RESEARCH PROTOCOL

TITLE

Effect of faba bean protein versus whey protein on human metabolic responses: a single-blind, randomised, acute, crossover study

SHORT TITLE (USED ON PARTICIPANT FACING DOCUMENTS)

Digestibility of broad bean (faba bean) protein compared to whey protein

BACKGROUND

Protein is the key provider of essential amino acids (EAA) required by the body for growth, repair and maintenance of bone, teeth, muscle and tissue, and for the production of functional components such as hormones, enzymes and immunoglobulins. Unlike carbohydrate and fat which can be stored within the body, daily dietary protein is essential to maintain the correct levels of amino acids for optimum health and to support growth.

Protein in the diet is derived from animal and plant sources and, with an increased focus on wider issues within the food system such as environmental sustainability, land-use and biodiversity, there has been increased focus on examining the role of different protein sources in promoting health. Protein quality, such as digestibility, bioavailability, absorption, EAA content, and the anabolic effect of dietary proteins can vary by source (1). The amino acid content of some common plant proteins (soy, pea and faba bean) compared to whey protein are shown in Table 1.

Table 1: Amino acid content of various dietary protein sources (g/100g raw material).

	Whey	Soy	Pea	Faba bean
Essential Amino Acids (EAA) (g/100g)				
Threonine	5.4	2.3	2.5	2.9
Methionine	1.8	0.3	0.3	0.7
Phenylalanine	2.5	3.2	3.7	4.0
Histidine	1.4	1.5	1.6	2.1
Lysine	7.1	3.4	4.7	5.4
Valine	3.5	2.2	2.7	4.3*
Isoleucine	3.8	1.9	2.3	3.9*
Leucine	8.6	5.0	5.7	7.1*
Sum of EAA	34.1	19.9	23.6	30.4
Non-essential amino acids (NEAA) (g/100g)				
Serine	4.0	3.4	3.6	4.2
Glycine	1.5	2.7	2.8	3.2
Glutamic acid	15.5	12.4	12.9	14.8
Proline	4.8	3.3	3.1	3.8
Cysteine	0.8	0.2	0.2	0.8
Alanine	4.2	2.8	3.2	3.6
Tyrosine	2.4	2.2	2.6	3.0
Arginine	1.7	4.8	5.9	7.6
Aspartic acid	Not measured	Not measured	Not measured	8.7#

Sum of NEAA	34.9	31.9	34.4	41.0

Values for whey, soy and pea are presented in g per 100g raw material. Whey, soy and pea values provided by Gorissen et al (1) which did not report tryptophan, aspartic acid, asparagine, and glutamine were not measured. Faba bean values, provided by Pulsin manufacturer, are reported as Average Amino Acid Composition [(*=Branch chain amino acids (BCAA)g/100g Powder]. #Not included in Sum of NEAA, as not available for other protein sources.

Animal proteins, such as milk or whey protein, are recognised as high biological value or 'complete' proteins as they provide all the EAA needed by the human body, have high digestibility, and can help transport other important nutrients such as calcium and iron. Proteins from animal sources (dairy, meat, fish, poultry) are the most bioavailable sources. Whey protein (WP) is widely used as an ingredient in foods or as a food supplement owing to its high nutritional value and versatility. WP is a high biological value protein, containing all the EAA, including the essential and is rich in branched chain amino acids (BCAA) leucine, isoleucine and valine, as well as bioactive peptides that are rapidly absorbed into the circulation. The digestibility and metabolic effects of WP have been widely studied in acute and some longer-term studies. In acute studies, it is known to stimulate the secretion of gut hormones (incretin peptides), particularly glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) which stimulate insulin secretion (2-4). The release of incretin peptides regulates the rate of gastric emptying and gastrointestinal transit of food (2-4). GLP-1 stimulates glucose-induced insulin secretion and reduces postprandial glycaemia. An increase in levels of GLP-1 can increase the synthesis of insulin stores in beta cells, increase beta cell mass and reduce the rate of beta cell apoptosis (5). Insulin can regulate hepatic glucose production and cross the blood brain barrier and may suppress appetite. WP is commercially available and often used to supplement a healthy diet. It has received much attention for muscle building, due to its high level of leucine, which acts as a signal to initiate the process of muscle protein synthesis. WP supplementation may serve as a simple, non-pharmaceutical method to improve postprandial glycaemia for health participants and those with T2DM (6-8).

The availability of plant-based protein supplements has grown substantially in the last few years as a result of increased consumer focus on health, ethical and environmental factors and an overall greater emphasis on plant rich diets for health and sustainability. The food industry has become more interested in plant-based protein sources due to greater sustainability, lower production costs, and health or ethical reasons. Plant-based foods can provide a good source of protein, antioxidants, vitamins, minerals and dietary fibre, supporting overall health and contributing to a balanced diet. Whilst there may be benefits of diets higher in plant-based protein, protein from plant sources is generally considered to be inferior in some respects compared to animal proteins as they are generally lacking in one or more of the EAA and have a lower Digestible Indispensable Amino Acid Score (DIAAS) (9). Most plant proteins are limited in their amino acid profile, with leucine, lysine and methionine found in lower concentrations in grains and legumes compared to meat, eggs and milk (10). EAA, especially BCAA such as leucine, are important in the regulation of muscle protein synthesis (MPS) (11). Whilst some evidence from human interventions indicates that in some cases animal protein may have a more favorable effect on lean body mass compared to plant protein, this evidence is limited by the number of studies that have directly compared animal and plant proteins and the limited types of plant proteins that have been studied (mainly soy protein) (12). Although there is some suggestion that plant-based proteins may be associated with better blood glucose control compared to animal protein, the evidence remains limited in terms of study quality and type of plant-protein tested, with most studies examining soy and pea proteins (13, 14).

There are a wide variety of plant-protein sources, such as legumes, pea, beans, oat, and soya protein and the EAA and non-EAA content differs according to the source (see examples in Table 1). As the popularity and demand for plant-based proteins grows, there has been an increase in the variety of commercially available sources. Many studies to date have focused on the functional and bioactive properties of soy protein compared to animal protein sources (15), whereas there is still limited knowledge of the physiological effects of other plant proteins such as pea and bean protein sources. Faba beans are emerging as sustainable and versatile plant protein source. Faba beans, also known as broad beans, have been grown across the UK for thousands of years. Approximately three quarters of a million tonnes of faba beans are harvested around the UK each year, with the majority being exported to the Middle East or used as animal feed. There is increased interest in faba bean as a potentially valuable crop to grow in Ireland. The 'Unlocking Protein Resource Opportunities To Evolve Ireland's Nutrition' (U-Protein) project aims to create new scientific and technological knowledge with long-term potential to re-engineer Ireland's agro-ecological system. It proposes to achieve this through greater diversification of protein resources, including faba bean, delivering sustainability, circularity and quality nutrition. As part of the U-Protein project, we aim to evaluate human metabolic responses to the consumption of faba bean protein (FP) sources which can be grown in Ireland.

Faba beans are a rich source of protein, vitamins, minerals and dietary fibre, and are low in fat. They are vegan, genetically modified organism (GMO) free and free of common allergens. Faba bean has a well-balanced amino acid profile, similar to that of the pea and soy (see Table 1) (16). Clinical studies have shown that regular consumption of pulses (including faba beans) can reduce blood levels of total cholesterol and low-density lipoprotein (LDL), and therefore may reduce the risk of CVD. One of the key benefits of FPs over many other plant-based protein powders, including pea protein, is that it has a less grainy (creamier) texture and is more palatable. Overall, there is very little known about the basic digestion of FP in terms of the postprandial amino acid profile and its effect on metabolism after ingestion. This information is fundamental to understand before embarking on longer term human studies. To the best of our knowledge, no other study has examined the specific bioavailability, digestibility, and metabolism of FP compared to WP.

AIM

The aim of this study is to assess the acute effect of FP vs WP, with and without a glucose load, on satiety hormones, postprandial amino acid bioavailability, glucose, insulin and a range of metabolomic markers in circulating blood in healthy volunteers.

MAIN RESEARCH QUESTIONS AND HYPOTHESES BEING ADDRESSED

Question 1 – What effect does FP have on glucagon-like peptide-1 (GLP-1), other satiety hormones, amino acid availability, glucose, insulin and a range of metabolomic markers compared to the same dose of WP in healthy adults?

Hypothesis 1 – A beverage containing FP protein will have a similar stimulatory effect on GLP-1 and satiety hormone secretion but will have lower amino acid availability compared to WP.

Question 2 – What effect does FP together with a glucose load have on GLP-1 and other satiety hormones, amino acid availability, postprandial glycaemic, and insulinaemic response and a range of

metabolomic markers compared to the same dose of WP together with a glucose load in healthy adults?

Hypothesis 2 – A beverage containing FP and glucose will attenuate the postprandial glycaemic response to a greater extent than a beverage containing the same dose of WP and glucose.

STUDY DESIGN OVERVIEW

The study is a single-blind, randomised, acute, crossover intervention trial.

STUDY DESIGN

Participants will complete four acute interventions (two pairs, as shown in Table 2 below) in a random order with at least a one week washout between studies (four study visits in total, each visit will last 2.5 hours). At each visit, participants will consume one of the four test beverages detailed in Table 2.

Table 2 Details of the four acute interventions provided to participants (one per study visit; four study visits in total)

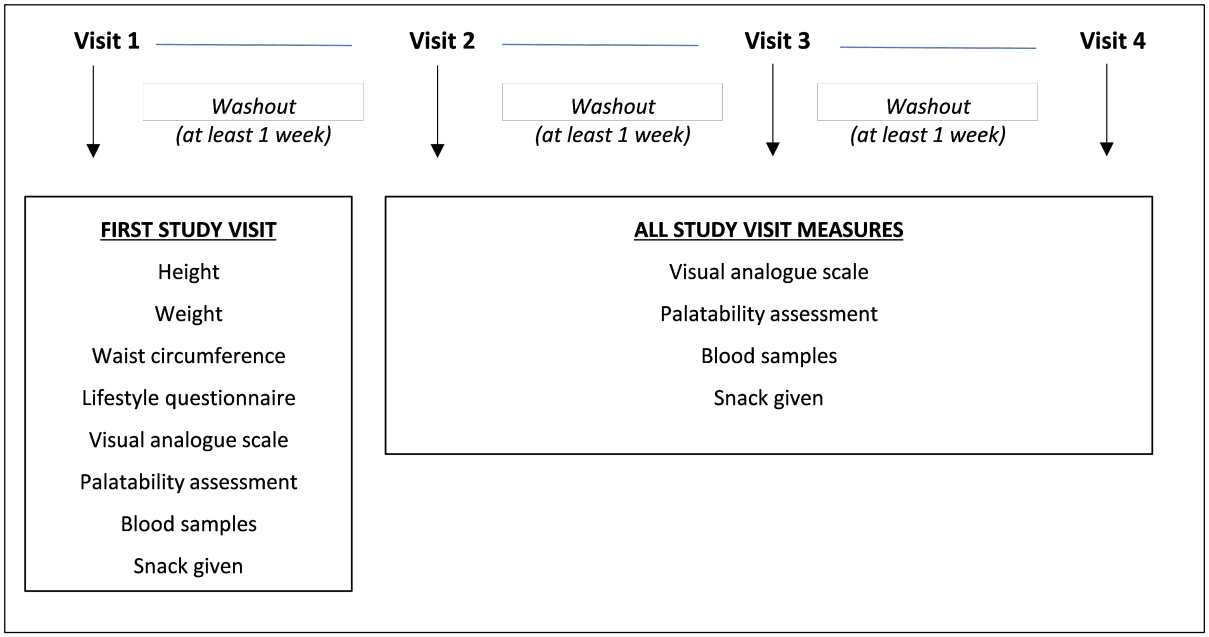
Pair 1 (WP vs FP)	Intervention 1	Whey protein, WP; 0.4g/kg body weight
	Intervention 2	Faba bean protein, FP; 0.4g/kg body weight
Pair 2 (WPG vs FPG)	Intervention 3	Whey protein and glucose load, WPG; 0.4g/kg body weight WP + 50g glucose
	Intervention 4	Faba bean protein and glucose load, FPG; 0.4g/kg body weight FP + 50g glucose

An overview of the study assessments and the participant's journey through the study is shown in Figure 1 and a detailed description of the methods is given below.

In brief, the study will require participants to attend the Northern Ireland Clinical Research Facility (NICRF) at the Belfast City Hospital U-floor on four occasions at least one week apart (Visits 1,2,3,4); each of these visits will last 2.5 hours and participants will consume a beverage consisting of one of the above interventions at each visit.

Figure 1: Overview of participant's journey throughout the study

Four interventions will be delivered in random order: intervention #1 - WP; intervention #2 - FP; intervention #3 – WPG; intervention #4 – FPG.



PARTICIPANTS

A total of 20 healthy participants will be recruited (see sample size section for details). The specific inclusion and exclusion criteria are as follows:

Inclusion criteria

- 18-40 years old
- Healthy volunteers
- Body mass index (BMI) ≥ 18.5 kg/m^2 and/or weight ≥50kg (7 Stone 12 pounds) [minimum weight in line with NI Blood transfusion requirements]
- Be willing to consume WP and FP beverages
- Be available to meet the requirements of the study

Exclusion criteria

- Medical condition or medication known to affect glucose regulation or appetite and/or digestion and absorption of nutrients
- Known history of diabetes mellitus or the use of antihyperglycemic drugs or insulin to treat diabetes and related conditions
- Use of steroids, protease inhibitors or antipsychotics
- Major medical or surgical event requiring hospitalisation within the preceding three months
- Any known food allergy or intolerance including lactose intolerance or milk allergy
- Pregnancy or lactating

- Donated blood to the NI Blood Transfusion Service in the last 12 weeks for men or 16 weeks for women

Eligible participants will be asked to fast from 21:00h (9pm) the night before and refrain from intensive physical activity for 24 hours prior to study visit. Intensive physical activity is defined as an activity that makes someone breathe hard and fast so it would be difficult to say more than a few words without pausing for breath, for example running, football, rugby, netball, hockey, aerobics, martial arts. Eligibility to participate in the study will be assessed using a screening questionnaire (Appendix 1.0) taking into consideration the above inclusion and exclusion criteria.

RECRUITMENT

Participants will be recruited from the student and staff population at Queen's University Belfast (QUB).

Students and staff will be made aware of the study in a number of different ways:

1. Poster advertisement (see Appendix 2.0)

With relevant permissions, an A4 poster will be placed around buildings frequented by staff and students from QUB, for example, the Medical Biology Centre (MBC), the School of Biological Sciences building, and the main site tower. The poster will include contact details so participants can contact the study team if interested to discuss the study further.

2. E-mail (see Appendix 3.0)

With permission from the relevant Head of School or Centre Director, an email will be sent to students enrolled for the 2023-2024 academic year within the School of Biological Sciences and the Centre for Biomedical Sciences Education, School of Medicine, Dentistry & Biomedical Sciences.

3. QUB Staff round-up (see Appendix 4.0)

Information about the study will be included in the QUB staff round-up.

4. Social Media (see Appendix 5.0)

With permission from the relevant gatekeeper, a post will be shared on the social media accounts of the Centre of Public Health and the Institute of Global Food Security and other relevant QUB social media streams.

Individuals who hear about the study via one of the above routes and are potentially interested in taking part will contact one of the researcher's, Dr Clare Kelly or Dr Eleni Spyreli, who will provide them with a Participant Information Sheet (see Appendix 6.0) and consent form (see Appendix 7.0). Participants will then be given at least 48 hours to read the information sheet and will have the opportunity to ask any questions they wish about the study before making a decision to participate. The researcher will make up to three attempts to contact individuals who have received the study PIS after which time it will be assumed they are not interested in taking part.

Written informed consent will be obtained when participants attend for the first study visit at the Northern Ireland Clinical Research Facility (NICRF).

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. As participants are to arrive fasted to the visit, they will be asked not to eat or drink after 21:00h the night before a test, although water will be allowed in moderation. Participants will be asked, with best efforts, to standardise and consume the same foods and drinks and quantities the day before each test and maintain the same physical activity the day before each test.

Participants will be given a £100 voucher after the first two study visits, and a further £100 after the final two study visits as a token of appreciation for the time they have dedicated to the research.

PLANNED INTERVENTIONS

Participants who meet the inclusion criteria will receive the four interventions detailed in Table 2 above (one at each visit, with at least a one week washout between interventions to minimise carry over effect) delivered in a random order.

The WP and FP powders will be obtained from a commercially available UK supplier (www.pulsin.co.uk; Pulsin Ltd 2023 | Pulsin Ltd, Unit 16, Brunel Court, Waterwells Business Park, Gloucester, GL2 2AL. Company registration number: 5466800). Glucose powder will be obtained from a commercially available UK supplier.

Preparation of beverages

Test beverages will be prepared fresh for each participant just before consumption and will be served in opaque containers. For interventions 1 and 2 (WP vs FP), beverages will be prepared using bottled water (450ml) together with protein powder equating to 0.4g/kg/d for the participant and flavoured with a sugar free flavouring (e.g. commercially available orange/blackcurrant/lemon cordial). The test beverages for interventions 3 and 4 (WPG vs FPG) will be prepared in the same way but with the addition of 50g glucose. Participants will be asked to consume beverages within 15 minutes of serving.

DOSAGE

UK and World Health Organization (WHO) dietary guidelines recommend a protein intake of 0.75g/kg/d (17) and 0.8g/kg/d, respectively, for the maintenance of good health. For this series of acute studies, a dose of 0.4g/kg/d has been chosen as this will provide approximately 50% of daily protein requirements and has been shown in previous studies to elicit an effect on insulin, satiety hormones and glucose in acute studies with whey or plant proteins as described below. For participants with a weight ranging from 55-90kg, this would equate to 22-36 grams of protein per test beverage.

In an acute dose response study, Claessens et al. tested three doses (0.3, 0.4 and 0.6g/kg of BW) of intact soya protein (SPI) and soya protein hydrolysate (SPH) in 12 healthy male participants, and compared this to the effects of three doses (0.3, 0.4 and 0.6g/kg of BW) of intact whey protein (WPI) and whey protein hydrolysate (WPH) in another group of 12 healthy male participants with blood samples collected at six time points within a two hour period (18). They found there was a dose response for insulin and glucagon area under the curve (AUC) for all four proteins. SPI induced a higher total AUC for insulin and glucagon than SPH, while there was no difference in area under the curve for

insulin and glucagon between WPI and WPH. There was no difference in the AUC for glucose between any form of protein. (18).

Giezenaar et al reported that a 60 minute intraduodenal infusion of 24g or 48g of WP significantly increased plasma GLP-1 and insulin concentrations (and lowered plasma ghrelin concentrations) in healthy older and younger men relative to a saline control in a randomised crossover study (19). Blood glucose concentrations were lower after 24g or 48g of whey compared to the saline control in younger, but not older, men.

Compared to a 50g glucose control, Thondre et al. showed that there was a dose response effect of pea protein (25g and 50g) on postprandial glycaemia and stimulated insulin release over a three hour period in healthy adults (14).

RANDOMISATION AND BLINDING

The study will be a four-visit, single-blind, acute crossover study; with at least a one week washout period between visits. A computer-generated randomisation scheme will be prepared by a statistician who is independent of the study team and provided to the researcher who is preparing the test beverages (CK).

The researcher will prepare beverages for each visit for each participant according to the randomisation list. To maintain participant blinding, beverages will be provided in identical opaque containers labelled with the participant number and the intervention period. Thus, the participants will be unaware which beverage they are taking during each visit.

PROPOSED OUTCOMES

Primary endpoint: Circulating concentrations of intact GLP-1 in blood.

Secondary endpoints: Circulating concentrations of other satiety hormones, glucose and insulin concentration, amino acid availability in blood.

Exploratory endpoints: Metabolomic and proteomic markers in blood.

SAMPLE SIZE

Based on variability data from previously published studies in similar populations (6, 8), as well as unpublished data from our own group, a sample size of 16 participants will give the study 80% power to detect a significant difference of 10-15% in total AUC GLP-1 between treatment conditions (WP vs FB; or WPG vs FPG) or a difference in AUC Glucose of 5-15% between treatment conditions (WPG vs FPG).

To allow for dropout, it is anticipated that 20 participants will be recruited.

STUDY ASSESSMENTS

All assessments will be carried out at the Northern Ireland Clinical Research Facility, U Floor, Belfast City Hospital tower block.

Questionnaires (paper copies provided for completion):

All participants will be asked to complete a short lifestyle questionnaire (sex, age, food and drinks, alcohol, physical activity and smoking status (adapted from Leeds Short Form Food Frequency Questionnaire (20)); see Appendix 8.0) at their first visit.

Visual analogue scales (see Appendix 9.0) to rate feelings of hunger will be completed during each study visit at T0 (before the drink is consumed), T30, T60, T90 and T120 (minutes).

At the end of each visit, participants will be asked to complete an assessment of palatability of the test beverage they consumed (see Appendix 10.0). Using the 9 point hedonic scale (21), we will ask participants to rate the initial and after taste, the texture/feeling in their mouth, and the overall acceptability of the beverage.

Anthropometric data:

At the first visit, weight (kg) and height (m) will be measured using calibrated scales and a stadiometer respectively (see Appendix 11.0). Body mass index (BMI) will be calculated – weight in kilograms divided by height in meters squared.

Waist circumference (inches) will be measured using standard equipment (See Appendix 11.0). It will be measured using a flexible measuring tape designed for this purpose.

Blood samples:

At the start of each visit to the NI Clinical Research Facility, a cannula will be inserted by a trained nurse. Blood samples will be taken at T0 (before the drink is consumed), T30, T60, T90 and T120 (minutes). At each time point, a 4ml EDTA plasma, 2ml Sodium Fluoride plasma and 6ml Serum tube will be collected giving a total volume of 60mls for each study visit.

Participants will be supervised at all times by the research nurse taking the blood samples and the researcher. They will be asked to remain in the NICRF for 15 minutes after the last blood sample has been taken.

All blood samples will be taken from the antecubital vein by the trained nurse at NICRF. Samples will be separated into plasma and serum and stored at -80^{o}C for batch analysis once the study has been completed. Samples will be processed, stored and analysed in the nutrition labs at the Centre for Public Health (CPH).

Participants will be given a snack and a drink to take away at the end of study visit.

<u>LABORATORY METHODOLOGY (Nutrition laboratories, Centre for Public Health)</u>

Assessment of gut hormones

The assessment of gut hormones will be measured by enzyme-linked immunosorbent assay (ELISA). GLP-1 will be carried out using an ELISA kit purchased from EMD Millipore Corporation. Other gut hormones (including GIP, Leptin, Ghrelin and Adiponectin) will be measured by Quantikine ELISA kits (R&D Systems, Minneapolis, MN, USA) as per the manufacturer's instructions. In assay validation, multiple freeze/thaw cycles will be tested

for stability. All assays were performed in duplicate using kits with the same batch number, by operators blinded to sample identity.

Assessment of insulin and glucose levels

Fasting insulin will be measured by Biochemistry labs at the Royal Victoria Hospital and fasting glucose will be measured using an automated glucose oxidase method using a Beckman glucose analyzer. Fasting glucose and insulin will be used to calculate Homeostatic model assessment (HOMA), a measure of insulin resistance and beta-cell function (22).

Targeted metabolomics analysis (School of Biological Sciences laboratories, Prof B Green)

Targeted metabolomics profiling will be performed using a commercially available MxP Quant500 XL kit (Biocrates Life Science AG, Innsbruck, Austria), which quantifies more than 1,000 metabolites and lipids from 26 analyte classes. This process also permits the calculation of >400 "metabolism indicators". These are pre-determined sums and ratios comprising the quantified metabolites and pertain to specific biological pathways or syntheses.

All frozen plasma or serum samples (-80°C) will be thawed on ice before preparation, according to the instruction from the kit manufacturer. In brief, 10 µL of phosphate-buffered saline, calibrators, quality controls (QCs, lyophilized plasma spiking with metabolites at three known concentrations), and plasma samples will be added to a 96-well plate which contains isotopic-labelled internal standards, followed by adding phenyl isothiocyanate (PITC) to derivatize amino acids and biogenic amines. Metabolite separation will be performed using an ultra-performance liquid chromatography (UPLC) system (AB SCIEX ExionLC system, California, USA) with a reversed-phase MxP Quant 500 UHPLC column and analysed using a triple-quadrupole mass spectrometer (Xevo TQ-S, Waters Corporation, Milford, USA) operating in the multiple reaction monitoring (MRM) mode. All the other metabolites (acylcarnitines, hexoses, glycerophospholipids, and sphingolipids) will be quantified using the same mass spectrometer without column separation by the flow injection analysis (FIA) operating in MRM mode.

For quantitation, both LC and FIA data will be converted and imported directly into the Biocrates software, MetIDQ Oxygen, and quantified. MetIDQ includes an automated simple target normalization procedure based on QC or sample pool for batch-to-batch and kit plate-to-plate correction for sample cohort across several kit plates. Metabolite concentrations will be calculated and expressed as micromole (µM).

Whenever ≥20% of measurements for a metabolite are lower than the limit of detection (LOD), the metabolite will be described as "undetectable" and will be excluded from the analysis. Any metabolism indicators involving undetectable metabolites will not be calculated. The LOD of each metabolite will be based on the Quant500 kit methodology in accordance with the manufacturer's instructions.

Proteomics analysis (School of Biological Sciences laboratories)

A relative quantification for each of the most abundant ~600 proteins in blood plasma will be performed. Data will be acquired in diaPASEF mode (data independent acquisition – parallel accumulation serial fragmentation) on a timsTOF Pro mass spectrometer equipped with nanoElute chromatography system (Bruker) established in by Dr Ben Collins' at QUB

(23). This method uses trapped ion mobility separations to increase sensitivity and resolving power combined with data independent acquisition to ensure quantitative robustness and data completeness. Current data from plasma samples show we can robustly quantify approximately 600 plasma proteins from only 10µl of human plasma. A commercial 96 well plate format sample preparation kit (Preomics iST) will be used to extract proteins from plasma, digest these to peptides, and clean-up by solid phase extraction resulting in injection-ready samples. Analysis of mass spectrometry data to produce protein-level quantitative data matrices will be performed using Spectronaut software.

STATISTICAL ANALYSIS

Statistical Analysis will be carried out using SPSS software for Windows, version 29. Statistical advice will be given by Professor Chris Cardwell, a medical statistician from the Centre for Public Health.

The data will be presented as mean, standard deviation (SD) and standard error of the mean (SEM) values. Before statistical analysis, the normality of the data was tested using the Shapiro–Wilk statistic.

Primary and secondary endpoints will be analysed using repeated measures ANOVA (for normally distributed data) and non-parametric Friedman test (where data were not normally distributed) to compare concentrations at each time point. Incremental Area under the Curve (iAUC) (at 30, 60, 90 and 120 min) will be calculated and reported along with peak concentration and time of peak concentration for relevant endpoints in response to each of the interventions. Post hoc analyses will be performed using the Bonferroni correction for parametric data and the Wilcoxon signed-rank test for non-parametric data. Statistical significance will be set at p value ≤ 0.05 for all tests.

Metabolomic and proteomic analysis will be considered exploratory analysis. P-values will not be altered for multiple testing, but findings will be treated as hypothesis generating rather than hypothesis testing.

PROPOSED TIMELINE FOR THE STUDY

January – February 2024 – Obtain ethical approval; study set-up

February – March 2024 – Recruitment

February – June 2024 – Completion of dietary interventions

July-December 2024 – Laboratory analysis, statistical data analysis and write-up

STUDY PHONE NUMBER AND EMAIL ADDRESS

To ensure personal telephone numbers are not given out we have obtained a mobile phone unique to the study.

Contact information for study participants:

Email address: clare.kelly@qub.ac.uk or eleni.spyreli@qub.ac.uk

Telephone number: 07810 810491

ETHICAL CONSIDERATIONS

The study will be carried out in accordance with the declaration of Helsinki. Ethical approval will be obtained from the Faculty of Medicine, Health and Life Sciences Research Ethics Committee at Queen's University Belfast. Participants will be given full details of the study and will have the opportunity to ask questions. All participants will provide written informed consent prior to participation and will be informed that they can withdraw from the study at any time without having to give a reason. Participants will be informed that any data or samples provided up to the point of withdrawal may be included in the study unless they request otherwise. Participants can also request withdrawal of their data or samples at any time after completion of the study by contacting the Chief Investigator; any data or samples that have been analysed or reported at the time of request will not be able to be retracted.

The main ethical considerations specific to the study protocol are in relation to the consumption of the study beverages and collection of blood samples at each study visit.

In relation to the study beverages, the WP and FB are both commercially available in the UK. The brand purchased will be Pulsin (www.pulsin.co.uk) which is produced to high safety and quality standards and is stocked by many of the UK main supermarkets and other major retailers. It is very unlikely that a participant would experience any undesirable effects from consuming these protein drinks, however, we will ask participants to rate the palatability of the beverages and will make it clear to participants that they can withdraw from the study at any time should they not wish to continue.

In relation to the blood samples, in total 60mls will be collected over a 2.5 hour period at each visit. If a participant completes all 4 visits, that means providing 240mls in total over a minimum of a one month period. This is well below the volume of blood collected during an NHS blood donation (450ml). As multiple blood samples will be collected at each visit, a trained phlebotomist at the NI Clinical Research Facility will insert a cannula at the start of each visit which will make collection of samples thereafter painless. Participants will be informed about the risk of minor discomfort and bruising associated with the insertion of the cannula and collection of blood.

Participants will present for each study visit in the morning after an overnight fast. They will consume the protein beverage during their visit, and we will also provide them with a cereal bar and a carton of juice at the end of the visit to ensure they are not excessively hungry before they leave.

CONFIDENTIALITY AND DATA STORAGE

The study participant information sheet describes how we will look after the data provided by participants and signposts to the QUB research privacy notice for research participants - www.qub.ac.uk/privacynotice/Research/ListofResearchPrivacyNotices/PrivacyNoticeforResearchParticipants

We will ensure that participant confidentiality is maintained throughout the study. When a participant consents to join the trial, they will be allocated a unique participant study ID which will be used to pseudonymise sensitive personal data collected during study visits with the key held by the research team in a password protected file.

Electronic data will be stored on the QUB OneDrive cloud storage which requires QUB password protected login and multifactor authentication to access and will only be accessible to the research

team. Paper based data will be stored in locked cupboards in locked offices in QUB buildings that have keypad entry and are alarmed outside of normal working hours. Identifiable information (e.g. consent forms and contact details) will be stored separately from other data. Participants will not be identifiable from any published report from the study.

Any personal communication with people who are interested in taking part in the study but are identified as ineligible or are eligible to participate but do not proceed to provide their consent, will be deleted (e.g. email communication) or shredded and disposed of as confidential waste for any paper based records.

Data for consenting participants will be stored, as described above, for 5 years until the research has been completed and after final publication of study results. Data access will be limited to only individuals necessary for quality control, audit and analysis. After this time, documents that contain personal data will then be shredded and disposed of as confidential waste or deleted in the case of electronic records.

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