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The MEDGICarb-Study: Design of a multi-center randomized controlled trial to determine the differential health-promoting effects of low- and high-glycemic index Mediterranean-style eating patterns

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ABSTRACT

Adults with central adiposity and other features of the metabolic syndrome have a markedly elevated risk of developing type 2 diabetes (T2D) and cardiovascular disease (CVD). A Mediterranean-style healthy eating pattern (MED-HEP) and consumption of foods with a lower glycemic index (GI) are potential dietary approaches to curb the T2D and CVD epidemic. However, experimental evidence of the effectiveness of MED-HEP and of the contribution of GI towards improving indices of glucose homeostasis, especially among non-diabetic people, are lacking. Therefore, we developed the MedGI-Carb trial, a multi-center (Italy, Sweden, and United States) intervention in adults with at least two components of the metabolic syndrome (elevated waist circumference + one other component) that aims to improve markers of glucose homeostasis through dietary modification. All participants were randomized to consume an isocaloric high- or low-GI MED-HEP for 12 weeks. We hypothesized that indexes of insulinemia (primary outcome: postprandial insulin and glucose after standardized breakfast and lunch; secondary outcomes: fasting plasma glucose and insulin, HbA_{1c}, 24-h continuous glucose monitoring) would be improved more with the low-GI versus the high-GI MED-HEP. Additionally, we hypothesized that consumption of a MED-HEP would improve other markers of cardiometabolic health and well-being (fasting blood pressure, fasting lipid profile, sleep quality, satiety, global metabolic alterations in the plasma metabolome, changes in the gut microbiota, subjective health and well-being), with no difference between groups. Collectively, the design of MEDGI-Carb allows several different research questions to be explored. TRIAL REGISTRATION: ClinicalTrials.gov identifier: NCT03410719.

1. Introduction

The global burden of type 2 diabetes mellitus (T2D) ballooned from 30 million in 1985 to 463 million in 2019 [1,2]. This is particularly troubling given the close association between T2D and cardiovascular disease (CVD) — the leading cause of morbidity and mortality in the United States and in many other Western countries [3]. The traditional medical establishment is struggling to handle this burden, necessitating redoubling research efforts to develop effective and sustainable lifestyle interventions — of which consuming healthier diets feature prominently — to curb this epidemic [4–8]. Postprandial glycemic and insulinemic responses are firmly implicated in the development of T2D and CVD [9,10]. Among factors influencing postprandial glycaemia, the glycemic index (GI) of the carbohydrate source is posited to play a central role [11]. However, the utility and relevance of the GI, particularly in the

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Abbreviation list

Abbreviation	*Meaning*
CGM	Continuous glucose monitoring
CV	Coefficient of variation
CVD	Cardiovascular disease
ESS	Epworth Sleepiness Scale
GI	Glycemic index
HEP	Healthy eating pattern
IPAQ	International Physical Activity Questionnaire
MED	Mediterranean
MGTT	Meal glucose tolerance test
NDSR	Nutrient Data System for Research
OGTT	Oral glucose tolerance test
PSQI	Pittsburgh Sleep Quality Index
TP	Time point
T2D	Type 2 diabetes
SF-36v2	Medical Outcomes Study 36-item short-form questionnaire

context of a mixed diet for people without T2D, has been a subject of debate for decades [12–15]. Specifically, GI has been questioned on counts of: failure to consider the insulin response [16], the high intra- and inter-subject variation in glucose response to a food [17], and a loss of discriminating power when foods are combined in a mixed meal [18]. Given the shift towards increased focus on eating patterns rather than specific foods or nutrients, it is of relevance to determine the impact of different GI foods in the context of a healthy eating pattern (HEP), especially considering the benefits of a low GI diet may be less pronounced in the presence of other health-promoting dietary features [19].

One such healthy eating pattern is a Mediterranean-style (MED) HEP — a pattern broadly emphasizing intake of fruits, vegetables, olive oil, and moderate intake of animal products — which has recently been touted as a fount of cardiometabolic wellness and is recommended by the World Health Organization [20] and other governmental bodies [21]. There is ample evidence that the MED HEP effectively promotes cardiometabolic health and wellness [22,23], and reduces risk of developing T2D [24,25]. However, there is currently limited experimental evidence supporting a MED HEP, underscored by a notable paucity of evidence supporting this eating pattern in populations other than from the MED region. To our knowledge, there have been no studies where low GI foods have been combined with a MED HEP.

Therefore, we developed a multi-center intervention study in adults with components of metabolic syndrome, i.e. at risk for development of diabetes, that aims to improve glycemic responses through modification of carbohydrate quality in the context of an overall healthy MED HEP. The purpose of the present study was to investigate differential effects of a low- vs high-GI MED HEPs on cardiometabolic health and well-being among participants with at least two features of the metabolic syndrome, as they are at greater risk of developing T2D and CVD [26]. During the intervention, all participants consumed an energy-sufficient MED HEP (based on criteria to be designated as a USDA Healthy Mediterranean Eating Pattern), differing only in the GI of half of the carbohydrate-foods provided in the diet. We hypothesized that the reduction in postprandial insulin and glucose responses would be greater for the low-GI versus high-GI MED HEP after the 12-week intervention. Additionally, we hypothesized that consumption of a MED HEP would result in comparable improvements in indices of cardiometabolic risk factors including fasting blood pressures, fasting serum lipid profile, glucose, insulin, hemoglobin A_{1c} (HbA_{1c}), and 24-h continuous interstitial glucose monitoring. Further, we plan to assess the potential for low-GI HEPs to differentially improve global metabolic alterations as detected in the plasma metabolome and composition of gut microbiota.

2. Methods/design

The MEDGI-Carb trial is an international multi-center randomized, controlled, parallel group, 15-week trial including a 3-week baseline testing period followed by 12 weeks of controlled dietary intervention (Fig. 1). This research study was initiated in January 2018 and the trial continued through December 2019. This study consisted of three centers at 1) Federico II University - Naples, Italy 2) Chalmers University of Technology - Gothenburg, Sweden, and 3) Purdue University - West Lafayette, IN, USA. This design allowed us to investigate the effects of GI in the context of a HEP in different settings and food environments. The study protocol was approved by the institutional review board of Federico II University, the Regional Ethical Review Board, Gothenburg, Sweden, and the biomedical institutional review board at Purdue University. This study is registered in the public trial registry Clinicaltrials.gov as NCT03410719.

2.1. Experimental design

During the intervention period, subjects consumed a controlled, isocaloric weight-maintenance diet and were instructed to consume intervention-specific foods to achieve a low-glycemic or high-glycemic MED HEP (low-GI or high-GI, respectively). During baseline and post-intervention assessments, the diet, general health, sleep quality, fecal microbiota profile, and blood pressure were assessed. Outcome measurements were obtained on testing days to determine markers of glucose homeostasis by completion of an 8-h meal glucose tolerance test (MGTT) including both breakfast and lunch resembling food choices of the assigned diet, an oral glucose tolerance test (OGTT), and continuous glucose monitoring (CGM) at baseline, mid-point (only in USA), and post-testing. Follow-up data of eating pattern, sleep quality, and general health and well-being were collected either personally or via email or regular postal services 3 months after completion of the 12-week controlled intervention period. All subjects were instructed to maintain their habitual types and levels of physical activities.

2.2. Eligibility criteria

The eligibility criteria were designed to select middle-aged and older adults at risk for developing CVD or T2D. Therefore, adults with waist circumference >102 cm (males) or > 88 cm (females) and one additional feature of the Metabolic Syndrome according to ATP [III] [27]: including blood pressure >130/85 mmHg or taking medication to control high blood pressure, fasting plasma glucose 100–126 mg/dL, fasting triglycerides 150–400 mg/dL, HDL cholesterol <40 mg/dL (males) or <50 mg/dL (females), were recruited. Additional study inclusion criteria were as follows: age 30–69 y, BMI 25–37 (kg/m^2), weight stable (±3 kg in previous 3 months), no acute illness, no cardiovascular events (myocardial infarction or stroke) during the 6 months prior to the study, not anemic (hemoglobin >12 g/dL), no renal or liver failure (creatinine <1.7 mg/dl and alanine aminotransferase/aspartate aminotransferase <2 times than normal values, respectively), not diabetic, not pregnant or lactating, not a vegetarian or consuming a diet incompatible with protocol diets, not smoking >20 cigarettes per day, not involved in a stable intensive physical activity regimen (<3 h/week of moderate or high intensity resistance or aerobic exercise training), Subjects taking antihypertensive and statin drugs were asked to keep the type and dosage of medication unchanged throughout the study period, or to notify research investigators soon after physician-directed changes were made.

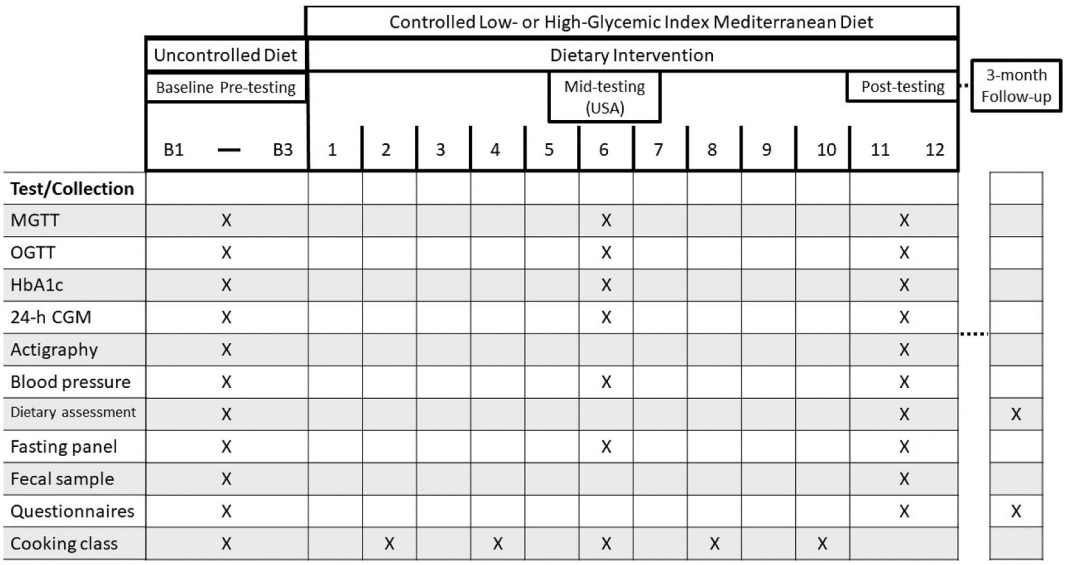

Fig. 1. Study Schematic of the MedGI-Carb-trial, a randomized single-blinded controlled trial assessing the effects of low-versus high-glycemic index in the context of a Mediterranean-style healthy eating pattern. CGM, continuous glucose monitoring; MGTT, meal glucose tolerance test; OGTT, oral glucose tolerance test. Blood panel includes complete metabolic panel (fasting glucose, GFR, etc.) 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 Tool.

2.3. Recruitment

Due to the different environments of the three centers, the recruitment and onboarding process varied by site.

The Italian center: To reach potential participants, information on the study was sent to all University employees through the administration's profiling database, and by displaying some specific leaflets around the "Federico II" University Hospital. In addition, participants of previous experimental studies were also invited to participate, as well as patients from hypertension, dyslipidemia and obesity clinics of the University Hospital. Participants who responded to advertisement were invited to the testing facilities of the Diabetes, Nutrition and Metabolism Unit for a screening visit in order to verify inclusion and exclusion criteria. The potential participants were invited to participate in the study.

The Swedish center: Participants from the Swedish site were recruited via internet postings and advertising in the Gothenburg area. Participants were sought via the press, announcements and leaflets. Further detailed information about the study were sent by email or regular mail to all people who responded to advertisements. If possible, people interested in participation were contacted by e-mail or telephone for a pre-screening according to the inclusion/exclusion criteria and any interested volunteer who expressed willingness to participate in the study was scheduled for a screening visit at the clinical laboratory of the Department of Clinical Nutrition, Sahlgrenska Academy, University of Gothenburg, where the study was conducted. Anthropometric and blood pressure measurements were taken. A blood sample was drawn for analysis of health parameters needed for the evaluation of inclusion/exclusion. Potential participants completed also a medical history questionnaire and demographic form.

The United States center: Numerous mediums were used to reach potential participants. Physical advertisements such as flyers were posted around the greater Lafayette, IN area. Online advertisements on Purdue University websites were posted. A professional study recruitment service specializing in social media marketing was contracted (Trialfacts©, Australia). A multi-step process was used to recruit potential participants in this research study. Participants who responded to advertisement were sent a pre-screening form and the opportunity to provide consent for us to send them a medical history questionnaire to obtain more in-depth information. After reviewing both the prescreening form and the completed medical history questionnaire, participants who qualified were invited to the testing facilities on the Purdue University campus in West Lafayette, IN for a screening visit. Should participants qualify based on the additional information gained from the anthropometric and blood results collected from the screening visit, all information was sent to a study physician. Should the study physician deem it safe for a potential participant to proceed, the potential participant would be invited to enroll in the study.

2.4. Informed consent procedure

All procedures for obtaining consent were reviewed and approved by the Federico II University Institutional Review Board, Naples, Italy (IRB Protocol #175/17), the Regional Ethical Review Board, Gothenburg, Sweden (IRB Protocol #663–17), and the Purdue University Biomedical Institutional Review Board (IRB Protocol #1610018310).

All potential participants were screened for eligibility and given opportunities to voice any questions or concerns. If a potential participant qualified based on the results from the screening visit and physician review, they were invited to join the study. If the participant accepted the invitation, a study orientation day was scheduled where participants first reviewed the study-consent documentation with a researcher and were given opportunities to voice any questions or concerns. Pending any concerns, participants then signed a consent form with a data clause allowing researchers to analyze samples for variables not specified in the primary aims (*e.g.* this consent form would permit determination of triglycerides).

2.5. Randomization

A member of the research team at each of the three testing sites who was not involved in data collection or analysis generated the random allocation sequence and assigned subjects to the interventions. Each subject was randomly assigned to one of two dietary groups using either a stratified block pattern (Italy & US; n = 8, 10 blocks; 4 randomized to each group per block of 8, using an online randomization plan generator; http://randomization.com/) or mixed size of the block pattern (Sweden; 4, 6, and 8 subjects per block in random block order; using Rstudio

software version 2.4.0 with package 'blockrand' version 1.3). The randomization code remained unrevealed until all participant testing and analyses of samples for *a priori* cardiometabolic outcomes were completed.

2.6. Dietary assessment

All participants completed a 14-item MED Diet Assessment Tool, adapted from the PREDIMED trial [28], to determine adherence to the MED HEP while consuming self-selected diets. Center-specific procedures for dietary assessments are provided below.

The Italian center: Dietary intakes were assessed by a four-day dietary record (three working days and one weekend day) at baseline and at weeks 4, 8, and 12 during the intervention, and 3 months after the intervention. Dietary-record data were entered into the Metadieta 4.0 software (METEDA S.r.l., San Benedetto del Tronto, Italy) for determination of food and nutrient intake assessed based on the Italian food composition table.

The Swedish center: The subjects reported their food intake by four-day food records (three working days and one weekend day) with portion estimation at baseline and after 4-week of intervention, at post-testing week and 3 months after the intervention. To determine the nutritional composition of the reported food intake, the Dietist Net Pro software (Kost och Näringsdata AB, Bromma, Sweden) with nutrient information from the Swedish Food Composition Database, was used.

The United States center: Dietary intakes were assessed via three-day dietary recalls on non-consecutive days during baseline weeks and 3 months after completing the intervention. A registered dietitian at the Purdue Bionutrition center contacted participants at unannounced times to attenuate the observer effect (aka Hawthorne effect). Dietary recall data were entered into the Nutrient Data System for Research (NDSR; University of Minnesota, NCC. Minneapolis, MN) for determination of food and nutrient intakes. Healthy Eating Index of participant dietary recalls and sample menus were calculated by inputting NDSR outputs into SAS program code provided by the United States National Institutes of Health, National Cancer Institute (https://epi.grants.cancer.gov/hei/sas-code.html).

2.7. Dietary compliance

During the 3-week baseline period, all subjects consumed their habitual, self-chosen, unrestricted diets. Throughout the 12-week intervention period, each subject was counseled to follow their assigned iso-caloric MED HEP using a combination of prescribed menus (breakfast, lunch, and snack eating occasions) and an item specific version of the 'Dinner Recipe Builder' for dinner. The 'Dinner Recipe Builder' bestowed greater freedom to participants with the goal of reducing burnout, allowing them to mix and match ingredients while still following a MED HEP. A sample 'Dinner Recipe Builder' (low-GI group) is provided in Supplemental Table 1. The two group-specific diet plans contained primarily the same foods and beverages typically included in MED HEPs (adjusted to local eating habits and food preferences), except for substitutions of major sources of starch in the meals: high-GI – jasmine rice, potato, mashed potatoes, cous-cous, wholegrain bread and rusks; low-GI – pasta, brown rice, flat bread, and wheat plus rye bread and seeds. All diet-related activities and assessments were performed in conjunction with the Indiana Clinical Research Center Bionutrition Facility at Purdue University, the Diabetes, Nutrition and Metabolism Unit at Federico II University of Naples and at the Clinical Nutrition facilities at Sahlgrenska Academy, University of Gothenburg.

The overarching goals were to have all participants in both groups consume a MED HEP with the same quantities of metabolizable carbohydrate (270 g/d) and fiber, and sufficient total energy for weight maintenance. Higher or lower energy content was achieved through modulation of dietary fat and protein. One-half of daily carbohydrate (135 g) was the same between low-GI and high-GI groups, including carbohydrates in fruits, vegetables, and other foods that all subjects consumed. The other one-half of daily carbohydrate intake (135 g) was specific to the low-GI and high-GI groups. Specifically, 135 g of carbohydrate in the low-GI group came from foods with GI values < 55, while 135 g of CHO in the high-GI group came from foods with GI values > 70. The intervention specific carbohydrates were distributed as 35 g at breakfast, 40 g at lunch, and 60 g at dinner. Dietary fiber was held at 35 g/d (range of 32–38 g/d) for both intervention groups. The specific means of achieving dietary control varied slightly among centers, dependent upon their respective research infrastructure and participant-lifestyle considerations. Center-specific procedures to achieve dietary control are described below.

The GI values of starchy foods included in the low- and high-GI diets were determined following the method described by the Food and Agriculture Organization/World Health Organization [29] and, later, applying the guidelines set up by the International Standards Organization [30]. All the analyses were performed at the Department of Food Science (Nutrition Unit) of the University of Parma, Italy. A list of the GI of starchy foods utilized in the study is provided in Supplemental Table 2.

The Italian center: Dietary counseling included group dinner meal preparation sessions every other week. All starchy foods (pasta, wheat plus rye bread and seeds, flat bread, brown rice for low GI diet, and wholegrain bread, jasmine rice, whole meal rusks, potatoes and mashed potatoes for high GI diet) and the extra virgin olive oil to be consumed throughout the study period were provided to participants free of charge. The individualized menus were developed by a registered dietitian, using Metadieta software (Meteda s.r.l., Italy). Menus were designed to meet criteria to be classified as a MED HEP [31]. Sample menus for both the low-GI and high-GI diets are provided in Supplemental Table 3. Menu booklets provided to participants to help their food choices in agreement to the prescribed diet consisted of 7-d rotating menus with food items (in gram amounts) grouped into breakfast, lunch, dinner, and snack categories. The target energy intake for each participant during the study is based on the energy equation for resting energy expenditure (MJ/d) according to LARN 2014 [32] multiplied with sedentary physical activity (PAL 1.45). Finally presented in total energy expenditure kcal/day. Five variants of the standard menu, ranging from 1700 kcal/d to 3000 kcal/d, were available to address different energy needs of the participants. Energy adjustments were performed in case of $\pm \Delta$ 3 kg weight change at any point of intervention period.

The Swedish center: Dietary counseling included group dinner meal preparation sessions every other week. All starchy foods (pasta, wheat plus rye bread and seeds, flat bread, brown rice, all bran flakes for low GI diet, and wholegrain bread, frasrost white bread, jasmine rice, wholemeal rusks, cornflakes, mashed potatoes and potatoes for high GI diet) and the extra virgin olive oil to be consumed throughout the intervention period were provided to participants free of charge during biweekly cooking sessions. Also, snacks such as: almonds, hazel nuts, shredded coconut, sunflower seeds, dried apricots, raisins and dried bananas were provided free of charge in connection with starchy foods. Subjects were limited to 3 servings of alcohol per week. Water and non-energy, caffeine-containing beverages were allowed ad libitum during the intervention. The individualized menus were developed by a registered dietitian, using Dietist Net Pro software (Kost och Näringsdata AB, Bromma, Sweden). Sample menus for both the low-GI and high-GI diets are provided in Supplemental Table 4. Menu booklets provided to participants to help their food choices in agreement to the prescribed diet consisted of 7-d rotating menus with food items (in portion estimation) grouped into breakfast, lunch, dinner, and snack categories. The target energy intake for each participant during the study is based on the energy equation for resting energy expenditure (MJ/d) according to the Nordic Nutrition Recommendations 2012 [33,34] multiplied with sedentary physical activity (PAL 1.4). Finally presented in total energy expenditure kcal/day. Nine variants of the standard menu, ranging from 1800 kcal/d to 3600 kcal/d, were available to address different energy

needs of the participants. Energy adjustments were performed in case of ± Δ 3 kg weight change at any point of intervention period.

The United States center: All study food was provided to participants during the 12-week intervention period. Dietary counseling included group dinner meal preparation sessions at baseline, conducted at a demonstration kitchen and using the 'Dinner Recipe Builder' as an instruction tool. Subjects were provided with group-specific instructions for food preparation. Energy intake prescription was not modified (except in extreme cases; ± Δ 3 kg) during the first 4 weeks of the intervention period to allow for weight stabilization upon adoption of a new diet. The individualized menus were developed by a registered dietitian, using NDSR software (University of Minnesota, NCC. Minneapolis, MN). Menus were designed to meet criteria to be classified as a USDA Healthy Mediterranean Eating Pattern. Sample menus for both the low-GI and high-GI diets are provided in Supplemental Table 5. The menu booklets provided to participants consisted of 7-d rotating menus with food items (in gram amounts) grouped into breakfast, lunch, afternoon snack, dinner, and evening snack categories. Participants were instructed to weigh the foods out and check them off in the menu booklets when they were consumed. Participants were permitted an optional serving of 5 oz. of red wine daily (not prescribed). Herbal seasoning of food, water, and non-energy, caffeine-containing beverages were allowed ad libitum during the intervention. At the evening meal, participants were instructed to circle one item from each row (or two, whereby they would split the gram amount) to consume from the 'Dinner Recipe Builder'. Each subject's total energy requirement was estimated using the sex-specific equations for normal weight, overweight or obese adults with a low activity level (physical activity coefficients; male = 1.12; female = 1.16) [35]. The baseline menu was established with a daily energy intake of 2400 kcal/d. Five variants of the base menu, ranging from 2100 kcal/d to 3300 kcal/d, were offered to address different energy needs of participants.

Intervention-specific foods were provisioned through an online supermarket grocery ordering service [36]. Due to the flexibility built into the 'Dinner Recipe Builder' meal, participants were in frequent contact with a research staff member on their grocery requirements. Participants were given a grocery order sheet with a pre-filled in column of anticipated grocery needs to adhere to the diet, as well as a column for actual needs to be filled in by the participant weekly. The research staff member would then review and process the grocery order into the online service. At a designated time, the research participant would pull into a parking space in the supermarket and their entire grocery order would be brought to their car. Subjects were provided with all foods, along with scales, measurement cups, measurement spoons, and a digital food scale.

2.8. Compliance

The center-specific procedures to document dietary compliance are described below.

The Italian center: Adherence to diets was evaluated by a four-day dietary record at intervention weeks 4, 8, and 12 and was reinforced by the dietitians through bi-weekly counseling and weekly phone calls. Compliance for participants allocated to high or low GI diets were calculated as a percentage of the quantity of starchy foods ingested among those prescribed for the high or, respectively, the low GI diet, with partial credit assigned to incomplete ingestion of items.

The Swedish center: Adherence to diets was evaluated by a four-day dietary records (after 4-week and at 12-week) and through the use of daily menu compliance checklists. Participants were instructed to check-off boxes next to the items they consumed during each day of the study in the 7-d rotating menu booklets. Participants were instructed to write in any deviations from the menus. Compliance was calculated as a percentage of all food items ingested, with partial credit assigned to incomplete ingestion of items.

The United States center: During the intervention, dietary compliance was assessed formally using daily menu compliance checklists and more crudely in real time through review of online grocery pickup service orders. The grocery orders were reviewed weekly for consistency with the study diet menus. Discrepancies were addressed by discussion with participants. Participants were provided with scales and measuring cups to weigh out portion sizes for each item on the menu (in grams). Participants were instructed to check off boxes next to the items they consumed during each day of the study in the 7-d rotating menu booklets. If an item was not completely consumed, participants were instructed to indicate what percentage of the item was ingested. Participants were instructed to write in any additional items consumed not part of the provided menus. Menu booklets were reviewed in full at the mid-point and post-testing weeks. Compliance was calculated as the percentage of all food items ingested, with partial credit assigned to incomplete ingestion of items.

2.9. Cooking classes

Participants were invited to cooking classes to help them learn to cook MED meals and increase self-efficacy in ability to follow an intensive dietary intervention.

The Italian and Swedish centers: Participants were scheduled for their first cooking class in the baseline run-in weeks (after MGTT) in order to teach them the basic features of the prescribed MED HEP. Thereafter, the class was aimed at instructing participants on how prepare a wide variety of recipes utilizing the starchy foods appropriate to the assigned diet (i.e. pasta, rice, flat bread, cous-cous) in combination with other ingredients (i.e, fat, vegetables, protein sources, herbs) in amounts and combinations apt to get the necessary energy and nutrients intake.

The United States center: Participants were scheduled for their first cooking class in the baseline run-in weeks. The class was designed to help participants prepare the 'Dinner Recipe Builder' meal. Participants were taught how to prepare a wide variety of recipes utilizing starchy foods appropriate to the assigned diet, and how to mix and match ingredients on the 'Dinner Recipe Builder' to make a suitable meal. The first class was mandatory, whereby participants would demonstrate that they could effectively prepare meals to adhere to the dietary intervention. Given the wide spread of culinary experience among participants, the classes could be considered helpful or basic. Due to this variance, attendance to cooking classes beyond the first was encouraged, if helpful to participants, but optional.

2.10. Clinical assessments

Visits for clinical assessments included a meal glucose tolerance test (MGTT), and an oral glucose tolerance test (OGTT) (Fig. 2). Blood pressure, anthropometric assessments including waist circumference and body composition via air displacement plethysmography (USA) or bio-electrical impedance (Italy) were also scheduled to occur while participants were at the testing facilities. Prior to all testing days, participants were instructed not to eat or drink anything (except a small amount of water) from 10:00pm the evening before the visit. Participants were counseled to refrain from vigorous physical activity for 48 h (24 h in Sweden) prior to testing days, avoid alcohol 24 h prior to testing days, and avoid caffeinated beverages the morning of testing days. After arriving at the testing facilities, participants would be seated in a chair/bed to rest. A catheter was placed in an antecubital vein that remained in place for the remainder of the testing day. Blood pressure was taken in duplicate after 15 min of rest. If blood pressure measurements differed by more than 5 mmHg, a third measurement was taken. The blood pressure measurements were then averaged.

2.10.1. Meal glucose tolerance test

Participants reported for an 8 h MGTT during baseline, mid-testing (USA center only), and post-testing. Double-baseline fasting blood

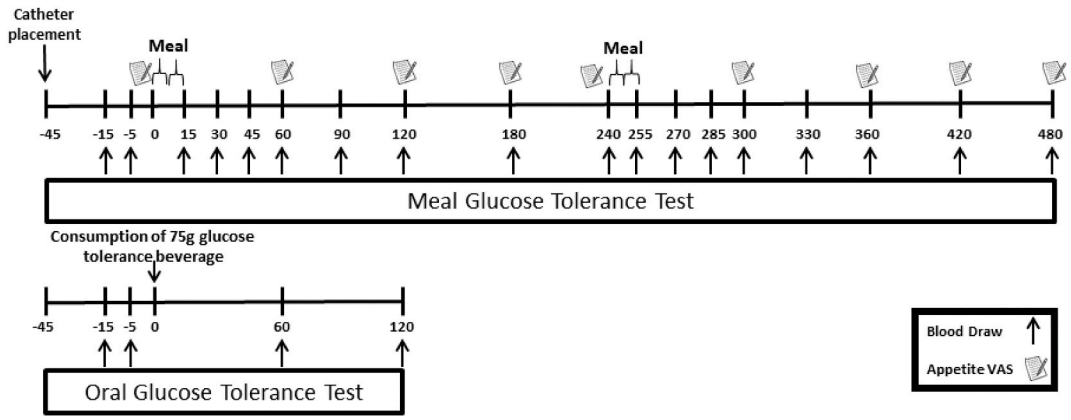

Fig. 2. Meal- and Oral Glucose Tolerance testing day schematic. VAS, visual analog scale.

samples were collected at the −15 min time point (TP) and −5 TP following 15 min of rest. At TP 0, the test meal was consumed in two parts, to better control pace of meal consumption. Subjects had 7.5 min to consume the first half of the meal, and 7.5 min to consume the second half of the meal. Eight ounces of water was included as part of the test meals in which subjects were required to consume during the 15 min. While the two group-specific dietary plans are adjusted to local eating habits and food preferences, the low-GI and high-GI test meals were strictly standardized across all three centers. Full test meal food and nutrient contents of the test meals are provided in Table 1.

Blood samples were collected immediately following the test breakfast meal (TP+15) and then at intervals progressing from 15 min to 1 h between blood sampling: TP+30, 45, 60, 90, 120, 180, and 240. The second test meal was provided following the TP+240 blood draw, and subjects had 15 min to complete the meal (7.5 min per half). The blood draw time pattern was then repeated after the second meal was consumed.

Standard visual analog scales were administered hourly, with the first administration at the −5 TP. Subjects responded to prompts to assessing subjective feelings of such as their level of hunger, desire to eat, and fullness by marking on a 100 mm scale with end descriptors ranging from “Not at all” to “Extremely” [37,38].

Table 1
Food composition of standardized meal glucose tolerance test meals.

Low-GI		High-GI	
Breakfast	Quantity	Breakfast	Quantity
Flat bread	75 g	Cornflakes	30 g
Olive Oil, Extra Virgin	10 g	Olive Oil, Extra Virgin	18 g
Eggs, whole, raw	50 g	Eggs, whole, raw	50 g
Ham, dry cured (country style)	38 g	Ham, dry cured (country style)	85 g
Apple, fresh without skin)	150 g	Apple, fresh without skin	150 g
Milk, 1% fat or lowfat, lactose free	244 g	Milk, 1% fat or lowfat, lactose free	244 g
		Bread, wholegrain	24 g
Lunch		**Lunch**	
Spaghetti noodles, white,	90 g	Rice, jasmine	88 g
Chicken, breast, skinless	58 g	Chicken, breast, skinless	70 g
Tomato sauce, plain, regular	50 g	Tomato sauce, plain, regular	50 g
Olive Oil, Extra Virgin	10 g	Olive Oil, Extra Virgin	10 g
Broccoli, cooked from frozen	90 g	Broccoli, cooked from frozen	90 g
Carrots, cooked from frozen	100 g	Carrots, cooked from frozen	100 g
Apple, fresh, without skin	150 g	Apple, fresh, without skin	150 g

2.10.2. Oral glucose tolerance test

Participants reported for a 2-h OGTT during baseline, mid-testing (USA center only), and post-testing. Prior to OGTT, blood pressure and a hemocue test (Sweden) to rapidly estimate fasting glucose was performed for exclusion of diabetic participants and for safety reasons. A fasting blood sample was collected after 15 min of rest at the −15 TP. A second baseline blood draw was taken 10 min later at the −5 TP (USA, Italy). At TP 0 the glucose tolerance test beverage, containing 75 g glucose dissolved in water (USA: Trutol 75 Glucose Tolerance Beverage, Thermo Scientific, Middletown VA; Sweden: TopStar75, TopLabs; Italy: Bioindustria, L.I.M.) was consumed. Subjects consumed the test beverage within 5 min. Subjects were not permitted additional fluid consumption during the test. Blood samples were collected 60 min, and 120 min after consumption of the test glucose beverage.

2.10.3. Blood collection and analysis

Blood samples were obtained from an antecubital vein and placed in tubes containing a clot activator to obtain serum or sodium/lithium heparin to obtain plasma. Serum tubes were held at room temperature for at least 15 min and then centrifuged at 4,000×*g* at 4 °C for 15 min (3000×*g* at 4 °C for 10 min in Sweden). EDTA-plasma, serum, and heparinized-plasma samples were immediately refrigerated/kept on ice, processed, and 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. Interim analyses for insulin and glucose were not conducted; all samples will be analyzed at the end of the study to minimize batch effects. EDTA-plasma samples will be utilized to assess insulin and glucose. The center-specific procedures to analyze blood samples are described below.

The Italian center: Serum, heparin, and EDTA-plasma tubes collected at fasting and during the MGTT and OGTT testing days were sent to the Diabetes Nutrition and Metabolism research laboratories of the Federico II University for determination of all relevant cardiometabolic parameters. Glucose was measured by enzymatic colorimetry using an oxidase method on an ABX Pentra 400 Autoanalyzer (ABX Diagnostics, Montpellier, France). Insulin was measured by electrochemiluminescence immunoassay method by ELISA (DIAsource ImmunoAssays, Nivelles, Belgium) on a Triturus Analyzer (Diagnostics Grifols, Barcelona, Spain).

The Swedish Center: Serum tubes and EDTA-plasma tubes collected at fasting and during the MGTT and OGTT testing will be analyzed after collection from all participants. Aliquoted plasma and serum samples will be analyzed for all relevant cardiometabolic parameters at the certified laboratory of the Department of Clinical Chemistry at Uppsala University Hospital. HbA_{1c} was analyzed in blood after collection (stored at +4 °C for maximum of one week) by HPLC method at Klinisk Kemi, Laboratoriemedicin, Sahlgrenska Universitetsjukhuset in Gothenburg.

The United States Center: Serum tubes from the baseline measurement of each MGTT and OGTT testing day were sent to MidAmerica Clinical Laboratories (Indianapolis, IN) for determination of fasting serum lipid-lipoprotein profile and a complete metabolic panel (fasting glucose, GFR, etc.). EDTA-plasma tubes from the baseline measurement were sent to MidAmerica Clinical Laboratories (Indianapolis, IN) for determination of HbA_{1c}. Glucose was measured by enzymatic colorimetry using an oxidase method on a COBAS Integra 400 analyzer (Roche Diagnostic Systems, USA, Indianapolis, IN). Insulin was measured by electrochemiluminescence immunoassay method on the Elecsys 2010 analyzer (Roche Diagnotic Systems USA, Indianapolis, IN).

2.10.4. Anthropometric assessments

Weight was measured at screening, baseline, and post-testing with the participant wearing light clothing and the shoes removed. Body weight was measured biweekly in connection with cooking session. BMI was calculated as body weight (in kilograms) divided by height (in meters) squared. Waist circumference was measured (Gulick II Tape Measure, Country Technology Inc., Gays Mills, WI or Seca201 measuring tape, Germany) to the nearest 0.5 cm during screening, baseline and post-testing at the point of the umbilicus in standing participants with arms relaxed along the sides. The participant was asked to take a deep breath, and the measurement is performed after the exhalation. Waist circumference measurements were performed in duplicate, and then averaged.

The Italian Center: At baseline and after 12-week of intervention whole body mass, fat mass, and lean mass were measured using Bioelectrical Impedance Analysis (Bodygram, AKERN s.r.l., Italy). The measurements were performed according to a standard protocol in the morning of the experiment after a 12-h fast, in supine position after 15 min of rest [39]. Resistance (R) and reactance (X) were measured at the flow of a 50-kHz injected mono-frequency current with a coefficient of variance $<1.5\%$.

The Swedish Center: No body composition assessments were performed.

The United States Center: At baseline and post-testing, fasting state whole body mass, fat mass, and lean mass were measured using air displacement plethysmography (BOD POD, COSMED USA, Concord, CA, USA). BOD POD-derived body weights (participants wearing a robe of known weight) were obtained at baseline and post-testing. Participants were also given a bathroom weight-scale for home use.

2.10.5. Twenty-four-hour continuous glucose monitoring

A Medtronic iPro2 Professional CGM device (Northridge, CA, USA) was used to obtain 24-h continuous interstitial glucose concentration data from study subjects during 6 days (5 days in Italy) of baseline, midpoint (USA center only), and post-intervention. The glucose oxidase-based sensor was inserted into the abdominal area at least 5 cm away from the umbilicus to obtain an interstitial glucose measurement every 10 s. A recorder stored these data and provided a filtered average of these values every 5 min. Self-monitoring glucose readings (finger sticks) were performed (Contour®NEXT USB, Ascencia Diabetes Care, US) three times per day by subjects to calibrate CGM sensor data. We considered 24-h glucose data valid when two or more self-monitoring glucose readings were documented [40]. CGM data will be used to calculate 24-h interstitial glucose peak, mean, coefficient of variation (CV) and total area under the curve (AUC). The group mean and peak glucose will be calculated from the individual subjects' means of 2–4 days of useable CGM data. Similarly, group CV will be calculated as the mean of the ratio of the individual subjects' standard deviation to mean. Additionally, data will be entered into EasyGV platform (University of Oxford, Oxford, England) for calculation of glycemic variability and mean glucose across each 24-h period.

2.11. Physical activity assessment

Participants completed the International Physical Activity Questionnaire (IPAQ) during baseline weeks, post-intervention, and 3 months post-intervention. The IPAQ was used to estimate daily ambulation and physical activity and has been shown to have acceptable validity and reliability in various nationalities [41].

2.12. Sleep quality assessment

Sleep quality and quantity was evaluated by the Pittsburgh Sleep Quality Index (PSQI) [42], the Epworth Sleepiness Scale (ESS) (in USA), and by data obtained from a wrist actigraphy monitor (Actiwatch 2, Phillips Respironics, Murrysville, PA) in USA and Sweden [43]. The PSQI and ESS were administered at baseline, post-testing, and three months post-intervention. Actigraphy data were collected for 7 days during baseline and post-testing. A sleep log questionnaire to quantify sleep and wake times was completed by participants during all 7 days of Actiwatch usage (USA).

Actigraphy data will be processed as previously described by O'Connor et al. [44]. Briefly, actigraphy measurements were taken at the default 30-s intervals. The actigraphs were scored using the manufacturer's algorithm set at medium sensitivity. Rest intervals can be determined in a variety of ways; the most preferable and reliable measurements come from the participant manually pressing the indicator button prior to sleeping and immediately upon waking. If there was no button press detected, the technician would rely on the recorded sleep and wake times provided by the participants. Lastly, if no recorded times can be retrieved, the Phillips Actiware (v6.0.7) software default algorithm would estimate sleep and wake times. Time spent in bed, time spent sleeping, sleep efficacy (percentage of time spent in bed sleeping), onset latency (amount of time to fall asleep once in bed), and total number of minutes awake after sleep onset will be assessed and reported.

Subjective data from the PSQI and ESS were used to supplement the more objective actigraphy data. With regard to the PSQI, a higher global sleep score (0–21 arbitrary units [AU]) indicates poorer sleep; a global sleep score ≥ 5 AU is classified as "poor sleep" [42,44]. With regard to the ESS, a higher ESS score (0–24 AU) indicates greater daytime sleepiness; a score > 10 AU indicates excessive daytime sleepiness [45]. The ESS and PSQI are found to be weakly (but significantly) associated with each other, and are posited to evaluate different aspects of sleep [46].

2.13. Subjective health and well-being assessment

The Medical Outcomes Study 36-item short-form questionnaire (SF-36v2), administered at baseline, post-testing, and at the 3 month follow-up, was used to measure perceived physical and emotional well-being [47,48]. Raw SF-36v2 questionnaire data will be entered into Optum ProCoRE (Optum Inc., Eden Prairie, MN) for algorithmic transformation into domains of perceived health and wellness including: physical functioning, role limitations due to physical health, bodily pain, general health, vitality, social functioning, role limitations due to emotional health, and mental health. Scores are presented on a 0–100 scale, with higher scores indicative of greater perceived health and wellness.

2.14. Fecal microbiota

Spot fecal samples were collected at baseline and post-testing for all participants. The samples were collected in specific devices (Easy-Sampler CollectionKit, GP Medical Devices ApS, Denmark) and were placed in the freezer right after collection and brought to the clinic frozen. Microbial DNA from fecal samples will be extracted for analysis of the gut microbiota by deep 16S rRNA sequencing using Illumina MiSeq [49]. The Bristol stool scale was used to assess defecation habits and bowel function during the stool collection day [50]. Additionally,

participants specified recent usage of antibiotics on the day of the fecal sample collection. The composition of the gut microbiota will be analyzed to provide possible mechanistic explanations underlying differential responses of participants (identifying responders/non-responders) on higher-versus lower- GI dietary interventions and effects on gut microbiota composition caused by the interventions.

2.15. Metabolomics assessment

Plasma samples collected at baseline and post-testing will be analyzed for untargeted metabolomics at the Swedish center. Untargeted metabolomics will be performed using 0.2 mL heparin-plasma from samples taken at TP-15, +120, 240, and 480 min from test meals on MGTT test days. Plasma samples will be processed and analyzed in four different modes (reverse phase/HILIC chromatography in positive/negative ionization, respectively) on a LC-QTOF-MS instrument according to an established protocol [51]. This will ensure the most comprehensive collection of metabolite features. Data will be pre-processed and analyzed according to a recently developed data-treatment pipeline developed by the Swedish group [52,53].

2.16. Study outcomes

The primary outcome of the MEDGI-Carb trial were changes in postprandial insulin concentrations during the 8-h MGTT. Postprandial insulin, of which power calculations were based on, was chosen as the primary endpoint because, in the presence of a well-functioning beta cell, any improvement in plasma glucose concentrations due to a dietary intervention would slowly be compensated by variation in insulin secretion triggered by glucose values. We prioritized assessments of insulin over C-peptide because C-peptide is not extracted by the liver [54], and therefore is not a good marker of derangements in postprandial insulin sensitivity. Assessment of postprandial insulin was complemented by the principle secondary outcome – postprandial glucose, of which power calculations indicated a similar number of participants to be recruited. Secondary outcomes of relevant parameters of blood glucose metabolism were operationalized as fasting glucose, fasting insulin, HbA_{1c}, and 24-h CGM responses, collected at baseline, mid-testing (USA-only), and post-testing. Fasting plasma glucose, HbA_{1c}, 2-h OGTT plasma glucose, homeostatic model assessment of insulin resistance (HOMA-IR) [55], postprandial blood glucose and insulin responses and CGM all measure different aspects of glucose metabolism [56]. We consider this to be a strength of the current research. While these measures are strongly correlated [57], inclusion of multiple measures of glucose control add additional context. For example, HbA_{1c} not only accurately predicts CGM-derived glucose values, but also adds additional information to the model for more accurate prediction of average glucose [58]. Conversely, it may not be sufficient to evaluate changes in postprandial glucose and insulin metabolism in non-diabetic people [59].

There are strengths and limitations to each of these measurements in isolation. Long considered a "gold-standard" for diagnosis of glucose intolerance and diabetes, the OGTT is the most sensitive measure of impaired glucose homeostasis, as increases in postprandial glucose responses precede increases in fasting glucose [60]. However, the OGTT suffers from a well-documented high degree of intra-individual variability (CV = 16.7%) [61]. Fasting plasma glucose has considerably less variability than 2-h OGTT glucose values and is considered the preferred diagnostic test by the American Diabetes Association [62]. However, there is still large biological variability in fasting plasma glucose relative to HbA_{1c} [61]. While HbA_{1c} measurements may present with the greatest reliability (CV < 1%) [63], there are notable discrepancies in readings according to iron status [64] and race/ethnicity [65], independent of glucose concentrations. Further, HbA_{1c} would not be expected to respond completely to our dietary-intervention of a length (~84 days) less than the average lifespan of hemoglobin (~120 days). Moreover, it is not sufficiently sensitive to appreciate small changes in glucose metabolism in non-diabetic individuals. With respect to CGM, glucose data derived from CGM may be less accurate than venous plasma glucose on a point-by-point basis [66], but it provides valuable information on glucose excursions and overall variability, which may contribute more to cardiometabolic disease risk than chronically elevated glucose [67].

We recognize the importance of study measurements translating to situations individuals find themselves in the real world. Therefore, the MGTT provides additional context and serves as an important practical translation of this work by documenting the glycemic and insulinemic responses to complete mixed meals. The MGTT serves as a 'nested acute study' within the overall trial, whereby the absolute difference in glycemic responses to high- and low-GI meals at any individual timepoint is not of particular importance, but the changes in the *relative* glycemic response over the course of the intervention can elucidate the importance of the background diet in modulating the glycemic response to acute feeding.

Effects of the high vs low GI MED-style HEP on the plasma metabolome and gut microbiota will be analyzed as exploratory analyses. Moreover, we also plan to address the role of gut microbiota composition and plasma metabolome at baseline as determinants of the response/non-response to intervention with regards to acute glycemic and long-term cardiometabolic risk factors. Molecular effects of the MED diet on cardiometabolic risk factors in these aspects has recently been reviewed by Tuttolomondo et al. [68].

2.17. Data analysis and sample size estimates

Both "intention-to-treat" and "per-protocol" data analyses will be performed. The primary analyses will follow the intention-to-treat plan, where participants will be analyzed regardless of compliance or completion of study procedures. *A priori* sensitivity analyses using a 'per protocol' plan will be conducted, where noncompliant participants will be excluded. A repeated measures, two-way ANCOVA will be used to assess main effects of diet, time, and diet by time interactions plus center. Potential covariates used in models include age, sex, height, weight, BMI, baseline dependent variables, medication usage, and smoking status. All completers (goal of 60 subjects per group, 180 subjects total) will be tested — providing greater than 80% power to detect a 30% differential response between the dietary interventions for the primary endpoint (postprandial insulin) with similar variation reported in the study by Giacco et al. [69]. An equal number of study participants are anticipated to be recruited in each of the three centers. Further analyses will be performed after identification of appropriate subgroups taking into consideration several parameters, i.e. sex, severity of metabolic derangements, degree of overweight, relevant features of the baseline diet, and operating center. All statistical analyses will be done with consultation from the NIH Indiana Clinical and Translational Biostatistics Core services staff (www.indianactsi.org). All statistical analyses will be performed with SAS statistical software version 9.4 (SAS Institute, Cary, NC).

3. Discussion

The MEDGI-Carb trial aimed to evaluate the relevance of the GI in the context of a MED-style HEP. The clinical utility of the GI is still under debate, despite decades of research and a plethora of trials [12–14]. Conversely, there is relatively less evidence — particularly experimental evidence — on the health-promoting effects of a MED-style HEP [70]. The results of the MEDGI-Carb trial have the potential to answer important questions pertaining to both Mediterranean-style eating patterns and the relevancy of the long-term effects of GI in a non-diabetic population. The results of the MEDGI-Carb trial have the potential to inform health care providers and the public regarding the importance of

consuming low GI carbohydrate foods as part of a Mediterranean-style eating pattern.

The MEDGI-Carb trial utilized several strategies to successfully implement dietary control, including dietary counseling, provision of specific study foods to participants during the intervention, frequent checks and documentation of food intake (Sweden, Italy) or completion of itemized menu checklists (Sweden, USA), and offering cooking classes to enhance participant self-efficacy. Further, the robustness of the primary manipulated variable (GI of the carbohydrate foods) was ensured through independent analyses to ensure that the GI of the carbohydrate foods were sufficiently different between the low- and high-GI groups. Additional strengths of the MEDGI-Carb study include strong clinical design features such as randomization, blinding, provision of tools to participants to enhance compliance to study protocols (weight scales, measuring tools, etc. In US), and an appropriate sample size with respect to effect size and power based on previous research. These features place the current research in a strong position to investigate GI and dietary patterning in a meaningful way.

In addition to the numerous avenues we have taken in assessing glucose homeostasis, we are positioned to answer other emerging questions in nutrition science such as, 1) to what extent the gut microbiome and specific metabolic profiles may moderate or indicate dietary intervention-induced changes in cardiometabolic health via collection of blood and stool samples, 2) the impact of dietary interventions on indices of sleep quality via objective (Actiwatch) and subjective (PSQI, ESS) measures, and 3) ratings of subjective health and well-being via collection of self-rated health assessments (SF-36v2). Collectively, the current research has the potential to provide a wealth of knowledge which can address outstanding questions in regard to GI and Mediterranean-style eating patterns, as well as generate promising new leads and hypotheses.

There may be important implications from this research regardless of whether the results support or refute our hypothesis. If our hypothesis is supported and there are greater improvements in indices of glucose control in the low-GI group relative to the high-GI group, then these novel findings would provide additional support for the potential relevancy of the GI in a complete mixed diet among non-diabetic populations. Low GI and low glycemic load are inherent elements of a traditional Mediterranean diet [71]. If the more traditional low-GI version of the Med HEP in this study is more effective than the high-GI version, this would support the inclusion of low-GI foods as part of a Med HEP. Conversely, if the results are inconsistent with our hypothesis, this may suggest that differences in GI are not robust or masked in the context of an overall healthy eating pattern. Therefore, the study design and hypotheses put forth are unconditionally robust, as results supportive or unsupportive of the hypothesis provide new directions for future research and recommendations.

Declaration of competing interest

G.Riccardi is member of the Health and Wellbeing Advisory Board of the Barilla company; remuneration for this activity goes to his University Department. R. Landberg is the project leader for the Nordic Rye Forum (www.nordicryeforum.org), for which funding is provided by industrial partners and NKJ (Nordic Committee of Agricultural Research); R. Landberg is also principal investigator in research projects funded by Lantmännen and Barilla. He did not receive any remuneration, salary, or any other financial recompense from the food industry. W. Campbell reports no competing interests.

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Appendix A. Supplementary data

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