The impact of a dried vegetable fibre on glucose metabolism and gut microbiota composition – VEZEL studie

RESEARCH PROTOCOL

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PROTOCOL TITLE

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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**
AR	**Adverse Reaction**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CGM	**Continuous Glucose Monitoring**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
GLP-1	**Glucagon-like peptide-1**
HOMA-ir	**Homeostatic model assessment of insulin resistance**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
NQplus	**Nutrition Questionnaires plus study**
PYY	**Peptide YY**
(S)AE	**(Serious) Adverse Event**
SCFA	**Short Chain Fatty Acid**
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**
Wbp	**Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)**
WF	**Whole Fiber**
WMO	**Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: Dietary fibre intake is usually lower than recommended and dried vegetable can be a useful additional source. This may improve glucose metabolism by changes in microbiota composition.

Objective: To study the effect of WF Fibre on (continuous) glucose metabolism and potential underlying mechanisms, body weight and waist circumference, microbiota composition and fasting GLP1/PYY, in subjects with pre-diabetes.

Study design: Randomised placebo controlled single-blind parallel study

Study population: Human volunteers with pre-diabetes (n=60)

Intervention (if applicable): One group receives daily a dose of 30g of WholeFiber (WF fibre) and the other group 16g maltodextrin (placebo) for 21 days.

Main study parameters/endpoints: Changes in fasting insulin and HOMA-ir are the primary outcomes of the study. Changes in mean glucose and glycemic variability from continuous glucose monitoring, body weight, waist circumference, microbiota composition, breath hydrogen, GLP-1/PYY and SCFAs are the secondary outcomes.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: The intervention is therapeutic for half of the participants and non-therapeutic to the other half of the participants. The risk associated with the participation is negligible and the burden can be considered as minimal. At screening the following measurements and questionnaires will be taken: inclusion and diabetes risk score questionnaire (1x) food frequency questionnaire for fibre intake (1x), height (1x), weight (1x), fasting blood glucose (finger prick)**.** During the intervention period over 3 weeks, with an additional 2 week run-in period and a 2 week wash-out period, subjects will take dietary supplements on the morning of each day of the intervention period, and come to the university on 4 occasions. During this 7 week period the following measurements will be taken: body weight (3x), fasting blood sample (2x), fasting breath sample (2x), continuous glucose monitoring (28 days), faecal samples (4x), wellbeing diary (49 days).

1. INTRODUCTION AND RATIONALE

Obesity and type 2 diabetes mellitus (T2DM) are worldwide expected to rise exponentially, with increasing health care costs and reduction in life expectancy and quality of life. We have recently shown that diabetes prevention is possible in real-life settings, by improving diets (such as whole-wheat bread and pasta) and increasing physical activity over a one-year period (Duijzer et al., 2017; den Braver et al., 2017)

Despite national recommendations to increase vegetable and fruit intake and intake of whole grain foods the habitual intake of dietary fibre is still low in the Netherlands (20-25 g/d compared to 35 g/d or more recommended) as well as in many other Western countries. Several observational studies, including our own (InterAct Consortium, 2015), have shown that subjects with higher fibre intake at reduced risk of developing obesity, and T2DM.

The underlying mechanisms have not been completely elucidated, but the intestinal microbiota may play an important role, for example by fermentation of fibre, modulation of microbiota composition and its activities, and specifically the resulting production of anti-inflammatory short chain fatty acids (SCFA) that via the release of incretins (GI hormones) PYY and GLP-1 directly stimulate insulin secretion by the beta-cell (DeClerq et al., 2017). Note that animal experiments have shown that modifying rodents' microbiota through faecal transplantation results in alterations of GI hormones and subsequently an altered metabolism (DeClerq et al., 2017). However, whether and to what these results relate to human metabolism is yet unclear. Earlier work of our group has shown that fibres, such as pectin, impact satiety and energy intake on the short-term (Wanders et al., 2011; Wanders et al., 2014).

Recent studies have indicated that there is a strong correlation between the intestinal microbiota and response to specific dietary compounds. A comparative study with various fibres in obese men with metabolic syndrome showed a marked individual response that related to their faecal microbiota composition (Salonen et al., 2014). A multicentre analysis revealed specific microbiota signatures that explained the responses of metabolic syndrome subjects to a variety of interventions, including resistant starch and inulin (Korpela et al., 2014). A further development derived from a large intervention study that used a machine-learning approach to mine personal health profiles that included microbiome data to predict the postprandial glycaemic response (Zeevi et al., 2015). A recent start-up is exploiting this and providing dietary advice based on the microbiota composition (daytwo.com). Hence, in fibre intervention studies it is of great interest to determine the faecal microbiota signatures that are present in the volunteers before and after the treatment in order to advance our knowledge and link these to the intervention success.

Recently, an interesting and natural food product (WholeFiber™) (WF) has been developed by WholeFiber Holding INC, that is based on whole vegetables, consisting of prebiotic fibres (85% on solids, consisting of inulin, pectin, hemicellulose and cellulose). This could be a useful addition to the general (Western) diet. It has a low ecologic footprint, and we propose that it can be used as a functional ingredient and a dietary supplement, alleviate deficiencies in dietary fibre intake and support a diverse gut microbiome, with subsequent prevention of delay of pre-diabetes. We propose as dose of 30g per day (approximately 25g fibre, the majority of which is inulin), in line with previous studies with inulin or inulin-type fructans (Dewulf et al.,

2013; Guess et al., 2016). To increase tolerance a dose escalation run-in period will be implemented (Guess et al 2016). We selected a parallel randomised intervention study in metabolic syndrome adults since cross-over studies suffer from intestinal microbiota adaptations (Salonen & De Vos, 2014).

This study will show whether WF can affect insulin resistance, continuous glucose metabolism and/or the microbiome during a three week period in subjects with elevated risk for developing type 2 diabetes mellitus. This will provide more insight in the potential health effects of a dried vegetable fibre and leads for the potential underlying metabolic changes including microbiota changes.

2. OBJECTIVES

The objective of the VEZEL-studie is to study the effect of WF fibre on insulin resistance, (continuous) glucose metabolism, body weight, waist circumference, microbiota composition and breath hydrogen in subjects with pre-diabetes.

Primary Objective:
To compare 3-week changes in fasting insulin level and HOMA-ir between subjects with pre-diabetes consuming either 30g of WF or 16g of placebo carbohydrate (maltodextrin).

Secondary Objective(s):
To compare 3-week changes in mean glucose and glycemic variability from continuous glucose monitoring (CGM), body weight, waist circumference, fasting GLP-1/PYY level, fasting SCFAs, breath hydrogen and microbiota composition between subjects with pre-diabetes consuming either 30g of WF or 16g of placebo of ‘control’ carbohydrate (maltodextrin).

3. STUDY DESIGN

The study is designed as a single blind, randomised placebo controlled parallel trial in which 2 groups of 30 subjects receive either 30g of WF or 16g of control carbohydrate (maltodextrin, in iso-caloric amount) for a period of 3 weeks (21 days). To reduce potential gastrointestinal side effects of WF, the study will start with a 2 week run-in period during which time WF will be taken at 15g/day for 2 weeks before the actual intervention starts. The control arm will receive MD 8g/day during this period, an iso-caloric amount. The study schedule is summarized below. During the pre-study screening visit a fasting blood glucose measurement will be done using finger-prick and the Dutch diabetes risk score (DRS) will be assessed (http://www.kijkopdiabetes.nl/professionals/diabetes-risicotest-3).

Table 1. Study schedule VEZEL studie

	Pre study screening	*Before run-in*	*Run-in 14 days*		*Intervention 21 days*		*Wash-out 14 days*		
		Day -17	*Day -14*		*Day 1*		*Day 22*		*Day 35*
Visit number →	***1***	***2***			***3***		***4***		
Home visit number →			*1*						*2*
Activity									
Informed consent	X								
Health questionnaire	X								
Diabetes risk score	X								
Finger prick (fasting glucose)	X								
Food frequency questionnaire	X								
Wellbeing diary			X	X	X	X	X	X	X
Handing out study sachets			X		X				
Return study sachets – compliance check					X		X		
Fasting blood sampling		X					X		
Stool collection			X		X		X		X
Fasting breath sample		X					X		
Continuous glucose monitoring		X	X	X until day -3	X	X until day 14			
Body weight / Waist circumference	X	X			X		X		
Body height	X								
Breakfast	X	X					X		
Post study screen									X
End of trial									X

X: CGM will be placed on the upper arm, device works for 14 days

4. STUDY POPULATION

4.1 Population (base)

Sixty non-diabetic men and women aged 40-75, at elevated risk for type 2 diabetes, will be recruited regionally from the general population.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following inclusion criteria:

- Age 40-75 yr
- Fasting blood glucose between 5.6 and 6.9 mmol/L (pre-diabetes according to American Diabetes Association 2016)
- Or fasting blood glucose between 5.0 and 5.6 mmol/L and diabetes risk score (DRS) ≥ 9.

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Having a history of medical or surgical events that may significantly affect the study outcome: IBS or IBD patients,
- Medical drug use: for diabetes
- Medical drug use: antibiotic use within 3 months of the study screenings day or chronic use of antacids
- Mental status that is incompatible with the proper conduct of the study
- Reported unexplained weight loss or weight gain of > 5 kg in the month prior to pre-study screening
- Reported slimming or medically prescribed diet
- Reported vegan or macrobiotic life-style
- Consumption of pre-probiotics or fibre supplements as of 1 month before the screening
- Sensitive to medical skin adhesives
- Not willing or afraid to give up blood donation during the study
- Personnel of Wageningen University, department of Human Nutrition, their partner and their first degree relatives
- Current participation in other research from the Division of Human Nutrition
- Not having a general practitioner

4.4 Screening visit

After an evening visit to the research centre for an information session about the study, interested participants will be invited for a subsequent morning visit to the research centre where fasting blood glucose (finger prick), body height, weight and waist circumference will be measured and 2 questionnaires will have to be filled out, followed by a small breakfast. Informed consent form will be handed out at the information session, and asked to be signed during the screening visit.

4.5 Sample size calculation

The sample size calculation for the study is based on results from literature (Guess et al., 2016), where a mean within-person reduction in fasting insulin of 37 pmol/L was observed, with t-statistic of 2.45 and n=7 equal to an effect size of 0.92 (=t/√n). A recent intervention study from our group showed that subjects at risk for diabetes had a mean fasting insulin of 85 pmol/L, with SD 50 mmol/L, and $SD_{change\ from\ baseline}$ of 37 pmol/L (Duijzer et al, 2017).
Based on an effect size of 0.92 as per Guess et al, and SD_{change} of 37 pmol/L in a parallel design, 40 (two times 20) subjects will be needed to achieve 80% power at a two-sided alpha of 5% (G*Power 3.1.9.2 T-test, two independent means, reduction of 34 pmol/L in intervention and 0 pmol/L in control group). To allow for a smaller effect size of 0.78 based on an estimated reduction of 29 pmol/L instead of 34 pmol/L, 54 (two times 27) subjects will be needed. To account for potential drop-out (10%) 60 (two times 30) subjects will be randomised in the study.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

WF fibre is a product with very high levels of prebiotic dietary fibres (~85%): inulin, pectin, hemi-cellulose and cellulose. The dose will be 30g product per day, corresponding with approximately 25g fibre and 3.5 gram other macro-nutrients (proteins, sugars, organic acids) and 1.5g water per day. The energy content will be ~ 2 kcal/g for fibres and 4 kcal/g for others, i.e. 64 kcal per day.
The 'control' arm will receive 16 g of maltodextrin per day, which will provide with 4 kcal/g also 64 kcal per day. Maltodextrin is a polysaccharide derived from starch. It has been used as placebo in an inulin trial before (DeWulf et al., 2013).

5.2 Use of co-intervention (if applicable)

Medication other than mentioned at the Exclusion criteria is allowed during the study. Subjects will be instructed to maintain their habitual lifestyle.
With respect to the visit to the study site, the subjects are asked to remain fasting (no eating or drinking other than water, after 21.00 hr).

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

WholeFiber™ is a high fibre product consisting of mainly inulin, some pectin and hemicellulose and cellulose that is derived from chicory roots. Besides dietary fibres, it contains low levels of proteins (5%), fat (<1%), mono- and disaccharides (3%), organic acids (2%) and minerals (2%). Chicory roots have a long history of safe use for coffee substitute, 'witlof', inulin extraction and their toxicological evaluation has been reported and states that there were no observed adverse effects and the NOAEL for the extract is 1000 mg/kg/g administered orally for 28 days (Schmidt et al., 2007). The product will be provided to the participants in a granulated form that highly palatable and can easily be mixed with yoghurt or other semi-liquid food products.
Maltodextrin will be provided as a powder and obtained from a commercial provider (Body&Fit Pure Maltodextrin).

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

The subjects will be instructed to add WF or maltodextrin to their yoghurt

6.6 Preparation and labelling of Investigational (Medicinal) Product

WF fibre will be delivered to the test site by the sponsor and will be stored at recommended condition. The design, production, and delivery of the test product WF fibre will be performed by the sponsor. Both products will be packed by the research team and labelled A and B.

6.7 Drug accountability

WF fibre will be delivered to the test site at Wageningen University by WholeFiber Holding INC. Maltodextrin will commercially obtained by the research team. Study personnel on site will supervise and register the compliance of taking the products.

7. NON-INVESTIGATIONAL PRODUCT

Not applicable.

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

Fasting insulin and HOMA-ir (homeostatic model assessment of insulin resistance, derived from fasting insulin and fasting glucose levels).

8.1.2 Secondary study parameters/endpoints (if applicable)

- Continuous Glucose Monitoring (CGM): mean glucose and glycemic variability
- Body weight
- Waist circumference
- Microbiota composition
- Breath hydrogen
- Fasting GLP-1/PYY
- Fasting SCFA
- Adverse events (bloating, belching, flatulence)

8.1.3 Other study parameters (if applicable)

Age, gender, body height (for calculating BMI), cigarette smoking, highest education level, family history of diabetes; for women, history of gestational diabetes.

8.2 Randomisation, blinding and treatment allocation

- Subjects will be stratified to sex and fasting blood glucose group (between 5.6 and 6.9 mmol/L or between 5.0 and 5.6 mmol/L) and within these strata subjects will be randomly assigned to arm A or B. Randomisation will be performed using a random number generator by a researcher unrelated to the study. The randomisation code will be broken after data-analysis.

8.3 Study procedures

The volunteers will have a pre-study screening before the start of the intervention. This will involve:

- assessment of medical history
- assessment of Diabetes Risk Score (kijkopdiabetes.nl)
- assessment of regular fibre intake measured with a specific food frequency questionnaire
- measuring height, weight, waist circumference
- measuring fasting glucose from finger prick

For the timing of the procedure, see Table 1 in the Chapter Study Design. For logistic reasons subjects will be divided in two groups, starting their treatment period on two consecutive days. For both series the first day of treatment will be specified as Day 01.

During the main part of the intervention the subjects will consume 30 grams of WF fibre or 16 grams of placebo per day, for 21 consecutive days. Before starting, a fasting venous blood sample (total 30ml) will be taken to determine fasting blood glucose and insulin concentrations, and body weight and waist circumference will be measured. Subjects are asked to provide a stool sample for microbiota determination. For this, the participants will receive a home sampling kit with instructions from the WU Laboratory of Microbiology. Also a continuous blood glucose meter will be placed. Also a fasting breath sample will be obtained.
A run in period of 2 weeks on 15 gram WF fibre or 8 grams of placebo will precede the trial to avoid gastro-intestinal discomfort. After that, and before starting the main phase of the intervention, another stool sample will be asked for.
After 21 days of intervention with the full dose, a fasting blood sample, a stool sample, a breath sample and data on body composition will be obtained. After another 14 days of wash-out a final stool sample will be obtained. During the 49 days study period (2+3+2 weeks of run-in, intervention and wash-out resp.) a diary on abdominal side effects will be kept.

Compliance will be assessed by asking subjects to return unused and opened non-empty sachets.

8.4 Details on measurements

All measurements will be done using validated methods developed for their purpose, and by experienced laboratories.

During screening, capillary blood sampling will be used for determination of fasting blood glucose using HemoCue B-glucose device (HemoCue AB, Ängelholm, Sweden).

During the intervention fasting insulin and fasting glucose will be measured in serum and NaF-plasma, respectively, as obtained by venepuncture, by Laboratory Clinical Chemistry of ZGV Hospital Ede. HOMA-ir will be calculated from fasting glucose and insulin levels using software from Matthews et al. (1985) (https://www.dtu.ox.ac.uk/homacalculator). For GLP-1 and PYY measurements blood will be collected in tubes with aprotinin.

Abott's FreeStyle Libre Flash will be used as CGM (see Bolinder et al, 2016). It includes a very tiny glucose sensor worn under the skin and connected to a water resistant, plastic on-body patch the size of a coin. The sensor remains inserted for max. 14 days and does not require fingerstick calibrations. The sensor can be inserted by the subject

him/her-self. To obtained read outs a reader device is being used which is held near the sensor patch. The results (glucose value, trend and graph of last eight hours) can be seen immediately (in this case the screen will be covered for the participants) or downloaded to PC software. EasyGV software will be used to assess measures of glycemic variability (Hill et al., 2011) (m-value, mean amplitude of glycaemic excursions, lability index, average daily risk range, j-Index, low blood glucose index and high blood glucose index, continuous overall net glycemic action, mean of daily differences, glycaemic risk assessment diabetes equation, mean average glucose).
Body weight will be measured to the nearest 0.1 kg. Body height will be measured to the nearest 0.1 cm. Waist circumference will be measured to the nearest 0.5 cm.
Microbiota analysis will be performed by the WU Laboratory of Microbiology on faecal samples essentially as described previously (Salonen et al., 2014) with the modification that extracted DNA will be subject to next generation 16S rRNA and/or metagenomic sequencing as described (Korpela et al 2016; Li et al 2016).
Breath hydrogen is an end product of fermentation in the large bowel and correlates strongly with colonic fermentation. Hydrogen will be measured by collecting breath samples using 250-ml sample holding bags (Quintron, Milwaukee, USA). Samples will be collected during the study visits in the fasting state. Breath hydrogen will be measured the collected breath samples using Quintron Microanalyzer (Quintron Instruments, Milwaukee, US).
Serum will be stored at -80˚C until laboratory analyses. The analyses for fasting GLP-1/PYY and SCFA will be done later, the laboratory has not yet been identified.

8.5 Withdrawal of individual subjects

Subjects may discontinue the trial at any moment without the obligation to state the reason for discontinuation. Subjects may be withdrawn from the study by the principal investigator if they do not comply with the rules and regulations of the study. Subjects may be withdrawn from the study by the medical supervisor in case of reported serious adverse events or in case of other medical/social/psychological events as evaluated by the medical investigator and discussed with the principal investigator.

8.5.1 Specific criteria for withdrawal (if applicable)

Not applicable

8.6 Replacement of individual subjects after withdrawal

Subjects not completing the study for any reason will be considered as drop-outs and will not be replaced.

8.7 Follow-up of subjects withdrawn from treatment

Not applicable.

8.8 Premature termination of the study

Since all ingredients of the products are suitable for human consumption and are microbiologically safe, we do not expect any negative or adverse events. However, a very high intake or an unusual high dose of dietary fibres may result in uncomfortable though harmless effects as increased flatulence or bloating. Research has shown that these adverse events are highly variable among people. Participants will be notified on these side effects in the participant information. The study will be terminated immediately if there would be another problem with the products when they appear to be harmful to the participants' health. In that case, all participants will be informed about this as soon as possible. Furthermore, we will inform the accredited METC.

9. SAFETY REPORTING

9.1 Section 10 WMO event

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 jeopardise subject 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 further review by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

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

9.2.2 Serious adverse events (SAEs)

As of 1 January 2010, submitters of investigator-initiated research are required to report serious adverse events through the web-portal Toetsing Online within 15 days. This requirement also applies for studies not involving a medicinal product.
A serious adverse event is any untoward medical occurrence or effect that at any dose:

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgement, the event may jeopardize the subject or may require an intervention to prevent one of the outcomes listed above.

The sponsor will report the SAEs through the web portal Toetsing Online to the accredited METC that approved the protocol, within 15 days after the sponsor has first knowledge of the serious adverse events.
SAEs that result in death or are life threatening should be reported expedited. The expedited reporting will occur not later than 7 days after the responsible investigator has first knowledge of the adverse event. This is for a preliminary report with another 8 days for completion of the report.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Not applicable

9.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the product under investigation.

9.4 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.

The follow-up of AE’s will also be reported in the annual progress report.

9.5 Data Safety Monitoring Board (DSMB) / Safety Committee

Not applicable.

10. STATISTICAL ANALYSIS

The statistical analyses will be carried out by SPSS (Version23; 2015, IBM SPSS Statistics) or a comparable software program. Statistical test will be done two-sided with a significance level of 5%. Normality will be checked for all continuous outcome variables by inspecting Q-Q plots.
For the descriptive analyses results will be presented as mean ± SD, or median and interquartile range, or percentage, depending on their distribution.

10.1 Primary study parameter(s)

Fasting insulin, and related/correlated HOMO-ir, derived from fasting glucose and fasting insulin (Matthews et al., 1985), will be analysed as continuous outcome variables. Descriptive statistics will be used to summarize values and changes from baseline by visit. The main analysis will consist of ANCOVA with end-line fasting insulin (or HOMA-ir) as outcome variable, treatment group as factor, and baseline insulin (of HOMA-ir) and age-group, BMI group and gender as covariates. The differences in least square means between the treatment groups and corresponding 95% confidence interval and p-value will be presented.

10.2 Secondary study parameter(s)

Differences in change between intervention and control group regarding mean glucose and glycemic variability from CGM, BMI and body weight, waist circumference, breath hydrogen and serum GLP-1/PYY and SCFA levels will be analysed using similar methods as the primary study parameters.
The data on microbiota composition will be analysed by the Laboratory of Microbiology (prof. Willem de Vos) according to established methods (Korpela et al., 2014). Data on wellbeing, i.e. hunger feelings, adverse events (bloating, belching, flatulence and frequency and consistence of faeces by means of the Bristol Stool Chart (Lewis and Heaton, 1997)) will be derived from the wellbeing diary. The data will be expressed as number of days with complaints, and will be compared between the intervention and control group using non-parametric statistics.

10.3 Other study parameters

Age, gender, body height, cigarette smoking, highest education level, family history of diabetes; for women, history of gestational diabetes will be described by intervention/control group, using descriptive statistics, to characterise the study population.

10.4 Interim analysis (if applicable)

Not applicable.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (Fortaleza, Brasil 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

11.2 Recruitment and consent

The focus of the recruitment will be on the database of volunteers of our department HNE, with inclusion of participants of an earlier longitudinal observational study NQplus. An invitation to participate will be sent to all subjects with reference to the age range. Subjects that are willing to participate will receive the information brochure including an informed consent and are invited for information meeting on the study. The informed consent must be signed in at the screening visit. The subject will sign the informed consent first, followed by the research assistant (in such a way that the subject can visually establish that the investigator does not change anything on the form after he/she signed it), and the subject will receive a copy of the informed consent form directly after the signing.
Personal data will be stored in a closed locker and a password-protected file, to which only the database manager (or his/her replacement) has access.
All the data will be destroyed 15 years after finishing the study.

11.3 Benefits and risks assessment, group relatedness

- To increase the fibre intake
- To decrease the number of diabetes patients
- To get information about some of their health outcomes; glucose levels, insulin levels and microbiota composition
- The intervention is therapeutic to the participants in the intervention group. The fibre intake including the 30g WF does not exceed 75g, a dose which may lead to uncomfortable side effects. Uncomfortable side effects as bloating and flatulence sometimes occur after ingestion of some types of fibre, they are however harmless. Participants are informed on these possible side effects beforehand. As the fibres used in the study are safe to use for human consumption the risk associated with participation is negligible and the burden can be considered moderate.
- Venapunctures can occasionally cause a local hematoma or bruise and some participants may report pain or discomfort. Placing the continuous glucose meter can cause minimal pain or discomfort.
- Screening visit 20 min, baseline visit 30 min, endline visit 30 min, questionnaires at home, total 60 min.

11.4 Compensation for injury

Wageningen University has a liability insurance which is in accordance with article 7, of the WMO.

Wageningen University (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 24 November 2014). This insurance provides cover for damage to research subjects through injury or death caused by the study.

1. € 650.000,-- (i.e. six hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2. € 5.000.000,-- (i.e. five million Euro) for death or injury for all subjects who participate in the Research;
3. € 7.500.000,-- (i.e. seven million five hundred thousand Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as 'verrichter' in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.
Wageningen University has this insurance at:
HDI- Global SE, the Netherlands
Westblaak 14
3012 KL Rotterdam

11.6 Incentives (if applicable)

Participants will receive €200,- after completing the study. When participant withdraw during the study they receive an incentive in proportion to the duration of the study.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

All data will be handled confidentially. Data will be stored in a password protected file. Only members of the project team can access this file. The informed consents will be stored separately from all other information. Human material, which is sampled during the study will be stored anonymously. All human material will be stored for 15 years after finishing the study.

12.2 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.
Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.3 Annual progress report

Not applicable

12.4 End of study report

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit. If the end of study is defined otherwise, this new definition should be given.
In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority 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.

12.5 Public disclosure and publication policy

Results will be presented in agreement with the CCMO publication statement.

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

Not applicable

13.2 Synthesis

Not applicable

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