Protocol for glycaemic response, insulin response, gastric emptying and satiety testing (3 hours)

Aim

The study aims to assess the glycaemic response (GR), insulinaemic response (IR) and gastric emptying (GE) time after the consumption of a millet-based muffin in pre-diabetic and healthy participants in order to establish whether this lowers GR and IR compared to the consumption of a non-millet-based, control muffin.

PROPOSED METHOD:

This study will investigate the effect of polyphenol-rich millet-based muffin on GR, IR and GE in healthy and pre-diabetic participants (people with prediabetes have blood glucose levels that are higher than normal but not yet high enough to be diagnosed as diabetes - the normal fasting blood glucose level is below 6.1 mmol/l or 108 mg/dl).

After potential participants have read the PIS and signed the consent form, they will be screened during the first visit to measure fasting blood glucose (FBG; fasting blood glucose should be between 6.1 to 6.9 mmol/l for pre-diabetic participants and < 6.1 mmol/l for healthy participants).If eligible for the study, body weight and height and blood pressure will be taken in the Functional Food Centre. If the fasting blood glucose test is less than 6.1mmol/l (participants will be informed of their fasting blood glucose result and given the choice either to do Oral glucose tolerance test (OGTT) to check for Impaired Glucose Tolerance (IGT), particpate in the healthy group or to be excluded). If the participant agrees to continue and has one or more of the pre-diabetes inclusion criteria,they can complete an oral glucose tolerance test (OGTT) over 2 hours.(An oral glucose tolerance test involves taking a fasting sample of blood and then taking a very sweet drink containing 75g of glucose. After having this drink participants need to stay at rest until a further blood sample is taken after 2 hours.) If their 2 h blood glucose result is between 7.9 to 11.0 mmol/l, they will be considered eligible to participate in the study as a prediabetic participant, and will be asked to come for a subsequent visit in order to begin the study (and therefore the first visit will act as a 'screening visit') (please, see chart below for more detail).If the participant does not have IGT, they have the option to take part in the study as a healthy participant.

Interested participants (pre-diabetic group) will be invited for screening test to check their fasting blood glucose level and will go through the following protocol. Testing is completely voluntary to the participant:

Screening visit
Fasting blood glucose test (FBG) for all volunteers

If the result of FBG is between 6.1 to 6.9 mmol/l

Participants will be informed of their fasting blood glucose result and their eligibility to participate in the study will be confirmed.
Moreover, It will be made very clear to the participant that the diagnosis of pre-diabetes is never made on the basis of a single abnormal blood glucose value.
To avoid any anxiety, they will be given the choice either to participate in the study directly or to seek GP advice if they wish and then will be allowed to join the study.

If the participant agree to participate

The following measurment will be taken:

1. Anthropometric measurement (weight, height and BMI), Blood pressure measurement **(in first visit only).**
2. GR, IR & GE tests **(in both visits**).

Then, the 2nd visit will be booked

Note: 2 sessions in total

If the result is less than 6.1 mmol/l and participant has one or more from criteria in the pre-diabetic inclusion criteria.

Participants will be informed of their fasting blood glucose result and have the choice either to be excluded or to do an oral glucose tolerance test (OGTT).

Why OGTT? If participant have one or more of the inclusion criteria and FBG test is less than 6.1 mmol/l, they will be asked to do OGTT in the near future to confirm whether they are prediabetic or not (Diabetes,UK).OGTT , can show if your body is having problems processing glucose.

If the participant agree to continue, Oral glucose tolerance test (OGTT) at 2 hours will be carried out.

If the participant disagree to continue they will be excluded and a snack will be given as thank you.

If the result at 2 hours is between 7.9 to 11.0 mmol/l, participants will be eligible to take part in pre-diabetic group and and they will be informed of their OGTT result
To avoid any anxiety, they will be given the choice either to participate in the study directly or to seek GP advice if they wish and then will be allowed to join the study.

If the result at 2 hours is less than 7.8 mmol/l the participants will be excluded from the study and snack will be given as thank you
OR the participant can take part in the healthy group if eligible and meets the other inclusion criteria.
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If the participant agree to participate

Then, 2 visits will be booked and snack will be given as thank you.

The following measurment will be taken:

1. Anthropometric measurement (weight, height and BMI), Blood pressure measurement (**in first visit only**).
2. GR, IR & GE tests (**in both visits**).

Note: 3 sessions in total

1- Glycaemic response (GR)

The method to be used is adapted from that described by Brouns et al. (2005) and is in line with procedures recommended by the FAO/WHO (1998) and ISO 26642:2010 guidelines (ISO 26642:2010 Food products – Determination of glycaemic index).

The control and millet muffins will be administered to 15 pre-diabetic participants and 15 healthy participants in a two-arm randomised, repeated measure, crossover design. The sample size is based on previous research conducted within the Functional Food Centre and has sufficient power to detect differences in blood glucose and insulin levels. All participants will consume control muffin (wheat) or test muffin (millet) in random order on separate occasions with at least one-day between each test and any day during the recruitment periods.On the evening prior to a test, the participant will be asked to avoid the consumption of caffeine, alcohol and nicotine, to avoid unusual, strenous exercise and to fast for 12 hours (overnight). At the beginning of the first test session, the following measurements will be taken:

- Body composition (using the Tanita BC-418MA segmental body composition analyser).
- Blood pressure (using an automated sphygmomanometer) - if blood pressure is higher than normal, they will be advised to contact their GP for further tests if necessary (high blood pressure is considered to be 140/90mmHg or higher)
- It will take approximately 15 minutes to complete these measurements.

Blood samples will be taken at -5 and 0 min before consumption of the control/test muffin and at 15, 30, 45, 60, 90,120,150 and 180 min after starting to eat. Finger-prick blood samples will be taken using a single-use lancing system (Unistick 3, Owen Mumford, Woodstock, UK). Blood glucose will be immediately measured using an automatic blood glucose analyzer (Glucose 201+, Hemocue AB, Sweden). The accuracy of the analyzer will be checked daily using control solutions. Each measurement requires only 5 µl blood, therefore the finger-prick will be small, with minimal discomfort, and the participants will not experience any negative consequences except for a possible slight bruising on finger tips. All blood samples will be destroyed immediately after reading and recording, according to Standard Operating Procedures. For each product, the incremental AUC, ignoring area beneath the baseline, will be calculated geometrically (FAO/WHO, 1998) and will include the area above the fasting level only; any area beneath the fasting level will be ignored.

Satiety

Immediately before each blood sample, participants' subjective feelings of satiety/hunger will be recorded on 7-anchored bidirectional scales.

2- Insulinaemic response

The determination of insulin will be undertaken at the same time as the blood glucose measurements (procedure is as given below).

1. Finger-prick blood samples will be taken using a single-use lancing system (Unistick 3, Owen Mumford, Woodstock, UK). For insulin, blood samples will be taken at the same time points and using the same finger-prick for glycaemic response. Each insulin measurement requires 300 µl blood. The finger-prick will be small, with minimal discomfort, and the participants and the participants may have some bruising in thier fingers. Samples will be collected in EDTA-coated tubes and kept on ice and will be centrifuged and the plasma frozen at -40°C until analysis.
2. Plasma insulin will measured by immunoassay (Cobas e411, Roche, UK).
3. The IR will be calculated geometrically by measuring the incremental AUC, ignoring the area beneath the baseline (FAO/WHO, 1998) and only include the area above the fasting level.

3- Gastric emptying

100 mg of 13C sodium acetate will be added to the muffins (control and millet) in order to measure gastric emptying rate. 13C is a naturally abundant stable isotope of carbon that is completely safe. Sodium acetate is regularly used in the food industry as seasoning. Breath samples for measurement of gastric emptying will be taken by blowing into a small glass tube through a straw. They will be collected every 15 minutes for 4 hours after breakfast (in the same time points of glycaemic and insulinaemic response plus at, 210 and 240 minutes).

Statistical Analysis

Data will be analysed using Microsoft Excel 2010 and SPSS 21.0 . Means, standard deviations, descriptive statistics and repeated measures Anova will be used to determine differences in glycaemic and insulinaemic response and gastric emptying between the control muffin and millet muffin.

References:

Brouns F, Bjorck I, Frayn KN, Gibbs AL, Lang V, Slama G and Wolever TMS. Glycaemic index methodology. *Nutr Res Rev* 2005;18:145-171.

FAO/WHO. *Carbohydrates in Human Nutrition*. Rome: FAO, 1998

ISO 26642:2010 Food products – Determination of glycaemic index.