Protocol for Pilot Studies

Effectiveness of Bioactive Enriched Foods (BEF) on markers of Metabolic Syndrome

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1. Background – Introduction

Many naturally-occurring compounds in dietary plants and animal products possess a variety of physiological functions which promote human health and wellbeing, and contribute to better diet-related-disease (DRD) prevention and particularly the Metabolic Syndrome (MS). These compounds, known collectively as bioactives (natural components of foods that possess biological activity in addition to their nutritional value), normally occur at very low concentrations in foods, and their effects on human health are being studied intensively for their possible contribution toward reducing the risk of many DRDs. Identifying bioactives, establishing their mechanisms of actions and health effects are all active areas of scientific inquiry and, through industrial exploitation, potential societal benefit.

Scientific understanding of the role and mechanisms of bioactives is fragmented. Research often addresses the theoretical possibility of health improvement effects rather than their real, practical use for everyday diets. Bioactives cannot be considered as discrete chemical compounds and research must focus on bioactive-enriched foods (BEF), if consumer demands for foods delivering appropriate health and wellbeing benefits are to be fulfilled. The general objective of PATHWAY-27, a pan-European interdisciplinary team of 16 life/social scientists and 10 high tech/ food processing SMEs, addresses the exploitation of bioactive compounds as ingredients of foods that, within the common diet, could significantly benefit human health and wellbeing. PATHWAY-27 will evaluate the effectiveness of docosahexaenoic acid (DHA) alone or in combination with two other bioactives, beta-glucan (BG) and anthocyanins (AC), chosen for known/claimed effectiveness in reducing some risk factors of MS. These compounds will be considered as ingredients of BEFs enriching three different widely consumed food matrices (dairy-, bakery-, egg products) and not pure compounds. This will allow us a better understanding of possible synergisms and bioactive matrix interactions.

BEFs to be tested in PATHWAY-27 clinical studies will be designed and selected in preceding work packages of this European FP7 project. SMEs will be deeply involved in the elaboration of BEFs. The purpose of pilot clinical studies will be to select, for each of the three food matrices, the BEF delivering the greatest reduction in serum triglycerides (TG) or increase in HDL-cholesterol (HDL-C). Three pilot studies will be conducted in three clinical research centres (Max Rubner Institute, Karlsruhe, Germany; University of Leeds, United Kingdom; and Human Nutrition Research Centre of Auvergne, France), each participating centre focusing on a specific food matrix. Each pilot study will be randomized and double blind and aim to identify the most active BEF within a specific food matrix. The BEFs selected after pilot studies will then be tested in the subsequent, larger interventional study, on their ability to modulate a wider range of metabolic outcomes.

2. Aim

The aim of this multi-centre, randomized, double blind, parallel pilot dietary intervention study is to identify the BEF achieving the greatest effect on lipid parameters. Three different matrices, dairy-, bakery-, and egg products, containing DHA, BG and AC given alone or DHA associated with BG or AC will be tested. Thus, 15 products, five in each centre will be tested.

3. Study population

3.1 Population

Three hundred participants will be included. Men and women aged 18 to 80 yrs, at risk for metabolic syndrome, will be eligible.

3.2 Sample size calculation

In each pilot intervention, 100 participants will be divided in 5 subgroups of 20 participants, each receiving a BEF within a specific food matrix (dairy-, bakery-, egg products) enriched with DHA, BG or AC alone, or DHA+BG, or DHA+AC. In this exploratory study, within each matrix, 20 subjects per arm would be sufficient to obtain measurable changes for the parameters that will be tested.

3.3 Inclusion criteria

MS is defined when three of the following criteria are met:

- elevated waist circumference (men ≥ 102 cm; women ≥ 88 cm)
- elevated fasting triglycerides (≥ 150 mg/dL)
- reduced fasting HDL-cholesterol (men ≤ 40 mg/dL; women ≤ 50 mg/dL)
- elevated blood pressure (systolic ≥ 130 mmHg and/or diastolic ≥ 85 mmHg) or hypotensive treatment
- elevated fasting glucose (≥ 110 mg/dL)

Subjects will be eligible to the pilot study if they present with two to four of the MS criteria, at least one of them being alteration of fasting triglycerides or HDL-C cholesterol.

3.4 Exclusion criteria

Participants are excluded if three or more clinical criteria for metabolic syndrome are met. Additionally, major exclusion criteria are:

- regular drug therapy with impact on serum lipids;
- diabetes (fasting glucose > 1.26 g/L, or anti-diabetic treatment);
- recent history of cancer or cancer treatment (less than 2 years);
- active or recently diagnosed intestinal malabsorption or disorders associated with malabsorption: Crohn's disease, short bowel syndrome, Pancreatic insufficiency, cystic fibrosis, Tropical Sprue, whipple's disease, chronic pancreatitis, gastrojejunostomy, surgical treatments for obesity, cholestasis, biliary atresia, parasite infections, HIV/AIDS
- familial dyslipidemia;
- use of medication known to cause malabsorption: tetracycline, cholestyramine, thiazide diuretics, aluminium/magnesium hydroxide, cochicine, neomycin, methotrexate, methyldopa, and allopurinol, and laxatives
- illegal drug use, chronic alcoholism or active smoking;
- consumption of nutritional supplements containing DHA, BG or AC;
- history of allergy or intolerance to any components used in BEFs, celiac disease, lactose intolerance, allergy to milk or egg proteins;
- institutionalised patients, those who lack autonomy to consent or are unable to meet all examinations;
- women who are pregnant, lactating or actively trying to conceive;
- participation in other clinical trials that may impact on outcome;
- subjects deprived of their liberty by judicial or administrative decision.

3.5 Recruitment

Possible participants will be identified from databases of interested volunteers. Those who meet eligibility criteria will be invited to participate. Additionally, recruitment will include interviews in local radio stations and advertisement in local newspapers if necessary. Waist circumference and history of hypertension, dyslipidemia and hyperglycemia will be included into the advertisement to be shared by the three centres. Interested persons will be contacted by

phone/email to answer an eligibility questionnaire to be shared by the three centres. Eligible persons will be invited for baseline examinations. Written consent will be obtained before any examination takes place.

3.6 Subject withdrawal criteria

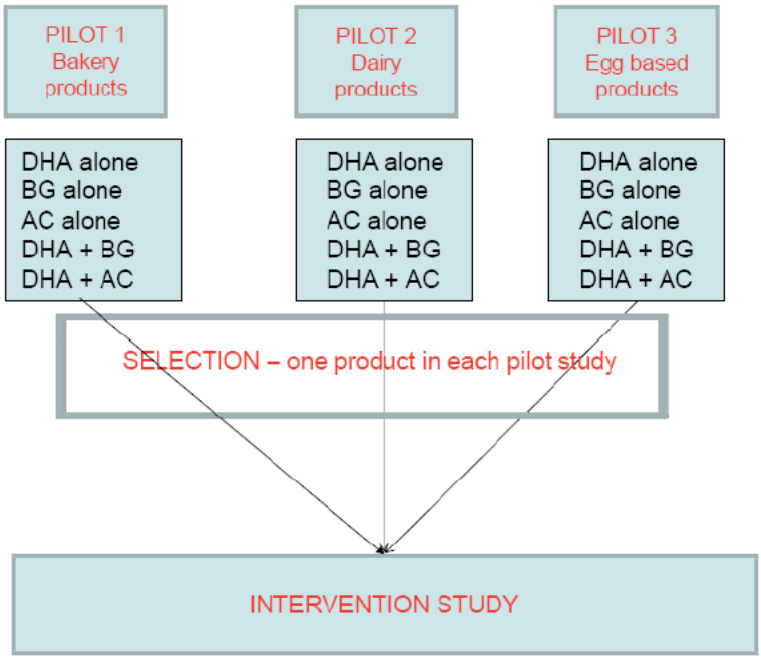
Subjects may discontinue their participation in the study at any time. A subject may be withdrawn from the study for any of the following reasons:

- withdrawal of consent - any subject may withdraw from the study at any time
- significant deviation from the protocol
- lost of follow-up
- death
- incidental illness or condition interfering with protocol
- occurrence of a serious adverse event or significant alteration in clinical and/or laboratory parameters. In these cases, appropriate actions will be taken. The sponsor will be notified immediately.

4. Study design

This is a multi-centre, randomized, double blind, parallel pilot dietary intervention study without a placebo. In total, 300 men and women at risk for metabolic syndrome will be recruited. Each of the three participating study centres (MRI, ULE, CRNH) will recruit 100 volunteers. Participants will be divided in five groups of 20 each receiving BEF enriched with DHA, BG, or AC alone or DHA+BG, or DHA+AC based on bakery, dairy or egg. In each pilot, BEF belonging to a specific food matrix will be studied (Figure 1). Participants will be required to consume the allocated BEF daily for a period of four weeks. At baseline and after 4 weeks of intervention, fasting blood samples will be collected for further analysis. Additionally, blood pressure and anthropometric data will be collected.

Figure 1 Scheme of pilot studies



5. Intervention

5.1 BEF delivery

Bakery, dairy or egg product-based BEFs will be produced by SMEs involved in the project, according to the protocols and formulations established in other work packages of the PATHWAY-27 program. BEF will be stored and supplied to participant centres according to shipment and storage rules given by producers. For each matrix, five different BEFs will be prepared and enriched with DHA, BG, or AC alone or DHA+BG, or DHA+AC. The putative amounts of supplied bioactives are given in table 1, and will be the same regardless of the food matrix.

Table 1 Putative bioactive supplies

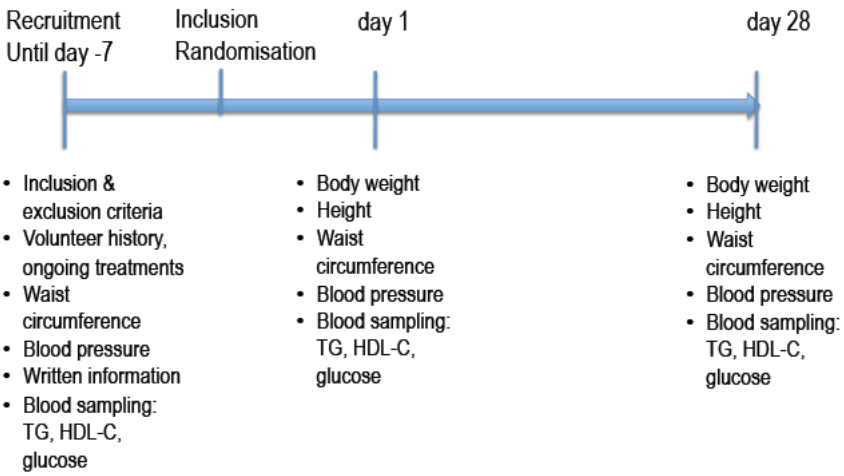
Bioactive	Docosahexaenoic acid (DHA)	Beta-glucan (BG)	Anthocyanins (AC)
Daily supply	250 mg	3 g	320 mg

Participants will be advised to consume their product daily. No placebo or control product will be given. The BEF will be consumed in replacement of similar foods, and not in addition to the usual diet.

5.2 Study protocol (Figure 2)

Recruitment will be performed until day -7. Eligible participants will be included and randomly allocated to one of the five groups. On days 1 and 28 after inclusion, each participant will come in to the laboratories for clinical and biochemical investigations.

Figure 2 Study protocol



Participants are advised to consume the tested products daily in replacement of the similar "common" food they consume normally, by including them in their regular meals. Besides BEF consumption, participants will be on a free diet, however a list of foods to be consumed no more than once per day will be provided to each participant. Dietary supplements containing DHA, BG or AC must not be taken.

5.3 Outcome assessment

Primary outcome: fasting serum TG and HDL-cholesterol will be measured at baseline and at the end of the study by standardized enzymatic methods.

Secondary outcomes (days 1 and 28):
- blood pressure
- body height and weight, BMI
- abdominal circumference
- blood glucose

At the beginning of the study participants will be asked to complete a food frequency questionnaire specific to the foods described on the restriction list. The purpose of this is to collect information about exposure to the bioactive ingredients from natural sources and highlight high/low level consumption that could be a potential confounding factor.

Participants at Leeds will also complete a 24 hour dietary recall at the baseline and end visit to collect information about energy, nutrient and alcohol intake. The purpose of this exercise is two-fold. Firstly to look for potential confounding factors, and secondly to investigate whether this type of dietary assessment tool is appropriate to capture the information required to perform an effective dietary assessment.

At the baseline and final visit participants will provide information about abdominal symptoms that they experience. This has been designed to assess whether the BEF have an impact on appetite and intestinal behaviour.

Participant acceptability of the BEF will be assessed at days 1 and 28 of the intervention using a food preferences questionnaire to collect data.

5.4 Safety assessment

- An adverse event (AE) is any untoward medical occurrence in a subject consuming a study product and which does not necessarily have a causal relationship with the study product.

- A serious adverse event (SAE) is any untoward medical occurrence that at any dose: results in death; is life-threatening (at the time of the event); requires inpatient hospitalisation or prolongation of existing hospitalisation; results in persistent or significant disability or incapacity. In the present study, expected adverse events may be linked to venous punctures for blood samples and digestive discomfort associated with BEF consumption. No serious adverse event is expected.

5.4.1 Declaration of AE/SAE

Any AE/SAE will be notified to the principal investigator who will ask for medical advice if necessary. The study coordinator will be informed of any SAE. In addition, AE/SAE will be reported in the Electronic Data Capture (EDC) System. Anybody who has access to the EDC System will have to report AE by selecting the patient and completing the AE/SAE form (annex 9). The investigators with then receive email notification of the AE/SAE which will be handle according to the seriousness of the event: in case of SAE a report will be sent within 24 hours to the sponsor; monthly reports will be created about non-serious events.

To detect possible non-reported AE, checks will be inserted into eCRF forms (e.g. alert and redirect the physician to the AE/SAE form if he/she enters an abnormal finding). The monitors will also have to raise data clarification queries if they encounter suspicious data. In the latter case the principal investigator will have to confirm the data and to complete an AE/SAE report or corrects the data.

Participants will be informed to contact the person responsible for their inclusion in the study (principal investigator) in case of any unexpected health event. On day 28, the clinical examination will look for any adverse effect, and if necessary, complementary investigations will be performed.

5.5 Methods of measurement

Blood pressure will be measured in participants after being at rest for five minutes in seated position followed by two additional measurements separated by two minutes each. A calibrated sphygmomanometer (e.g. *Boso-Carat professional*) will be used for the pilot study and appropriate sized cuffs are applied after measurement of arm circumference.

Body weight will be measured using a calibrated balance with the participants barefoot and dressed in underwear.

Height will be determined using a calibrated stadiometer.

Waist circumference will be measured in standing position between the iliac crest and the lowest rib using a measuring tape (WHO guideline).

All measurements will be performed by trained study nurses strictly adhering to SOPs.

Serum markers (serum TG, HDL, fasting glucose) will be determined by standardized enzymatic methods.

5.6 Harmonization of methodologies and standard operating procedures (SOPs).

The SOPs to be applied by the three centres are given in the following annexes.

1. SOP Recruitment of participants for clinical trials
2. Information for participants
3. Consent form
4. SOP Collection of blood by venepuncture from fasted subjects and preparation of serum
5. SOP Quantification of HDL-cholesterol in serum samples
6. SOP Quantification of triglycerides in serum samples
7. SOP Quantification of glucose in blood samples
8. SOP Physical examination
9. Adverse event/serious adverse event declaration
10. Composition of pancakes
11. Risk assessment
12. Pancake consumption record
13. Clinical Research File – Screening – Visit 1
14. Clinical Research File – Baseline – Visit 2
15. Clinical Research File – Endpoint – Visit 3
16. Recruitment advert
17. Recruitment email
18. Telephone screening questionnaire
19. Letter to participant GP
20. Restricted food list
21. Abdominal symptoms questionnaire
22. Study instructions for participants
23. Food preferences questionnaire
24. 24 hour dietary recall
25. Visit 1 Invitation letter
26. Visit 2 invitation letter
27. Food frequency questionnaire – restricted foods
28. References

5.7 Extraction, processing, and storage of biological samples

Blood will be sampled after an overnight fast (10 to 14 hours). Serum will be obtained by centrifugation at 4°C 20 min 1800g, immediately shipped to the accredited analysing lab or aliquoted and stored at -80°C until further analysis. Cellular fractions will be discarded and destroyed by incineration. Triglycerides, HDL-cholesterol and glucose will be measured by internationally validated standard enzymatic assays. The analysing lab is responsible for proper disposal of the biological material according to local rules. Biological materials will be kept until the publication of the study in order to be able to perform eventual control measurements. Thereafter, the analysing lab is responsible for proper disposal of the biological material according to local rules.

5.8 Compliance assessment

Compliance will be assessed after collection of all food packages on day 28 and using a record that participants are asked to complete during the trial.

6. Statistical considerations

6.1 Randomization

The study will be double-blinded (blinded for the study subjects as well as for the investigator). Throughout the trial the blind must not be broken, only in case of emergency, in which case sealed emergency envelopes containing the name of the enrichment material will be available. The consumption of experimental product must be discontinued if the code has been broken for any reason. Every code breaking has to be reported to the WP5 leader.

A total number of 300 participants will be enrolled into the study. The complete sample size will be equally distributed between three centres responsible for either bakery, dairy or egg products, which results in a sample size of 100 per centre. In each centre participants will be divided in 5 groups of 20 each receiving BEF formulated using a specific matrix (bakery, dairy or egg-based), and enriched with DHA, BG, or AC alone or DHA+BG, or DHA+AC. The allocation of participants to the different groups will be carried out based on predefined randomization lists created separately for each pilot study. All three randomization lists will be prepared with a sample size of 120, with a block size of 5 and with an allocation ratio of 1:1:1:1:1. In case of withdrawn of a volunteer, he/she will be substituted by a new one. For this purpose, an additional sample size of 20 will be applied for each pilot study. Gender will be considered as a stratum with a ratio near to 1:1. Each randomization list will consist of a three-digit randomization number from 101 to 160 for male and from 201 to 260 for female participants with the name and code of the assigned enrichment material. The prepared randomization lists will be sent to the manufacturers of each product, who will pack and label the products according the list and send them to the centres.

A three-digit unique randomization number will be assigned to each individual recruited volunteer, which will determine the allocation of the products. Randomization numbers will be distributed in ascending order with the starting number of 101 in each centre. Centres will also get a unique one-digit centre identifier. Therefore volunteers will be identified by a centre and a participant codes.

6.2 Data Management

6.2.1 Summary

Data management consists of tasks required to capture all study data into electronic form. Data management also includes tasks designed to validate the entered data by means of a variety of edit checks (e.g., subjecting the data to range checks, valid value checks, crosschecks, and manual review) that provide feedback to those entering/providing the data. The data management guidelines are incorporated into the Data Management Plan and Data Validation Plan. Both plans will be prepared together with the electronic data capture system and will be finalized before starting to enter data into the database system.

The goal is to have an electronic clinical database that accurately reflects the data collected and is able to be used for purposes of analyzing study data for regulatory submissions and professional publication. The data capturing process and the electronic system that captures clinical data for purposes of analysis and reporting will adhere to GCP guidelines for data management systems.

6.2.2 Electronic Data Capture (EDC) System

The proposed EDC system for use in this study is Mythos CDMS v2.0. The system is validated according to GCP and 21 CFR Part 11 regulations to ensure accuracy, reliability, consistent

intended performance, and the ability to discern invalid or altered records. The EDC system is hosted by AdWare Research Ltd.

Mythos is a web application based on Oracle 10gR2 database and accessible through secure Internet connection via a common modern web browser. Statistical analysis is performed with SAS 9.2. Data transfer tool used to access data from statistical analysis software is SAS Oracle Data Transfer. Database and daily backups are stored on mirrored hard drives at a secured location with access control. Disaster Recovery Plan, uninterruptible power supply and comprehensive malware protection are also provided.

Site staff will be trained on CRF best practices and system use. Users are authorized by their electronic signature. All study participants are provided different levels of access based on the participant's role:

- Level 1 access (clinical researcher): enrol/exclude patients, complete/modify eCRFs, answer queries, upload in the system data of patient enrolled by him/her, can access patient data enrolled by him/her.
- Level 2 access (study monitor): verify/approve eCRFs, raise/close queries, can view all data, but can't modify them.
- Level 3 access (coordinator and WP5 leader): can view all patients data, but cannot modify them.
- Level 4 access (principal investigator - one in each centre): same as clinical researcher. In addition he/she can access all data and can modify them if needed. All modification must be recorded, and coordinator and WP5 leader must be informed before data modification.
- Level 5 access (administrator): can't view any patient data; can upload files of shared documents (eg. protocol outline, printable informed consent form and adverse event/serious adverse event forms, etc.), can manage sites, user accounts, can open/close study or parts of the study.

Audit trail of all database transactions – including names and dates corresponding to each entry-of or change-to a field – is managed automatically by the EDC system.

The data entry into EDC system is performed by investigators from source documents. The EDC system provides real-time data quality control (like range- and validity checks, etc.). Site monitor access to clinical data is facilitated to enable source document verification. Data managers provide status reports for enrolment, CRF completion, outstanding queries, query overrides, perform medical coding of adverse events and concomitant medications, conduct SAE and AE database reconciliation, perform data cleaning, query generation, query resolution, update database based on query responses, perform audit of final database, lock database and archive database files, completed CRFs, and query records. Disaster Recovery Plan will be provided as an annex of the Data Management Plan.

Data management processes and activities are controlled by the standard operating procedures of AdWare Research Ltd.

6.3 Statistical Evaluation

6.3.1 Analysis Datasets

- The full analysis dataset (FAS) is defined as all randomized subjects who received at least one dose of experimental product and have primary outcome data both at baseline and at the end of the study.

- The safety analysis dataset (SD) is defined as all randomized subjects who received at least one dose of experimental product.

A total number of 300 participants will be enrolled into the study. The complete sample size will be equally distributed between three centres responsible for either bakery, dairy or egg products, which results in a sample size of 100 per centre. In each centre participants will be divided in 5 groups of 20 each receiving BEF from a specific matrix, and enriched with DHA, BG, or AC alone or DHA+BG, or DHA+AC. The allocation of patients to the different groups will be carried out based on predefined randomization lists created separately for each pilot study.

6.4 Statistical Analysis

The primary and secondary analyses will be performed on the FAS dataset. Descriptive statistics for both of the primary and secondary variables will also be provided on the SD dataset. The evaluation will be done using the SAS 9.2 (or later) program package version.

Mean change in blood TG and HDL-cholesterol (primary endpoints) will be analyzed with a mixed linear model including food matrix and type of enrichment as fixed effects. If the model indicates significant differences among groups, post-hoc tests will be applied for pair wise comparisons.

Secondary endpoint variables will be analyzed similarly. Descriptive statistics for both of the primary and secondary parameters will be provided including the mean, median, minimum, maximum, standard deviation, standard error of the mean and number of cases. Mean changes (and their 95% CI) from baseline to end-of-study visit will be presented.

For safety data, descriptive statistics and individual listings of eventual adverse events (adverse events linked to venous punctures, digestive troubles following BEF ingestion, symptoms of allergy or any unexpected health event) will be also presented.

7. Ethics

Prior to initiating the pilot study, ethical approval will be obtained from the relevant local research ethics committees of the participating countries. Each recruitment centre will be responsible for obtaining ethical approval from their respective ethical committee. Adverts for recruitment, screening questionnaires and letters for doctors will be harmonised, but each centre may use their participant information sheets and consent forms when the local ethical committee requires that.

Before submission to the local Ethic Committee, the PATHWAY-27 Ethical Advisory Board will approve all documents. All protocols for experiments with human subjects will require approval by the members of PATHWAY-27 Ethical Advisory Board before experiments can start, and this process will take place prior to submission of those protocols to the relevant local research ethics bodies. In case of disagreement, the PATHWAY-27 Ethical Advisory Board will make the final decision as to the ethical suitability or otherwise of the studies proposed. No experiments involving human subjects will proceed without approval of the PATHWAY-27 Ethical Advisory Board and the relevant local ethics committee. Copies of approval from the REC (Research Ethics Committee) will be provided to the European Commission prior to commencing the research.

During the research period, any change of protocol must be reported to the PATHWAY-27 Ethical Committee and local ethics committees.

8. Study registration

This multi-centre study will be registered in clinicaltrials.gov.

9. Annexes

9.1 SOP Recruitment of participants for clinical trials

Standard Operating Procedure (SOP) Recruitment of participants for clinical trials

Purpose and scope

This SOP describes the procedures that all study personnel will use in recruiting participants while fulfilling ethical responsibilities for protecting the rights and welfare of participants as part of PATHWAY-27 WP5.

Responsible persons

Principal Investigator

In each investigating centre, the Principal Investigator will be responsible and accountable for:
1. Getting approval from the PATHWAY-27 and relevant ethics committees of the methods to be used to recruit participants, including review and approval of recruiting materials.
2. Assuring that all participants understand the purpose of the study, its risks and benefits.
3. Assuring that all participants understand what is expected of them (number of visits, types of examinations, dietary and lifestyle restrictions, intervention).
4. Assuring that the participants understand that they may withdraw from the study at any point, but need to inform the research team.
5. Assuring that all participants meet eligibility criteria as specified in the study protocol.
6. Assuring that all participants have given informed consent prior to study procedures being performed.
7. Maintaining accurate and complete records of potential and enrolled study participants.
8. Informing participants of the termination or cancellation of the research trial.
9. Informing participants of any approved deviation from the protocol.
10. Informing participants of any remuneration to recompense their participation, or reimbursement of expenses, when applicable.
11. Reporting accurately to the work package leader for continuing review.

The principal investigator may delegate responsibility for recruiting and enrolling participants to other qualified researchers involved in the study, but may not delegate accountability.

Clinical Researcher

The clinical researcher may be given the responsibility for:
1. Monitoring the progress in recruiting patients.
2. Keeping records of subject recruitment.
3. Informing the principal investigator about progress toward recruitment goals.
4. Verifying and reviewing each participant's information/history for eligibility criteria, and informing the principal investigator if the participant's information changes during the trial.

Procedures

Recruitment strategies

1. Participants can be recruited from a variety of sources including internet and paper postings, written media advertisements, television/radio advertisements, from support groups, health fairs, or from local hospitals and clinics via clinician deferral.
2. The principal investigator or the clinical researcher will enter in the EDC system subjects satisfying to entry criteria.

3. When applicable, researchers who get referrals from clinician colleagues may not contact these referrals directly. The clinicians may inform their patients of the research and advise their patients to contact the researcher.
4. Financial or other incentives provided to research staff or physicians by sponsors based on numbers of participants recruiting or enrolled are strictly prohibited.
5. Recruitment Materials
6. Any materials directed to participants or the general public with the intent of recruiting them to participate in clinical research must be approved by the PATHWAY-27 ethics committee and submitted to the relevant ethics committee for review and approval. Similarly these materials will have to be approved by the local ethics authorities. These include announcements, advertisements, flyers/posters, phone scripts for screening, radio and television announcements, notice board tear-offs, Internet postings.
7. Copies of approval from the REC (Research Ethics Committee) will be provided to the European Commission prior to commencing the research.
8. Advertisements may include:
    a. A statement that the study involves research
    b. A brief description of the purpose of the study, including details of the clinical disorder that the study is investigating
    c. Eligibility criteria (in summary form)
    d. A truthful description of potential benefits, if any, to the subject from study participation
    e. The name of the institution conducting the study
    f. The name of the sponsor providing finance for the study
    g. The name and phone number of person to be contacted for further information
9. Advertisements may not include:
    a. Any expressed or implied claim that the research will improve the subject's medical condition
    b. Expressed or implied statement that the research is endorsed by the medical profession or any medical or government or professional bodies
10. Any questions about criteria should be referred to the principal investigator. All such contacts/discussions must be recorded.

Enrolment Procedures

1. Before enrolment, the Clinical Researcher must ensure that the subjects meet the eligibility criteria (inclusion and exclusion criteria).
2. The Clinical Researcher will keep records of recruitment and monitor and will inform the principal investigator of progress in recruiting participants. Every person who is considered a potential candidate for the study should be entered in the Screening and Enrolment Log (based on study inclusion and exclusion criteria). It should be noted whether individuals have enrolled in the study and, if not, the reason should be documented.
3. At the screening visit, the potential participant must give informed written consent prior to any screening procedures. Signed informed consent forms from all subjects who entered the screening process should be retained for the duration of the study including the ones who terminated their participation in the study during the screening process.
4. After screening and randomization, the participant's code/ID number should be entered in the Screening and Enrolment Log. The Enrolment Log can serve as the coded subject list, which must be archived at the end of the study. The Screening and Enrolment Log must include details of all subjects randomized in the trial, with subject name, address, and year of birth, code.
5. Recruitment rates should be regularly evaluated during the recruitment period, with reassessment of the strategy when recruitment targets are not being met.

9.2 Information for participants

Information for participants

Project Title

Pivotal assessment of the effects of bioactives on health and wellbeing from human genoma to food industry – PATHWAY-27

Pilot clinical study

Effects of bioactive enriched foods on markers of the metabolic syndrome.

You are invited to take part in a research study at the University of Leeds. Before you decide to take part, it is important that you understand the purpose of the study and what it will involve. Please take the time to read the following information carefully. Please ask us any questions that you may have.

What is the purpose of the study?

The aim of the study is to improve the nutritional quality of common foods (egg, dairy and bakery products) by enriching them with natural ingredients, and to test whether consuming these enriched foods, on a daily basis as part of a normal diet, decreases the risk of a common condition clinically referred to as metabolic syndrome.

The food product being tested in this study is a pancake. The pancakes will be enriched with either docosahexaenoic acid (DHA) – an unsaturated lipid extracted from egg yolk, beta-glucan – a type of dietary fibre extracted from oats, or anthocyanin – a pigment extracted from grapes.

Metabolic syndrome is the name given to a group of conditions that together are risk factors for heart disease and diabetes. Metabolic syndrome is defined when three of the following criteria are present:

- Elevated waist circumference (men ≥ 102 cm/40 inches; women ≥ 88 cm/35 inches),
- Elevated fasting blood triglycerides (≥ 150 mg/dL),
- Reduced fasting blood HDL-cholesterol (men ≤ 40 mg/dL; women ≤ 50 mg/dL),
- Elevated blood pressure (systolic ≥ 130 mmHg and/or diastolic ≥ 85 mmHg) or hypotensive treatment,
- Elevated fasting glucose (≥ 110 mg/dL).

This study is part of a project that has been approved and supported by the European Commission. Centres in Germany and France will be testing dairy and bakery products.

Why have I been asked to participate?

You have been invited to participate in the study because we are looking for adults presenting the conditions associated with metabolic syndrome; these may include elevated waist

circumference, altered blood lipids, elevated levels of fasting glucose or mild hypertension. It is up to you to decide whether you wish to participate in the study, and you may withdraw from the study at any point. Your choice will not affect the care that you receive from your doctor or nurse.

What will I have to do if I take part in the study?

If you decide to take part, the researcher will explain the study in detail and will ask you some questions about your medical history, to check your eligibility to take part against a set of criteria. This may be done at the research centre or by telephone. If you are eligible and you decide to take part, you will be asked to come in to the research centre in person and sign a consent form to show that you have agreed to take part in the study. You will be given a copy of this information sheet and the consent form for your records. You will also have the opportunity to ask questions.

Provided that you meet the eligibility criteria and have signed the consent form, you will be invited to come to the research centre for an initial screening session. We will ask you some more questions about your health, take some physical measurements (height, weight, waist circumference and blood pressure), and we will collect a blood sample. You should come to the screening session fasted, your last meal the night before should be before 10 pm, and you should refrain from doing strenuous exercise and drinking alcohol the night before, and on the morning of the screening session. You will be asked to undress down to your underwear and wear a gown during the physical examination. We will analyse your blood sample for the levels of triglycerides, HDL-cholesterol ("good" cholesterol), and glucose. These tests will allow us to confirm that you meet the criteria for the study. We will inform you by telephone and in writing about whether you are eligible to carry on with the further stages of the study. If we detect elevated levels of glucose in your blood that indicate the presence of intermediate hyperglycemia or diabetes, we will inform your GP.

If your tests show that you are eligible to continue with the study, we will invite you for another session at the research centre around 10 days after the screening session. If necessary, we will collect another fasted blood sample and repeat the physical measurements. We will also give you a supply of the test food product. These foods have been made by the food industry according to Food Hygiene, and Health and Safety regulations. You will be required to eat these foods on a daily basis as part of your normal diet, replacing your usual foods with the food provided. For example, you will be asked to replace your usual snack with the pancake. You will be asked to wash and keep all packaging of the food consumed in a bag provided.

We will provide you with a regular supply of foods so that you will have enough to be consumed daily for 4 weeks. We require you to return the packaging of all consumed food and any remainder food to the centre each time you are provided with new food. At the end of the 4-week period, you will be asked to come to the research centre, bringing any empty packaging and/or remaining foods in the bag provided. We will collect another fasted blood sample and repeat the physical measurements for a last time. We will also ask you questions about your experience of consuming these foods. Blood samples will be used to analyse metabolic changes induced by the diet that you consumed.

What are the benefits and risks of taking part in the study?

Benefits

You will receive a clinical and biological nutritional assessment. The information we obtain from the study will allow us to evaluate the efficacy of these foods for reducing the risk of metabolic syndrome.

Risks

There is a slight risk associated with blood collection; however this will only be carried out by a qualified phlebotomist. There is a possibility that you might suffer an adverse effect associated with consuming the food although the amount of added natural ingredients is within limits approved by the European Commission.

To compensate for your effort and time spent, we would like to offer a £20 gift voucher.

Can I withdraw from this study at any time?

Yes, you can withdraw from the study at any time without giving a reason. However, we kindly request that you let the research team know. This will not affect the care that you receive from your doctor or nurse. Your blood samples will be kept but your personal data will be destroyed after your withdrawal from the study.

Will the information collected be kept confidential?

Yes, all the information provided will be kept confidential. The information collected about all the participants will be number coded and therefore cannot be identified by any other person apart from the principal investigator. Information about all the participants will be kept safe in a locked location at the University of Leeds, and the coding could be broken by the principal investigator or the general coordinator of the whole project for safety reason only. Files will be destroyed 10 years after the end of the study according to National regulations.

Anonymous findings may be published in a scientific journal or presented at scientific meetings. If you are interested in receiving information about the findings of this study, please let us know and we will send you a copy of the research findings.

Who has reviewed this study?

The study has been reviewed by the ethical committees of the PATHWAY-27 project consortium, the University of Leeds faculty of Mathematics and Physical Sciences and Engineering (MEEC 13-027), and Leeds Teaching Hospitals NHS Trust R&D (CP14/11315). The whole project has been approved and granted by the European Community (FP7 – Grant agreement no: 311876).

Research team

If you would like more information, have any questions, or concerns about the study, please contact a member of the research team:

Dr Samantha Sutulic
Telephone: 0113 343 0064
Email: food27@leeds.ac.uk

Complaints

If you have any complaints, please contact:

University of Leeds
School of Food Science and Nutrition
Woodhouse Lane
Leeds
LS2 9JT

Thank you for taking the time to read this information

9.3 Consent form

Consent form

Project Title: Pivotal assessment of the effects of bioactives on health and wellbeing from human genoma to food industry – PATHWAY-27

Pilot clinical study: Effects of bioactive enriched foods on markers of the metabolic syndrome.

Please read the following information and mark with tick (✓) if appropriate:

1. I confirm that I have read and understood the information provided on the information sheet dated 03 September 2014 v1.4 for the above study. I have had the opportunity to consider the information and ask questions. ( )

2. I understand that my participation is entirely voluntary and that I am free to withdraw at any time without giving any reason, without my medical or legal rights being affected. ( )

3. I understand that all the information about me, collected as part of this Study, will be kept securely and that my personal details will not be available to anyone outside the research team (principal investigator, clinical researcher, manager, nurse). The use of a code instead of my name will guarantee the anonymity unless the code is broken for some safety reasons. ( )

4. I understand that the samples and/or information collected from/about me will be used to support other research in the future, and may be shared anonymously with other researchers. ( )

5. I agree to my General Practitioner being informed of my participation in the study. ( )

6. Please send me a copy of the results of the above study. ( )

7. I agree to take part in the above study. ( )

Name of participant	Date	Signature
_____	_____	_____
Name of researcher	Date	Signature
_____	_____	_____

When completed: one copy for participant, one copy for researcher site file.

9.4 SOP Collection of blood by venepuncture from fasted subjects and preparation of serum

Standard Operating Procedure (SOP) Collection of blood by venepuncture from fasted subjects AND preparation of serum

Purpose and scope

This SOP describes the procedures that all study personnel will use for collecting peripheral blood samples by venipuncture from fasted study subjects, and preparation of serum needed for laboratory testing, as specified in the PATHWAY-27 WP5 human study protocol, with proper specimen identification and handling, while ensuring the safety of subjects and staff.

Responsible persons

Principal Investigator

The Principal Investigator is responsible and accountable for:

1. Checking that the phlebotomist has the necessary and up-to-date training required by the local authorities for blood collection procedures.
2. Ensuring that all materials and equipment required for the procedure are available and in good working order for use by the phlebotomist.
3. Ensuring that a suitable dedicated area is available for blood collection.

Clinical Researcher

The clinical researcher may be given the responsibility for:

1. Ensuring that subjects are aware of the prerequisite conditions.
2. Ensuring that all samples are correctly labelled.
3. Keeping records of samples collected including subject's code, date, storage and analysis information.
4. Informing the principal investigator about any issues that may arise during the collection of blood samples.

Phlebotomist

The phlebotomist will be responsible for:

1. Ensuring that all blood collections are done in a manner that maximizes the safety of the subject, himself and any other staff or person present during blood collection.
2. Reporting any accidents or incidents that may occur during blood collection.

Materials and equipment

Disposable gloves
Disinfecting spray (isopropyl alcohol) for disinfecting surfaces
Disinfecting swaps (chlorhexidine gluconate, isopropyl alcohol, isopropyl alcohol)
Tourniquet
Vacutainer/Sarstedt specimen tubes
Vacutainer/Sarstedt holders
Appropriate size sterile disposable needles
Cotton balls/swabs
Sharps disposal container for clinical waste and clinical waste bags
Markers

Refrigerator
Refrigerated centrifuge

Procedures

Subject prerequisites

The clinical researcher must ensure that subjects are aware of the pre-requisite conditions for blood collection, and should check with the subject that these were complied with using a checklist. Conditions include:

1. The subject should be fasted for at least 10 hours, and not more than 14 hours, prior to blood collection.
2. The subject should refrain from consuming alcohol during the evening before blood collection.
3. The subject should refrain from partaking in strenuous exercise the evening before and in the morning before blood collection.

Before blood collection

- Ensure the area designated for blood collection is clean. Disinfect tables and trays as defined by local hygiene plan before each blood draw.
- Ensure that all materials and equipment is available, assembled and in good working order.
- Ensure that only new sterile, single use needles and tubes are used for each blood draw, ensure that the packaging has not been opened or tampered with. They are never to be cleaned and reused for any purpose.
- Check what the blood is to be used for to confirm the quantity of blood to be drawn and which kind of tubes to use.
- Verify the identity of the subject before any procedure commences.
- Install the subject in a comfortable chair or on a medical bed with the extremity from which blood will be drawn supported on a sturdy table or other support. The preferred sites for phlebotomy are the median antecubital veins of the upper extremity. Veins on the dorsum of the hand and other forearm veins are possible alternative sites.
- Explain the blood drawing procedure to the subject and reassure him/her for safety.
- Tubes or other specimen containers should be labelled immediately before sample collection. Do not prepare tubes for more than one subject at a time.
- Blood collection:
- The phlebotomist should wear disposable gloves and use an aseptic technique during phlebotomy. Gloves should always be worn when handling or transporting specimens if there is any possibility of direct contact with blood or other body secretion.
- A tourniquet may be used to transiently distend veins prior to drawing blood. Do not leave the tourniquet longer than it is necessary.
- Using the tip of the index finger, examine the phlebotomy site, feel the vein, and decide exactly where to place the puncture.
- Disinfect the phlebotomy site by swabbing the skin in small outward circles with an alcohol swab. Do not touch the prepared puncture site with your fingers after disinfecting the skin.
- Using aseptic technique, insert the needle of the vacutainer/sarstedt device into the vein. If possible, always allow the full amount of blood to be drawn by each evacuated tube when using the vacutainer/sarstedt system tubes.
- After drawing, carefully mix the blood in tubes containing additives by inverting the tubes several times. After serum preparation, place the tubes in suitable bags, and if possible store at 2-8 °C until further use.
- After drawing the required blood samples, release the tourniquet. Remove the needle from the vein, cover the puncture site with a cotton swab, and hold until adequate haemostasis is visible.

- After completion, needles must be properly disposed of in a puncture resistant container to be destroyed by incineration. Any other non-sharp material that has been in contact with blood fluids must be disposed by incineration in a suitable holding bag.

Discontinuation of the procedure during blood collection

- The procedure may be stopped if:
- The subject becomes distressed. In which case remove the needle only when it is safe to do so.
- There is any breach of protocol or event that compromises the safety of the subject or the phlebotomist. Inform the clinical researcher immediately.

Labelling

Appropriate labelling of the tubes is essential for appropriate handling and analysis of the samples, ensuring that samples are not wasted unnecessarily.

Each tube must be clearly labelled with:

- Date of collection
- Time of collection
- Subject's code
- Type of analyses to be performed or barcode that includes these informations according to operating lab.

In LIGHT, tubes must be placed inside polyethylene bags, sealed and labelled.

Preparation of serum

Some analyses require the use of serum. Serum will be prepared according to the following procedure:

1. Blood samples will be allowed to clot for 30 minutes at room temperature in the collection tubes (no heparin).
2. Centrifuge the tubes for 10 min at 820 RCF at 4°C.
3. Collect the supernatants in suitable new, sterile and tubes labelled as described above.
4. Store at -80°C if storage is required.
5. Discard old tubes with blood cells and remnants in a clinical waste bag and destroy by incineration.

Reporting

Any accident or mishap will be reported to the principal investigator.

9.5 SOP Quantification of HDL-cholesterol in serum samples

Standard Operating Procedure (SOP) Quantification of HDL-cholesterol in serum samples

Purpose and scope

This SOP describes the procedures that all study personnel will use when analyzing serum samples for the quantitative determination of serum HDL-cholesterol as part of PATHWAY-27 WP5, while ensuring the safety and welfare of subjects and staff involved in the study.

Responsible persons

Principal investigator

The principal investigator is responsible and accountable for:
1. Ensuring that the equipment is fit for purpose
2. Ensuring that all staff is suitably trained in all aspects of the procedure, including the safe handling of blood-derived samples and chemicals.
3. Notifying the WP leader of any deviations, safety breaches or abnormal results.
4. Keeping record of the code of all subjects entering the study, guaranteeing their data will be anonymous

Clinical researcher or manager

The clinical researcher or manager is responsible for Reporting to the principal investigator the results of analysis and logs of all procedures undertaken, highlighting any deviations, safety breaches or abnormal results

Operator

The operator is responsible for:

1. Following all procedures according to the SOP and instructions provided, including the keeping of logs of calibration and control procedures.
2. Notifying the clinical researcher/manager or principal investigator of any deviations or abnormal results.

Procedures

Name of the method

Automated enzymatic colorimetric method for determination of HDLcholesterol.

Purpose

For the in vitro diagnostic use in the quantitative determination of HDL-cholesterol from serum using an automated blood analyser.

Principle

The method is a two-stage reaction. In the first step, non-HDL cholesterol is enzymatically released and eliminated. In the second step, HDL-cholesterol is specifically released using a surfactant. Cholesterol is de-esterified using cholesterol esterase to form free cholesterol and fatty acids. Cholesterol is then enzymatically oxidised to cholesten-3-on and hydrogen peroxide (catalysed by cholesterol oxidase). Hydrogen peroxide can be measured following a Trinder

endpoint reaction (catalysed by catalase), the products of which can be detected by colorimetry at wavelengths >505 nm.

Equipment

Automated blood analyser. Manufacturer varies between countries therefore cannot be harmonized. However, the analyses will focus on variations of this parameter from d0 to d28 that would not be affected by the lack of analyser harmonisation.

Reagents

Reagents will be purchased, prepared and stored according to the equipment manufacturer's instructions.

Calibration of the equipment

The equipment should be calibrated according to manufacturer's instructions. Calibrations will be performed:

- Every time that the equipment is switched off
- Every time that the equipment is cleaned or maintained
- Every time that any trouble shooting procedures have been undertaken
- Every time that any of the reagents or optical components are changed
- Each instrument brand/model will have a maximum calibration stability that should be complied to. Calibration standards will be applied according to manufacturer Instructions. A log of calibrations will be kept including the date, the lot number of the reagents used, the date the reagents were first opened and their expiry date.

Quality control

Appropriate control tests will be routinely performed using control samples (upper and lower limit controls). Each laboratory may have its own quality control procedures, but as a minimum, these control tests will be performed:

- Twice daily
- After each calibration
- If there are any issues with analyses that suspect deviations from the normal range of analysis
- Quality control standards will be purchased and used according to the manufacturer's instructions. A log of quality control tests will be kept including the date, the lot number of reagents used, the date the reagents were first opened, and their expiry date.

Samples

Serum will be used for analysis.

- Sera will be collected, prepared and stored according to SOP.
- Before analysis, the operator will check that check the tubes for signs of leakage or contamination, and that the labelling is correct for the type of test requested. The operator shall keep a record of the samples analysed by subject's code.
- Minimum volumes drawn will be in accordance with manufacturer instructions.
- There are no significant interfering molecules within serum, but some chemical substances may interfere with catalase activity (e.g. sodium azide), therefore unprocessed/unadulterated serum will be used.
- For details of how the serum should be handled and loaded onto the machine, please adhere to manufacturer's instructions.

Range of analysis:

For serum samples, the method is linear for the following range:

5–115 mg/dL or 0.1–3.0 mmol/L

Conversion factor: mg/dL x 0.0259 = mmol/L

Precision

Each instrument will have specific performance ranges, but generally the percentage coefficient of variation (%CV) should be below 3%. Replicate analyses will be performed for each sample.

Expected values

Low (undesirable, high risk): < 40 mg/dL (< 1.0 mmol/L) for men < 50 mg/dL (< 1.0 mmol/L) for women

There is no clinical significant value at which medical team should be contacted.

HDL-cholesterol values are not affected by fasting.

Recording of results

Results will be collected by the clinical researcher and entered into the database for statistical analyses. Any abnormal results will be reported to the principal investigator.

9.6 SOP Quantification of triglycerides in serum samples

Standard Operating Procedure (SOP) Quantification of triglycerides in serum samples

Purpose and scope

This SOP describes the procedures that all study personnel will use when analyzing serum samples for the quantitative determination of serum triglycerides as part of PATHWAY-27 WP5, while ensuring the safety and welfare of subjects and staff involved in the study.

Responsible persons

Principal investigator

The principal investigator is responsible and accountable for:
1. Ensuring that the equipment is fit for purpose.
2. Ensuring that all staff is suitably trained in all aspects of the procedure, including the safe handling of blood-derived samples and chemicals.
3. Notifying the WP leader of any deviations, safety breaches or abnormal results.
4. Keeping record of the code of all subjects entering the study, guaranteeing their data will be anonymous

Clinical researcher or manager

The clinical researcher or manager is responsible for:
1. Reporting to the principal investigator the results of analysis and logs of all procedures undertaken, highlighting any deviations, safety breaches or abnormal results
2. Operator
3. The operator is responsible for:
4. Following all procedures according to the SOP and instructions provided, including the keeping of logs of calibration and control procedures.
5. Notifying the clinical researcher/manager or principal investigator of any deviations or abnormal results.

Procedures

Name of the method

Automated enzymatic colorimetric method for determination of triglycerides.

Purpose

For the in vitro diagnostic use in the quantitative determination of triglycerides from serum using an automated blood analyser.

Principle

The method relies on the enzymatic hydrolysis of triglycerides to fatty acids and glycerol. Glycerol is then oxidized to dihydroxyacetone and hydrogen peroxide. Hydrogen peroxide can be measured following a Trinder endpoint reaction, the products of which can be detected by colorimetry at wavelengths >505 nm. The single reagent procedure measures total triglycerides including the mono and diglycerides and the free glycerol fractions.

Equipment

Automated blood analyser. Manufacturer varies between countries therefore cannot be harmonized. However, the analyses will focus on variations of this parameter from d0 to d28 that would not be affected by the lack of analyser harmonisation.

Reagents

Reagents will be purchased, prepared and stored according to the equipment manufacturer's instructions.

Calibration of the equipment

The equipment will be calibrated according to manufacturer's instructions. Calibrations will be performed:

- Every time that the equipment is switched off
- Every time that the equipment is cleaned or maintained
- Every time that any trouble shooting procedures have been undertaken
- Every time that any of the reagents or optical components are changed
- Each instrument brand/model has a maximum calibration stability that must be complied to. Calibration standards will be applied according to manufacturer Instructions. A log of calibrations will be kept including the date, the lot number of the reagents used, the date the reagents were first opened and their expiry date.

Quality control

Appropriate control tests will be routinely performed using control samples (upper and lower limit controls). Each laboratory may have its own quality control procedures, but as a minimum, these control tests will be performed:

- Twice daily
- After each calibration
- If there are any issues with analyses that suspect deviations from the normal range of analysis
- Quality control standards will be purchased and used according to the manufacturer's instructions. A log of quality control tests will be kept including the date, the lot number of reagents used, the date the reagents were first opened, and their expiry date.

Samples

Serum will be used for analysis.

- Sera will be collected, prepared and stored according to SOP.
- Before analysis, the operator will check that check the tubes for signs of leakage or contamination, and will check that the labelling is correct for the type of test requested. The operator shall keep a record of the samples analysed by subject's code.
- Minimum volumes drawn will be in accordance with manufacturer instructions.
- Unconjugated bilirubin and haemoglobin interfere significantly (>10% interference) with the assay. Haemolysis should be avoided during blood collection and serum preparation.
- Details of how the serum should be handled and loaded onto the machine will adhere to manufacturer's instructions.

Range of analysis:

For serum samples, the method is linear for the following range:

0 to 550 mg/dL (0 to 6.22 mmol/L)

Conversion factor: mg/dL x 0.0113 = mmol/L

Precision

Each instrument will have specific performance ranges, but generally the percentage coefficient of variation (%CV) should be below 4%. Replicate analyses will be performed for each sample.

Expected values

Normal fasting < 150 mg/dL (<1.70 mmol/L)
Normal non-fasting: < 250 mg/dL (< 2.83 mmol/L)
Borderline high: 250–500 mg/dL (2.83–5.65 mmol/L)
Hypertriglyceridemia: > 500 mg/dL (> 5.65 mmol/L)
High risk for pancreatitis: > 1000 mg/dL (> 11.30 mmol/L)
Triglyceride levels > 1000 mg/dL will be notified to the participant medical doctor.

Recording of results

Results will be collected by the clinical researcher and entered into the database for statistical analyses. Any abnormal results should be reported to the principal investigator.

9.7 SOP Quantification of glucose in blood samples

Standard Operating Procedure (SOP) Quantification of glucose in blood samples

Purpose and scope

This SOP describes the procedures that all study personnel will use when analyzing blood samples for the quantitative determination of glucose as part of PATHWAY-27 WP5, while ensuring the safety and welfare of subjects and staff involved in the study.

Responsible persons

Principal investigator

The principal investigator is responsible and accountable for:

- Ensuring that the equipment is fit for purpose.
- Ensuring that all staff is suitably trained in all aspects of the procedure, including the safe handling of blood-derived samples and chemicals.
- Notifying the WP leader of any deviations, safety breaches or abnormal results.
- Keeping record of the code of all subjects entering the study, guaranteeing their data will be anonymous.

Clinical researcher

The clinical researcher or manager is responsible

- Reporting to the principal investigator the results of analysis and logs of all procedures undertaken, highlighting any deviations, safety breaches or abnormal results

Operator

The operator is responsible for:

- Following all procedures according to the SOP and instructions provided, including the keeping of logs of calibration and control procedures.
- Notifying the clinical researcher/manager or principal investigator of any deviations or abnormal results.

Procedures

Name of the method

Automated enzymatic colorimetric method for determination of glucose.

Purpose

For the in vitro diagnostic use in the quantitative determination of glucose from blood using an automated blood analyser.

Principle

The method relies on the enzymatic oxidation of glucose to gluconic acid and hydrogen peroxide. Hydrogen peroxide can be measured following a Trinder endpoint reaction, the products of which can be detected by colorimetry at wavelengths >505 nm. It is a single-reagent, endpoint procedure.

Equipment

Automated blood analyser. Manufacturer varies between countries therefore cannot be harmonized. However, the analyses will focus on variations of this parameter from d0 to d28 and would not be affected by the lack of analyser harmonisation.

Reagents

Reagents will be purchased, prepared and stored according to the equipment manufacturer's instructions.

Calibration of the equipment

The equipment should be calibrated according to manufacturer's instructions. Generally calibrations should be performed:

- Every time that the equipment is switched off
- Every time that the equipment is cleaned or maintained
- Every time that any trouble shooting procedures have been undertaken
- Every time that any of the reagents or optical components are changed
- Each instrument brand/model will have a maximum calibration stability that should be complied to. Calibration standards will be applied according to manufacturer Instructions. A log of calibrations should be kept including the date, the lot number of the reagents used, the date the reagents were first opened and their expiry date.

Quality control

Appropriate control tests should be routinely performed using control samples (upper and lower limit controls). Each laboratory may have its own quality control procedures, but as a minimum, these control tests should be performed:

- Twice daily
- After each calibration
- If there are any issues with analyses that suspect deviations from the normal range of analysis
- Quality control standards should be purchased and used according to the manufacturer's instructions. A log of quality control tests should be kept including the date, the lot number of reagents used, the date the reagents were first opened, and their expiry date.

Samples

Blood will be used for analysis.

- Blood should be collected, prepared and stored according to SOP.
- Before analysis, the operator should check that check the tubes for signs of leakage or contamination, and should check that the labelling is correct for the type of test requested. The operator shall keep a record of the samples analysed by subject's code.
- Minimum volumes drawn will be in accordance with manufacturer instructions.
- Unconjugated and conjugated bilirubin, lipids and ascorbic acid are some of the interfering substances (>10% interference).
- For details of how the serum should be handled and loaded onto the machine, please adhere to manufacturer's instructions.

Range of analysis:

For blood samples, the method is linear for the following range:

to 750 mg/dL (0.3 to 41.6 mmol/L)

Conversion factor: mg/dL x 0.0555 = mmol/L

Precision:

Each instrument will have specific performance ranges, but generally the percentage coefficient of variation (%CV) should be below 3%. Replicate analyses should be performed for each sample.

Expected values:

Normal range: 74–106 mg/dL (4.1–5.9 mmol/L)
High fasting: ≥ 110 mg/dL (> 6.1 mmol/L)

For the first glucose measurement (the one for setting if the volunteer is eligible), glucose levels showing diabetes or hypoglycemia will be notified to the participant and to his medical doctor and the participant will not be included in the trial. If unexpected high glucose level is found at the end of the trial the volunteer will be alert after his/her code is broken. In such case, the volunteer will not be excluded.

Recording of results:

Results will be collected by the clinical researcher and entered into the database for statistical analyses. Any abnormal results should be reported to the principal investigator.

9.8 SOP Physical examination

Standard Operating Procedure (SOP) Physical examination

Purpose and scope

This SOP describes the procedures that all study personnel will use for undertaking physical examinations of subjects (aged over 18 years), as specified in the PATHWAY-27 WP5 human study protocol, while fulfilling ethical responsibilities for protecting the rights and welfare of participants.

Responsible persons

Principal Investigator

The Principal Investigator is responsible and accountable for:
1. Ensuring that all materials and equipment required for the procedures are available and in good working order.
2. Ensuring that a suitable dedicated area is available for the physical examination to take place. All examinations should be performed in privacy and maintain the safety of both subjects and staff.
3. Informing the WP leader and relevant ethics committee of any significant issues arisen or any deviations to the protocol
4. Keeping record of the code of all subjects entering the study, guaranteeing their data will be anonymous

Clinical Researcher

The clinical researcher may be given the responsibility for:
1. Keeping records of all examinations including details of subject's code, date, storage and measurement information in the appropriate case report form (CRF).
2. Informing the principal investigator about any issues that may arise during the procedures.

Research nurse

The research nurse may be given responsibility for:
1. Ensuring that all parts of the examination are carried out in a manner that ensures the dignity of the subject, and the safety of the subject and themselves.
2. Reporting any accidents or incidents that may occur during the examinations.

Materials and equipment

Disinfectant wipes
Hand soap and disposable paper towels for hand washing
Cuffs of various sizes
Non-elastic tape measure
Devices to be calibrated and certified according to local rules:
- Floor electronic scale (e.g. SECA or other brand) accurate to nearest 0.1 g
- Calibration weights
- Standard stadiometer with vertical headboard and moveable horizontal headboard (e.g. SECA or other brand)
- Calibration rod for height calibration

- Electronic sphygmomanometer blood pressure monitor (e.g. *Boso-Carat professional or Omron*)

Procedures

Before the examination:

1. All examinations should be carried out with two members of staff present (e.g. clinical researcher and research nurse)
2. Ensure the area designated for the examination is clean.
3. Ensure that all materials and equipment are available, assembled and in good working order. Calibrate all equipment.
4. Clean all equipment with disinfectant wipes.
5. Ensure that all necessary information and paperwork is available (e.g. participant details and CRFs).

During the physical examination:

- Introduce yourselves and the purpose of the examination.
- Confirm the identity of the subject and record their subject code on the CRF.
- Confirm that the subject understands what is going to happen and are happy to proceed.
- Ask the subject to take shoes and clothes off, apart from their underwear and socks/stockings/tights, and to wear the disposable gown provided. A screened area should be provided for this purpose.
- Undertake the examination in the following order: body weight, body height, waist circumference, and blood pressure.

Measurement of body height:

- Instruct subject to remove any headwear or hair ornaments that may interfere with the measurement.
- Instruct the subject to stand on the base of the stadiometer and to position their feet flush against the foot gauge, hills together with toes pointing outward by about 30o angle.
- Ensure that the participant is standing flat on both his/her feet, straight with shoulders back and relaxed, trunk above the waist vertical, and preferably the back of the head, shoulder blades, buttock and health touching the backboard.
- Instruct the subject to align their head so that the line between the ear canal and the bottom border of the eye orbital is parallel to the floor.
- Instruct the subject to take a deep breath to extend the spine.
- Move the horizontal headboard so that it touches the top of the head, with sufficient pressure to compress the hair.
- Take the measurement and record to nearest 0.1 cm on the CRF.
- Instruct the subject to stand away from the stadiometer.
- In the event of someone having issues with stature (e.g. curvature of the spine), record this on the CRF as ‘not straight’.

Measurement of body weight:

- Ensure the scale is on a flat, non-carpeted surface.
- Turn on the electronic scale, tare, and wait for the display to show ‘0’.
- Instruct the subject to stand on the scale on both feet.
- Wait until the measurement is stable, and record to nearest 0.1 g on the CRF.
- Instruct the subject to stand off the scale and switch scale off.

Measurement of waist circumference:

- Instruct the subject to stand upright with arms and shoulders relaxed.
- Stand to the right, behind the subject and locate the top of the right ileum with the fingers.
- Stand to the right of the subject and located the bottom of the lowest rib.
- Place the measuring tape between the top of the right ileum and the bottom of the lowest rib.
- Ensure that the measuring tape is horizontal to the floor all around the subject.
- Hold the zero value on the tape below the measurement value, without compressing the skin.
- At the end of a normal expiration, take the measurement and record on the CRF to nearest 0.1 cm.

Measurement of blood pressure:

- Switch the blood pressure monitor on.
- Instruct the subject to lie down on the medical bed, or to sit down with the right arm rested on the arm rest at heart level.
- Instruct the subject not to talk during the entire procedure.
- Select appropriate sized cuff according to arm circumference.
- Connect the cuff to the monitor.
- Place the cuff around the arm around 2 cm from the inner side of the elbow joint, with the artery position mark aligned with the brachial artery.
- Wrap the cuff snugly around the arm and secure with the Velcro fastening.
- Instruct the subject to relax their arm and open their hand with palm facing upwards.
- Press the on button and wait until the measurement is given. Record both diastolic and systolic pressure to the nearest 0.1 mmHg on the CRF.
- If you need to stop the measurement at any point, press the STOP button to deflate the cuff instantly.

At the end of the examination:

a. Ensure that you are satisfied with the quality and accuracy of all measurements recorded. If not, then repeat as necessary.
b. Instruct the subject to get dressed.
c. Thank them for their participation.

9.9 Adverse event/serious adverse event declaration

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Adverse Event / Serious Adverse Event Description	
Is the adverse event serious? *AE/2/AESER*	○ yes ○ no
AE Number *AE/2/AESPID*	
What is the Adverse Event term *AE/2/AETERM*	
What is the DATE the adverse event started? *AE/2/AESTDAT*	
At what time did the adverse event start? *AE/2/AESTTIM*	
Is the Adverse Event still ongoing? *AE/2/AEONGO*	○ No ○ Yes
What is the DATE the adverse event ended? *AE/2/AEENDAT*	
At what time did the adverse event end? *AE/2/AEENTIM*	
What was the severity of the adverse event? *AE/2/AESEV*	○ Mild ○ Moderate ○ Severe
Is the adverse event associated with a congenital anomaly or birth defect? *AE/2/AESCONG*	○ No ○ Yes
Did the adverse event result in Persistent or significant disability or incapacity? *AE/2/AESDISAB*	○ No ○ Yes
Did the adverse event result in death? *AE/2/AESDTH*	○ No ○ Yes
Date of death *AE/2/AESDTHDAT*	

Electronic printout generated @ 2013-08-21 10:12:07	Investigator's signature: .....
Date of monitoring:	Monitor's signature: .....

Page 1 of 2

Did the adverse event result in initial or prolonged hospitalization for the subject? *AE/2/AESHOSP*	○ No ○ Yes
Is the adverse event Life Threatening? *AE/2/AESLIFE*	○ No ○ Yes
Is the adverse event associated with other serious or important medical events? *AE/2/AESMIE*	○ No ○ Yes
What was the relationship to study treatment? *AE/2/AEREL*	○ Not Related ○ Unlikely Related ○ Possibly Related ○ Related
What action was taken with study treatment? *AE/2/AEACN*	○ DOSE INCREASED ○ DOSE NOT CHANGED ○ DOSE REDUCED ○ DRUG INTERRUPTED ○ DRUG WITHDRAWN ○ NOT APPLICABLE ○ UNKNOWN
What other action was taken in response to this adverse event? *AE/2/AEACNOTH*	
What was the outcome of this adverse event? *AE/2/AEOUT*	○ FATAL ○ NOT RECOVERED/NOT RESOLVED ○ RECOVERED/RESOLVED ○ RECOVERED/RESOLVED WITH SEQUELAE ○ RECOVERING/RESOLVING ○ UNKNOWN
Did the adverse event cause the subject to be discontinued from the study? *AE/2/AEDIS*	○ No ○ Yes

9.10 Food consumption record

Food consumption record

Screening ID		**Randomisation ID**	

You are required to eat 2 pancakes from each packet every day.

Please use this sheet to record each day that you eat the pancakes, and note any days that you do not eat them. Also write down if you only eat part of the portion.

The researcher will highlight your start and end dates on this sheet.

Today's date	.....	Researcher signature	.....

	Mon	Tue	Wed	Thu	Fri	Sat	Sun
Week 1							

	Mon	Tue	Wed	Thu	Fri	Sat	Sun
Week 2							

	Mon	Tue	Wed	Thu	Fri	Sat	Sun
Week 3							

	Mon	Tue	Wed	Thu	Fri	Sat	Sun
Week 4							

	Mon	Tue	Wed	Thu	Fri	Sat	Sun
Week 5							

Thank you for your help!

9.11 Clinical Research File – Screening – Visit 1

Visit 1 Screening CRF

Contact details	
ID	
First name	
Surname	
Telephone number	
Mobile number	
Email	
Home address	

General information

Gender	MALE	FEMALE
Date of Birth		<18 or >80 y = exclude (<January 1934 to September 1996>)

Date & Time of examination: ……

Physical examination	Measurement	Fits MS Criterion? (Y / N)	MS Criterion
Height (m)			
Weight (kg)			
Waist circumference (cm)			Male ≥102 cm Female ≥88 cm
Systolic BP (mm Hg)			≥130 mm Hg
Diastolic BP (mm Hg)			≥85 mm Hg

Date & Time of blood collection: ……..

Blood analysis	Analyte concentration	Fits MS Criterion? (Y / N)	MS Criterion
Triglycerides (mg/dL)			≥150 mg/dL
Total cholesterol (mg/dL)			
HDL-cholesterol (mg/dL)			Male ≤40 mg/dL Female ≤50 mg/dL
Glucose (mg/dL)			≥110 mg/dL

MS criteria met (please tick those which apply)

Waist Circumference	Blood Pressure (SBP &/or DBP)	Triglycerides	HDL-cholesterol	Glucose	Number of MS criteria met

Continue to page 2

GP contact details	
GP name	
Surgery name	
Telephone number	
Surgery address	

End of Visit 1 Screening CRF

9.12 Clinical Research File – Baseline – Visit 2

Visit 2 Baseline CRF

Randomisation ID

Screening ID

Date & Time of examination: ……

Physical examination	Measurement	Fits MS Criterion? (Y / N)	MS Criterion
Height (m)			
Weight (kg)			
Waist circumference (cm)			Male ≥102 cm Female ≥88 cm
Systolic BP (mm Hg)			≥130 mm Hg
Diastolic BP (mm Hg)			≥85 mm Hg

Date & Time of blood collection: ……..

Blood analysis	Analyte concentration	Fits MS Criterion? (Y / N)	MS Criterion
Triglycerides (mg/dL)			≥150 mg/dL
Total cholesterol (mg/dL)			
HDL-cholesterol (mg/dL)			Male ≤40 mg/dL Female ≤50 mg/dL
Glucose (mg/dL)			≥110 mg/dL

MS criteria met (please tick those which apply)

Waist Circumference	Blood Pressure (SBP &/or DBP)	Triglycerides	HDL-cholesterol	Glucose	Number of MS criteria met

Continue to page 2

Questions about your lifestyle			
Females only: Are you pregnant, lactating or actively trying to conceive?	YES	NO	Yes = exclude
Females only: Are you currently using any contraceptive treatment or hormone replacement therapy?	YES	NO	
Do you smoke?	YES	NO	Yes = exclude
Do you use recreational drugs?	YES	NO	Yes = exclude
Do you drink alcohol?	YES	NO	
If yes, how much?	(day / week)		Male ≥5 units/d = exclude Female ≥4 units/d = exclude
As a guide, 1 pint of beer / 1½ bottles of lager / 1 medium (175 mL) glass of wine = 2-3 units			
Do you take any dietary supplements?	YES	NO	
If yes, give details.			DHA, AC, Fibre = exclude
Are you on any kind of diet?	YES	NO	
Do you regularly play sport or exercise?	YES	NO	
If yes, how often and for how long?			

Questions about your health			
Have you ever been told that you have any of the following?			
High blood pressure	YES	NO	
High blood triglycerides	YES	NO	
Altered blood cholesterol	YES	NO	
High blood glucose	YES	NO	

Continue to page 3

Questions about your health continued			
Do you have a family history of high blood lipids?	YES	NO	Yes = exclude
Do you have an allergy or intolerance to any food?	YES	NO	
If yes, give details.			Lactose, milk, egg, any other ingredient of BEF = exclude
Have you ever been diagnosed with any of the following?			
Celiac disease	YES	NO	Yes = exclude
Intestinal disorders (Crohn's disease, short bowel syndrome, pancreatic insufficiency, cystic fibrosis, tropical sprue, whipple's disease, chronic pancreatitis, gastrojejunostomy, surgical treatment for obesity, cholestasis, biliary atresia, parasite infections)	YES	NO	Yes = exclude
Diabetes	YES	NO	Yes = exclude
Heart disease	YES	NO	Yes = exclude
Liver disease	YES	NO	Yes = exclude
Kidney disease	YES	NO	Yes = exclude
Cancer	YES	NO	
If yes, when did treatment end?			End of treatment <2 y = exclude
Any other disease in the last 2 years	YES	NO	Yes = check if relevant
If yes, give details.			
Are you currently taking or do you regularly take any prescription or non-prescription medicine?	YES	NO	
If yes, give details.			Statins, fibrates, tetracycline, cholestyramine, thiazide diuretics, aluminium/magnesium hydroxide, cochicine, neomycin, methotrexate, methyldopa, allopurinol, warfarin, other anticoagulant/lipid lowering, laxatives = exclude
Have you had antibiotic treatment within the last 3 months?	YES	NO	

Continue to page 4

Questions about your ability to participate			
Are you currently taking part in any other clinical trial?	YES	NO	Yes = exclude
Are you able to travel to the University of Leeds on 3 occasions during the next 8 weeks?	YES	NO	No = exclude
Are you free to make your own decisions and not under the care of the state, by judicial or administrative decision?	YES	NO	No = exclude

End of Visit 2 Baseline CRF

9.13 Clinical Research File – Endpoint – Visit 3

Visit 3 Endpoint CRF

Randomisation ID

Screening ID

Date & Time of examination: ……

Physical examination	Measurement	Fits MS Criterion? (Y / N)	MS Criterion
Height (m)			
Weight (kg)			
Waist circumference (cm)			Male ≥102 cm Female ≥88 cm
Systolic BP (mm Hg)			≥130 mm Hg
Diastolic BP (mm Hg)			≥85 mm Hg

Date & Time of blood collection: ……..

Blood analysis	Analyte concentration	Fits MS Criterion? (Y / N)	MS Criterion
Triglycerides (mg/dL)			≥150 mg/dL
Total cholesterol (mg/dL)			
HDL-cholesterol (mg/dL)			Male ≤40 mg/dL Female ≤50 mg/dL
Glucose (mg/dL)			≥110 mg/dL

MS criteria met (please tick those which apply)

Waist Circumference	Blood Pressure (SBP &/or DBP)	Triglycerides	HDL-cholesterol	Glucose	Number of MS criteria met

End of Visit 3 Endpoint CRF

9.14 Telephone screening questionnaire

Telephone screening questionnaire

Thank you for volunteering to take part in this study to investigate the effects of bioactives on metabolic health.

To help me determine whether you are eligible to take part I need to ask you some questions about your lifestyle and health, and take some general information about you.

Contact details	
First name	
Surname	
Telephone number	
Mobile number	
Email	
Home address	

General information about you		
Gender	MALE	FEMALE
Height (units)		(cm / metres / feet & inches)
Weight (units)		(kg / lbs / stones & lbs)
Date of Birth		<18 or >80 y = exclude (<January 1934 to September 1996>)

Continue to page 2

Questions about your lifestyle			
Females only: Are you pregnant, lactating or actively trying to conceive?	YES	NO	Yes = exclude
Females only: Are you currently using any contraceptive treatment or hormone replacement therapy?	YES	NO	
Do you smoke?	YES	NO	Yes = exclude
Do you use recreational drugs?	YES	NO	Yes = exclude
Do you drink alcohol?	YES	NO	
If yes, how much?	(day / week)		Male ≥5 units/d = exclude Female ≥4 units/d = exclude
As a guide, 1 pint of beer / 1½ bottles of lager / 1 medium (175 mL) glass of wine = 2-3 units			
Do you take any dietary supplements?	YES	NO	
If yes, give details.			DHA, AC, Fibre = exclude
Are you on any kind of diet?	YES	NO	
Do you regularly play sport or exercise?	YES	NO	
If yes, how often and for how long?			≥5 h per week = exclude

Questions about your health			
Have you ever been told that you have any of the following?			
High blood pressure	YES	NO	
High blood triglycerides	YES	NO	
Altered blood cholesterol	YES	NO	
High blood glucose	YES	NO	

Continue to page 3

Questions about your health continued			
Do you have a family history of high blood lipids?	YES	NO	Yes = exclude
Do you have an allergy or intolerance to any food?	YES	NO	
If yes, give details.			Lactose, milk, egg, any other ingredient of BEF = exclude
Have you ever been diagnosed with any of the following?			
Celiac disease	YES	NO	Yes = exclude
Intestinal disorders (Crohn's disease, short bowel syndrome, pancreatic insufficiency, cystic fibrosis, tropical sprue, whipple's disease, chronic pancreatitis, gastrojejunostomy, surgical treatment for obesity, cholestasis, biliary atresia, parasite infections)	YES	NO	Yes = exclude
Diabetes	YES	NO	Yes = exclude
Heart disease	YES	NO	Yes = exclude
Liver disease	YES	NO	Yes = exclude
Kidney disease	YES	NO	Yes = exclude
Cancer	YES	NO	
If yes, when did treatment end?			End of treatment <2 y = exclude
Any other disease in the last 2 years	YES	NO	Yes = check if relevant
If yes, give details.			
Are you currently taking or do you regularly take any prescription or non-prescription medicine?	YES	NO	
If yes, give details.			Statins, fibrates, tetracycline, cholestyramine, thiazide diuretics, aluminium/magnesium hydroxide, cochicine, neomycin, methotrexate, methyldopa, allopurinol, warfarin, other anticoagulant/lipid lowering, laxatives = exclude

Have you had antibiotic treatment within the last 3 months?	YES	NO	

Continue to page 4

Questions about your ability to participate			
Are you currently taking part in any other clinical trial?	YES	NO	Yes = exclude
Are you able to travel to the University of Leeds on 3 occasions during the next 8 weeks?	YES	NO	No = exclude
Are you free to make your own decisions and not under the care of the state, by judicial or administrative decision?	YES	NO	No = exclude

Thank you for answering these questions.

**Give the relevant response from the following.*

***Eligible**

I can confirm that you are eligible to take part in the study and I would like to invite you to come into the research centre for a screening examination. This will involve measuring your height and weight, waist circumference and blood pressure, and taking a small amount of blood to analyse for glucose, cholesterol and triglycerides. If you would still like to take part would you like to make an appointment now?

YES Date | | Time | |

I will send you a letter to confirm your appointment and a Participant Information Sheet with more details about the study. If you want to withdraw or change your appointment at any time please feel free to contact me. Thank you for taking the time to answer these questions.

NO, please call me later Date | | Time | |

Thank you for taking the time to answer these questions.

NO, I don't want to take part Thank you for taking the time to answer these questions.

***Not eligible**

I'm sorry but you have met one or more of our exclusion criteria so I am not able to invite you to participate. Thank you for taking the time to answer these questions.

End of Telephone Screening

9.15 Restricted food list

Restricted food list

You are not required to exclude any foods from your diet. The following is a list of foods that should not be eaten more than once each day.

FRUIT (fresh/frozen/tinned)
Apple – pink / red
Blackberries
Blackcurrants
Blueberries
Cherries
Grapes – black / red
Orange – blood
Peach / Nectarine
Plums
Pomegranate
Raspberries
Strawberries

DRIED FRUIT
Apple – pink / red
Cherries
Cranberries
Currants
Fig
Peach
Prune
Raisins

VEGETABLES (fresh/frozen/tinned)
Aubergine / Eggplant
Beans – red kidney
Cabbage – red
Onion – red
Radicchio / Red endive
Radish
Sweet potato / Yam

BREAD / CEREALS / BAKED GOODS
Brown / wholemeal bread / rolls
Wholemeal scones / cakes
Wholemeal biscuits (e.g. Digestives)
Wholegrain crackers
Wholegrain breakfast cereals (e.g. Branflakes, Weetabix, Shreddies)
Cereal bars
Bran fibre or wheatgerm
Oat biscuits (e.g. Hob Nobs)
Oatcakes
Porridge oats
Oat based breakfast cereals (e.g. Oatibix, muesli)
Barley grain (e.g. pearl barley)
Fruit / malt loaf (e.g. Soreen)

PASTA / RICE / NOODLES
Wholewheat pasta shapes
Wholegrain rice
Wholewheat spaghetti / noodles

DAIRY

FISH

Yoghurt containing wholegrains or fruit listed above	All

DRINKS

Blackcurrant juice (e.g. Ribena)
Fruit juice / smoothie containing items listed above
Port
Wine – red / rose

9.16 Abdominal symptoms questionnaire

Abdominal symptoms questionnaire

Screening ID | | **Randomisation ID** | |

Please answer the following questions by placing an X in the appropriate box.

In the last 7 days, how often have you experienced the following?

		Never	Once	Several times	Every day
1	Abdominal pain				
2	Watery / loose stools				
3	Constipation / straining to pass stools				
4	Lumpy / hard stools				
5	Feeling bloated or feeling that your abdomen was swollen after meals				
6	Flatus / gas / belching				
7	Unusually increased hunger / appetite				
8	Unusually decreased hunger / appetite				

Thank you for your help!

9.17 Food preferences questionnaire

Food preferences questionnaire

Randomisation ID

Screening ID

Please read the following questions and place an X in the appropriate box.

1. In general, would you say your health is:

Very bad

Bad

Moderate

Good

Excellent

2. How often do you eat these types of products?

	Every day	**Every week**	**Every two weeks**	**Once a month**	**Less than once a month**	**Never**
Biscuits						
Milkshake						
Pancake						

3. What do you think about the **appearance** of the pancakes?

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
I dislike extremely								**I like extremely**

Comments: positive: ……..

negative: ……..

4. How would you rate the **overall aroma** / **odour** of the pancakes?

1	2	3	4	5	6	7	8	9
I dislike extremely								**I like extremely**

Comments: positive: ……..

negative: ……..

5. How would you rate the **overall taste** of the sample?

1	2	3	4	5	6	7	8	9
I dislike extremely								**I like extremely**

Comments: positive: ……..

negative: ……..

6. What do you think about the **texture** of the pancakes?

1	2	3	4	5	6	7	8	9
I dislike extremely								**I like extremely**

Comments: positive: ……..

negative: ……..

7. How much do you like the pancakes **overall?**

1	2	3	4	5	6	7	8	9

I dislike extremely								**I like extremely**

Comments: positive: ……..

negative: ……..

8. How much do you like the pancakes **overall** if they contribute to improving your health?

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
I dislike extremely								**I like extremely**

Comments: positive: ……..

negative: ……..

Thank you for your help!

9.18 24 hour dietary recall

24 hour dietary recall

Randomisation ID | |

Screening ID | |

We would like you to record all the food and drinks you have consumed during the last 24 hours. Begin with the most recent, this could be today, and work back to the same time yesterday.

Please answer the following questions:

1. Enter todays date.

		/			/		
Day			Month			Year	

2. Which day(s) of the week does this record?

Sun		Mon		Tue		Wed		Thu		Fri		Sat	

3. Is this a typical day?

Yes		No	

If not, give an example of a typical day after yesterday's record, if you wish.

Continue to page 2

24 HOUR RECORD		
Time	Quantity eaten	Details of food and drink

Continue to page 3

24 HOUR RECORD		
Time	Quantity eaten	Details of food and drink

Continue to page 4

24 HOUR RECORD		
Time	Quantity eaten	Details of food and drink

Thank you for your help!

9.19 Food frequency questionnaire – restricted foods

Food Frequency Questionnaire

About the questionnaire

This questionnaire contains some questions about how often you have consumed certain foods/drinks in the past four weeks.

Please read the following instructions before answering the questions.

How to complete the questionnaire

Put a tick (✓) in the box to indicate how often, on average, you have eaten the specific amount of each food/drink over the last four weeks. This may include processed products such as frozen, canned, dried and ready-meals.

Example

For apple, the quantity is one medium serving (i.e. 1 apple), so if you ate one serving a day, you should put a tick in the column headed 'once a day'.

FOOD & QUANTITY	AVERAGE CONSUMPTION IN LAST FOUR WEEKS								
FRUIT **1 adult portion**	**Never or less than once/month**	**1-3 per month**	**Once a week**	**2-4 per week**	**5-6 per week**	**Once a day**	**2-3 per day**	**4-5 per day**	**6+ per day**
Apple – pink / red						✓			

Note

Estimate your average food consumption as best you can.

Please put a tick on every line.

Continue to page 2

FOOD & QUANTITY	AVERAGE CONSUMPTION IN LAST FOUR WEEKS								
FRUIT **1 adult portion** (fresh/frozen/tinned/dried)	**Never or less than once/month**	**1-3 per month**	**Once a week**	**2-4 per week**	**5-6 per week**	**Once a day**	**2-3 per day**	**4-5 per day**	**6+ per day**
Acai berries									
Apple – pink / red									
Arctic bramble berries									
Avocado (raw=0.33)									
Banana									
Bilberries									
Blackberries									
Blackcurrants									
Blueberries									
Cherries									
Chokeberries									
Cranberries (dried=0.60, sauce=0.1, juice=0.41)									
Dates									
Elderberries									
Fig (raw=0.5)									
Grapes – red									
Gooseberries									
Lingonberries									
Mango (raw=0.1)									
Nectarine									
Orange – blood									
Peach									
Pear									
	Never or less than once/month	**1-3 per month**	**Once a week**	**2-4 per week**	**5-6 per week**	**Once a day**	**2-3 per day**	**4-5 per day**	**6+ per day**

Continue to page 3

FRUIT **1 adult portion**	**Never or less than once/month**	**1-3 per month**	**Once a week**	**2-4 per week**	**5-6 per week**	**Once a day**	**2-3 per day**	**4-5 per day**	**6+ per day**
Plum – black / purple / red									
Pomegranate									
Prunes									
Raisins (0.03)									
Raspberries									
Redcurrants									
Strawberries									
Products made from any of the above (e.g. jam, compote, desserts)									
VEGETABLES **1 adult portion**									
Aubergine / Eggplant (cooked=0.02)									
Cabbage – red									
Cabbage – pak choi (0.02)									
Green beans (0.02)									
Lettuce - red									
Onion – red									
Peas (cooked=0.03)									
Radicchio									
Radish									
Red endive									
Sweet potato / Yam									
	Never or less than once/month	**1-3 per month**	**Once a week**	**2-4 per week**	**5-6 per week**	**Once a day**	**2-3 per day**	**4-5 per day**	**6+ per day**

Continue to page 4

NUTS **1 adult portion**	**Never or less than once/month**	**1-3 per month**	**Once a week**	**2-4 per week**	**5-6 per week**	**Once a day**	**2-3 per day**	**4-5 per day**	**6+ per day**
Almonds									
Hazelnuts									
Pecans									
Pistachios									
Walnuts									
LEGUMES **1 adult portion**									
Black beans									
Red kidney beans									
BREAD / CEREALS / BAKED GOODS **1 adult portion**									
Brown / wholemeal bread / rolls									
Wholemeal scones / cakes									
Wholemeal biscuits (e.g. Digestives)									
Wholegrain crackers									
Wholegrain breakfast cereals (e.g. Branflakes, Weetabix, Shreddies)									
Muesli									
Cereal bars									
Bran fibre or wheatgerm									
Oat biscuits (e.g. Hob Nobs)									
Oat cakes									
Porridge oats									
	Never or less than once/month	**1-3 per month**	**Once a week**	**2-4 per week**	**5-6 per week**	**Once a day**	**2-3 per day**	**4-5 per day**	**6+ per day**

Continue to page 5

BREAD / CEREALS / BAKED GOODS **1 adult portion**	**Never or less than once/month**	**1-3 per month**	**Once a week**	**2-4 per week**	**5-6 per week**	**Once a day**	**2-3 per day**	**4-5 per day**	**6+ per day**
Oat based breakfast cereals (e.g. Oatibix, muesli)									
Barley grain (e.g. pearl barley)									
Fruit / malt loaf (e.g. Soreen)									
Other: please specify….									
PASTA / RICE / NOODLES **1 adult portion**									
Wholewheat pasta									
Wholegrain rice									
Wholemeal noodles									
FISH **1 adult portion**									
Salmon									
Tuna									
Sardines									
Mackerel									
Anchovies									
Herring									
Eel									
Halibut									
Catfish									
Products containing any of the above									
	Never or less than once/month	**1-3 per month**	**Once a week**	**2-4 per week**	**5-6 per week**	**Once a day**	**2-3 per day**	**4-5 per day**	**6+ per day**

Continue to page 6

DAIRY **1 adult portion**	**Never or less than once/month**	**1-3 per month**	**Once a week**	**2-4 per week**	**5-6 per week**	**Once a day**	**2-3 per day**	**4-5 per day**	**6+ per day**
Yoghurt with wholegrain									
Yoghurt containing fruits listed above									
DRINKS **1 adult portion**									
Blackcurrant juice (e.g. Ribena)									
Fruit juice / smoothie containing fruits listed above									
Port									
Wine – red / rose									
	Never or less than once/month	**1-3 per month**	**Once a week**	**2-4 per week**	**5-6 per week**	**Once a day**	**2-3 per day**	**4-5 per day**	**6+ per day**

End of Questionnaire

Average portion sizes for adults

FRUIT (fresh/frozen/tinned) – Approximately equivalent to 80g in weight	
Apple – pink / red	1 medium
Blackberries	1 handful (9 to 10 blackberries)
Blackcurrants	2 handfuls (4 heaped tablespoons)
Blueberries	2 handfuls (4 heaped tablespoons)
Cherries	Tinned = 11 or fresh = 14 (3 heaped tablespoons)
Grapes – black / red	1 handful
Orange – blood	1 orange
Peach / Nectarine	1 medium
Plums	2 medium
Pomegranate	1 pomegranate
Raspberries	2 handfuls (20 raspberries)
Strawberries	1 handful (7 to 9 strawberries)

DRIED FRUIT – Approximately equivalent to 30g in weight	
Apple – pink / red	4 rings
Cherries	1 heaped tablespoon
Cranberries	1 heaped tablespoon
Currants	1 heaped tablespoon
Fig	2 figs
Peach	2 halves
Prune	3 prunes
Raisins	1 tablespoon

VEGETABLES – Approximately equivalent to 80g in weight	
Aubergine / Eggplant	1/3rd
Beans – red kidney	3 heaped tablespoons
Beetroot	3 baby whole or 7 slices
Cabbage – red	1/6th small cabbage or 2 handfuls sliced or 3 heaped tablespoons shredded
Onion – red	1 medium
Radiccio / Red endive	1 cereal/dessert bowl
Radish	10 radishes
Sweet potato / Yam	1 large

PASTA / RICE / NOODLES	
Wholewheat pasta shapes	3 handfuls uncooked or 1 small bowl cooked
Wholegrain rice	⅓ cup uncooked or 1 cup cooked
Wholewheat spaghetti / noodles	2½ oz (65g) uncooked or one small bowl cooked

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BREAD / CEREALS / BAKED GOODS	
Brown / wholemeal bread / rolls	2 slices or 1 medium roll
Wholemeal scones / cakes	1 medium scone or 1 medium slice
Wholemeal biscuits (e.g. Digestives)	2 biscuits
Wholegrain crackers	5 crackers
Wholegrain breakfast cereals (e.g. Branflakes, Weetabix, Shreddies)	1 small bowl or 2 Weetabix
Cereal bars	1 bar
Bran fibre or wheatgerm	2 tablespoons
Oat biscuits (e.g. Hob Nobs)	2 biscuits
Oatcakes	2 oatcakes
Porridge oats	½ cup uncooked or 1 small bowl cooked
Oat based breakfast cereals (e.g. Oatibix, muesli)	1 small bowl or 2 biscuits
Barley grain (e.g. pearl barley)	2 tablespoons uncooked
Fruit / malt loaf (e.g. Soreen)	2 medium slices

FISH	
All	5 oz (140g) cooked weight

DAIRY	
Yoghurt	One small pot (150g)

DRINKS	
Blackcurrant juice (e.g. Ribena)	Diluted 1 part (50ml) squash with 4 parts water to give a 250ml serving
Fruit juice / smoothie	1 medium glass (150ml)
Port	1 small glass (50ml)
Wine – red / rose	1 medium glass (175ml)

Thank you for your help!

9.20 References

European Food Safety Authority. 2009. Scientific Opinion on the substantiation of health claims related to beta-glucans and maintenance of normal blood cholesterol concentrations (ID 754, 755, 757, 801, 1465, 2934) and maintenance or achievement of a normal body weight (ID 820, 823) pursuant to Article 13(1) of Regulation (EC) No 1924/20061. *EFSA Journal*. [Online]. **7**(9), p.1254. [Date accessed 07 July 2014]. Available from: http://www.efsa.europa.eu/en/efsajournal/doc/1254.pdf

European Food Safety Authority. 2010. Scientific Opinion on the substantiation of health claims related to docosahexaenoic acid (DHA) and maintenance of normal (fasting) blood concentrations of triglycerides (ID 533, 691, 3150), protection of blood lipids from oxidative damage (ID 630), contribution to the maintenance or achievement of a normal body weight (ID 629), brain, eye and nerve development (ID 627, 689, 704, 742, 3148, 3151), maintenance of normal brain function (ID 565, 626, 631, 689, 690, 704, 742, 3148, 3151), maintenance of normal vision (ID 627, 632, 743, 3149) and maintenance of normal spermatozoa motility (ID 628) pursuant to Article 13(1) of Regulation (EC) No 1924/20061. *EFSA Journal*. [Online]. **8**(10), p.1734. [Date accessed 07 July 2014]. Available from: http://www.efsa.europa.eu/en/efsajournal/doc/1734.pdf

European Food Safety Authority. 2011. Scientific Opinion on the substantiation of health claims related to beta-glucans from oats and barley and maintenance of normal blood LDL-cholesterol concentrations (ID 1236, 1299), increase in satiety leading to a reduction in energy intake (ID 851, 852), reduction of post-prandial glycaemic responses (ID 821, 824), and “digestive function” (ID 850) pursuant to Article 13(1) of Regulation (EC) No 1924/20061. *EFSA Journal*. [Online]. **9**(6), p.2207. [Date accessed 07 July 2014]. Available from: http://www.efsa.europa.eu/en/efsajournal/doc/2207.pdf

European Food Safety Authority. 2012. Scientific Opinion on the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA)1. *EFSA Journal*. [Online]. **10**(7) p.2815. [Date accessed 07 July 2014]. Available from: http://www.efsa.europa.eu/en/efsajournal/pub/2815.htm

European Commission. 2014. Food additives database. [Online]. [Date accessed 07 July 2014]. Available from: https://webgate.ec.europa.eu/sanco_foods/main/?event=display

National Health Service. 2012. Metabolic syndrome. [Online]. [Date accessed 14 July 2014]. Available from: http://www.nhs.uk/Conditions/metabolic-syndrome/Pages/Introduction.aspx

Qin, Y. et al. 2009. Anthocyanin supplementation improves serum LDL- and HDL-cholesterol concentrations associated with the inhibition of cholesteryl ester transfer protein in dyslipidemic subjects. *Am J Clin Nutr.* **90**(3), pp.485-492

Toufektsian, M.C. et al. 2011. Dietary flavonoids increase plasma very long-chain (n-3) fatty acids in rats. *J Nutr*. **141**(1), pp.37-41

Wei, X. et al. 2011. Cyanidin-3-O-beta-glucoside improves obesity and triglyceride metabolism in KK-Ay mice by regulating lipoprotein lipase activity. *J Sci Food Agric.* **91**(6), pp.1006-1013