). Acetate has been linked to blood pressure regulation through mechanisms like modulation of the renin-angiotensin system and activation of G-protein-coupled receptors (21, 201). However, contrary to expectations, no associations were found between butyrate, propionate, or IPA and cardiometabolic risk factors. The metabolic response to SCFA and IPA may vary by individual characteristics such as age, sex, ethnicity, gut microbiota, and genetics. While human intervention studies support SCFA and IPA's metabolic effects, these effects may be modest and influenced by other factors (21).

5.5.4 Differences in microbial abundance and diversity between healthy and impaired glycemic control groups

A significantly higher abundance of *Ruminococcus gnavus group*, *Clostridium sensu stricto 1*, *Sellimonas*, *Cetobacterium*, and *Intestinibacter* were observed in the healthy group compared to the impaired glycemic control group (all adjusted p-values < 0.05). In contrast, *Lachnospiraceae UCG-001* was significantly more abundant in the impaired glycemic control group compared to the healthy group (adjusted p-value = 0.006) (**Table**

7). *Clostridium sensu stricto 1* is known for producing butyrate, a short-chain fatty acid associated with enhanced insulin sensitivity and protective effects against obesity and type 2 diabetes (202). In this study, this genus was more abundant in the healthy group, reinforcing its potential role in supporting metabolic health. Nonetheless, despite these microbial differences, there was no observed difference in circulating butyrate levels between the groups. This suggests that butyrate production may exert more localized effects within the gut, or that other factors might influence systemic butyrate levels

Table 7. Differences in microbial abundance between diabetes class (healthy and impaired glucose control)

Genus	**Mean**[1]	**Log2FC**[2]	**SE**[3]	**p-value**[4]	**p-adjusted**[5]
Lachnospiraceae UCG-001	165	1.2	0.3	<0.001	0.006
Ruminococcus gnavus Group	57	-2.0	0.5	<0.001	0.01
Clostridium sensu stricto 1	86	-1.1	0.3	<0.001	0.03
Sellimonas	2	-1.3	0.3	<0.001	0.01
Cetobacterium	13	-1.8	0.5	<0.001	0.03
Intestinibacter	101	-0.9	0.3	<0.001	0.03

Log2FC: Log2FoldChange. [1] Normalized read counts. [2] Log2 fold change between healthy and impaired groups, with positive values indicating greater abundance in the impaired group and negative values indicating greater abundance in the healthy group. [3] Standard error of the log2 fold change. [4] P-value from the Wald test. [5] Adjusted p-value (corrected for multiple testing)

A significant difference in the Shannon index was found. The impaired glycemic control group exhibited higher microbial diversity compared to the healthy group (p = 0.025). The Simpson index showed a borderline significant difference, with the impaired group having slightly higher diversity (p = 0.053). However, no significant difference was observed in the Chao1 index between the groups at baseline (p = 0.76) (**Table 8**). Interestingly, although the impaired glycemic control group showed greater microbial diversity (Shannon index), the higher presence of beneficial bacteria in the healthy group suggests that specific taxa, rather than overall diversity, may be more crucial for regulating glucose homeostasis.

Table 8. Microbial diversity between the diabetes classes at baseline

Index	**Diabetes class**	**Median**[1]	**IQR**[1]	**p-value**[3]
Shannon	Healthy	3.29	0.56	
	Impaired	3.59	0.31	0.025
Chao1	Healthy	66504	3268	
	Impaired	66141	4610	0.763
Simpson	Healthy	0.93	0.06	
	Impaired	0.94	0.02	0.053

[1] Median diversity index at baseline, [2] IQR: interquartile range, [3] p-value from Kruskal-Wallis test between the groups

In summary, the Mediterranean diet intervention, irrespective of low- or high GI, increased postprandial acetate levels but did not affect propionate, butyrate, or IPA levels. Baseline acetate levels were linked to reductions in systolic blood pressure, suggesting a role in cardiometabolic health, while no direct associations were found between other SCFAs/IPA and glycemic control. Analysis of gut microbiota indicated that specific microbial taxa, rather than overall diversity, were more abundant in individuals with healthy glucose control, highlighting the potential influence of certain bacteria on metabolic health.

5.6 CarbHealth, β-glucans and metabolic control

A total of 194 participants (116 female, 78 male) were included in the analyses of the effectiveness of β-glucans on glycemic control.

No significant difference between the oat and control groups post-intervention was found for the primary endpoint of the trial, i.e. difference in change in HbA1c between the two groups (**Table 9**). Similarly, changes in fasting glucose, insulin, HOMA-IR, and QUICKI did not differ between the groups. An ad hoc analysis, based on a median split of bread consumption, also showed no effect on the results (all $p > 0.05$). The lack of difference in glycemic control measurements contrasts with findings from efficacy studies, suggesting that real-world effectiveness may differ from effects observed under more strictly controlled conditions. Several studies have been investigated the effects of β-glucans on glycemic control under controlled conditions (83, 84). While these findings highlight the benefits of β-glucans in controlled conditions, our study suggests effects may not be sufficient to make a difference under real-world settings. This aligns with previous studies on dietary components, which frequently reveal a gap between laboratory efficacy and real-world effectiveness (161, 203). In a real-world context, factors such as adherence, individual variability, and habitual diet significantly influence outcomes.

An additional potential explanation for the lack of effectiveness in the study is the relatively healthy metabolic profile of the participants. Meta-analyses have shown that β-glucans have a more pronounced effect among individuals with manifest type 2 diabetes compared to healthier individuals (84). Additionally, no changes were observed in the blood lipid profile (all $p > 0.05$), and the results remained unchanged when accounting for lipid-lowering drug treatment ($n = 16$). Furthermore, there were no significant differences in changes in fatty liver index, hepatic steatosis index, or C-reactive protein between the groups post-intervention (all $p > 0.05$) (**Table 9**).

Over the 16-week intervention, there were no significant differences between the oat and the control group in terms of changes in body weight, body fat mass, or fat-free mass.

Table 9. Clinical measurements at baseline and after 16 weeks. (Oat = 102, Control= 92, unless otherwise stated).

Variable	Timepoint	Oat mean (SD)*	Control mean (SD)*	Δ (95% CI)†	P-value‡
Hba1c (%)	Baseline	5.6 (0.28)	5.6 (0.27)	*NA*	
(mmol/mol)		37.7 (3.0)	38.0 (3.0)		
	16 weeks	5.6 (0.15)	5.6 (0.15)	-0.01 (-0.03, 0.06)	0.49
		38.0 (1.7)	38.1 (1.7)	-0.16 (-0.30, 0.63)	
Glucose (mmol/L)	Baseline	5.6 (0.7)	5.5 (0.7)	*NA*	
	16 weeks	5.5 (0.4)	5.5 (0.4)	-0.02 (-0.11, 0.14)	0.77
Insulin (pmol/L)	Baseline	13.7 (10.2)	12.5 (10.1)	*NA*	
	16 weeks	12.5 (6.2)	13.3 (6.2)	-0.76 (-0.99, 2.5)	0.39
HOMA-IR	Baseline	3.6 (3.1)	3.2 (3.1)	*NA*	
	16 weeks	3.2 (1.6)	3.4 (1.6)	-0.26 (-0.20, 0.71)	0.28
QUICKI	Baseline	0.15 (0.02)	0.14 (0.02)	*NA*	
	16 weeks	0.14 (0.01)	0.14 (0.01)	0.00 (-0.00, 0.00)	0.71
Cholesterol (mmol/L)	Baseline	5.5 (1.0)	5.5 (1.0)	*NA*	
	16 weeks	5.5 (0.7)	5.5 (0.7)	0.08 (-0.26, 0.10)	0.39
LDL-C (mmol/L)	Baseline	3.6 (1.0)	3.6 (1.0)	*NA*	
	16 weeks	3.6 (0.6)	3.5 (0.6)	-0.11 (-0.27, 0.05)	0.19
HDL- C (mmol/L)	Baseline	1.5 (0.4)	1.5 (0.4)	*NA*	
	16 weeks	1.5 (0.2)	1.5 (0.2)	-0.00 (-0.05, 0.07)	0.78
Triglycerides (mmol/L)	Baseline	1.5 (0.6)	1.4 (0.6)	*NA*	
	16 weeks	1.4 (0.5)	1.4 (0.5)	0.00 (-0.13, 0.13)	0.98
FLI	Baseline	10.7 (15.3)	10.4 (15.3)	*NA*	
	16 weeks	10.1 (4.7)	10.2 (4.7)	-0.06 (-1.25, 1.37)	0.93
HSI	Baseline	42.6 (5.5)	42.3 (5.5)	*NA*	
	16 weeks	42.4 (2.6)	42.6 (2.6)	-0.17 (-0.55, 0.89)	0.63
CRP§ (mg/L)	Baseline	1.7 (1.0)	1.6 (1.0)	*NA*	
	16 weeks	1.9 (0.9)	1.8 (1.0)	0.09 (-0.4, 0.23)	0.58

HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment insulin resistance; QUICKI, quantitative insulin sensitivity check index; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol, FLI fatty liver index, HSI hepatic steatosis index. * Mean adjusted for study cite, † Δ between groups represents the difference in adjusted means control - oat between baseline and 16 weeks, ‡Generalized linear model. Δ week 16 – week 0 as dependent variable, treatment group as factor, week 1 value and center as included covariates. § Values above ≥ 4.1 mg/L removed. Analyses are based on n = 137 (Oat = 75, Control= 62).

5.6.1 Bread consumption, acceptance and adherence

Adherence to the protocol was not measured in the CarbHealth study due to its pragmatic design. However, based on the consumption journals, participants in the oat group consumed an average of 3.4 slices per day (SD: 0.6), providing approximately 6.8 grams of β-glucans daily over 16 weeks. The control group consumed an average of 3.6 slices per day (SD: 0.8), corresponding to 0.07 grams of β-glucans daily. The consumption pattern was similar between the groups. In the oat group, nine individuals (8.8 %) and in the control group, seven individuals (7.3 %) did not meet the instructed intake of at least three slices per day. The majority (79 %) of participants in the oat group reported consuming between 3 and 4 slices daily, while 76 % of the control group did the same. Around 8 % in the oat group and 11% in the control group reported consuming between 4 and 6 slices per day (**Table 10**). Adherence is based on self-reported data, and it is known that adherence to interventions varies significantly among individuals. A complement to self-reported data could be the use of oat biomarkers such as avenanthramides and avenacosides. Due to the pragmatic design of the intervention, all participants were included in analyses independent of adherence to the protocol. This reflects the real-world context, where perfect adherence is rare, and thus the results may better represent the typical effectiveness of the intervention.

Table 10. Average number of slices of bread consumed per day (ITT, oat = 102, control = 92)

Group	Average/day[1]	β-glucans[2]	≥ 3 slices[3]	3-4 slices[4]	4-6 slices[5]	< 3 slices[6]
Oat	3.4 (0.6)	6.8 g/day	88.3 %	79.0 %	8.0 %	8.8 %
Control	3.6 (0.8)	0.07 g/day	89.9 %	76.2 %	11.3 %	7.3 %

[1] Mean number of slices consumed per day (SD), [2] Mean daily intake of β-glucans, [3] Percentage of participants consuming ≥ 3 slices/day, [4] Percentage of participants consuming 3-4 slices/day, [5] Percentage of participants consuming 4-6 slices/day, [6] Percentage of participants consuming < 3 slices/day.

Overall, both types of bread were equally accepted at the start of the trial and at week 8. However, acceptance varied between countries. German participants showed significantly higher acceptance of the oat bread at week 8 (mean score = 5.9) compared to Norwegian participants (mean score = 3.7). Participants also reported significantly higher overall satiety (mean score = 4.6) and satiation (mean score = 6.9) after consuming oat bread compared to the control bread (mean scores of 4.3 and 6.2, respectively). No differences in satiety or satiation were observed between day 1 and week 8, or between countries (data not shown).

In summary, while efficacy studies have shown benefits of β-glucans for glycemic control, this real-world effectiveness study did not demonstrate significant metabolic improvements from β-glucan-enriched bread in a high-risk population. Variability in adherence, baseline metabolic health, and daily consumption patterns may influence outcomes, suggesting that real-world application of β-glucan-enriched foods requires further exploration to optimize effectiveness.

6 General discussion

In the MEDGI-Carb study (**Paper I)** it was found that low-GI foods improved postprandial glucose control, and indices of glycemic variability among non-diabetic individuals compared to high-GI foods. These findings suggest that incorporating low-GI foods into a daily diet can improve postprandial glucose response, a factor linked to an increased risk of developing type 2 diabetes and CVD.

Additionally, this study showed that the Mediterranean diet lowered daily blood glucose levels, regardless of GI group. This provides additional evidence of the health benefits of the Mediterranean diet, which has consistently been associated with a lower risk of developing cardiometabolic disease. Therefore, recommending the Mediterranean diet, particularly with an emphasis on low-GI foods, may further improve blood glucose control and reduce cardiometabolic risk. This combination improves the Mediterranean diet's inherent health benefits while optimizing glycemic control, offering an effective approach to promoting long-term metabolic health among individuals at cardiometabolic risk.

In **Paper II,** a mechanistic model was used on data from a MMTT from the MEDGI-Carb trial and successfully identified two response clusters, with different postprandial responses. The different response clusters were differently associated with risk factors of type 2 diabetes. These findings provide valuable insights into the field of precision nutrition, underscoring the need for personalized dietary strategies due to large variability in individual dietary responses. Tailored approaches that account for these individual differences could provide more effective ways to optimize health compared to generalized dietary recommendations. Furthermore, the model used in the analysis only included glucose data making it an option when measurements of insulin are missing such as for continuous glucose device measurements.

Results in **Paper III** showed that while the Mediterranean diet increased postprandial plasma acetate concentrations, it did not significantly affect propionate, butyrate, or IPA levels. A study from Seethaler et al (204) examined the association between the Mediterranean diet, SCFA concentrations, and intestinal barrier integrity. After three months, fecal SCFA levels increased in the Mediterranean diet group compared to controls. Unlike the current study, which focuses on plasma SCFA levels, Seethaler et al. used fecal SCFA concentrations. However, studies indicate that fecal and plasma SCFA concentrations are not directly correlated (205, 206). There are benefits of measuring SCFA in plasma compared to fecal samples, while fecal concentration mostly indicates local production in the gut, circulating SCFA reflects the SCFA absorbed and available for peripheral tissues (207, 208). Furthermore, circulating SCFAs have been shown to be more directly associated with metabolic health outcomes compared to fecal SCFAs

(209).

While previous studies suggest that butyrate has a role in improving glycemic control, we did not find a significant difference in plasma concentrations between healthy vs individuals with impaired glucose control. This discrepancy highlights the complexity of diet-microbiota-host interactions, suggesting that factors like individual variability, diet, and/or baseline gut microbiota composition might influence the link between SCFA and glycemic outcomes. Furthermore, gut microbiota analysis revealed that certain bacterial taxa, rather than overall diversity, were more abundant in individuals with healthier glucose control, underscoring the influence of specific microbes on metabolic health. Together, these findings emphasize the importance of further investigating the role of gut microbiota metabolites in glycemic regulation, particularly among adults at increased cardiometabolic risk.

Baseline acetate concentrations were linked to lower systolic blood pressure, which is in line with previous research (201). However, contrary to expectations, no associations were found between butyrate, propionate, or IPA and cardiometabolic risk factors. The metabolic effects of SCFAs and IPA can vary widely due to factors like age, sex, ethnicity, gut microbiota composition, and genetics. External factors, including diet, lifestyle, and medication, may also modulate these effects (21) highlighting the complexity of these interactions.

While efficacy studies have demonstrated benefits of β-glucans for glycemic control, the CarbHealth trial, a real-world effectiveness study, did not observe significant metabolic changes caused by β-glucans (**Paper IV**). The beneficial glycemic and blood lipid response from consuming β-glucans has resulted in an authorized health claims from EFSA (15). However, the health claim is based on the effect of β-glucans on acute short-term studies. Furthermore, there has been some criticism of the EFSA claim on oat β-glucans that primarily revolves around the varying effectiveness of β-glucans in different contexts. For example, the EFSA claim in reducing postprandial glucose is based on 4 grams of β-glucans per 30 grams available carbohydrates (15). In **Paper IV** the daily dose of β-glucans was 6 grams per day, assuming a consumption of three slices of bread per day, yet a significant effect on glycemic control or blood lipid concentration was not observed. However, the effect of β-glucans vary depending on molecular weight, solubility and food matrix (78), impacting their ability to reduce blood glucose levels in real-world settings.

One other example is an application to use the health claim on processed breakfast cereals (1.3 g β-glucans per 25 g available carbohydrates), which EFSA rejected due to insufficient evidence at lower doses, and for β-glucans in a product that had been extensively processed (210). This demonstrates the challenge of translating β-glucan

efficacy from controlled studies to diverse food applications where processing may alter β-glucan properties. Unlike controlled environments, real-world settings involve diverse dietary habits, and variability in β-glucan intake, which may all influence glycemic outcomes. More studies should be designed with the aim of evaluating the effect of an intervention in a real-world context.

Overall, the findings from this thesis underscore the complexity of dietary effects on metabolic health, especially for populations at high cardiometabolic risk. This thesis highlights the importance of carbohydrate quality, even in the context of a healthy diet pattern. A healthy diet does not fully compensate for consuming foods of low carbohydrate quality. Furthermore, the findings on gut microbiota metabolites in relation to glycemic control demonstrate the complexity of the relationship between glycemic regulation and gut microbiota derived metabolites. Moreover, the demonstration of differential responders of a standardized meal provides further evidence of the large inter-individual variably, highlighting the need for more personalized guidelines to improve metabolic effects and potentially subsequently also public health. Finally, this thesis demonstrates the challenges of translating health effects observed in controlled trials to everyday context, and thereby the potential impact of these findings in the real-world settings.

7 Strengths and Limitations

The studies presented in this thesis have several strengths and limitations, with key methodological considerations discussed below.

The thesis is based on two large-scale, international, multicenter dietary intervention trials with large sample sizes and robust study designs. Both trials employed a randomized controlled design, considered the gold standard for evaluating the effect of an intervention. Additionally, the international setting, covering several countries in Europe and the USA, allowed for the inclusion of local dietary habits from different nations with different habitual dietary and lifestyle patterns. Other strengths include long intervention periods, and the provision of intervention products, which increased the likelihood of adherence to the study protocols.

Participants in both trials were blinded to their allocated groups, which is considered a methodological strength. However, blinding in dietary interventions can be challenging, as it is often difficult to fully conceal the nature of what participants are consuming. In the MEDGI-Carb study, the diets were identical in terms of protein and fat content, with the groups sharing half of the carbohydrate types, while the other half differed. As a result, it is possible that some participants were able to guess their assigned group. In the CarbHealth trial, there was a slight difference in color between the oat and whole grain wheat breads. While participants were not allowed to directly compare the breads and did not see both types simultaneously during clinic visits, the subtle color variation may have allowed some participants to assume their group allocation. However, the research staff conducting clinical examinations and collecting samples were unaware of the participants' group allocation. Additionally, group allocation remained blinded during the analyses and was not revealed until after the data analysis was completed.

Additional strengths in both trials are the choice of comparator. In the MEDGI-Carb trial both groups consumed a healthy Mediterranean diet allowing us to distinguish the relative contribution of GI in cardiometabolic risk markers. Furthermore, the robustness of the primary manipulated variable, i.e., the glycemic index of carbohydrates, was ensured through independent analyses to confirm a sufficient difference between the groups, allowing for the detection of a significant effect. Additionally, the GI values of starchy foods were determined using the method outlined by the Food and Agriculture Organization/World Health Organization and following the guidelines established by the International Standards Organization. In the CarbHealth trial the comparator was a whole-grain wheat bread, which aligns well with everyday dietary habits in Sweden, Norway, and Germany, where whole-grain and mixed-grain breads are commonly consumed.

A potential limitation in both trials is that both study samples consisting almost exclusively of participants of Caucasian ethnicity. As a result, caution should be made when applying the findings to other ethnic groups.

Additionally, analyses in **Paper II** and **Paper III** were exploratory and no prior power calculation was performed. While this may limit the statistical power to detect smaller effects, the exploratory design enables a broader examination of potential trends and associations that could inform future, more targeted studies. Findings from these analyses should therefore be interpreted with caution, as they may be influenced by sample size limitations and variability within the data. In the CarbHealth trial, although the recruitment goal of 250 participants was not achieved, which could be seen as a potential limitation in detecting significant effects, the drop-out rate was notably lower than expected, approximately 19 % compared to an anticipated 45 %. This lower drop-out rate helped maintain a sample size that met the requirements for statistical power. Consequently, despite recruitment challenges, including those posed by the COVID-19 pandemic, the study likely retained sufficient power to detect meaningful outcomes. The CarbHealth trial was conducted during the COVID-19 pandemic, which involved varying restrictions across the participating countries. Fortunately, the trial proceeded as planned, albeit with significant delays. However, it remains difficult to assess the pandemic's impact on participants' habitual dietary habits and physical activity and thereby the effectiveness of the intervention.

In **Paper IV**, differential responders were identified through postprandial glucose measurements using a mechanistic model. While the model effectively captured most physiological responses, it has some simplifications within the model, such as the assumption of a fasted state before each meal. This limitation suggests the model may be less accurate when applied to multiple sequential meals where glucose dynamics become more complex such as the second meal effect. Despite these limitations, the model effectively identified major differences in glucose regulation among clusters, illustrating a nuanced depiction of individual variability in glucose response.

8 Conclusions

In conclusion, this thesis investigated how carbohydrate quality, dietary fiber and gut microbiota interactions affect glycemic control and metabolic health among adults at elevated cardiometabolic risk. The findings highlight the benefits of low-GI foods within a Mediterranean diet, the lack of impact of β-glucan-enriched foods in real-world settings, and the role of gut-derived metabolites and microbial composition in metabolic health. Together, these findings underscore the importance of individualized dietary strategies and the challenges of translating controlled trial results into everyday applications.

More precisely, the results showed that:

- The sustained improvement in postprandial glucose control over time among non-diabetic individuals consuming low-GI foods highlights the benefits of these foods within a healthy Mediterranean diet.

- A Mediterranean diet can improve daily glycemia regardless of glycemic index. However, only the low glycemic index diet produced meaningful improvements in daily glycemic variability.

- A simplified model was effectively used to characterize glucose responses to a standardized mixed meal tolerance test, identifying two distinct response clusters. These clusters demonstrated different associations with type 2 diabetes risk markers and gut microbiota profiles, contributing insights into how individual metabolic responses relate to diabetes risk and microbial composition.

- Following a 12-week Mediterranean diet led to a significant increase in postprandial acetate levels after a standardized meal, while no changes were observed for the gut microbial metabolites propionate, butyrate, or for IPA. Baseline acetate concentrations were linked to reductions in systolic blood pressure, suggesting potential benefits for cardiometabolic health.

- A difference in gut microbiota composition was observed between individuals with healthy glucose control and those with impaired glucose control. While this finding may suggest the importance of specific microbial taxa, rather than overall microbial diversity, in influencing metabolic outcomes, it remains unclear whether these microbial changes drive impaired glucose control or result from it.

- No significant improvements in glycemic control were observed after 16 weeks of

consuming β-glucan-enriched bread in individuals at elevated risk of developing type 2 diabetes. This suggests that replacing a healthy control whole grain bread with β-glucan enriched alternative may not be enough to improve glycemic control in this population.

9 Future perspectives

- Given the large inter-individual variability in metabolic responses to standardized meals, future studies should first aim to better characterize individual metabolic profiles and gut microbiota composition. This knowledge could then support the development of personalized guidelines tailored to these profiles. Additionally, research should explore how personalized recommendations might be integrated into broader dietary guidelines.

- Future studies should investigate if glucose response clusters can be identified using the mechanistic model using data from continuous glucose monitoring devices in a home setting. The mechanistic model should also be validated in different populations such as women with gestational diabetes or individuals with manifest type 2 diabetes.

- Future studies are needed to examine how diet, host factors, and gut microbiota collectively impact circulating SCFA and IPA levels and thereby influence their potential metabolic benefits.

- Specific bacterial taxa have been associated with glycemic control, and future studies should investigate how these taxa interact with dietary components that influence glycemic outcomes. Understanding these interactions can lead to interventions targeting host gut microbiota, such as specific dietary fibers or probiotics that can enhance the benefits from a healthy diet pattern such as the Mediterranean- or the Nordic diet.

- While short-term clinical trials support the benefits of β-glucans for blood glucose response, pragmatic, real-world trials are needed to assess how food processing and consumption patterns impact their effectiveness. Research in these areas could identify optimal food matrices and processing methods, making β-glucans more effective in everyday dietary contexts.

- Since both trials included in this thesis primarily included Caucasian participants, future trials should be conducted in other ethnic groups to capture potential variations in metabolic responses and ensure that findings are applicable across diverse populations.

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