PERSonalized glucose Optimization through Nutritional intervention

The PERSON study

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Date	**24-06-2019**
Coordinating investigator/project leader	**Prof. Dr. E. E. Blaak** T: +31(0)43 38 81503 E: e.blaak@maastrichtuniversity.nl Dept. of Human Biology, MUMC+, Maastricht, The Netherlands
Principal investigator(s) (in Dutch: hoofdonderzoeker/uitvoerder) **Multicenter research: per site**	**Prof. Dr. E. E. Blaak** **Principle investigator MUMC+** T: +31(0)43 38 81503 E: e.blaak@maastrichtuniversity.nl Dept. of Human Biology, MUMC+, Maastricht, The Netherlands **Prof. Dr. Ir. E.J.M. Feskens** **Principle investigator WUR** T: +31(0)31 74 82567 E: edith.feskens@wur.nl Division of Human Nutrition, WUR, Wageningen, The Netherlands
Sponsor (in Dutch: verrichter/opdrachtgever)	**Maastricht University**
Subsidising party	**Top Institute Food and Nutrition (TIFN)** P.O. Box 557 6700 AN Wageningen The Netherlands T: + 31 88 022 8000 E: info@tifn.nl

	Website: www.tifn.nl **NWO, TKI Agri&Food, FrieslandCampina, Danone, DSM**
Independent expert(s)	**Prof. Dr. Ir. A.M.W.J. Schols** **Dept. of Respiratory Medicine, MUMC+, Maastricht, The Netherlands**
Laboratory sites	**Maastricht University Medical Center+, Dept. of Human Biology** **Wageningen University & Research, Division of Human Nutrition**
Pharmacy	**Not applicable**

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Sponsor or legal representative: n.a. For non-commercial research, Head of Department: Prof. Dr. E. E. Blaak Project leader TIFN - Principle investigator MUMC+ T: +31(0)43 38 81503 E: e.blaak@maastrichtuniversity.nl Dept. of Human Biology, MUMC+, Maastricht, The Netherlands	Prof. dr. E.E. Blaak	24/6/19
Coordinating Investigator/Project leader/Principal Investigator: Prof. Dr. E. E. Blaak Principle Investigator MUMC+ T: +31(0)43 38 81503 E: e.blaak@maastrichtuniversity.nl Dept. of Human Biology, MUMC+, Maastricht, The Netherlands Prof. Dr. Ir. E.J.M. Feskens Principle Investigator WUR T: +31(0)31 74 82567 E: edith.feskens@wur.nl Division of Human Nutrition, WUR, Wageningen, The Netherlands	Prof. dr. E.E. Blaak Prof. dr. Ir. E.J.M. Feskens	24/6/19

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)**
AE	**Adverse Event**
AGEs	**Advanced glycation end-products**
ALAT	**Alanine Transaminase, a liver enzyme which can be used to see if there is injury to the liver**
ANOVA	**Analysis of variance**
AR	**Adverse Reaction**
ASAT	**Aspartate aminotransferase**
AT	**Adipose tissue**
AUC	**Area under the curve**
BMI	**Body Mass Index, calculated as weight (kg) / (lengthxlength) (m)**
CA	**Competent Authority**
CAR	**Carotid artery reactivity**
CCA	**Common carotid artery**
CCMO	**Central Committee on Research Involving Human Participants; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CGM	**Continuous glucose monitor**
CIW	**Clinical Investigation Week**
CPT	**Cold pressor test**
DIW	**Dietary intervention week**
DXA	**Dual-energy X-ray Absorptiometry**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
HbA1c	**Glycated hemoglobin, a marker for long-term plasma glucose concentration**
HFMM	**High-fat mixed-meal**
^{1}H-MRS	**Proton magnetic resonance spectroscopy**
HOMA	**Homeostatic model assessment**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IR	**Insulin resistance**

IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
LIR	**Liver insulin resistance**
MAGE	**Mean Amplitude of Glycemic Excursion**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
MIR	**Muscle insulin resistance**
MRI	**Magnetic Resonance Imaging**
MUMC+	**Maastricht University Medical Centre+ (In Dutch: Maastricht Universitair Medisch Centrum+)**
NSAID	**Non-steroidical Anti-inflammatory Drug**
NWO	**Netherlands Organisation for Scientific Research (Dutch: Nederlandse Organisatie voor Wetenschappelijk Onderzoek)**
OGTT	**Oral glucose tolerance test**
RQ	**Respiratory quotient**
(S)AE	**(Serious) Adverse Event**
SD	**Standard Deviation**
SPC	**Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
T2DM	**Type 2 diabetes mellitus**
TIFN	**Top Institute Food and Nutrition; the public-private partnership of which this project is part of the full project plan**
UM	**Maastricht University (in Dutch: Universiteit Maastricht)**
Wbp	**Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)**
WMO	**Medical Research Involving Human Participants Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen)**
WUR	**Wageningen University & Research**

SUMMARY

Rationale:

A healthy lifestyle is an essential element to release the physical and mental potential of every individual and is able to prevent the epidemic development of overweight, and cardio-metabolic diseases. Unfortunately, most people do not manage to incorporate or maintain the recommended changes in their daily lifestyle. This may be due to the fact that people do not perceive the benefits of a healthy lifestyle in the short term, nor the adverse effects of an unhealthy lifestyle. It is increasingly recognized that maintaining well-controlled blood glucose concentrations is essential for remaining healthy and preventing chronic metabolic diseases. Additionally, there is evidence that well-controlled blood glucose concentrations - by boosting physical and mental energy - may be an important determinant of well-being, mental and physical performance. The link between blood glucose and the latter factors has hardly been studied. Moreover, it is not known to what extent these relationships differ in healthy participants and participants with an impaired glucose metabolism. When people feel better, fitter and/or otherwise motivated to follow a dietary advice, for example, by personalized feedback on physiological measures of glucose control or other indicators of health status, the implementation of a healthy lifestyle is expected to be more successful.

Furthermore, despite being compliant to lifestyle advice, the metabolic flexibility to respond to dietary intervention may vary between individuals. Recent evidence indicates that insulin resistance and metabolic inflexibility may develop separately in different organs, representing different etiologies towards cardio-metabolic diseases. Interestingly, these tissue-specific sub-phenotypes may have a differential response to diet. In a recent ground-breaking study, it was shown that, despite high inter-individual variability in glycemic response, responses to individual meals in daily life could be more accurately predicted by means of an algorithm that included lifestyle factors (diet, physical activity) and microbial composition as compared to a prediction by common practice. The above data suggest that successful lifestyle interventions may require a more personalized approach. Therefore, we hypothesize that phenotype-based dietary intervention optimizes beneficial effects on blood glucose regulation, metabolic health and subsequently mental and physical performance and well-being.

Objective:

To obtain insight into the metabolic and lifestyle determinants of postprandial blood glucose responses and to establish the effect of macronutrient manipulation of a 12-week dietary intervention on blood glucose homeostasis in metabolically different groups and its relationship to physical and mental performance and well-being.

Study design:

Two-center dietary intervention study with a double-blind, randomized, controlled parallel design. The metabolic phenotype will be blinded to the participants and researchers.

Study population:

Two hundred and forty overweight/obese (BMI 25-40 kg/m^2) Caucasian men and women (age 40-75y) insulin resistant predominantly in muscle (MIR) or liver (LIR) will be randomized by means of minimization into a metabolic phenotype targeted dietary intervention. The MIR and LIR phenotype will be determined based on the glucose response after an oral glucose tolerance test (OGTT). Of the total study population, 80 participants will be selected for more extensive metabolic phenotyping. The in- and exclusion criteria will be described in more detail elsewhere in the protocol (4.2 and 4.3).

Intervention:

Two diets will be implemented, one that is optimal for the MIR group and one that is optimal for the LIR group with respect to improvements in the primary outcome measure, disposition index. The disposition index is a composite marker of first phase insulin secretion and insulin sensitivity and is a strong determinant of blood glucose homeostasis. We hypothesize that the optimal diet for the MIR group is a diet high in monounsaturated fatty acids while the optimal diet for the LIR group is a diet high in protein and fibre and low in fat. All participants are randomly assigned to one of the two diets, which will either be an optimal or suboptimal diet for their specific phenotype. Participants will follow their allocated diet during a period of 12 weeks. Both diets are in line with the Dutch dietary guidelines (Gezondheidsraad, 2015).

Main study parameters/endpoints:

The primary outcome parameter is change in disposition index from pre to post intervention. This will be determined from insulin and glucose concentrations during a 7-points OGTT. Secondary outcomes include tissue specific insulin sensitivity, changes in body composition and body fat distribution, blood pressure, metabolic response to a high fat mixed meal, energy metabolism and substrate oxidation during a hyperinsulinemic-euglycemic clamp, blood lipid spectrum, fecal and oral microbiota, targeted metabolomics, mental and physical performance and well-being, gene and protein expression of skeletal muscle and adipose tissue, carotid artery reactivity, accumulation of advanced glycation end-products (AGEs) and immune function. Changes in these secondary outcomes in the optimal vs suboptimal diets within the LIR and MIR groups will also be meausred. Additionally, DNA will be analyzed at baseline.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

Burdens that participants may experience include the time they need to invest in the study and the dietary restrictions during the 12-week intervention period. The total time investment during CIW1 and 2, measurements will be around 30 hours (sub-study: 50 hours) divided over six university visits and six days of assessments at home. Additionally, the participants will be asked to report to the university weekly and record dietary intake at home, investing approximately 7.5 additional total hours. The total study period with the intervention will be approximately 13 weeks. The following burdens or risks may be associated with participation:

- The continuous glucose monitoring method requires calibration four times per day by using a capillary blood glucose meter. Although determining blood glucose by fingerprick is daily routine in diabetic individuals, participants could experience both psychological burden and local sensitivity of the fingers. However, previous experience has shown that most participants do not experience this as a burden.
- The collection of feces may be considered a burden. However, based on previous experience, this procedure is quite feasible.
- Throughout the study, questionnaires will be completed and food intake will be recorded periodically by means of an app or computer during daily life, which requires an extra time investment.
- During the test days, blood will be collected via a venous catheter. Venipunctures can occasionally cause a local hematoma or bruise. Some participants in previous studies reported pain during a venipuncture.
- Adipose tissue biopsies will be taken twice (pre- and post intervention), and for extensive phenotyping, a skeletal muscle biopsy (*m. vastus lateralis)* will be taken before the hyperinsulinemic-euglycemic clamp pre- and post intervention. Both adipose tissue and skeletal muscle biopsies may cause local hematoma. Compressing the biopsy site for approximately 10 minutes will reduce the risk of developing hematoma. After the muscle biopsy, some participants may report stiffness or pain (comparable to muscle ache) for a couple of days. Discomfort during the procedure itself is minimized due to the use of local anesthetics, although participants may experience pressure during the introduction of the needle. The incision will leave a small scar (~3 mm for adipose tissue biopsy and ~8 mm for skeletal muscle biopsy). To promote good wound healing, the incision will be sealed with steristrips and a waterproof band-aid. The site of the muscle biopsy will additionally be sealed with a compression bandage.
- During the hyperinsulinemic-euglycemic clamp, there is a small risk of hypo- or hyperglycemia. However, from the extensive experience in our research group, these

conditions rarely occur and can be reversed immediately. A medical doctor will always be available during the clamps.

- No risks are known about the oral glucose tolerance test, high-fat mixed-meal test and indirect calorimetry. These measurements are routinely applied in human metabolic research, and SOPs are available in the database of the department.
- The total radiation dose participants will be exposed to during the two DXA scans is <20 µSv. Since the average yearly radiation dose per person in the Netherlands is approximately 2.5 mSv, the amount of radiation exposure during DXA scans is negligible.
- Participants with claustrophobia may experience the MRI scan as uncomfortable. However, due to the duration and clear instructions this discomfort will be minimized.
- Dietary products provided during the intervention are widely used and freely available to consumers. Both the composition of the diets and individual food products will not cause discomfort for the participants.

Aside from receiving information about their health status, participants are provided with specific foods that may be beneficial. Furthermore, since the prevalence of overweight and cardio-metabolic disorders is continuing to rise, study outcomes could provide future health benefits for the general public. In addition, the diets that the participants will follow are advantageous to overall health.

1. INTRODUCTION AND RATIONALE

A healthy lifestyle is essential for optimal health and well-being and the prevention of chronic metabolic diseases. Indeed, lifestyle interventions including individual guidance on the general recommendations for a healthy diet and increased physical activity have been shown to effectively reduce the cumulative incidence of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) by more than 50% (1). Around 30% of the population does not respond to the intervention, which is even higher in a setting where only general advice is provided. Currently, the prevalence of overweight and overweight-related metabolic disturbances is increasing at an alarming rate. Worldwide, more than 50% of the adults are overweight (>1 billion individuals) and a further 12% (475 million) can be classified as clinically obese. Every year, at least 2.8 million adults die due to overweight/obesity. In parallel, blood glucose concentrations are rapidly increasing in the population with a steep incline in the prevalence of prediabetes and impaired glucose homeostasis, thereby increasing the risk for T2DM and CVD. Thus, maintaining well-controlled blood glucose concentrations is essential for remaining healthy and preventing chronic metabolic diseases. With the global increase in prevalence of these chronic metabolic diseases, much effort and budget are spent by governmental and public health bodies as well as by organizations involved in research implementation to increase awareness of the burden of chronic metabolic diseases and to educate the general public on the importance of a healthy lifestyle. Nevertheless, many people do not manage to incorporate or to maintain the recommended changes in their daily lifestyle. Thus, strategies to increase the response and/or adherence to a healthy lifestyle are urgently warranted.

An important explanation for the limited success of implementation of a healthy lifestyle may be that people do not perceive the benefits of a healthy lifestyle in the short term. Interestingly, there is evidence that a well-controlled blood glucose level - by boosting physical and mental energy - may also be an important determinant of general wellbeing, fitness, self-control and cognitive function (2-4). Indeed, relationships have been reported between blood glucose concentrations and measures of self-control such as controlling attention, regulating emotions and overriding aggressive impulses. Currently, it is not known whether the measures of self-control relate to magnitude of glucose levels or variability over the day, or both. Indeed, low glucose concentrations have been shown to be related to greater aggression in married couples (3). The link between blood glucose and the above factors have, so far, hardly been studied in the context of diet or physical activity-induced control of blood glucose concentrations. Moreover, it is not known to what extent these relationships differ in relatively healthy individuals and in indivuduals with an impaired glucose metabolism. Thus, there is an urgent need for combining dietary, lifestyle and

physiological glycemic dynamics in order to define optimal health, not only from a metabolic point of view but also from a mental and behavioral perspective. When people feel better and fitter, and are motivated to adhere to a dietary advice - for example, by personalized feedback on physiological measures of health status - the implementation of a healthy lifestyle is expected to be more successful.

On the other hand, despite being compliant to lifestyle advices, metabolic flexibility (defined as the ability to adapt substrate oxidation to substrate availability) (5, 6) to certain dietary and/or lifestyle patterns may vary between individuals, resulting in differential effects on blood glucose homeostasis. Recent evidence indicates that metabolic inflexibility and insulin resistance may develop separately in different organs (7), representing different etiologies towards diabetes and cardio-metabolic risk. Interestingly, the insulin-resistant metabolically inflexible phenotype (being predominant in muscle or liver) has been shown to interact with diet to determine changes in metabolic outcome (8, 9). Additionally, participants with LIR showed a higher postprandial triglyceride response as compared to individuals with MIR (10). Noteworthy, tissue-specific insulin sensitivity (adipose tissue, liver, skeletal muscle) may be modeled based on the glucose curve and metabolic response after an oral glucose or an oral lipid load (11). Finally, groundbreaking recent data showed that dietary habits, anthropometrics, physical activity, and gut microbiota can accurately predict personalized metabolic responses (12, 13). The above studies illustrate that the response to an intervention depends on a variety of factors including overall diet composition (i.e. protein, carbohydrate, fat and dietary fiber), food products, the physiological metabolic phenotype and microbial profile as well as lifestyle factors including sleep, stress and physical activity, and which each cannot be isolated from their context.

2. OBJECTIVES

Primary objective:

The primary objective of this study is to establish the effect of a metabolically targeted, optimal versus suboptimal macronutrient manipulated 12-week dietary intervention on the change in disposition index (composite marker of first phase insulin secretion and insulin sensitivity). One diet will be optimal for the muscle insulin resistant phenotype (MIR, half of the study population) and suboptimal for liver insulin resistant phenotype (LIR, half of the subject population), whereas the other diet will be optimal for the LIR phenotype and suboptimal for the MIR phenotype.

Secondary objectives:

Additionally, we aim to study the effects of 12-weeks of targeted macronutrient manipulation on changes in:

1. Tissue-specific insulin sensitivity, glucose tolerance, 24-hour glucose values
2. Body composition and body fat distirbution
3. Circulating metabolites after a high fat mixed meal under fasting and postprandial (high fat mixed meal) conditions
4. Energy metabolism and substrate oxidation during a hyperinsulinemic-euglycemic clamp (2 steps)
5. Baseline blood lipid spectrum
6. Fecal microbiota composition
7. Oral microbiota composition
8. Targeted metabolomics (baseline and during the clamp)
9. Physical and mental performance and well-being.
10. Blood pressure
11. Gene and protein expression in skeletal muscle and adipose tissue.
12. Advanced glycation end-products (AGE) accumulation
13. Carotid artery reactivity
14. Fasting immune metabolism (PBMCs)
15. Outcomes 1-14 listed above in the optimal versus suboptimal diets within the LIR and MIR groupsDNA analysis (buffy coat collection, pre-intervention only)

3. STUDY DESIGN

Overview

The study will be a double-blinded randomized controlled trial based on a dietary intervention in two metabolic phenotypes, evaluating the effects of targeted diets on glucose homeostasis. Before entering the study, a screening procedure will take place to assess eligibility. A 7-points OGTT will be performed during the screening, which determines their metabolic phenotype (MIR or LIR). If all results from the screening visit are in compliance with the inclusion/exclusion criteria, participants will be suitable for the intervention. See a graphical representation of the study design in Figure 1.

Before and after the dietary intervention, participants will undergo several measurements over six subsequent days (‘*characterization week’)*. After the baseline (pre-intervention) mearsurements, in a parallel design, each individual will follow either an optimal or a sup-optimal diet for their phenotype (LIR or MIR), varying in macronutrient content and quality for a 12 week period (as described previously on page 10, ‘intervention’). All participants will be asked to report to the university weekly.

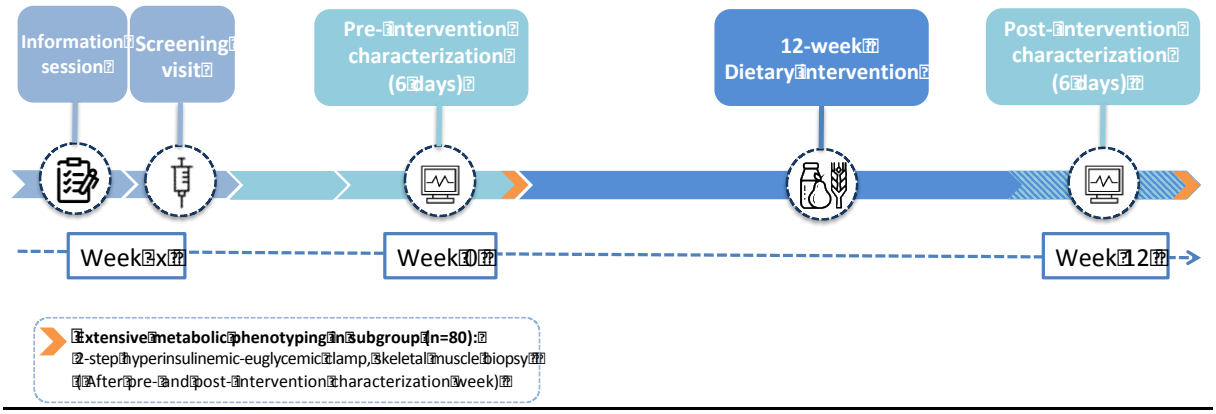

Figure 1. Study design.

Screening procedure

Participants showing interest from advertisements will receive written information via the particular website or email. When people are interested in participation in the study, they will be invited for either a group information session at the university, or an individual telephone call during which the researchers will explain the study and assess eligibility by checking basic in- and exclusion criteria (e.g. age, health status, use of medication and dietary habits). If interested and eligible, after at least one week following the information session with the researchers, potential participants will decide upon definite application for the study. The informed consent will then be signed during the screening visit, which will be planned after the week of reflection.

Characterization weeks (CIW1 and CIW2)

The 6-day characterization period will be planned the week before the dietary intervention period (CIW1) and during week 12 of the intervention period (CIW2). In short, participants will undergo study procedures according to the following schedule (see also Table 1):

- **Visit 1 (CIW1 and 2):** The continuous glucose monitor (CGM) and physical activity monitor will be applied after a MRI (at UM) and DXA scan and anthropometric measurements. Participants will wear the CGM monitor continuously until visit 3 and the physical activity monitor until the first visit during the intervention. This will provide detailed information about the participant's daily blood glucose responses and physical activity. A dietary intake consultation will take place at the end of the visit.
- **Visit 2 (CIW1 and 2):** A 7-points OGTT will be used to determine glucose tolerance and insulin sensitivity (tissue-specific insulin sensitivity, Matsuda Index and disposition index will be calculated). A fasting saliva sample will be collected before the OGTT. After a standardized lunch, participants will complete a set of computerized cognitive tests and Macronutrient and Taste Preference Ranking Task (MTPRT) to determine mental performance and food preferences.
- **At home days:** During three days of each characterization period, participants will be monitored (CGM, physical activity and food intake) in their daily living conditions. In addition, they will be asked to complete online questionnaires via an app (if needed on paper) asking about dietary intake, mood, fatigue, stress, hunger. The investigators will provide participants with two standardized breakfast meals, and additional standardized lunch, evening meal and snacks the day before visit 3.
- **Visit 3 (CIW1 and 2):** A HFMM test will be performed to characterize postprandial metabolic responses. Blood will be collected for determination of e.g. circulating metabolites. At WUR, also AGEs will be measured. Furthermore, an adipose tissue biopsy will be taken, blood pressure will be measured, and participants will be asked to bring their feces and 24-hour urine samples collected at home. The CGM monitor will be removed at the end of visit 3. A fasting blood sample will be taken to determine inflammatory, oxidative stress and nitrosative stress profile by targeted metabolomics, for baseline blood lipid spectrum and PBMCs (WUR).
- **Visit 4 (CIW1 and 2):** During the characterization week, 80 UM participants will be asked to consume a standardized meal the day before visit 4. Adipose tissue, liver/peripheral insulin sensitivity, energy and substrate oxidation (2-step hyperinsulinemic-euglycemic clamp, indirect calorimetry), protein/gene expression (skeletal muscle biopsy) and targeted metabolomics (blood samples) will be determined. The 80 participants will be evenly distributed across the two insulin resistance phenotypes (MIR/LIR) and diets (HMUFA/LFHP), as depicted in figure 2.

NOTE: The MRI scan at WUR wll take place during an additional short visit before visit 1. No extensive phenotyping will take place at WUR.

Table 1. Example overview of measurements and time investment.

	Pre + post 12- week intervention measurements							Extensive phenotyping (UM)
	Screening Visit 0	Day 1 Visit 1	Day 2 Visit 2	Day 3 At home	Day 4 At home	Day 5 At home	Day 6 Visit 3	Day 8 *Visit 4
University visit	✓	✓	✓				✓	✓
****Questionnaires**	✓	✓	✓	✓	✓	✓	✓	
Standardized meal • **Breakfast** • **Lunch** • **Dinner**		 ✓	 ✓		✓	✓ ✓ ✓		
Blood collection	✓		✓				✓	✓
Blood pressure	✓						✓	
Anthropometrics	✓	✓						
DXA scan		✓						
MRI/MRS scan* **		✓						
OGTT	✓		✓					
HFMM							✓	
Cognitive tests** **			✓					
AT biopsy							✓	
Feces/urine collection						✓		
Saliva collection			✓					
Indirect calorimetry								✓
Clamp								✓
SM biopsy								✓
PBMCs (WUR only)	✓						✓	
AGEs (WUR only)							✓	
Carotid artery ultrasound (WUR only)		✓						
CGM		✓	✓	✓	✓	✓	✓	
PA monitoring		✓	✓	✓	✓	✓	✓	✓
Food intake				✓	✓	✓		
Time investment (h)	3	2	4	1	1	1	6	10
Total time investment per characterization week = 15h (excluding extensive phenotyping, which will add an extra 10h)								

* Participants (n=80) will consume standardized meal evening before visit 4.
** See section "F" for questionnaire timing and examples.
*** WUR: MRI/MRS on extra visit before visit 1
**** Also possible during visit 1

Dietary intervention

After CIW1, participants will start with the 12-week dietary intervention (Figure 2). CIW2 will take place in week 12. For more details about the dietary intervention, we refer to chapter 5 “Treatment of Participants.” In short, participants are instructed to follow their randomly assigned diet. Compliance will be checked with a web-based food diary (app) and weekly university visits. Participants will receive food products during the weekly visits to the university. As the diets will be isocaloric, maintenance of body weight will be monitored during the weekly visits. Daily kilocalories for each participant will be calculated according to energy needs, based on estimated basal metabolic rate and physical activity level using the validated Schofield equation.

The diet will be known to the investigators and participants. However, the metabolic phenotypes and thus whether assigned diets are either optimal or suboptimal will be blinded for both investigators as well as participants. The participants will be allocated for their diet using minimization (described on page 26, paragraph 7.2) using metabolic phenotype (MIR and LIR), age and sex as minimization prognostic variables. The duration of the intervention is selected based on previous experience with dietary intervention studies (14, 15).

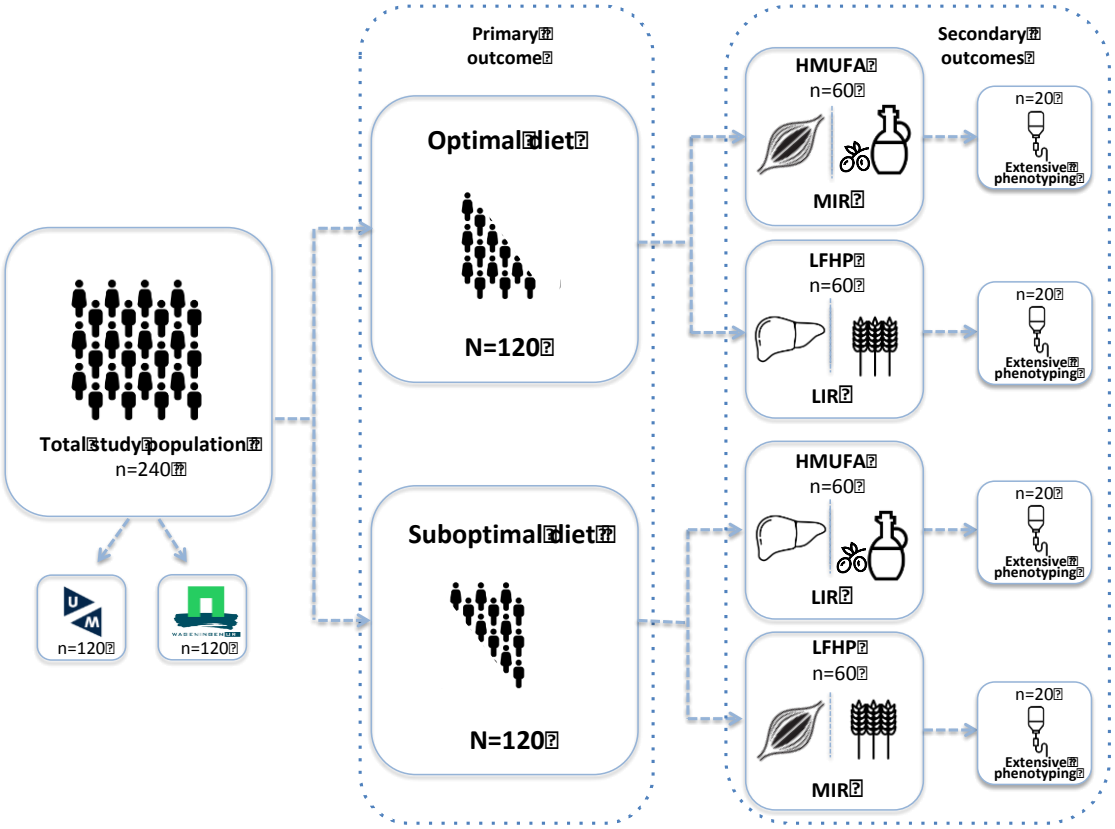

Figure 2. Flowchart dietary intervention. Participants receive either a high mono-unsaturated fatty acid (HMUFA) or a low fat/high protein (LFHP) diet. Whether the diet is considered optimal or suboptimal depends on the metabolic phenotype.

4. STUDY POPULATION

4.1 Population (base)

Two hundred and forty overweight/obese (BMI ≥ 25 kg/m^2 < 40 kg/m^2) Caucasian men and women with insulin resistance aged between 40-75y will be included from the general population. At UM, 80 participants will be included for the extensive metabolic phenotyping. Participants will be recruited either in the region of Maastricht or in the region of Wageningen via posters, advertisements in local newspapers and on targeted websites and social media. Both study centers aim to include an equal number of participants (120 participants per center). After determining the metabolic phenotype of the individuals, allocation into dietary intervention groups will be completed at UM and WUR separately by using minimization with metabolic phenotype, age and sex as prognostic variables.

Since we will include both men and women with overweight or obesity, we foresee no difficulties with the recruitment of study participants. Women, regardless of their menopausal state, can participate in the study. During the screening phase, hormonal status will be assessed by self report (questionnaire). Only Caucasian individuals will be included as several studies showing that body composition and metabolic profile can vary between different ethnicities, even when having the same BMI (16).

4.2 Inclusion criteria

Caucasian men and women with a BMI ≥25 to <40 kg/m^2, aged 40-75 years, with predominant MIR or LIR will be included. Criteria for the presence of MIR and LIR will be based on tertiles for these parameters from the DioGenes (17), CODAM (18) and Maastricht studies. Also, participants should be weight stable for at least 3 months prior to participation (+/- <3 kg).

4.3 Exclusion criteria

Participants will be excluded from participation when one or more of the following aspects are present:

Table 2. Exclusion criteria

Category	Definition of criteria
Diseases	- Pre-diagnosis of type 1 or type 2 diabetes mellitus - Renal or hepatic malfunctioning (pre-diagnosis or determined based on ALAT, ASAT and creatinine values) - Gastrointestinal diseases or abdominal surgery (allowed i.e.:

	appendectomy, cholecystectomy) - Food allergies, intolerances (including gluten/lactose intolerance) and/or dietary restrictions interfering with the study (including special diets, vegetarians and eating disorders) - Cardiovascular diseases (e.g. heart failure) or cancer (e.g. non-invasive skin cancer allowed) - High blood pressure (untreated >160/100 mmHg, drug-regulated >140/90 mmHg) - Diseases affecting glucose and/or lipid metabolism (e.g. pheochromocytoma, Cushing's syndrome, acromegaly) - Anemia defined as Hb men <8.5 and women <7.5 mmol/l - Diseases with a life expectation shorter than 5 years - Major mental disorders - Drug treated thyroid diseases (well substituted hypothyroidism is allowed inclusion) - Other physical/mental conditions that may interfere with study outcomes
Medication	- Medication known to interfere with study outcomes (e.g. PPAR-α or PPAR-γ agonists (fibrates), sulfonylureas, biguanides, α-glucosidase inhibitors, thiazolidinediones, repaglinide, nateglinide and insulin, chonic use of NSAIDs) - Use of certain anticoagulants - Use of antidepressants (stable use ≥ 3 months prior to and during study allowed) - Use of statins (stable use ≥ 3 months prior to and during study allowed) - Use of β-blockers (only for the extensive phenotyping participants) - Chronic corticosteroids treatment (>7 consecutive days of treatment) - Use of antibiotics within 3 months prior to the study
Lifestyle	- Participation in regular sports activities (>4 hours per week) - Having a resctricted dietary pattern interfering with the study diets (e.g. vegan or Atkins diet) - Plans to lose weight - Abuse of alcohol (alcohol consumption >14 units/week) and/or drugs (cannabis included) - Not willing to limit alcohol consumption to 7 drinks per week - Regular smoking (including use of e-cigarettes) - Use of strong vitamins or other dietary supplements (e.g. pre- or probiotics) expected to interfere with the study outcomes
Other	- Pregnant or lactating women, or women who are planning to become pregnant - Inability to comply with the study diet - Blood donation within the last 3 months - Participation in possibly interfering studies within the last 3 months - Inability to understand study information and/or communicate with staff - Unwillingness to be randomized or sign informed consent - Unwillingness to save data for 15 years

4.4 Sample size calculation

The sample size calculation is based on the change in disposition index (DI), as determined from insulin and glucose concentrations during a 7-points OGTT, using data from the previously published DioGenes study (17). With a power of 90%, two-sided alpha of 5% and a standardized effect size of 0.46, a total sample size of 202 was calculated using the statistical analysis software R. Taking into account a drop-out rate of 15%, 240 subjects will be included. Data of the DioGenes study was used to determine the standardized effect size; the standardized effect size was calculated from the difference in outcome values between the optimal and sub-optimal diet groups (difference = 1.07) divided by the standard deviation of the outcome (standard deviation = 2.34). The optimal diet group was defined as participants following a low GI diet that have liver insulin resistance (LIR); the sub-optimal diet was defined as participants following a high GI diet that have liver insulin resistance (LIR). Within the new intervention study, the same effect size is expected for optimal and sub-optimal diets for the MIR group, as well as when comparing a diet that is optimal/sub-optimal for the MIR group and sub-optimal/optimal for the LIR group. Based on observations in the Cordioprev study, we may expect disposition index changes by 55 and 67% in the LIR and MIR respectively on their optimal diet and by 20 and 26% respectively on their sub-optimal diet (8).

$$n = \frac{2(Z_{\alpha}+Z_b)^2}{(\frac{E}{S_d})^2}$$

$$101 = \frac{2(1.96+1.28)^2}{(\frac{1.07}{2.34})^2}$$

Also, for the other secondary outcome measures including body composition and body fat distribution (DXA and MRI), glucose tolerance, 24-hour blood glucose, fecal and oral microbiota composition, blood pressure, AGE accumulation, carotid artery reactivity, fasting immune function, baseline blood lipid spectrum, baseline targeted metabolomics, changes in physical and mental performance and well-being, metabolite profile after a HFMM as well as the changes in these outcomes in the optimal vs suboptimal diets within the LIR and MIR groups, a number of 120 subjects per diet group and 60 subjects when divided by diet and metabolic phenotype will be sufficient (15, 19-21).

Based on the tertiles that are developed to classify individuals into the MIR or LIR groups, and based on the in- and exclusion criteria, which are similar to the CODAM study, we

expect that approximately one in four screened individuals will be eligible for allocation to the dietary intervention. Therefore, we expect that approximately 240*4=960 particpiants will be screened to allocate 240 individuals. Because there is a possibility that the inclusion rates between MIR and LIR are not exactly the same, it is possible that near the end of the study, more participants have to be screened. It is expected that the difference in inclusion rate will not be more than 10%. Therefore, it is expected that a maximum of 960 * 10% = 1056 participants will be screened to reach 240 participants eligible for treatment allocation.

For the extended phenotyping group, which is a secondary outcome, a total of 80 participants will undergo extensive metabolic phenotyping to determine tissue-specific insulin resistance using a 2-step hyperinsulinemic-euglycemic clamp. For this study, we have powered for four different groups, taking into account both diet and phenotype. Phenotype is taken into account for this sample size calculation because the clamp may detect difference in tissue-specific insulin resistance (phenotype). Based on a previous study from this lab (19), a 20 percent difference in whole-body insulin resistance is considered physiologically relevant, therefore, the effect size is 0.2. The expected standard deviation is also 20%, therefore, the power is 0.80, and expected drop-out rate is 20% (MEC15-3-043 and (19)). The drop-out rate is slightly higher compared to the total group because of the higher burden of the extensive phenotyping measurements. Therefore, we will need n=20 per group, which leads to 80 individuals in total.

$$16 = \frac{2(1.96 + 0.84)^2}{(\frac{0.20}{0.20})^2}$$

For other secondary outcome measures in the extensive phenotyping including energy metabolism and substrate oxidation, targeted metabolomics during the clamp, the indicated number of 20 subjects per group will be sufficient to detect physiologically relevant changes as shown in previous studies (20).

5. TREATMENT OF PARTICIPANTS

5.1 Investigational product/treatment

The hypothesized optimal diet for MIR is a moderate fat content which is high in mono-unsaturated fatty acids (HMUFA) with a macronutrient breakdown of 38 E% from fat (20% MUFA, 10% PUFA, 8% SFA), 48 E% from CHO (35% complex), and 14 E% from protein (35-40% plant protein) (8). The hypothesized optimal diet for LIR is low in fat, high in protein (LFHP) and increased fiber with a macronutrient breakdown of <28 E% from fat (10% MUFA, 10% PUFA, 8% SFA), 48 E% from CHO (35% complex), and 24 E% from protein (35-40% plant protein), and an additional supplement of 6-12g of soluble fiber per day (8, 22, 23).

The dietary intervention will be employed using freely available commercial food products. In addition, some products (which will also be commercially available) will be provided by industrial partners and distributed at the university. To ensure that the main fat source for the HMUFA diet is MUFA, olive oil will be given to the participants. Additionally, a commercially available fiber supplement and food packs, including yogurt drinks, kwark, etc., will be provided for the participants. The metabolic phenotype will be blinded for the participants and researchers, thus it is unknown whether the provided diet is optimal or suboptimal.

5.2 Use of co-intervention (if applicable)

Participants will be asked to maintain their habitual lifestyle (e.g. their exercise regime, sleep patterns) during the full study period, and refrain from any type of extreme physical activity during the CIW. Any changes in use of medication, dietary supplements or other products should be reported directly to the investigators.

5.3 Escape medication (if applicable)

Not applicable.

6. NON-INVESTIGATIONAL PRODUCT

6.1 Name and description of non-investigational product(s)

The glucose drink (OGTT), lidocaine (biopsies), [6,6-$^{2}H_{2}$]-glucose, insulin and glucose 20% (clamp) are regularly used in clinical trials in the MUMC+ and are safe for human use. Please find detailed information in attachments D2.

6.2 Summary of findings from non-clinical studies

Not applicable.

6.3 Summary of findings from clinical studies

Not applicable.

6.4 Summary of known and potential risks and benefits

Not applicable.

6.5 Description and justification of route of administration and dosage

Not applicable.

6.6 Dosages, dosage modifications and method of administration

- Glucose drink (OGTT): oral administration of 75 g glucose in a total volume of 200 ml (ready to use). Participants will consume the glucose drink within five minutes using a straw. The drink will be available in the flavors lemon and orange (LemonGluc and OranGluc, Novolab, Belgium).
- Insulin and glucose 20% (clamp): i.v. glucose 20% strongly dependent on the insulin sensitivity of the participant.
- [6,6-$^{2}H_{2}$]-glucose (clamp): i.v. prime concentration of 2.4 mg/kg body weight and a continuous infusion of 0.04 mg/kg/min for t= -120 to 330 minutes. The infusion will be set at a concentration that has no metabolic effect in humans (tracer amounts).
- Lidocaine (AT and skeletal muscle biopsies): 4-6 ml for local subcutaneous injection.

6.7 Preparation and labeling of Non-Investigational Medicinal Product

Please find detailed information in the attached documents D2.

6.8 Drug accountability

Not applicable.

7. METHODS

7.1 Study parameters/endpoints

7.1.1 Main study parameter/endpoint

Primary outcome parameter: change in disposition index (first phase insulin secretion adjusted for insulin sensitivity) between metabolically targeted optimal and suboptimal macronutrient composition modulated diet after participants follow an optimal or suboptimal diet for their phenotype (MIR or LIR). We expect that the change in disposition index among the two phenotypes will be similar. The disposition index will be determined from insulin and glucose concentrations during a 7-points OGTT.

7.1.2 Secondary study parameters/endpoints (numbering linked to 2. Objectives)

Additionally, we aim to study the effect of 12-weeks of targeted macronutrient manipulation on change in:

1. Tissue-specific insulin sensitivity, glucose tolerance, 24-hour glucose values
2. Body composition and body fat distirbution
3. Circulating metabolites after a high fat mixed meal under fasting and postprandial (high fat mixed meal) conditions
4. Energy metabolism and substrate oxidation during a hyperinsulinemic-euglycemic clamp (2 steps)
5. Baseline blood lipid spectrum
6. Fecal microbiota composition
7. Oral microbiota composition
8. Targeted metabolomics (baseline and during the clamp)
9. Physical and mental performance and well-being.
10. Blood pressure
11. Gene and protein expression in skeletal muscle and adipose tissue.
12. Advanced glycation end-products (AGE) accumulation
13. Carotid artery reactivity
14. Fasting immune metabolism (PBMCs)
15. Outcomes 1-14 listed above in the optimal versus suboptimal diets within the LIR and MIR groups
16. DNA analysis (buffy coat collection, pre-intervention only)

7.2 Minimization, blinding and treatment allocation

Participants will be allocated to a optimal or suboptimal diet by means of minimization based on metabolic phenotype, age and sex in a double blind parallel design. The optimal or

suboptimal diets include a moderate fat/high mono-unsaturated fatty acid diet (HMUFA, optimal for MIR, suboptimal for LIR) or low fat/high protein diet (LFHP, optimal for LIR, suboptimal for MIR).

Whether this diet is optimal or suboptimal for an individual participant will be double-blinded. For the allocation of the participant to the diet we will use minimization (24, 25) based on phenotype, sex and age. Minimization ranks each new participant for each prognostic variable with the participants already assigned. This forces a renumbering of the previous participants. It then sums these rank numbers by treatment group with the new participant tentatively assigned to each. The final assignment is made to the treatment group where the participant minimizes the difference between the rank sums. We will use a randomization factor of 0.8 for age and sex and 1.0 for the LIR/MIR phenotype, and a base probability of 0.7 by means of biased-coin (26).

Minimization will be done separately for MUMC+ and WUR. An independent analist will analyse the results from the OGTT during screening to determine whether a participant is either MIR or LIR. This analist will also be responsible for minimization. The executing researchers will have no access to these results, and will therefore only know to which diet the participants is allocated. Participants will not know whether the diet they receive is optimal or suboptimal for them.

Minimization aims to ensure treatment arms are well balanced with respect to pre-defined patient variables as well as for the number of patients in each group, as we have done in previous studies (14). The investigators have no access to the allocation distribution. The principle investigators Prof. Dr. E. Blaak and Prof. Dr. E. Feskens will be informed of the treatment code to reveal the treatment in case of medical emergency. Furthermore, results of the CIW will become available to the researchers after the study has finished, preventing bias.

We will use the minimization program Minimpy (https://sourceforge.net/projects/minimpy/). Data backups are performed every minimization and stored in a separate location.

7.3 Study procedures

In this study, 240 men and women will be included. Participants will be recruited in the vicinity of either Maastricht or Wageningen by means of posters and advertisement in local newspapers or on websites/social media. Participation will be on a voluntary basis. People who are interested and respond to the advertisement will be sent information via website or email. When still interested after reading these resources, they will be invited for an informational meeting at the university or for an individual telephone call. During this session, the protocol will be explained by the researchers and basic inclusion criteria will be checked. If potential participants are eligible according to basic in- and exclusion criteria, they will be given a minimum of 7 days to decide upon definite application for the study. The informed

consent will be signed after at least 7 days during the screening visit. Participants have the right to end their participation at any time during the study. The maximum time between inclusion and minimization is 3 months.

For the university visits, participants will be asked to travel by car or public transport after an overnight fast. They will be instructed to follow their habitual diet and exercise pattern and consume a standardized meal provided by the investigators during the evening before university visit days. Furthermore, participants are instructed to refrain from drinking alcohol and performing strenuous exercise the day before the screening visit and during the full characterization weeks.

Prior to CIW 1~~+2~~

Before the start of the two characterization periods, participants record their food intake via a validated 3-day food record mobile app (Wageningen University, developed in 2016) on two home-days and one weekend-day. This will be done to calculate dietary intake and to use for developing personalized dietary advice during the intervention.

Screening visit

Visit 0

Duration: 3 hours

After giving informed consent, a screening will be performed. In the morning after an overnight fast, participants will come to the university. During the screening visit, the researcher will review the information about the study and participants will be given the chance to ask questions. In addition, the researcher will review the completed questionnaires with the participant to check for in- and exclusion criteria. The following measurements will take place:

- Body weight
- Height
- Waist-to-hip ratio
- Blood pressure
- Fasting levels of blood glucose, insulin, ALAT/ASAT, creatinine and Hb.
- 7 point oral glucose tolerance test (OGTT, from this muscle and hepatic insulin resistance will be modelled,)

The OGTT will be performed after an overnight fast. Blood will be collected via a venous catheter at time point 0 (12/16ml) and participants will be instructed to drink 200 ml of a ready-to-use 75g glucose solution (Novolab, Belgium) within five minutes. At WUR only, an

additional fasting blood sample of 16 ml will be collected for isolation of peripheral blood mononuclear cells (PBMCs). At time points 15, 30, 45, 60, 90 and 120 min, 6 ml of blood will be collected for measurement of plasma glucose and insulin. Muscle insulin sensitivity and hepatic IR were estimated using the methods of Abdul-Ghani et al. Both indexes were developed and validated against gold standard hyperinsulinemic-euglycemic clamp studies (11). At each time point, 2 ml of blood is discarded prior to drawing the blood samples.

The muscle insulin sensitivity index (MISI) will be calculated according to the following formula: MISI = (dG/dt) / mean plasma insulin (pmol/l) concentration during OGTT. Here, dG/dt is the rate of decay of plasma glucose concentration during the OGTT, calculated as the slope of the least square fit to the decline in plasma glucose concentration from peak to nadir (11). The decline in plasma glucose concentration after 60 min primarily reflects glucose uptake by peripheral tissues, mainly skeletal muscle.

The hepatic IR index (HIRI) will be calculated using the square root of the product of the area under curves (AUCs) for glucose and insulin during the first 30 min of the OGTT - i.e., SQRT (glucose0–30 [AUC in mmol/l·h] · insulin0–30 [AUC in pmol/l·h). This index has been developed and validated against the product of fasting plasma insulin and endogenous glucose production in clamp studies (11). Participants will be divided into tertiles according to HIRI and MISI scores classifying each participant in the MIR or LIR group. The lowest tertile of MISI represented individuals with muscle IR; the highest tertile of HIRI represented individuals with hepatic IR. The tertile cutoffs are described in 4.2.

Participants who are initially excluded based on metabolic phenotype, but with MISI/HIRI values close to the cut-off values for inclusion, will be approached within 6-18 months for re-evaluation of their MISI/HIRI valuesw by performing a new 7-pt OGTT.

Visit 1 (CIW1 and 2)

Duration: 2 hours

DXA scan and anthropometry

After an overnight fast and before the application of the PA and CGM monitors, a DXA scan (effective radiation dose: <20 µSv) will be performed as a validated measure to determine body composition. There is no discomfort during the DXA scan.

Carotid artery reactivity (WUR only)

(Peripheral) vascular function will be assessed by determining carotid artery reactivity (CAR) in response to a cold pressor test (CPT) (27). The CPT will be applied to stimulate the

sympathetic nervous system. This thermal stimulus is known to elevate blood pressure via sympathetic pathways, so it can be used to study the vascular response to sympathetic activation (28-31). The participant will submerge their left hand in a bucket of ice water (approximately 4˚C) for three minutes, which is reported to be sufficient to induce a maximal dilation in the common carotid artery (CCA) (32). Ultrasound will be used to continuously measure the diameter of the left CCA during a 1-min baseline assessment and during the 3-min CPT. The CCA will be imaged on the proximal 1.5cm straight portion of the artery using gray-scale imaging (33). Systolic blood pressure (sBp), diastolic blood pressure (dBp) and heart rate (HR) will be assessed with an oscillometric blood pressure monitor before, after, and during the CAR measurement with a 1 minute-interval. There is minimal risk to the participant during these assessments, as ultrasound is non-invasive and commonly used in clinical practice.

MRI scan (WUR: 1 day before visit 1)

Whole-body fat distribution will be determined via MRI scan. MRI is a non-invasive imaging technique commonly used in body composition research, and provides more detailed information about specific fat depots (e.g. liver fat). In Maastricht, participants will undergo a full body MRI scan with a 3T MR system (3T MAGNETOM Prisma fit, Siemens Healthcare), using a radiofrequency transmit/receive body coil (total scanning time: 6 minutes). Using a turbo spin echo sequence with a proton flip angle of 90 degrees, proton density weighted scans will be acquired. This will create an image characterizing the proton density of each tissue type. The images will be acquired using a matrix size of 320-512 pixels and a 55 cm field of view. Contiguous images of 10 mm thickness with no gap will be taken in four to six sequences of 38-40 images for defined body regions for each participant. Analysis of the MRI scan data will be performed by external experts (AMRA, Linköping, Sweden) to quantify both subcutaneous and visceral fat depots, and liver and muscle fat content.

At WUR, proton magnetic resonance spectroscopy (1H-MRS) will be used to quantify the lipid content in liver, while MRI will be used to measure abdominal fat distribution, i.e. intra-abdominal fat and subcutaneous abdominal fat. Magnetic resonance imaging (MRI) will also be used to guide the spectroscopic measurements. The procedures will be performed on a 3.0 T whole body scanner (Siemens). For measurements of abdominal fat distribution, axial T1-weighted spin echo images will be acquired using the body coil with the patient in supine position. Slices will be centred at the interspace L4-L5, with the other slices situated above and below to cover the whole abdomen. A breath–hold technique will be applied to avoid breathing induced artefacts. A semi-automated software program will be used to quantify VAT and SAT and their ratio. For the IHL measurement by 1H-MRS, a voxel will be selected

and fine shimming will be performed to optimize the magnetic field homogeneity within the region of interest before acquiring 1H-MRS spectra. Vascular structures and the proximity of subcutaneous fat will be avoided in localization of the voxel. In the 1H-MR spectra, the water signal will be suppressed using frequency-selective pre-pulses and the spectra will be fitted to quantify the lipid peak. A tissue-specific coil will be used to optimize data acquisition and optimize signal-to-noise ratio. Due to motion, liver measurements will be respiration triggered. All measurements will be performed as much as possible by the same technician and at the same time of the day. Measurements will be performed in collaboration with the Department of Radiology, Hospital Gelderse Vallei.

Continuous Glucose Monitoring

For a 6-day period, 24-hour glucose levels will be monitored in all participants with a glucose monitor (iPro2, Professional CGM MiniMed, Medtronic) connected to a glucose sensor (Enlite Glucose Sensor MiniMed, Medtronic). The sensor, containing a thin needle, will be inserted subcutaneously, 5 cm from the umbilicus on the right side of the abdomen. Glucose levels in the interstitial fluid of the subcutaneous tissue will be determined and sent to the monitor every five minutes. The CGM readings will not be visible during the monitoring period. For the calibration of the sensor, participants will be instructed to use a blood glucose meter to determine capillary glucose levels four times per day at standardized time points (before breakfast, before lunch, before dinner and before sleep). One hour after application of the monitor; the first calibration will be performed in the presence of the investigator. The blood glucose values can be read in a display and documented in the personal study diary.

The CGM data will be downloaded immediately after removal of the device on visit 3. The mean glucose concentration over 24 hours will be calculated as the average glucose concentration of 288 measurements equally spaced in time over 24 hours. The net incremental area under the curve (iAUC) will be calculated by the trapezoid rule, using fasting glucose value as baseline. The iAUC provides a summary measure of the net increase in glucose levels above the fasting level during the 24-hour period. The frequency and duration of hypo- and hyperinsulinemia will be monitored and defined as a glucose level of ≥10.0 mmol/l for hyperglycemia, whilst hypoglycemia will be defined as a glucose concentration ≤3.9 mmol/l.

Physical Activity Monitoring (ActivPAL)

During CIW1 and 2, participants will wear the ActivPAL3 micro monitor (PAL Technologies, Glasgow, Scotland) to measure 24-hour daily physical activity patterns. The ActivPAL will be

applied during visit 1 (CIW1) and dietary visit 10 (CIW2) , and will be worn simultaneously with the CGM to ensure adequate alignment of the data generated by the two devices. The device will be made waterproof with a small sleeve to cover the monitor, wrapped in one piece of adhesive dressing (Tegaderm, 3M), and will be attached to the right anterior thigh using Tegaderm. With the ActivPAL, the intensity, duration and type (walking, standing and sitting/lying) of physical activity can be monitored. In addition, data will provide information for estimation of total energy expenditure. Participants will be instructed to not remove the ActivPAL and to avoid temperatures higher than 40°C. Participants will record the time they wake up and go to sleep in a paper diary. Data will be downloaded immediately after removal of the device at the first visit of the intervention period (DIW1) or visit 3/4 during CIW2, and analyzed later with ActivPAL software.

Dietary intake

Either during visit 1 or 2 in CIW1, a dietary consultation intake will take place with the participants. The aim of this consulation is to acquire more insight into the dietary history of the partcipants for adequate consultation during the dietary intervention period.

Visit 2 (CIW1 and 2)

Duration: 4 hours

Saliva samples for oral microbiota analysis

Before consuming the OGTT drink, saliva samples will be collected for microbiological and metabolite analysis. The participants will be asked to collect their saliva in sterile, ice-chilled Falcon tubes. The saliva samples will be transferred to aliquots, stored at -80°C and analyzed later.

7-points OGTT

During visit 2, participants will undergo the 7-points OGTT as performed at screening in order to have all measurements directly prior to the dietary intervention. At fasting, blood will not be collected for liver function tests or hemoglobin, but will be collected for HbA1c (4ml). Participants will receive a standardized lunch meal after completing the test. Disposition index will be calculated as follows: [Insulin Sensitvity index (ISI) * ($AUC_{30\ min\ insulin}$ / $AUC_{30\ min\ glucose}$)], where $AUC_{30\ min}$ is the area under the curve between baseline and 30 minutes of the OGTT for insulin (pmol/l) and glucose (mmol/l), respectively, and ISI (34) is defined as: [10,000 ÷ square root of (fasting plasma glucose (mmol/l) x fasting insulin (pmol/l)) x (mean glucose (mmol/l) x mean insulin (pmol/l))]

Cognitive functioning tests

After the OGTT and consumption of the standardized meal, the Cambridge Neuropsychological Test Automated Battery (CANTAB) will be used to assess neurocognitive performance. These tests may also be performed during visit 1, after the consumption of the same standardized meal. Domains that will be addressed are attention and psychomotor speed, executive function and memory. The mean time for performing the total test battery will be approximately 45 minutes. Before each individual test, participants will be provided with standardized instructions in Dutch and a 'practice' test for familiarization. All tests of the CANTAB are computerized and presented on a touch screen (iPad 2017). Data are instantly and safely recorded. The test battery will consist of the following tests, presented in a fixed order:

Attention and psychomotor speed

- Motor Screening Task: provides a general assessment of whether sensorimotor deficits or lack of comprehension, will limit the collection of valid data from the participant. Duration: approximately 2 minutes.
- Reaction Time: provides assessments of motor and mental response speeds, as well as measures of movement time, reaction time, response accuracy and impulsivity. Duration: approximately 3 minutes.

Executive function

- Multitasking Test: assesses the participant's ability to manage conflicting information provided by the direction of an arrow and its location on the screen and to ignore task-irrelevant information. Duration: approximately 8 minutes.
- Spatial Span: assesses visuospatial working memory capacity. Duration: approximately 5 minutes.

Memory

- Delayed Matching to Sample: assesses both simultaneous visual matching ability and short-term visual recognition memory, for non-verbalisable patterns. Duration: approximately 7 minutes.
- Paired Associates Learning: assesses visual memory and new learning. Duration: approximately 8 minutes.

Food preference task

Following the CANTAB during either visit 1 or 2, participants will complete the computer-based Macronutrient and Taste Preference Ranking Task (MTPRT) (35), to determine the effects of dietary intervention on food preferences. The test contains a total of 32 images of food products, which will be presented to the participants in different sets of four (in total 28

comparisons). Participants are asked to rank the products according to “what they most desire to eat at this moment”. The presented food products differ in nutritional content (high fat, high protein, high carbohydrate and low energy) and taste (sweet and savory). All food products are solid foods that can be easily recognized in a picture. The participants will practice prior to the main task using different pictures to familiarize them with the ranking test.

In the second part of the task, food preference will be assessed by presenting all 32 products with the question: “How much do you like [product name]?”. Answers will be rated on a visual analogue scale (VAS) anchored by “do not like at all” and “like extremely”. A relative preference score for sweet versus savory products and for each macronutrient category can be calculated. The task will take approximately ten minutes to complete, and will be executed in EyeQuestion software (Logic8 BV), which facilitates participants to complete the task online.

Non-visit days (3)

At home

The three home days during CIW1 and 2 will provide more information on study outcomes (glucose regulation) in free-living conditions of the participants. During this time, participants will periodically (every two hours from 8:00h to 22:00h) be prompted via mobile app to respond to a Likert scale regarding mood, fatigue, hunger and stress. They will also be asked to record their food intake via validated 3-day food record mobile app (Wageningen University, developed in 2016). They will be asked to record all food and drinks consumed and will be prompted to enter type of food eaten, brand name, amount, cooking methods, and time of day eaten. Although use of the mobile app is preferred, the Likert scales and food diary may be filled in on paper if necessary. A standardized breakfast will be provided for the second and the third non-visit days. Additionally, on the day prior to visit 3, the participants will be given standardized (adjusted to their energy needs) lunch, evening meals and snacks to consume.

Feces collection

Fecal samples will be collected for microbial composition analysis (16S-RNA sequencing) preferentially the day before visit 3, but otherwise any other home-day of CIW1 and 2. Participants will be provided with collection containers and instructions to sample and store feces at home. They will be asked to store the feces at -20°C until they bring it to the university on visit 3. The feces will be collected in three pre-weighed containers. The fecal samples will be weighed and frozen at -80°C.

Urine collection

Collection of 24-hour urine will be done the day before visit 3 of CIW1 and 2. Urine will be used to calculate nitrogen excretion, which will be used for calculation of energy expenditure and substrate oxidation (see p. 36). Participants will be provided with collection containers and instructions to collect the urine and store it at home in the refrigerator or in a cool and dry place until they bring it to the university on visit 3.

Visit 3 (CIW1 and 2)

Duration: 6 hours

Adipose tissue biopsy

On visit 3, before the start of the HFMM, an adipose tissue biopsy will be taken to examine the effect of the dietary intervention on adipose tissue gene and protein expression. A small amount of abdominal subcutaneous tissue (~1g) will be collected under local anesthesia (2% lidocaine without adrenalin, 6-8 cm lateral from the umbilicus) using a needle biopsy (with the needle connected to a vacuum syringe). The procedure will be performed by investigators with extensive knowledge and experience under authority of a physician. Biopsies will be snap-frozen in liquid nitrogen and stored at -80°C. Based on the most recent literature, a set of interesting genes and inflammatory markers will be selected for determination of gene expression profiles (microarrays or RNA sequencing). Protein expression will be determined by Western blot.

Blood pressure

Before the start of the high-fat mixed-meal, blood pressue will be measured in a sitting position.

High-Fat Mixed-Meal Test (HFMM)

A high-fat mixed-meal test will be performed after an overnight fast to determine the effects of the targeted diets on postprandial glucose and lipid metabolism. Participants will receive a standardized dinner the evening before the challenge and thereafter will be instructed to remain fasted until they receive the test meal at the university. Drinking water will be permitted. Participants will receive a liquid high-fat mixed-meal (ingested at t=0), which provides 2.6MJ and a macronutrient content of 61 E% fat (35.5 E% SFA, 18.8 E% MUFA, and 1.7 E% PUFA), 33E% carbohydrates and 6.3 E% protein. Details are described in document D2 Productinformation HFMM.

A venous catheter will be inserted 30 minutes before the HFMM test. At t=0, 30, 60, 90, 120, 180 and 240 a total of ~135 ml will be collected to determine plasma insulin, glucose, FFA,

TAG, GLP1, PYY, metabolomics, free glycerol, HDL, total cholesterol, and short chain fatty acids (Figure 3). At WUR only, a 24 ml fasting blood sample will be collected for isolation of PBMCs. At each time point, 2 ml of blood is discarded prior to drawing the blood samples. A visual analogue scale (VAS) will be taken after every blood draw during the test, to assess feelings of hunger and satiety.

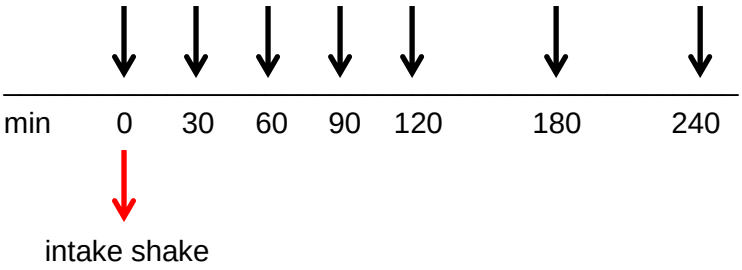

Figure 3. Time schedule of the HFMM test. Black arrows indicate blood collection and VAS for hunger and satiety.

Buffy coats

Buffy coats will be collected from a fasted blood sample that will be taken prior to the HFMM. DNA from the buffy coats will be analysed to investigate the relationship between certain gene polymorphisms (SNPs) and metabolic parameters (e.g. insulin sensitivity). The genetic profile of participants is likely an important determinant of personalized nutrition. Additionally, the DNA will be collected to expand our database of well-phenotyped participants for potential further analysis. Participants will be informed that buffy coats will be collected and will have the option to withdraw from this measure (as written on the informed consent form).

AGE accumulation (WUR only)

At WUR, advanced glycation end-products (AGE) accumulation will be measured by skin autofluorescence (AF) using the AGE reader (Diagnoptics, Groningen, The Netherlands), a non-invasive method to measure AGEs. The AGE Reader uses an ultra-violet-A black light tube, which illuminates approximately 4 cm^2 of the skin. Skin AF will be measured 3 times at the volar side of the arm and then averaged. During measurements, impurities of the skin like scars and birthmarks will be avoided as much as possible, and it will be made sure that participants do not have sunscreen on their skin since this could affect the results.

Monitor collection

At the end of visit 3, the CGM monitor will be removed.

Dietary consultation

At the end of the visit (CIW1 only), participants will receive dietary consultation. Participants receive a meal plan including a variation list to guide them with their dietary intake for the intervention.

Visit 4 (CIW1 and 2) - UM

Duration: 10 hours

Eighty participants (evenly recruited from each phenotype (MIR/LIR) and each diet (HMUFA/LFHP)) will undergo extensive metabolic phenotyping during CIW1 and 2. Visit 4 is at least 2 days after visit 3. During visit 4, a skeletal muscle biopsy, a 2-step hyperinsulinemic-euglycemic clamp and indirect calorimetry will be performed and blood will be collected. Also, targeted metabolome profile (protein, lipid and carbohydrate metabolism) will be determined. Participant are asked to follow the same preparations as during the other test days, and the researchers will provide them with a standardized evening meal that will be ingested during the evening before the visit (see D2 Productinformatie Avondmaaltijd).

Skeletal muscle biopsy

Before the start of the hyperinsulinemic-euglycemic clamp, a muscle biopsy will be taken from the *m. vastus lateralis* under local anesthesia by means of a Bergström biopsy needle method. During this procedure, skin and muscle fascia will be locally anesthetized using 2% lidocaine (with adrenalin). After ten minutes, a small incision will be made in the skin and fascia after which the biopsy needle will be inserted into the muscle. Suction will be applied to the needle and, with the needle kept *in situ*, several small muscle samples (total ~100 mg) will be collected. The samples will be snap-frozen in liquid nitrogen and stored at -80°C for later gene and protein expression analysis. Following the collection of the muscle biopsy, the skin will be closed using a steristrip and covered by waterproof bandage after which a pressure compress will be applied. After the 12-week intervention, muscle biopsies will be collected from the same leg compared to baseline (pre-intervention). An experienced medical doctor will perform the muscle biopsies.

2-step hyperinsulinemic-euglycemic clamp

Peripheral, hepatic and adipose tissue insulin sensitivity will be determined by a 2-step hyperinsulinemic-euglycemic clamp. Participants will be placed in a semi-recumbent position. Arterialised venous blood will be obtained through a cannula inserted into a superficial dorsal hand vein. Ten minutes before the blood draw, the participant's hand will be warmed in a hot-box, which will be maintained at 60ºC (36). In the contralateral arm, a second cannula will be

introduced anterogradely in an antecubital vein of the forearm for the infusion of glucose, [6,6-$^2H_2$]-glucose and insulin.

After collection of fasting blood samples (10 ml), a primed constant infusion of the glucose tracer ([6,6-$^2H_2$]-glucose) is initiated (t=-120). This is a naturally occurring isotope, therefore safe for participants. After 90 minutes, to allow isotopic equilibration, three additional blood samples will be drawn (6 ml at t=-30, -15 and 0 minutes). This allows calculation of rates of glucose appearance (Ra), glucose disposal (Rd) and hepatic glucose production (EGP) at plasma glucose concentrations of 5.0 mmol/l. In addition, adipose tissue insulin sensitivity will be determined by the insulin-mediated suppression of plasma free fatty acid (FFA) concentrations during the low-dose insulin infusion. During the last 30 minutes of this first period, basal substrate oxidation and energy expenditure will be measured with indirect calorimetry (ventilated hood, Omnical, Maastricht University) (37), and used to calculate non-oxidative glucose disposal (38). Participants will be placed in a resting position underneath the transparent hood placed over their head. Participants will be permitted to watch television while lying on the bed. The equations of Weir (39) and Frayn (37) will be used to calculate resting metabolic rate (RMR) and the total rate of fat and carbohydrate oxidation. Nitrogen (N) excretion will be calculated from 24 hours urine collection.

Calculations:

Energy Expenditure (EE) (kJ/min) = $(3.9*VO_2) + (1.1*VCO_2)$

Carbohydrate oxidation (CHO) (g/min) = $(4.55*VCO_2) – (3.21*VO_2) – (2.87*N)$

Fat oxidation (FAT) (g/min) = $(1.67*VO_2) – (1.67*VCO_2) – (1.92*N)$

Subsequently, a low primed constant infusion of insulin is started (10 mU/min/m^2, Actrapid, Novo Nordisk) to assess hepatic insulin resistance (t=0). Plasma glucose levels will be clamped at ~5 mmol/l by variable co-infusion of 20% glucose. Every five minutes a small amount of blood (1 ml) will be sampled from the dorsal hand vein to determine glucose concentrations immediately after sampling. The cathether will be flushed with a physiological salt solution, and before every blood draw this will be discarded. Radiometer blood glucose analyzer will be at bedside. When necessary, glucose infusion rate will be adjusted to obtain plasma glucose levels of ~5 mmol/l (euglycemia).

At t= 180 minutes, the primed constant infusion of insulin is increased (40 mU/min/m^2) to fully stop the hepatic glucose production and only study the rate of disapperance as a measure for skeletal muscle insulin resistance. Additional blood samples of 6 ml will be collected at t= 60, 120, 150, 165, 180, 300, 315 and 330 minutes. The total amount of blood collected

during the test day is approximately 200 ml. Insulin stimulated substrate oxidation will be measured with indirect calorimetry between t=150 to 180 and t=300 to 330 minutes.

The insulin infusion will be stopped after t=330 minutes and the participants will receive sandwiches and drinks to increase blood glucose concentration, which will be monitored every five minutes by a blood draw (total ~5ml). The glucose infusion will be continued for approximately 30-45 minutes to prevent hypoglycemia. The glucose infusion rate will be decreased in a step-wise fashion to maintain euglycemia. As soon as the blood glucose concentration rises, glucose infusion will be decreased and finally discontinued. A medical doctor will always be available during the clamp periods.

Dietary compliance during intervention period

During the 12-week intervention period, dietary compliance will be assessed by three unannounced 1-day food records per participant. The participants will use the same method that will be used for the 3-day food records.

Additionally, weekly visits with the research team will include a check-in with research personnel, measurement of body weight and distribution of new food products, if needed.

Monitor collection

At the first visit of the dietary intervention period, the ActivPAL physical activity monitor will be removed.

Flexibility in CIW1 and 2

- Visit 2 has to take place on the day after visit 1.
- The CANTAB, food preference task and dietary intake may take place during visit 1 or 2.
- The DXA and/or MRI scan may be performed during one of the other visits (UM).
- Visit 3 can be 3, 4 or 5 days after visit 2.
- The order of the home-days depends on the visits. The standardized home day (all meals provided) should be before visit 3. The third home day could also be one day after visit 3 (but participant has come to the university to hand in GCM).
- Visit 4 could be up to 14 days after or before visit 3.

7.4 Withdrawal of individual participants

A participant can withdraw from the study at any given time for any given reason. The primary investigator/physician will decide whether or not to withdraw a participant from the study if the project team becomes aware of any of the following conditions:

- Urgent medical reasons (adverse events, serious adverse events);

- Non-medical reasons (on participants request);
- Participant's inability to undergo any procedure measuring primary or secondary endpoints as outlined in the protocol;
- Illness or change in medication use.

7.4.1 Specific criteria for withdrawal

Not applicable.

7.5 Replacement of individual participants after withdrawal

For any patient withdrawn from the studies, an alternative candidate may be selected in order to ultimately meet the calculated sample size/power. The minimization procedure will take extra candidates into account.

7.6 Follow-up of participants withdrawn from treatment

The study will be considered completed for an individual when all treatments and testing periods have been completed. The study as a whole will be completed when all minimized participants have completed the double-blind treatment period and final measurements. The investigator will provide follow-up medical care for all patients who are prematurely withdrawn from the study, or will refer them to a medical doctor.

7.7 Premature termination of the study

In case of emergency the study will be terminated. Reasons for prematurely terminating the study will be explained clearly and justified in writing to the Medical Ethical Committee.

8. SAFETY REPORTING

8.1 Temporary halt for reasons of participant safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardize participant health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all participants are kept informed.

8.2 AEs, SAEs and SUSARs

8.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experiences occurring to a participant during the study, whether or not considered related to the experimental intervention or study procedures. All adverse events reported spontaneously by the participant or observed by the investigator or his staff will be recorded.

8.2.2 Serious adverse events (SAEs)

A serious adverse event (SAE) is any untoward medical occurrence or effect that:

- Results in death;
- Is life threatening (at the time of the event);
- Requires hospitalization or prolongation of existing inpatients' hospitalization;
- Results in persistent or significant disability or incapacity;
- Is a congenital anomaly or birth defect;
- Any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgment by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events. The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

8.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Not applicable.

8.3 Annual safety report

Not applicable.

8.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow-up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

8.5 Data Safety Monitoring Board (DSMB) / Safety Committee

Not applicable.

9. STATISTICAL ANALYSIS

SPSS and R software will be used to carry out the statistical analyses. Regarding descriptive statistics, numerical variables will be reported as mean ± SD. Categorical variables will be reported as numbers and percentages. To determine normality of the data, visual inspection of QQ-plots and results of the Kolmogorov-Smirnov test will be used. In case of abnormally distributed data, the data will be transformed. In general, a p-value <0.05 will be considered statistically significant using two-tailed tests.

9.1 Primary study parameter(s)

Change in disposition index between the optimal and the suboptimal diet as determined from insulin and glucose concentrations during a 7-points OGTT will be considered as our primary outcome parameter. Intention-to-treat and completer's analysis will be used. Differences between intervention groups will be analyzed using a linear mixed model analysis to take repeated measurements into account. Final value will be used as outcome, and baseline value will be included as co-variate, as recommended (40). The location will be included as random factor. Phenotype, age and sex will be added as covariates in the analysis.

9.2 Secondary study parameter(s)

The statistical approach for analyses of secondary outcome parameters is equal to what is described above. Several secondary study parameters will also compare the difference in outcomes between and within the tissue-specific insulin resistant subgroups (MIR versus LIR) (as described in 7.1.2.).

In addition, correlation and regression analyses will be used to determine cross-sectional relationships between 1) physical patterns, dietary patterns and blood glucose homeostasis, 2) metabolic factors and blood glucose homeostasis and 3) blood glucose homeostasis and markers of well-being and physical and mental performance. Confounders will be added as covariates to the models. Potential confounders will be based on knowledge and literature on risk factors for the outcome, and observed associations between these potential confounders and the exposure in the data. For longitudinal analyses, such as the association between changes in blood glucose homeostasis and performance, linear mixed models will be used.

9.3 Other study parameters

Not applicable.

9.4 Interim analysis (if applicable)

Not applicable.

10. ETHICAL CONSIDERATIONS

10.1 Regulation statement

The study is approved by the Medical Ethical Committee of the academic hospital Maastricht and Maastricht University (METC azM/UM). The study will be conducted according to the Declaration of Helsinki (64th WMA General Assembly, Farcaleza, Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO). The general principles of informed consent, ethics review and data management will be in line with GCP.

10.2 Recruitment and consent

Participants will be recruited by means of advertisements in local newspapers and posters, targeted websites, social media and from an existing cohort of participants that have previously agreed to be contacted by the investigator for future studies. People who are interested and respond to the advertisement will be sent more detailed information via website or email. The researchers will additionally plan either a group information session at the university or an individual session via telephone. After this session, potential participants will be given a minimum period of 7 days to decide upon definite application for the study. Every potential participant in Maastricht will be informed about the extensive phenotyping and will have the option to be involved in these measurements until n=80 is reached. Participation in these extended studies is voluntary.

The written informed consent will be signed before the start of the screening. Participants have a one week reflection time and can always contact the researchers if any questions arise. If desired, participants can contact an independent expert, who will not be involved in the study. The privacy of the participants who take part in the study will be protected by appointing a participant number which is kept separate from their actual identification.

10.3 Objection by minors or incapacitated participants (if applicable)

Not applicable.

10.4 Benefits and risks assessment, group relatedness

The general interest of this study is to obtain insight into the metabolic and lifestyle determinants of postprandial blood glucose responses and to establish the effect of macronutrient manipulation of a 12-week dietary intervention on blood glucose homeostasis in metabolically different groups and its relationship to physical and mental performance and well-being. Participants may have personal health benefits if intervention effects are according to expectations. Following the study completion, all participants will have access to

all results of the testing performed. These data can provide information about their health status and additional information about their metabolic phenotype, which may positively impact their health.

Specifically for the extensive metabolic phenotyping, the aim is to obtain further insight into the metabolic responses during and after the hyperinsulinemic-euglycemic clamp and relate this to tissue-specific insulin sensitivity, substrate oxidation, molecular markers and blood glucose homeostasis in participants with normal and impaired glucose homeostasis. The hyperinsulinemic-euglycemic clamp is the gold standard for determining tissue-specific insulin resistance and is a very accurate method. Ultimately, these measures will provide extensive understanding of the etiology and pathophysiology of impaired glucose homeostasis and how these may be affected by the dietary intervention. The hyperinsulinemic-euglycemic clamp will only be performed in n=80 participants as it not feasible to perform this in all participants (not possible at WUR and higher burden for participants). Additionally, 80 participants will be sufficient to answer the secondary objectives, due to the accuracy of the method. Participants will not experience additional benefits from the extra measurements performed, other than the extensive metabolic phenotyping information they will receive. The burden will be higher for participants (time inverstment for example), but additional risks will be relatively low (explained below).

Participants will have to invest approximately 40 hours in the study, plus an additional 20 hours for extensive phenotyping at UM (see for an overview table 1), therefore time is a potential burden. The dietary and healthy regimen they will follow can be considered a burden, but also an overall health benefit as both diets are considered healthy. Also the collection of fecal and urine samples can be experienced as a burden (collecting the samples storing them at home for up to 24 hours).

The MRI scan does not have any risks associated with it, however some patients can experience claustrophobia. The MRI scan at UM is only six minutes, which is a significant reduction of a normal MRI scan time, therefore this effect is minimized. Although scan time at WUR is longer (30 minutes), participants will be closely monitored.

In addition, participants will undergo a DXA scan. Thereby, they will receive a total radiation dose of <20 µSv (calculated by Heleen Huyten-Erkens, Radiation Expert, Randwyck, Maastricht). The average dose of each person in the Netherlands is 2,5 mSv per year, therefore the dose of the radiation is negligible (statement by Heleen Huyten-Erkens, Radiation Expert, Randwyck, Maastricht).

The placement of the CGM and ActivPAL, though quite non-obtrusive, can be considered a burden for the participants. The placing of each piece of equipment will be done by experienced researchers and will be secured by well-practiced measures to minimize issues the participant may encounter. The calibration of the CGM may be considered a burden due to the fingerprick that is required four times a day by a glucose meter.

During the test days, blood will be collected via a venous catheter. Venipunctures can occasionally cause a local hematoma or bruise to occur. Some participants report pain during venipuncture. During visit 3, an adipose tissue biopsy will be taken. The adipose tissue biopsy might cause a local hematoma. Visit 4, will require a skeletal muscle biopsy. After the muscle biopsy, some participants report pain, which is experienced as muscle pain. More often the muscle feels stiff for a couple of days after the biopsy. To minimize the risk for a hematoma, the area where the biopsy was taken will be compressed for approximately five minutes after placing the steristrips and a waterproof bandage. The place of incision will leave a small scar (~3 mm for adipose tissue biopsy and ~8 mm for skeletal muscle biopsy). During the hyperinsulinaemic-euglycemic clamp there is a small risk of hypo- or hyperglycemia. However, from our own extensive experience, these conditions do not occur very often and can be reversed immediately. A medical doctor is always available during the clamp. Concerning the other study procedures (OGTT (screening and visit 2), and HFMM (visit 3), there are no known risks and these measurements are routinely applied in human biology research. Standard operating procedures (SOPs) for each measurement are available on the UM Human Biology Department's server.

10.5 Compensation for injury

The sponsor/investigator has liability insurance, which is in accordance with article 7 of the WMO. The sponsor also has insurance, which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides coverage for damage to research participants through injury or death caused by the study.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study. The liability-insurance of each individual university applies for this study.

10.6 Incentives

Participants will receive a financial compensation for the time invested in the study. Participants will voluntarily undergo the study. After complete participation in the study compensation will be €550/€600 (UM/WUR respectively). Participants who complete

additional participation in the extensive phenotyping will receive a total compensation of €700. Also travel expenses will be covered (€0.19 per km; WUR only: with a maximum of 30 km one-way).Premature termination or non-compliance will result in a reimbursement relative to the duration of the participation. Participants will not receive any payment after completing only the screening, but their travel cost will be covered.

11. ADMINISTRATIVE ASPECTS AND PUBLICATION

11.1 Handling and storage of data and documents

The privacy of the participants who participate in the study will be protected. After inclusion, participants will be assigned a unique participant number that will not change during the study. This number is linked to personal information and is in a password protected file. Only members of the project team have access to this file. The participant number will be used for identification. All personal information, collected material and study results will be related to this unique participant number. The key that links the participant numbers to an individual participant will be maintained at the participating centers.

When participants give permission, collected body materials will be kept in a biobank for 15 years after completion of this study to allow the possibility of additional analyses based on new techniques and advanced insights. Body materials will only be used for additional analyses that are in line with the current study. Data will be kept by a data manager centrally at UM and will be stored for 15 years after completion of the study. A participant will be excluded if he/she does not consent to the timelines of storage. Only researchers directly involved in the research can request access to the data via a synopsis principle.

11.2 Monitoring and quality assurance

Monitoring depends on the risk classification, which will be determined in consultation with Clinical Trial Center Maastricht after approval of this research protocol.

11.3 Amendments

Amendments are changes made to the research after a favorable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favorable opinion.

11.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first participant, numbers of participants included and numbers of participants that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

11.5 Temporary halt and (prematurely) end of study report

The investigator will notify the accredited METC of the end of the study within a period of eight weeks. The end of the study is defined as the last participant's last visit. The investigator will notify the METC immediately of a temporary halt of the study, including the reason for such an action. In case the study is ended prematurely, the investigator will notify the accredited METC within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

11.6 Public disclosure and publication policy

There are no restrictions with respect to publication of the data. Both positive and negative results of the studies will be made public, preferably in peer-reviewed international scientific journals, according to the CCMO statement of publication policy.

12. STRUCTURED RISK ANALYSIS

12.1 Potential issues of concern

No potential issues foreseen.

12.2 Synthesis

Participants will be screened for potential health risks (see inclusion and exclusion criteria (paragraph 4.2 and 4.3). The test days will be performed at the Metabolic Research Unit Maastricht (MRUM) and at the test facility at WUR. There are strict guidelines on how to respond in case of emergency within these units. The guideline for each individual measurement is described in detail in the Standard Operating Procedures. Furthermore, a physician will be informed about the scheduled test days and will always be reachable via telephone. Moreover, the participants will get the phone number of the researchers and independent expert. In case of complications or questions, the participants can call the researcher and /or expert at any time.

Specifically, the dietary intervention will consist of food products that are part of a regular Dutch food pattern. Any potential health issues encountered will most likely not be a result of the dietary intervention.

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