

Protocol for Roquette: glycaemic response, insulinaemic response, GLP-1 and GIP study

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Aim

The aims of this study are to examine:

- Phase I – the glycaemic response (GR) and insulinaemic response (IR) to:
 - 50 g available carbohydrate
 - 50 g available carbohydrate + protein (dose 1)
 - 50 g available carbohydrate + protein (dose 2)
- Phase II – the GLP-1 and GIP response to:
 - 50 g available carbohydrate
 - 50 g available carbohydrate + protein (dose 1)
 - 50 g available carbohydrate + protein (dose 2)

Study design

A single-blind, randomised, repeat measure, crossover design trial will be used to study the response following consumption of three meals: one reference meal (50 g available carbohydrate) and two test meals (50 g available carbohydrate with protein dose 1 and 50 g available carbohydrate with protein dose 2). The study will be conducted in two phases:

- Phase I: glycaemic response (GR), insulinaemic response (IR)
- Phase II: GLP-1 response and GIP response

Phase II will be dependent on the outcomes from Phase I.

Participants will act as their own controls. Participants will be randomly assigned to test the products using a pseudo-random number generator. The trial will be conducted by the Oxford Brookes Centre for Nutrition and Health (OxBCNH). Ethical approval for the trial will be obtained from the University Research Ethics Committee (UREC) at Oxford Brookes University. Participants will be given full details of the study protocol and the opportunity to ask questions. All participants will give written informed consent prior to participation.

Participants

A sample size of 30 participants will be recruited. Healthy male and female adult participants (aged 18-60 years) will be recruited for the study. Participants will be excluded from the study if they meet any of the following criteria:

- Aged < 18 or > 60 years

- Pregnant or lactating
- Body mass index (BMI) $> 30\text{kg}/\text{m}^2$
- Fasting blood glucose value $> 6.1 \text{ mmol/l}$
- Any known food allergy or intolerance
- Medical condition(s) or medication(s) known to affect glucose regulation or appetite and/or which influence digestion and absorption of nutrients
- Known history of diabetes mellitus or the use of antihyperglycaemic drugs or insulin to treat diabetes and related conditions
- Major medical or surgical event requiring hospitalization within the preceding 3 months
- Use of steroids, protease inhibitors or antipsychotics (all of which have major effects on glucose metabolism and body fat distribution)

In addition, participants will be excluded if they are unable to comply with experimental procedures or do not follow GR and IR testing safety guidelines.

Products

Three meals will be administered:

- 50 g available carbohydrate
- 50 g available carbohydrate + protein (dose 1)
- 50 g available carbohydrate + protein (dose 2)

Protocol

The study will be conducted by Good Clinical Practise (GCP) certified researchers at OxBCNH. The protocol used by OxBCNH is based on ISO standards for the determination of the glycaemic index (GI).¹

On the day prior to a test, participants will be asked to restrict their intake of alcohol and caffeine-containing drinks and to restrict their participation in intense physical activity (for example, long periods at the gym, excessive swimming, running and aerobics). Participants will also be told not to eat or drink after 9.00 pm the night before a test, although water will be allowed in moderation.

The reference meal and two test meals will be administered to participants in a randomised, repeated measures design. In each participant, the meals will be tested in random order on separate days, with a one-week gap between measurements. Participants will be studied in the morning, before 10 am after a 12-hour overnight fast. Participants will consume the products at a comfortable pace, within 15 minutes and will remain sedentary during each session and will not consume any additional food or fluid.

Anthropometric measurements

All anthropometric measures will be taken, using standard methods, in the morning, after an overnight fast (12 hours):

- Height and weight
- BMI will be calculated using the standard formula: weight (kg)/height (m)²
- Waist and hip circumference

¹ Food Products – Determination of the glycaemic index (GI) and recommendation for food classification. ISO 26642: 2010(E)

- Body composition using a body composition analyser (Tanita BC-418 MA; Tanita UK Ltd)

Phase I

Glycaemic response (GR)

The glycaemic response method used by OxBCNH at Oxford Brookes University is carried out in accordance with the ISO 26642:2010 guidelines.¹

Blood samples will be taken at -5 min and 0 min before consumption of the reference product/test product and the baseline value taken as a mean of these two values. The product will be consumed immediately after this and within 15 min. Further blood samples will be taken at 15, 30, 45, 60, 90, 120, 150 and 180 min after starting to drink. The time between participants finishing drinking and the first postprandial blood sample will be recorded.

Blood will be obtained by finger-prick using the Unistik®3 single-use lancing device (Owen Mumford). Capillary blood sampling is preferred for reliable GR testing. Prior to a finger-prick, participants are encouraged to warm their hand to increase blood flow. Fingers are not squeezed to extract blood from the fingertip as this may dilute with plasma. Blood glucose will be measured using the HemoCue Glucose 201+ analyser (HemoCue® Ltd), which is calibrated daily using control solution from the manufacturer.

The incremental area under the blood glucose response curve (iAUC) will be calculated geometrically by applying the trapezoid rule, ignoring the area beneath the baseline.

Insulinaemic response (IR)

Blood samples in the fasting state will be obtained five minutes apart (-5 and 0 min) for measuring baseline insulin levels. Following the consumption of the reference product/test products, further blood samples will be collected at 15, 30, 45, 60, 90, 120, 150 and 180 min after starting to drink.

At each test time point, 300 µL of capillary blood (from finger pricks) will be obtained. Finger pricks will be made using the Unistik 3 single-use lancing device (Owen Mumford, Woodstock, UK) and blood will be collected into chilled Microvette® capillary blood collection tubes treated with di Pottassium EDTA (CB 300 K2E; Sarstedt Ltd, Leicester, UK). The Microvette® tubes will then be centrifuged and 200 µL of the supernatant plasma obtained. Insulin concentrations in the plasma samples will be determined by electrochemiluminescence immunoassay using an automated analyzer (Cobas® E411; Roche diagnostics, Burgess Hill, UK). The Cobas® system is a reliable method of plasma insulin determination². The unit of measurement is µU/ml.

² Siannidou T, Margeli A, Kappis A, Papassotiriou I & Mandyla H. Circulating visfatin levels in healthy preterm infants are independently associated with high-density lipoprotein cholesterol levels and dietary long-chain polyunsaturated fatty acids. *Metabolism* 2010;60(3):389-93

Phase II

GLP-1 and GIP

During Phase I of the study, a cannula will be fitted and baseline measurements (0 min) will be taken before participants are provided with the test meals. Blood samples will then be collected at 30, 60, 90, 120 and 180 min after the start of consumption.

Blood will be taken from the antecubital vein via cannulation and will be centrifuged to obtain plasma samples which will be stored at -80°C until analysis. Depending on the outcome of Phase I, samples will be subsequently analysed to determine the concentrations of GLP-1 and GIP.

Statistical analysis

Data will be analysed using the IBM Statistical Package for the Social Sciences (SPSS, version 24). Data will be presented as mean, standard deviation (SD) and standard error of the mean (SEM) values. The blood glucose, plasma insulin, GLP-1 and GIP iAUC (at 60, 90, 120 and 180 min) will be calculated geometrically by applying the trapezoid rule, according to the ISO standards. Prior to statistical analysis, the normality of the data will be tested using the Shapiro-Wilks statistic. Parametric or non-parametric tests will be used as appropriate to compare the following between the products:

- *Phase I*
 - blood glucose/insulin concentrations
 - peak blood glucose/insulin value and time of the blood glucose/insulin peak
 - glucose/insulin iAUC (at 60, 90, 120 and 180 min)
- *Phase II*
 - GLP-1 and GIP concentrations
 - GLP-1 and GIP iAUC (at 60, 90, 120 and 180 min)

Statistical significance will be set at $P < 0.05$.

Outcomes

- The primary outcome will be the GR and IR, as measured by the incremental area under the curve, blood glucose/insulin concentrations, peak and the time at which peaks occur
- The secondary outcome will be the GLP-1 and GIP incremental area under the curve and concentrations