Study Protocol

Full Title:	Time-restricted eating (TRE) and meal frequency to influence appetite control in adults with overweight or obesity: a randomized crossover trial
Study Acronym:	Time restricted eating and meal frequency for appetite control
Sponsor:	The University of Aberdeen
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Chief Investigator:	Professor Alexandra Johnstone Co-applicants Ms Nouf Alkhattabi and Professor Justin Rochford
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Protocol Approval

Time-restricted eating (TRE) and meal frequency to influence appetite control in adults with overweight or obesity: a randomized crossover trial

Signatures

By signing this document I am confirming that I have read, understood and approve the protocol for the above study.



ALEXANDRA JOHNSTONE		28th October 2022
Chief Investigator	Signature	Date

List of Abbreviations

2MPD	2 meals per day intervention diet
5MPD	5 meals per day intervention diet
BIA	Bio-impedance Analysis
BMI	Body Mass Index
BPAQ	Baecke Physical Activity Questionnaire
CGMS	Continuous Glucose Monitoring System
CI	Chief Investigator
EE	Energy Expenditure
ESQ	Epworth Sleepiness Questionnaire
FFM	Fat-Free Mass
FM	Fat Mass
GCP	Good Clinical Practice
HISU	Human Intervention Studies Unit
HP	High-protein diet
IF	Intermittent Fasting
MT	Maintenance Diet
OGTT	Oral Glucose Tolerance Test
PI	Principal Investigator
PIS	Participants Information Sheets
RCT	Randomized Crossover Trial
REC	Research Ethics Committee
RMR	Resting Metabolic Rate
SOP	Standard Operating Procedure
T2D	Type 2 diabetes
TBF	Total Body Fat
TEF	Thermic Effect of Food
TRE	Time-restricted eating
VAS	Visual Analog Scales

Summary

<table>
<tr><td>Trial Title</td><td colspan="2">Time-restricted eating (TRE) and meal frequency to influence appetite control in adults with overweight or obesity: a randomized crossover trial</td></tr>
<tr><td>Internal ref. no. (or short title)</td><td colspan="2">Study 820</td></tr>
<tr><td>Trial Design</td><td colspan="2">Randomized Crossover Trial (RCT)</td></tr>
<tr><td>Trial Participants</td><td colspan="2">Male and female adults aged 18-65 years</td></tr>
<tr><td>Planned Sample Size</td><td colspan="2">10 males and females classified as overweight and obese with BMI 25+ kg/m^2</td></tr>
<tr><td>Treatment duration</td><td colspan="2"><u>20 days total + 1 extra day for the last test day (21st day):</u>
12 days dietary period (all meals provided):
• Maintenance diet 1 (MT1) for 2 days, fed to energy balance
• Intervention diet 1 (2MPD, 2 meals/day or 5MPD, 5 meals/day) for 4 days
• Maintenance diet 2 (MT2) for 2 days
• Intervention diet 2 (5MPD, 5 meals/day or 2MPD, 2 meals/day) for 4 days
8 day washout period (no meals provided): between Intervention diet 1 and Maintenance diet 2
1 test day on the last day of the study</td></tr>
<tr><td></td><td>Objectives</td><td>Outcome Measures</td></tr>
<tr><td>Primary</td><td>- Does eating frequency influence appetite, in adults with overweight or obesity?</td><td>Participant appetite response measured with visual analogue scales (VAS).</td></tr>
<tr><td>Secondary</td><td>- Does eating frequency affect bio-markers of health such as glucose sensitivity and insulin response in adults with overweight or obesity?

- Does meal frequency affect basal (RMR) and postprandial (TEF) energy metabolism?

- Assess the impact of diet on physical activity

- Assess the impact of diet on sleep

- Assess body composition change</td><td>- Application of continuous glucose monitoring (CGMS), blood samples for oral glucose tolerance test (OGTT) at times 0 and 180 mins after a test meal

- Measure resting metabolic rate (RMR) before a test meal and the thermic effect of food (TEF) for 180mins after the test meal.

- Application of Baecke Physical Activity Questionnaire (BPAQ) and measure activity using an accelerometer (Actigraphy).

- Application of Epworth sleepiness questionnaire (ESQ) and three, 7day sleep diaries, (Actigraphy).

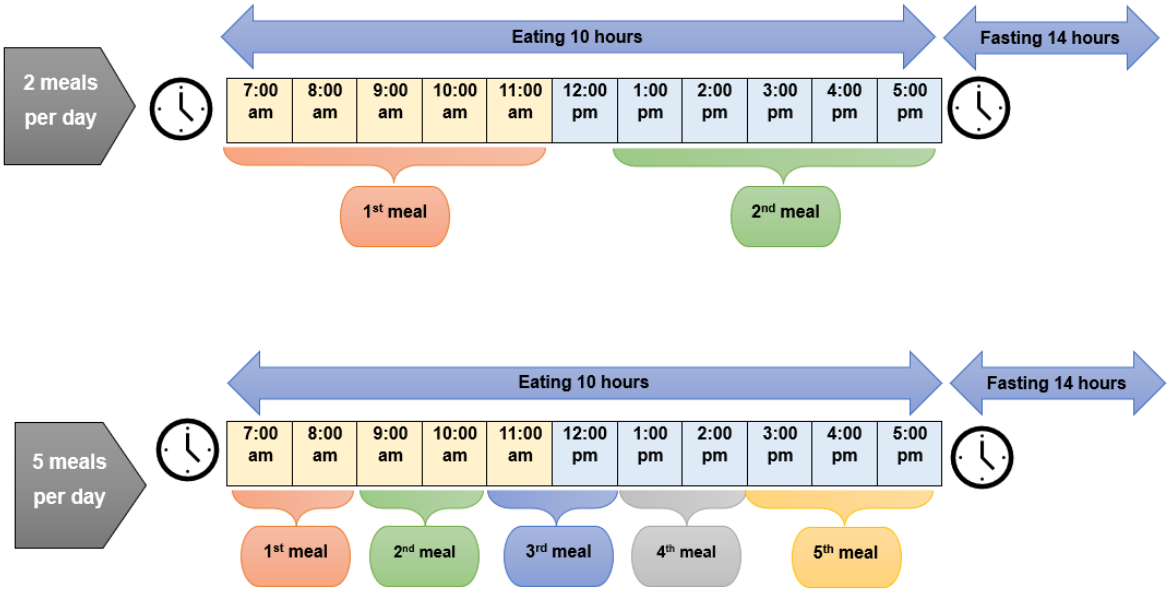
-measure body mass (kg); waist and hip circumference; fat mass and fat-free mass by bio-impedance analysis (BIA)</td></tr>
<tr><td>Diets</td><td colspan="2">• Maintenance diet 1 (MT1), fed to energy balance, for 2 days
• Intervention diet 1 (2MPD, 2 meals/day or 5MPD, 5 meals/day), fed to 100% RMR, for 4 days
• Washout period (no meals provided) for 8 days
• Maintenance diet 2 (MT2), fed to energy balance, for 2 days
• Intervention diet 2 (5MPD, 5 meals/day or 2MPD, 2 meals/day), fed to 100% RMR, for 4 days</td></tr>
</table>

1. Introduction

1.1. Background

Obesity is one of the biggest worldwide health problems (Windisch and Andrade, 2017). Increasing rates of overweight and obesity in recent years, with the percentage of overweight and obesity among adults exceeding 1.9 billion and 650 million, respectively (Sharma and Sharma, 2020). Overweight and obesity have been associated with an increase in the risk of type 2 diabetes (T2D), coronary heart disease, hypertension and other non-communicable diseases. There are multiple nutritional causes for obesity, including both the type and amount of food consumed. Other non-nutritional factors can be just as important to influence body mass, such as the number of meals eaten (Sjöholm, 2017), which is termed meal frequency. The changing food environment influences calorie consumption, such as increased food variety, increased availability and food processing, a nocturnal lifestyle that disrupts the circadian clock, meal timing and frequency (Zerón-Rugerio et al., 2021), irregular meal patterns, and prolonged duration of eating during a day (Flanagan et al., 2021). This results in increasing foods and beverage consumption throughout the day (Ha and Song, 2019). Meal frequency is commonly defined as, 'the number of foods or drinks a person consumes in one day' (Sjöholm, 2017; Zimmerman et al., 2018). Meals and snacks are less clearly defined (Zerón-Rugerio et al., 2021).

Meal is defined as any food providing≥ 50 kcal and separated by more than one hour (Bachman et al., 2011). In most cultures, the name meal is given to the three eating episodes in a day: breakfast, lunch, and dinner (Berg and Forslund, 2015); and breakfast, brunch, lunch, supper, and dinner (Murakami and Livingstone, 2016). In this study, we will use the definition of (Murakami and Livingstone, 2016), but it will be on restricted time: 10 hours eating and 14 hours fasting (from 7:00 to 17:00) and one type of diet (high protein diet). Others defined meals by the time of the day, such as early and late breakfast, lunch and dinner intake (Garaulet et al., 2013; Leech et al., 2015). These definitions differ according to culture (Leech et al., 2015; Murakami and Livingstone, 2016). Others defined meals based on timing, such as (6:00 am –10:00 am), (12:00 pm – 3:00 pm), and (6:00 pm – 9:00 pm) (Leech et al., 2015; Murakami and Livingstone, 2016). In this study, the definition (Garaulet et al., 2013; Leech et al., 2015) will be used, but the time of meals will be modified to comply with the nature of this project as:



Some studies have highlighted that there are factors that affect the number of meals which a person consumes.

	• Eating a high amount of energy during breakfast helps to feel full for a longer period (Paoli et al., 2019). • Time of eating and duration of eating or not eating (Jones et al., 2020; M. Correia et al., 2020). • The type of macronutrient intake; for example, high protein (HP) intake reduces ad libitum energy intake. The number of meals eaten per day; some studies have confirmed that consuming small frequent meals is better for health development such as appetite control in general population compared to consuming one huge meal a day (Speechly et al., 1999; Speechly and Buffenstein, 1999). This can assist to control appetite and plasma glucose concentrations (Ohkawara et al., 2013). On the other hand, recent research has indicated that consuming (≥6 meals/day) increases disease risk significantly more than consuming (1–2 meals/day). Some researchers believe that there is no relationship between meal frequency and weight (Paoli et al., 2019).
1.2.	**Rationale for Study**
	Rationale for Study **Research Hypothesis:** To investigate if meal frequency (2 or 5 meals a day) in a controlled time restricted eating regime can influence appetite and glycaemic control in adults with overweight or obesity. **Research Question:** Does meal frequency as 2 or 5 meals a day, impact on participant appetite and glucose excursion in adults with overweight or obesity?
2.	**Study Objectives**
2.1.	**Objectives**
	Explore the efficacy and physiological responses to a controlled time restricted eating regime in a human intervention study focused on food intake, energy balance, appetite control and glycaemic response.
2.2.	**Outcomes**
2.2.1.	**Primary Outcome**
	Comparing the influence of dietary interventions (2 or 5 meals per day) on the participant appetite response, measured with visual analogue scales (VAS). - on test days recorded at timepoints 0, 30, 60, 90, 120, 150 and 180 mins. - on all other study days recorded hourly during waking hours.
2.2.2.	**Secondary Outcomes**
	Comparing the influence of dietary interventions (2 or 5 meals per day) on the following: - Plasma fasting blood glucose and 3 hours post-prandially after a standardized test meal. Analysed by KONE for timepoints 0 and 180 mins. - Plasma fasting blood insulin and 3 hours post-prandially after a standardized test meal. Analysed by ELISA for timepoints 0 and 180 mins. - Continuous glucose monitoring System (CGMS) on all study days to assess free living glycaemic control in response to diet. - All components of energy expenditure and substrate utilization are monitored by ventilated hood (indirect calorimetry) as resting metabolic rate (RMR, 30 minutes before eating breakfast); thermic effect of food (TEF post prandial 3 hours, measured 10 minutes every 30 minutes by ventilated hood). - To assess changes in physical activity with accelerometry on all study days and the Baecke Physical Activity Questionnaire (BPAQ) completed on each test day. - To assess if meal frequency influences sleep using a sleep diary completed on all study days and the Epworth Sleepiness questionnaire (ESQ) completed on each test day. - To assess if meal frequency influences body mass and body composition (fasted body weight measured on all food collection and test days. Fasted waist and hip circumferences and fat mass by bio-impedance analysis (BIA) measured on all test days).

3. Study Design

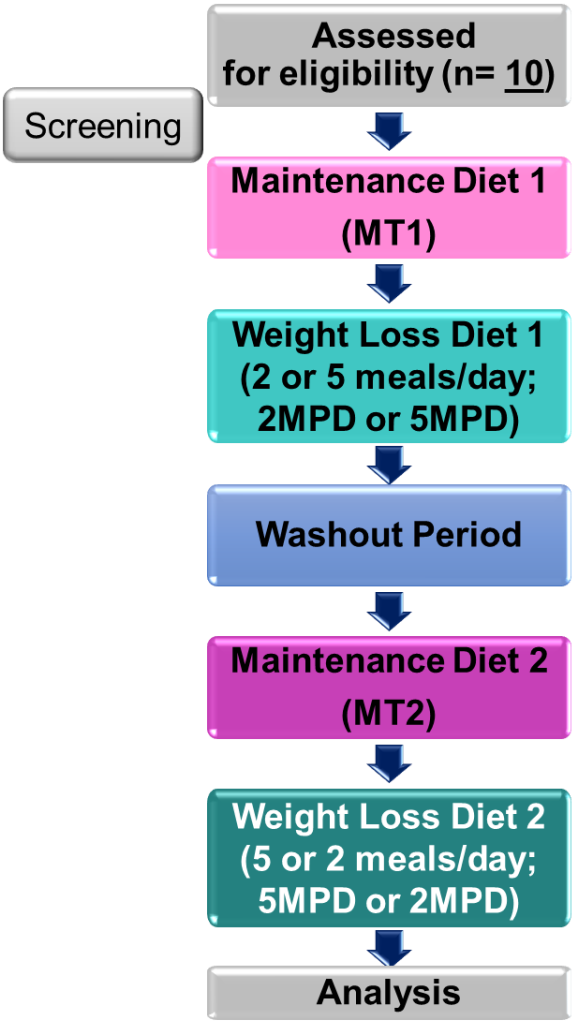
3.1. Study Description

A randomized crossover trial (RCT). Each participant will complete a 21 day study design as follows:

- 7 days food diary (recording at home, habitual diet) [Days -6 to 0]
- 2 days maintenance diet 1 (MT1). [Days 1-2]
- **Test Day on Day 3**
- 4 days intervention weight loss diet (2MPD, 2 meals/day or 5MPD, 5 meals/day). [Days 3-6]
- **Test Day on Day 7**
- 8 days washout period, no meals provided. [Days 7-14]
- 2 days maintenance diet 2 (MT2). [Days 15-16]
- **Test Day on Day 17**
- 4 days intervention weight loss diet (5MPD, 5 meals/day or 2MPD, 2 meals/day). [Days 17-20]
- **Test Day on Day 21**

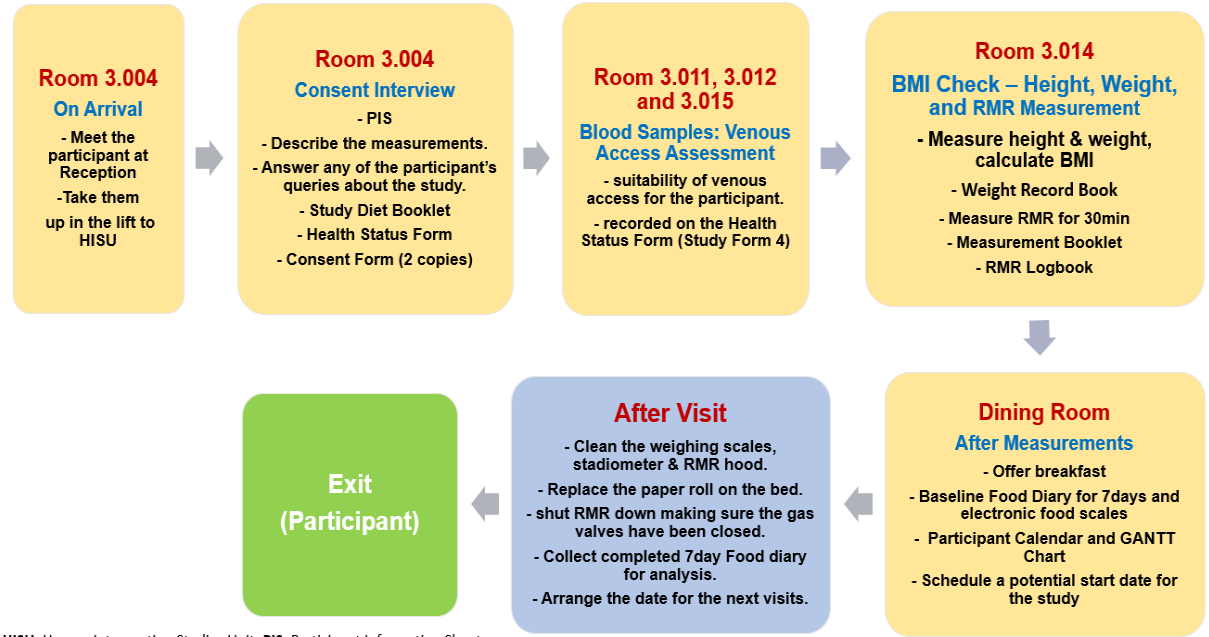
Protocol Diagrams for the study and the test days can be found in **Appendix 2** and **3** at the end of this document.

3.1.1. Study Flowchart



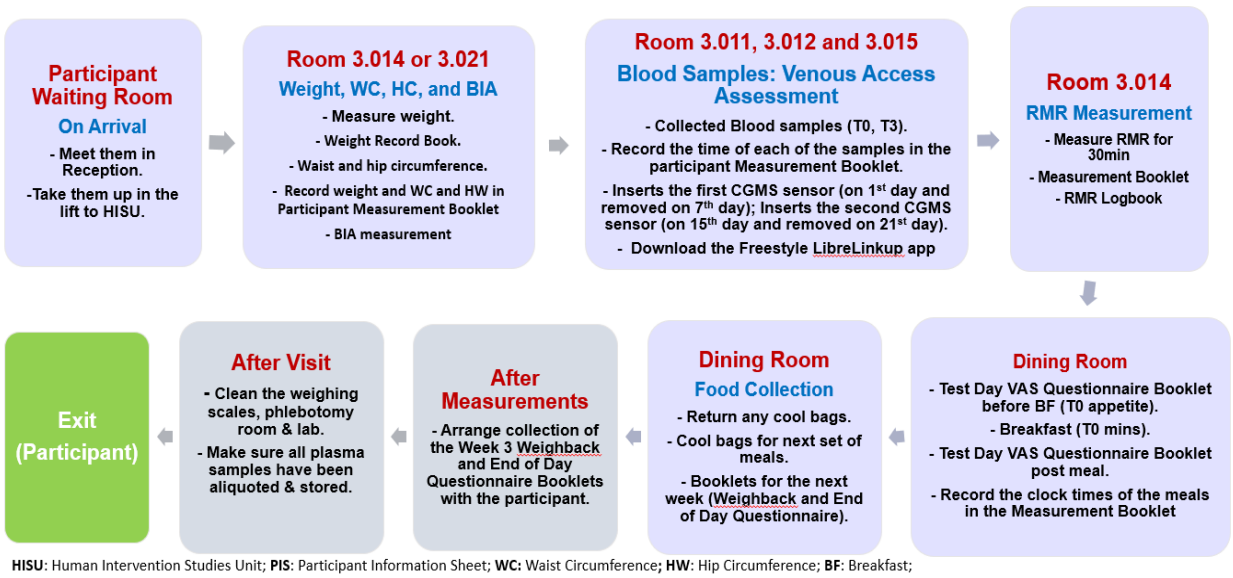
Procedures: As described in the three below-proposed flows of study site activities

Proposed flow of study site activities (screening visit)



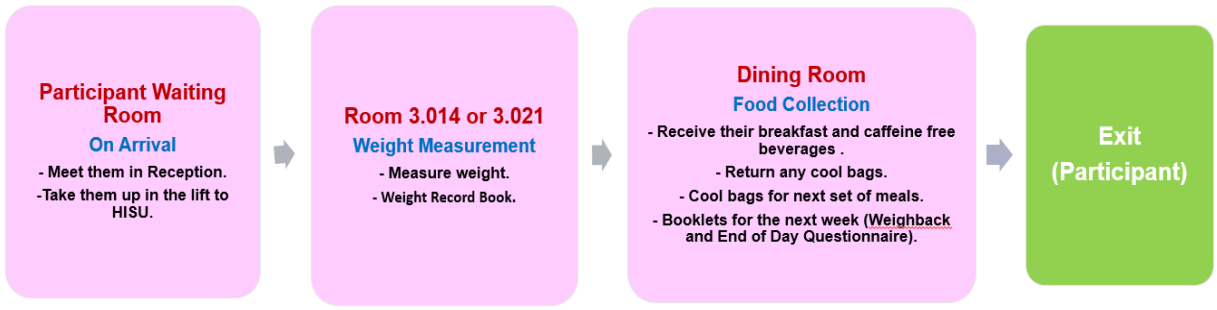
HISU: Human Intervention Studies Unit; **PIS**: Participant Information Sheet;

Proposed flow of procedures for the (Test Day Visit)



HISU: Human Intervention Studies Unit; **PIS**: Participant Information Sheet; **WC:** Waist Circumference; **HW**: Hip Circumference; **BF**: Breakfast;

Proposed flow of procedures for the (Study Food Collection Days)



HISU: Human Intervention Studies Unit

3.2.	**Study Diets**
	In the screening visit, RMR will be measured for each participant, and based on the results, each participant will receive a different meal portion, depending upon their RMR results. All food will be prepared at the Human Intervention Studies Unit (HISU), Rowett Institute. This study will include two types of diet: • a maintenance diet (MT, 15:30:55% of energy from protein, fat and carbohydrate respectively). On days 1-2 (MT1) and 15-16 (MT2) this diet will be fed to meet measured energy requirements (1.5 x Resting Metabolic Rate, RMR). • a high protein (HP) intervention weight loss diet (WL, 30:35:35% of energy from protein, fat and carbohydrate respectively). On days 3-6 (WL1) and 17-20 (WL2) this will be fed to 100% resting metabolic rate. The food intake during the intervention periods will be randomized for two patterns fed between 07:00 to 17:00; five small meals per day (5MPD) and two large meals per day (2MPD). The protein in the diets will come from different sources like meat, poultry, fish, soy, and beans. In addition, all meals on the diets will contain equal proportions of protein so that the daily protein is not all consumed in one meal.
3.3.	**Study Measurements**
	In total there will be nine study visits to the Human Intervention Studies Unit (HISU), Rowett Institute: one screening visit, which last approximately 2 hours, 4 visits for Test Days representing pre and post dietary phases (day 3rd, 7th, 17th, and 21st), which last approximately 4-5 hours, and 6 visits to collect food (day 1st, 3rd, 6th, 15th, 17th, and 20th), two of them are at the same day of test, which last approximately 30 mins except 2 visits will be 4-5 hours because it is the day of Test Days too. All visits details are described in the protocol (appendix 2). Researcher(s) carrying out the procedure: The study will be co-ordinated by a Research Team, consisting of Professor Alexandra Johnstone (PI), Prof Justin Rochford (CI), Ms Nouf Alkhattabi (PhD student), Claire Fyfe (Research Technician), David Bremner (Research Assistant), Claire Kidd (Research Assistant), Christina Petkov (Honorary Research Nurse).
3.3.1.	**Screening Visit**
	The participant should already have a copy of the PIS in advance of the consent and screening visit should already have a good understanding of the study and have sufficient time to read it before this visit. After discussion, the consent should always be obtained BEFORE the health status and eligibility. *n*=10 to complete All measurements will be conducted after a fast from 10pm the night before. Breakfast will be provided at the end of the screening visit. Screening will continue until study completion and involves • Completing written, informed consent paperwork (PIS and consent form) • A self-report general health screening questionnaire • RMR measurement for calculation of energy requirements (ventilated hood) • Height and weight measurements to calculate body mass index (BMI) The participants will then be given a 7-day weighed intake food diary and scales to take away with them to complete to establish their regular eating habits before the start of diet provision.
3.3.2.	**Test Day Visits** (on Days 3, 7, 17 and 21)

	All measurements will be conducted after a fast from 10pm the night before. On days 3 and 17 meals will be provided as breakfast, lunch, dinner, and snacks according to the intervention diet randomisation (2MPD or 5MPD), while on days 7 and 21 only breakfast will be provided.
	
	• Overnight fasted anthropometric measures
	  ○ Body weight and body mass index (BMI) calculation
	  ○ Total body fat (TBF), fat mass (FM) and fat-free mass (FFM) by bio-impedance analysis (BIA)
	  ○ Waist and hip circumferences measured using a tape measure.
	  ○ Resting energy expenditure (RMR) will be measured for 30mins in fasting state by indirect calorimetry.
	
	• The blood samples will be collected by multiple venepuncture (T0 and T180mins).
	• Venepuncture for fasted blood sampling (one 4ml LiHep tube) (Maha H Alhussain et al., 2022):
	  ○ T0mins Glucose (1x500µl plasma in KONE cup)
	  ○ T0mins Insulin (1x300µl plasma in 0.5ml Eppendorf)
	  ○ T0mins LiHep Spare (2x300µl plasma in 1.5ml Eppendorf)
	
	The total volume of blood taken will be as follows:
	  ○ In each visit, 8ml will be taken for each participant.
	  ○ The total blood taken for 4 visits will be 32ml for each participant.
	
	• Measure of fasted participant appetite by Visual Analogue Scales (VAS) at T0mins (Anderson et al., 2002).
	• Consumption of intervention breakfast meal at T0
	
	• After the test meal the thermic effect of food (TEF) will be calculated from indirect calorimetry measurements taken for the last 10 min of every 30 min up to 3 hours (T20, T50, T80, T110, T140 and T170mins) (Maha H Alhussain et al., 2022; Smeets and Westerterp-Plantenga, 2008).
	
	• Questionnaires
	  ○ A validated self-report questionnaire for physical activity Baecke Physical Activity Questionnaire (BPAQ) and the sedentary is categorized as, low, medium, or high as referred to in (Oyeyemi et al., 2016) study.
	  ○ Assessment of how meal frequency influences sleep using the Epworth Sleepiness questionnaire (ESQ)
	  ○ Post-prandial measurement of participant appetite by VAS at T30, T60, T90, T120, T150 and T180mins.
	
	• Venepuncture for postprandial blood sampling (one 4ml LiHep tube) (Maha H Alhussain et al., 2022):
	  ○ T180mins Glucose (1x500µl plasma in 0.5ml KONE cup)
	  ○ T180mins Insulin (1x300µl plasma in 0.5ml Eppendorf)
	  ○ T180mins LiHep Spare (2x300µl plasma in 1.5ml Eppendorf)
	
	• Food diary recorded for the remainder of the day

3.3.3.	**Other Measurements**
	• Continuous Glucose Monitoring System (CGMS) throughout the 3week study (two, 2week insertions per participant*) using Freestyle Libre™ devices. * sensors record data for 14days at a time. Freestyle Libre sensor: water resistant sensor (in up to 1m water for a maximum of 30mins) that continuously measures the glucose concentration in interstitial fluid, and stores 8 hours of data. Can be scanned by any mobile device capable of downloading the FreeStyle LibreLinkUp app. LibreLinkUp is compatible with Android and iOS smartphones which meet the following requirements: Android OS 6.0 (or higher) or iOS 11 (or higher) and an internet connection. Participants without a smartphone capable of using the app will be provided with a budget smartphone. Each sensor has a working life of 2 weeks. Participants will be supplied with the required number of sensors in order to complete the study. The FreeStyle Libre sensor is

	applied on to the back of the upper arm with a simple, disposable device called an applicator. When the sensor is applied, a thin, flexible and sterile fibre is inserted just under the skin. It is held in place with a small adhesive pad. In a study conducted by Abbott, users agreed that inserting the sensor was no more painful than a typical finger prick test. To obtain a glucose reading from this device, participants must perform a quick, painless 1-second scan of the reader over the sensor. This scan gives you more information than monitoring with blood glucose test strips, without the need for routine finger pricks. Each scan of the reader over the sensor gives a current glucose reading, the last 8-hours of glucose history, and a trend arrow showing if glucose is going up, down, or changing slowly. The reader can scan through clothing. • Temporal changes in appetite using VAS questionnaires throughout the 2 intervention phases of the study. Questionnaires completed hourly during waking hours. • Composition of all meals consumed during the 3weeks study. Recorded by participants using weighback sheets and the food scales provided at the screening visit. • Physical activity levels recorded by Actigraph accelerometers. The actigraph provides data for energy expenditure per day, the intensity of the activity carried out taken when the monitor was worn. • Assessment of how meal frequency influences sleep. Hours slept will be calculated from the data recorded by the Actigraph accelerometers and from two, 7day sleep diaries with questions the participants will answer every morning of the intervention phases.
4.	**Study Population**
4.1.	**Number of Participants**
	10 adults (males and females) who have expressed an interest in taking part and meet the inclusion criteria. Should there be any withdrawals, we will continue to replace participants until 10 have completed the trial.
4.2.	**Inclusion Criteria**
	• Adult (males and females), age 18-65 years • Who are healthy but overweight or obese (BMI 25+ kg/m^2) • With no history of kidney disease or bariatric surgery • Not following any specific type of medical or religious diet • Not taking any drugs that affect the rate of physical activity or metabolic circulating • Not pregnant or breastfeeding. • With a fluent understanding of the English language We will recruit ages 18 to 65 because those under 18 are considered adolescents, and those over 65 are considered old. We will recruit adults ages 18 to 65 as a similar age group used in the previous studies (Hampl et al., 2003; Zimmerman et al., 2018; O'Connor et al., 2022) and because the use of certain medications or occurrence of certain diseases which are in our exclusion criteria are more common above age of 65 (Chen et al., 2001; Onder et al., 2016). In other work (unpublished survey data), the inclusion age was 18 years and above. Survey results showed a significant effect on BMI among adults (18 years and above), and a systematic review, also included the age (18 years and above). Finally, previous studies have examined the effect of meal frequency and meal timing in similar age groups and obtained statistically relevant outcomes (Maha H. Alhussain et al., 2022; Perrigue et al., 2016; Ruddick-Collins et al., 2022). 1. A study including 12 participants aged 18-50 years and BMI ≥18kg/m^2 found that low eating frequency recorded a significant hunger score compared to the high EF eating frequency ($P < 0.001$). The desire to eat decreased significantly in low EF than in high EF

	(P < 0.001). The mean fullness was significantly higher after the higher meal compared with low meal frequency (P < 0.05) (Perrigue et al., 2016). 2. A study including 9 participants aged 18-45 years and BMI 28-40 kg/m2 found that TEF was significantly lower after the irregular meal patterns (3-9 meals/d) than regular (6 meals/d). Additionally, the GLP-1 was significantly higher after the irregular meal than the regular one (Maha H. Alhussain et al., 2022). 3. A study including 14 participants aged 20-60 years and a BMI of 31.7±3.0 kg/m2 found that TEF was significantly affected by meal timing, which was greater in the morning (Ruddick-Collins et al., 2022).
4.3.	**Exclusion Criteria**
	Potential volunteers will be asked to fill in a health questionnaire to assess their suitability for the study. This information will allow us to exclude and participant with: • Anyone with a BMI (in kg/m^2) under 25 • Anyone under 18 years or above 65 years. Medication exclusion criteria: • Anyone taking the statins (current), aspirin or anti-coagulants (current). Medical exclusion criteria: • Anyone with chronic inflammatory disorders such as rheumatoid arthritis or inflammatory bowel disease • Anyone with cardiovascular disease • Anyone with diabetes • Anyone who is are planning to be pregnant, are pregnant or breastfeeding • Anyone with food allergy, self-reported food sensitivity or intolerance • Anyone with coeliac disease or gluten intolerance • Anyone with a gastrointestinal disorder, kidney disease, liver disease or gout • Anyone taking medication which may affect appetite or circadian rhythm. • Anyone with an eating disorder • Anyone suffering from unregulated thyroid disease. • Anyone using Beta Blockers which can affect the rate of physical activity. • Anyone using some anti-depressants such as mirtazapine can affect the appetite. Other exclusion criteria: • Anyone following a vegetarian or vegan diet • Anyone following a weight loss programme (that may affect lifestyle, physical activity & diet) • Anyone with unsuitable veins for blood sampling • Anyone who is unable to fluently speak, read and understand English • Anyone who is unable to comply with an alcohol-free diet for 3 weeks • Anyone consumption of nutrition supplements • Anyone has the intention to donate blood within 2 months of the study. • Anyone intending to travel away from Aberdeen during the intervention phase. • Anyone who is a shifts worker. • Anyone smoking or e-cigs or vaping status. • Anyone recently participated in any other research studies during the last 4 weeks.
5.	**Participant Selection and Enrolment**
5.1.	**Identifying Participants**
	Recruitment of volunteers will be achieved by advertising the study on the University/Rowett Institute website, on social media, in the local press, on a public poster (to be displayed in the local Aberdeen area in libraries, community centres, gyms, shops and on the University Campus).

	We will also contact volunteers from previous studies who have consented specifically that they are willing to take part in future human intervention studies.
5.2.	**Consenting Participants**
	When they attend the HISU for their first visit they will meet a member of the Research Team who is trained in taking informed consent. This person will ensure the participant understands what the study entails and answer any queries they have. If the participant is happy to proceed, then they and the researcher will sign & date two copies of the study Consent Form. The participant will keep one copy of this form and the researcher the other.
5.3.	**Screening for Eligibility**
	Participants will be deemed eligible if (i) their health and wellbeing is not at risk by undertaking the study and (ii) they are on any medication or have any health condition or which will impact study outcomes. Only eligible volunteers based on meeting the inclusion and exclusion criteria (detailed in section four above) will be recruited for this study up to the required number (*n*=10). Once this is achieved, additional people enquiring about the study will be thanked for their interest and no data will be collected. The participant will have sufficient time to read the PIS and consent to take part or not for about a week. Participants who express an interest and attend the health screening will be required to sign the consent form before undertaking any of the screening measures. Participants who are deemed ineligible during the screening will be thanked for their interest and provided with their health screening results which will be kept for audit purposes. Ineligible participants will be replaced to ensure 10 participants are recruited to complete the study.
6.	**Randomization and Blinding**
6.1.	**Randomization Details**
	The treatment order for each participant number will be randomly generated by computer. Following the health screening and confirmation of eligibility each participant will then be assigned their individual participant number and relevant treatment order.
6.2.	**Blinding**
	The study will be conducted in a non-blinded method with diets colour coded.
6.3.	**Withdrawal Procedures**
	If a participant wishes to withdraw from the study at any point after recruitment, any data already collected with consent will be retained and used in the study. No further data will be collected, or any other research procedures carried out on or in relation to the participant after they withdraw. Unless the participant specifically withdraws consent to use any data already collected, all data to the date of withdrawal will be retained and used for analysis purposes. The anonymised data will be made available to other research teams. Details of participants information will not be published. We cannot be withdrawn after publication because we use anonymous data for publication. The withdrawal form will be completed and added to the participant's record.
7.	**Study and Safety Assessments**
	The risks associated with participation in this project are minimal. Minor risks are associated with the collection of blood samples. Blood Collection: Blood collection will be performed by trained and experienced phlebotomists or research nurses. The multiple venepuncture (T0 and T180mins) blood collection is not associated with risks greater than that associated with a routine blood test. There may be mild discomfort and a small amount of localised bruising as a result of blood collection and some participants may feel lightheaded or faint, but no more than is experienced with a routine blood test. Each member of the research team also has extensive experience in blood collection and handling. A phlebotomy checklist will be completed with each blood test to confirm any previous problems with blood tests recent blood donations and current illness to minimise risks.

The fitting of the Freestyle Libre continuous glucose monitor involves a minor prick of the skin. Participant will be asked to wear the continuous glucose monitor for up to 2 weeks at a time. When wearing the continuous glucose monitor participant should avoid wearing tight clothing on their arms as doing so may pull off the sensor. Showering/bathing: The sensor is water resistant, but participant should use extra care when cleansing around the sensor and when towelling off so that they do not catch or pull off the sensor.

Any medical values which fall outside of normal ranges will be reported to the Clinical Research Nurse and PI who will investigate and action accordingly. Only Serious Adverse Events and potentially repeated AE's are reported to the Sponsor. All measurements will be undertaken by trained research staff in accordance with authorised SOPS and nursing staff are on site to maintain participant health and safety. All adverse events will also be reported to the HISU unit manager and Sponsor.

8. Data Collection and Management

All data will be collected by the research team and bloods collected by the phlebotomists or research nurse. Data will be collected using validated questionnaires and biological samples (blood). Measures of human physiology will be collected with calibrated research equipment. The research team are trained in the appropriate data collection methods and data collection will be completed according to authorised SOPS.

The data will be collected and used during the project from:

1. **Questionnaires:**
    - Study Form 1 - General Information
    - Study Form 4 - Health Status
    - Test Day Questionnaires (Epworth Sleepiness Scale and Baecke Physical Activity Questionnaire)
    - Baseline 7d Food Diary
2. **Biological samples (blood):**
    - Glucose and insulin at T0 and T180hrs
3. **Measures of human physiology:**
    - Measurement Booklet for weight and height to calculate the BMI
    - Measurement Booklet for RMR and TEF
    - Measurement Info Actigraphy
    - Measurement Info tape
    - Measurement Info Sheet for bio-impedance analysis (BIA)
    - Measurement Info Sheet for continuous glucose monitoring (CGMS)
    - Measurement booklet for appetite visual analogue scales (VAS) and sleep Diary.

Participants will link the Freestyle Libre sensor with the FreeStyle LibreLink app which will also allow researchers to harvest real-time glycaemic data. This data will be stored electronically.

Data analyses will be reviewed by independent Statistician, Dr Graham Horgan from BioSS. The entire Research Team will be responsible for data analysis.

All data will be stored in anonymised form in locked cabinets at the Human Intervention Studies Unit (HISU), Rowett Institute or on secure shared drives accessible by the research team. Biological samples will be stored in secured -70°C degree freezers until analysis. Data will be identifiable only by the research team in order to link health outcomes and samples to participant. All electronic data being held indefinitely is anonymised or pseudonymised will be stored “ad infinitum” on the University of Aberdeen central server. Archiving will only occur once all relevant paper data has been published. It is expected that paper data will be archived after 10 years following publication of the project; in line with the institutional retention schedule (AKAF data).

9.	**Labs and Samples Analysis**
9.1.	On each test day blood samples will be collected by a trained phlebotomist or research nurse into 2 separate LiHep vacutainer tubes (T0 and T180 mins). Blood samples will immediately be spun in the centrifuge and plasma aliquots placed into Kone Cups (for glucose analysis) and Eppendorf tubes (for insulin analysis). All samples will be collected and stored in anonymised forms. Plasma samples will be stored in a -70°C degree freezer until analysis. All freezers are monitored by University of Aberdeen staff. All laboratory analysis of blood samples will be undertaken at the Rowett Institute by trained laboratory technicians in the analytical laboratories. Samples will be stored until the study has been completed and then for further 5 years to allow for potential additional analysis in future ethically reviewed and approved studies. After this period, all samples will be destroyed (under PI responsibility) or transferred to a bio-repository to allow for possible future use of these samples in ethically approved studies.
10.	**Statistics and Data Analysis**
10.1.	**Sample Size Calculation**
	The sample size in this study will be 10 participants and the reasons for justifying a sample size of 10 are (1) time of the intervention diet, (2) we will be looking for change in appetite ratings, blood glucose and insulin levels; these measurements can be affected by the intervention diet in a short time as in (Speechly and Buffenstein, 1999), (3) and most of the previous studies included from 8-12 participants (Speechly and Buffenstein, 1999; St-Onge et al., 2019). The main end point is differences in the participant appetite response by the intervention diet and time restriction feeding (TRF) including (5 or 2 meals/day). A previous study by (Ravussin et al., 2019) showed that the mean desire to eat had declined by 5±2 mm ($P = 0.08$) and the mean fullness had increased by 3±2 mm ($P = 0.06$) with eTRF using a visual analogue scale (VAS) score. In our study, we expect that the SD of increased fullness will be ≥ 3±2 mm ($P = 0.06$) and hunger and desire to eat will be decreased by ≥ 5±3 mm ($P = 0.08$) as reported before. In addition, using the G*power software with the 80% power and a 0.05 significance level will give a standardised effect size of 0.996 on fullness, which will be able to detect with 10 volunteers.
10.2.	**Missing Data**
	Data analysis will be performed using linear mixed models which enables the inclusion of all participants within the analysis even with missing time points. When entering data into excel spreadsheets, missing data will be left blank. Blank cells will be checked by statistician Dr Horgan during statistical analysis to confirm the data as uncollected/ erroneous as opposed to incorrectly transcribed. The loss of data will be minimised by immediate saving of results on test days and storage of data on the University central server which is backed up daily. Consistent reminders to participants of study protocol, adherence to SOPs and calibration of equipment will be used to minimise the chances of errors.
11.	**Study Management and Oversight Arrangements**
11.1.	**Study Management Group**
	The study will be co-ordinated by a Research Team, consisting of Professor Alexandra Johnstone (PI), Prof Justin Rochford (CI), Ms Nouf Alkhattabi (PhD student) and Claire Fyfe (Research Technician). Statistical analysis was carried out by Nouf Alkhattabi with the advice from Dr Graham Horgan and Dr Nicosha De Souza (BioSS). Regular meetings will be held with the team to ensure consistent communication between all parties. The PI and Rowett Institute shall permit study related monitoring, audits, and review. The PI agrees to allow the Sponsor or, representatives of the Sponsor, direct access to all study records and source documentation if required.

11.2.	**Study Management**
	This remains the overall responsibility of the PI. Any queries will be resolved by the PI or a delegated member of the Research Team. The study has a trial master file within the secure Rowett Shared Drive with versions of the Participant Information Sheet, consent, protocol, questionnaires, Ethical and HSMC approvals. A study-specific Delegation Log will be prepared, detailing the responsibilities of each member of staff working on the study.
12.	**Good Clinical Practice**
12.1.	**Ethical Conduct of the Study**
	The study will be conducted in accordance with the principles of good research practice (GRP) or good clinical practice (GCP). All research staff have GRP or GCP certificate that is up-to-date. Prior to commencement of the study a favourable Human Studies Management Committee (HSMC) opinion will be obtained, and approval from the Rowett Ethics Panel (REP) and University of Aberdeen Research Governance, for sponsorship.
12.1.1.	**Confidentiality**
	All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain participant confidentiality. All records will be kept in a secure storage area with limited access to study staff only. Clinical information will not be released without the written permission of the participant, except as necessary for monitoring and auditing by the Sponsor or its designee. The CI and study staff involved with this study will not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the Sponsor or its designee will be obtained for the disclosure of any said confidential information to other parties.
12.1.2.	**Data Protection**
	The PI and study staff involved with this project will comply with the requirements of the General Data Protection Regulations (GDPR) and the Data Protection Act 2018. The HRA recommended wording to fulfil transparency requirements under the GDPR for health and care research has been included in the PIS. The PI and study staff will also adhere, if appropriate, to the current version of the NHS Scotland Code of Practice on Protecting Patient Confidentiality. Access to collated participant data will be restricted to the PI and appropriate study staff. Computers used to collate the data will have limited access measures via usernames and passwords. Published results will not contain any personal data that could allow identification of individual participants.
12.1.3.	**Insurance and Indemnity**
	The University of Aberdeen is sponsoring the study. Insurance: The University of Aberdeen will obtain and hold a policy of Public Liability Insurance for legal liabilities arising from the study. Indemnity: The Sponsor does not provide study participants with indemnity in relation to participation in the Study but has insurance for legal liability as described above.
13.	**Study Conduct Responsibilities**
13.1.	**Protocol Amendments, Deviations and Breaches**
	The PI will seek approval for any amendments to the Protocol or other study documents from the Sponsor (via Research Governance) and REP. Amendments to the protocol or other study documents will not be implemented without these approvals. In the event that a PI needs to deviate from the protocol, the nature of and reasons for the deviation will be recorded in the master file, documented and submitted to the Ethics panel Chair for approval.

13.2.	**Study Record Retention**
	Archiving of paper study documents will be conducted in line the University of Aberdeen's archival policy and will be stored for 10 years after the end date of the study. An electronic copy of the data will be held indefinitely by the PI, within the secure University Shared Drive.
13.3.	**End of Study**
	The end of study visit for volunteers is defined as the last visit at study day 21 with the written consent to contact participants again with their feedback report. The feedback will be provided to participants either during or at the end of the project through printable information such as the BIA results. If they withdraw at the middle, they will receive half of the expenses only £25 but if they are completing all study procedures will be receive a gratuity of £50. Also, they will receive the travel expenses paid e.g. bus cost or diesel.
14.	**Reporting, Publication and Notification of Results**
14.1.	**Authorship Policy**
	Ownership of the data arising from this study resides with the study team and their respective employers. On completion of the study, the study data will be analysed and tabulated, and a clinical study report will be prepared.
14.2.	**Publication**
	The study report will be used for publication and presentation at scientific meetings. Investigators have the right to publish orally or in writing the results of the study. Summaries of results will also be made available to Investigators for dissemination within their clinical areas (where appropriate and according to their discretion).
14.3.	**Peer Review**
	This project will be reviewed by the Human Studies Management Committee (HSMC) at the Rowett Institute.

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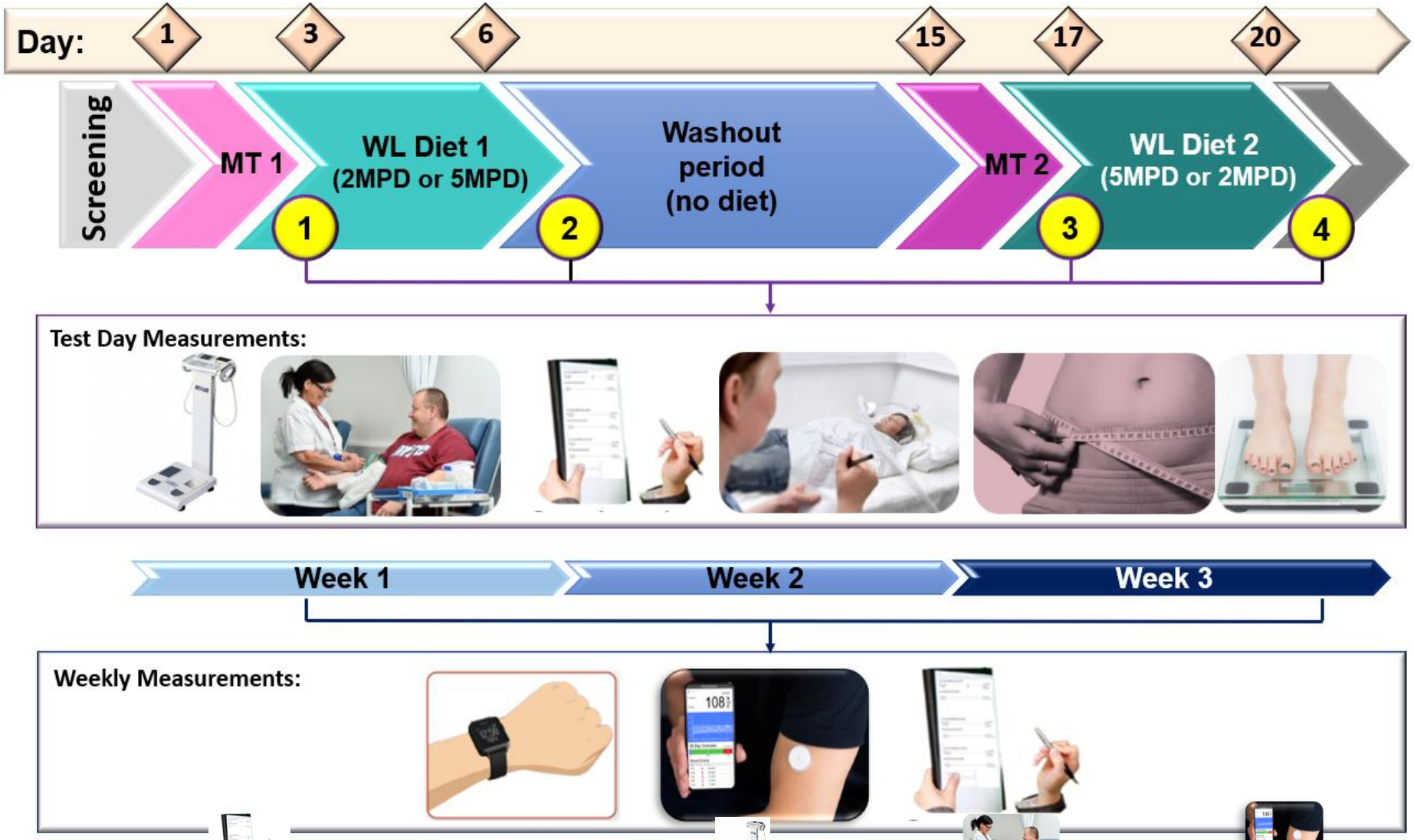
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APPENDIX 2: Test Day and Food Collection Days

Study Day	Screening	Diet	Test Meal	At HNU	Food Provision (d)
1	Wed	MTD1		Collect Food	2
2	Thu	MTD1			
3	Fri	Diet 1 (2/5meals)	MTD1	Collect Food	3
4	Sat	Diet 1 (2/5meals)			
5	Sun	Diet 1 (2/5meals)			
6	Mon	Diet 1 (2/5meals)		Collect Food	1
7	Tue	Washout (No Diet)	Diet 1 (2/5meals)		
8	Wed	Washout (No Diet)			
9	Thu	Washout (No Diet)			
10	Fri	Washout (No Diet)			
11	Sat	Washout (No Diet)			
12	Sun	Washout (No Diet)			
13	Mon	Washout (No Diet)			
14	Tue	Washout (No Diet)			
15	Wed	MTD2		Collect Food	2
16	Thu	MTD2			
17	Fri	Diet 2 (5/2meals)	MTD2	Collect Food	3
18	Sat	Diet 2 (5/2meals)			
19	Sun	Diet 2 (5/2meals)			
20	Mon	Diet 2 (5/2meals)		Collect Food	1
21	Tue	Study End	Diet 2 (5/2meals)		
Test Day					

APPENDIX 3A) Study Matrix



Activity measurement; Activity, appetite, food and sleep records; BIA measurement; Blood samples; CGMS measurement;

FC Food collection days; RMR and TEF measurements; TD Test days; Waist and hip measurement; Weight.

2MPD, 2 meals/day; 5MPD, 5 meals/day; BIA, bioimpedance; CGMS, continuous glucose monitoring system; RMR, resting metabolic rate; TEF, Thermic effect of food; WL, Weight Loss

APPENDIX 3B) Study Screening Visit Diagram



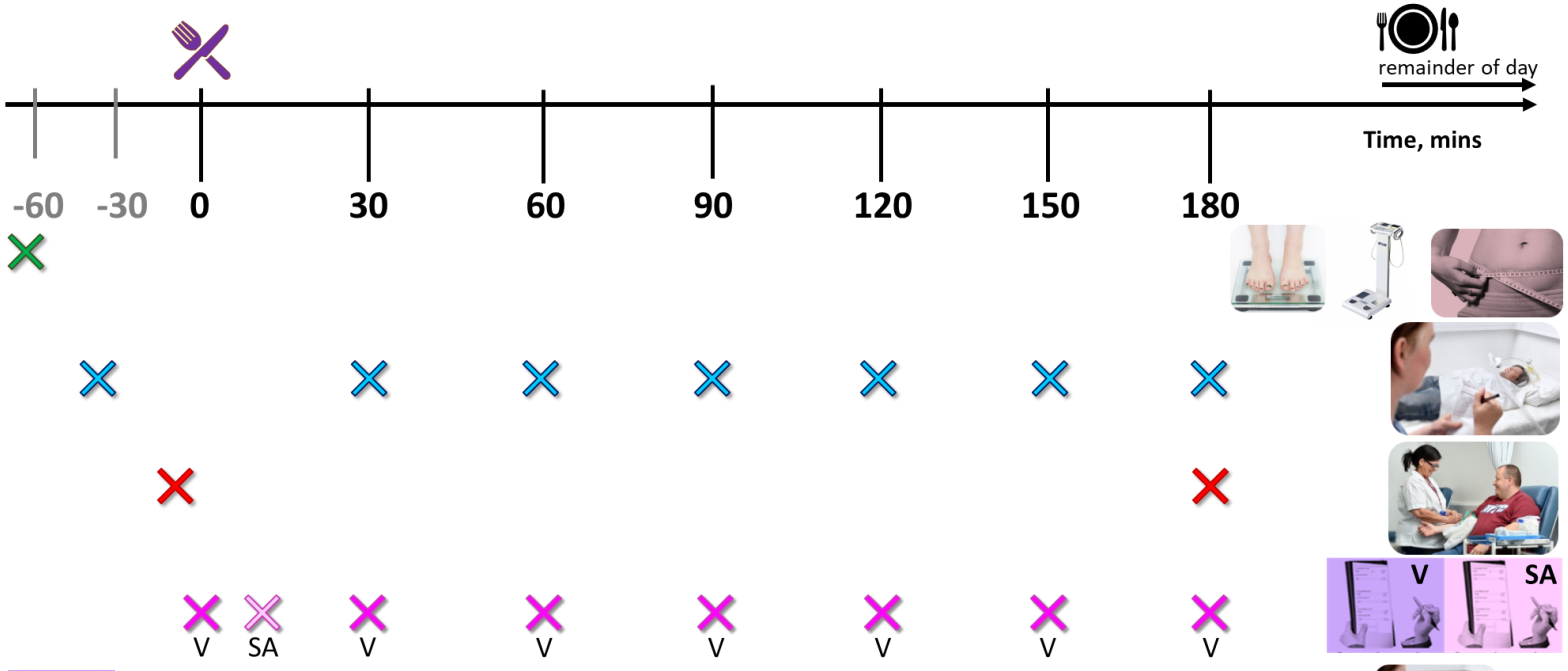
7d 7day food diary;

Anthropometric measurements (height, weight and BMI);

Consent paperwork and general health questionnaire;

Resting Metabolic Rate (RMR) measurement

APPENDIX 3C) Study Test Day Diagram



V Appetite VAS questionnaires; BIA measurement; Blood samples; Food record; RMR and TEF measurements;

SA Sleep and Activity questionnaires Test Meal; Waist and hip measurement; Weight.

BIA, bioimpedance; RMR, resting metabolic rate; TEF, Thermic effect of food

APPENDIX 4: PROTOCOL FOR STUDY 820 VISITS

1. Screening Visit:	**Relevant HISU Forms / SOPs***
1.1 Paperwork: • Study Diet Booklet for meal visualisation. • Participant Calendar of visits and Study GANTT Chart • Measurement Info Sheets (CGMS) • Study Form 1 - General Information • Study Form 2 - Participant Information Sheet (PIS, 2 copies) • Study Form 3 - Consent Form (2 copies) • Study Form 4 - Health Status • Measurement Booklet • Baseline 7d Food Diary with electronic food scales. (Scales need to be calibrated before the screening visit. Record the calibration results and scales serial number in the equipment log. Tell HISU staff the serial number of the scales assigned to the participant). • Study Weight Record Book • Food Scales Equipment Log	FORM463
1.2 Visit Instructions: **Equipment Set Up:** • Switch on the weighing scales, Quark RMR machine & computer. • If HISU staff are unavailable carry out two air & gas calibrations of the Quark machine. • Calibrate the Quark flowmeter.	
On Arrival: • Try to be in Institute at least 30mins before the scheduled appointment time in case the participant is early. • Meet the participant at Reception & take them up in the lift to HISU.	
Consent Interview: (Rm 3.004) • Discuss the Participant Information Sheet, describe the measurements & answer any of the participant's queries about the study. Give them a copy of the PIS to keep. • Use the Study Diet Booklet to explain about the study diets. • Ask them to complete the Health Status Form. The Investigator signs the eligibility section. • If the participant is happy to proceed get them to complete both copies of the Consent Form and give them a copy to keep.	HNU001 HNU002 HNU014
Venous Access Assessment: (Rm 3.012) • A trained member of the HISU staff assesses the suitability of venous access for the participant. The assessment outcome is recorded on the Health Status Form (Study Form 4)	FORM473
BMI Check - Height & Weight: (Rm 3.014 or 3.021) • Ask the participant to put one of the Unit dressing gowns on over their underwear. • Measure height & weight, calculate BMI to confirm it is 25+kg/m^2.Record the values in the study Weight Record Book and participant Measurement Booklet.	HNU018
RMR Measurement: (Rm 3.014)	

• Measure RMR for 30mins. Keep an eye on the $FeCO_2$ value & adjust blower if necessary. • Record the start & end times in the participant Measurement Booklet. • Record the measurement details in the RMR Logbook. • If you have access to the Study folder on the I drive save the RMR data to the appropriate Screening folder e.g. for vol 000 save file 820-000.xls to: *I:\HNU\study 820 Nouf\Chapter 5 – Human Study 820\820 Study Data\820 RMR*	HISU055 LOGBOOK 079
After Measurements: • Offer breakfast to the participant in the HISU Dining Room. • Provide the participant with their Baseline Food Diary and electronic food scales. Explain how to complete the Baseline Food diary for 7days. Use the Participant Calendar and GANTT Chart to highlight the study timetable. Check if the participant has any planned holidays. Schedule a potential start date for the study.	
After Visit: • Clean the weighing scales, stadiometer & RMR hood. • Replace the paper roll on the bed. • If no-one else has the RMR booked, shut it down making sure the gas valves have been closed. • Collect completed 7day Food diary for analysis.	

2. Test Day Visit (*Friday and Tuesday*)**:**	**Relevant** HISU **Forms / SOPs**
2.1 Paperwork: • Study Weight Record Book • Measurement Booklet • Test Day VAS Questionnaire Booklet • Participant Weighback Booklet • End of Day VAS and GI Questionnaire Booklet • Participant's Kitchen Weighback Booklet (stored in a folder on the shelf in the Kitchen) • CGMS Equipment Log • 820 CGMS Instructions • 820 Actigraph • 820 Test Day Questionnaires (Epworth Sleepiness Scale and Baecke Physical Activity Questionnaire) • 820 Form Measurement Information Sheets (CGMS)	
2.2 Visit Instructions: **Equipment Set Up:** • Switch on the weighing scales and lab centrifuge. • Make sure you have a tape measure and a timer.	
On Arrival: • Try to be in Institute at least 30mins before the scheduled appointment time in case the participant is early. • Meet the participant at reception and take them up in the lift to HISU.	

Weight Measurement: (Rm 3.014 or 3.021) • Ask the participant to put one of the Unit dressing gowns on over their underwear. • Measure weight. • Record the values in the study Weight Record Book and participant Measurement Booklet.	HNU018
Waist + Hip Measurement: (Rm 3.014 or 3.021) • Using a tape measure, measure Waist Circumference three times. • Measure Hip Circumference three times. • Record the values in the participant Measurement Booklet.	HNU005
Blood Samples: (Rm 3.011, 3.012 and 3.015) • See 820 Blood Sample Protocol for specific information on sample collection & aliquots. • **All biological samples (Whole blood and plasma) MUST be processed in the Biological Safety Cabinet (BSC) in the HISU Lab.** • Ensure Phlebotomy Form is completed • Blood samples are to be collected at T0, T3 (before and after breakfast). • Record the time of each of the samples in the participant Measurement Booklet.	HISU 069 LOGBOOK 070 FORM 304 HNU010 HISU011 HISU030 HNU061 FORM477 EQP005
RMR (TEF) Measurement: (Rm 3.014) • Measure RMR for 30mins. Keep an eye on the $FeCO_2$ value & adjust blower if necessary. • After the test meal the thermic effect of food (TEF) will be calculated for the last 10 min of every 30 min up to 3 hours (T20, T50, T80, T110, T140 and T170mins). • Record the start & end times in the participant Measurement Booklet. • Record the measurement details in the RMR Logbook. • If you have access to the Study folder on the I drive save the RMR data to the appropriate Screening folder e.g. for vol 000 save file 820-000.xls to: *I:\HNU\study 820 Nouf\Chapter 5 – Human Study 820\820 Study Data\820 RMR*	HNU055 LOGBOOK079
VAS Questionnaires: • Before breakfast give the participant the Test Day VAS Questionnaire Booklet to answer the T0 appetite questions. • Appetite questions are to be answered on the test day at T30, T60, T90, T120, T150 and T180mins.	
CGMS: (Rm 3.012) • Two CGMS sensors will be fitted during the study by a trained member of the HISU staff inserts the CGMS sensor to the back of the participant's arm: o on the 1st day of the study (removed on study day 7th). o on the 15th day of the study (removed on study day 21st). • Ask the participant to download the Freestyle LibreLinkup app. • If required a Smartphone is provided, with the serial number recorded in the participant Measurement Booklet. • All sensor log numbers and times of removal/insertion are recorded in the participant Measurement Booklet and CGMS Equipment Log.	MET0036

Test Meals: • Meals are provided in the HISU Dining Room at T0 mins on all test days. • Record the clock times of the meals in the Measurement Booklet. • Record any weighbacks from the meals in the Participant's Kitchen Weighback Booklet.	
Food Collection: • Return any cool bags from the previous visit to the kitchen staff. • After the participant has finished their dinner give them the cool bags containing their next set of meals (stored in walk-in fridge and freezer) and the Booklets they need for the next week (Weighback and End of Day Questionnaire).	HISU034 HNU035 HNU039 HNU040 HNU042 HNU043
After Measurements/Other: • Arrange collection of the Week 3 Weighback and End of Day Questionnaire Booklets with the participant.	
After Visit: • Clean the weighing scales, phlebotomy room & lab. Make sure all plasma samples have been aliquoted & stored.	

3. Study Food Collection Days (*Mon, Wed, Fri*)**:**	**Relevant HISU Forms / SOPs**
3.1 Paperwork: • Study Weight Record Book • Participant Weighback Booklet (provided weekly on Mon, Wed, Fri)	
3.2 Visit Instructions: **Equipment Set Up:** • Switch on the weighing scales.	
On Arrival: • Try to be in Institute at least 30mins before the scheduled appointment time in case the participant is early. • Meet the participant in Reception & take them up in the lift to HISU.	
Weight Measurement: (Rm 3.014 or 3.021) • Ask the participant to put one of the Unit dressing gowns on over their underwear. • Measure weight. • Record the value in the study Weight Record Book.	HNU018
Food Collection: • Take the participant to the HISU dining room where they will receive their breakfast. • Return any cool bags from the previous visit to the kitchen staff.	HNU034 HNU035 HNU039 HNU040

• The kitchen staff will give the participant a cool bag with their next set of meals, caffeine free beverages (if requested) and Weighback Booklet if it is a Friday. • The participant is free to leave the unit once they have their food bags & had breakfast.	HNU042 HNU043

4. End of Study:	Relevant HISU Forms / SOPs
4.1 Paperwork and Equipment to be returned: • Study Weight Record Book • Day 21 Participant Weighback Booklet • Day 21 End of Day Questionnaire Booklet • Electronic food scales • Smartphone (if provided for CGMS measurement) • Participant's Kitchen Weighback Booklet	N/A

List of Forms/SOPs

Code	Description
EQP005	Operation of Thermo – KONELAB 30
FORM304	Phlebotomy Sheet for Cannulation Blood Sampling
FORM448	BD PosiFlush NaCl 0.9% Competency Document Cannulists in the HNU
FORM463	Food Scale Assessment Log
FORM473	Venous Access Assessment Log
FORM477	POSIFLUSH 0.9% Sodium Chloride 3ml
HNU001	Research Governance and Quality Assurance Policy
HNU002	Procedure for the Implementation of Research Projects and studies Involving Human Volunteers
HNU004	Automated Blood Pressure Measurement Omron Blood Pressure Apparatus (HEM-705IT or M5-1)
HNU005	General Technique for Measuring Girths and Circumferences and Breadths
HNU010	Blood Sampling Including How to Deal with Haematomas and Bruises
HISU011	Obtaining Emergency or Non-emergency Medical Assistance for HNU Volunteers
HISU014	Exclusion Criteria and Medical Screening of Volunteers
HNU018	Procedure for Measurement of Weight (Body Mass) and Height (Stature)
HNU019	Procedure for Intravenous Cannulation and Multiple Blood Sampling using VENFLON Cannular
HNU027	Guidance on Handling Enquires by Potential Study Volunteers or Contacting Prospective Volunteers
HISU030	Procedure for the Monitoring and Recording of Adverse Events
HISU034	Personal Hygiene and food handling training
HNU035	Stock Control and Food Transportation in HNU
HNU039	Food Temperature Control and Monitoring Checks
HNU040	HNU Kitchen Cleaning
HNU042	Control of cross contamination
HNU043	Maintenance of HNU kitchen/food storage equipment
HISU055	Operation of the Quark Metabolic Monitor - CLSM A4314
HNU060	Recording and Analysing a Weighed Food Intake Record
HNU061	Procedures to implement in event of needlestick injury during phlebotomy
HNU068	Kitchen cleaning COVID-19
HISU069	HNU laboratory Biological Safety Cabinet (BSC) operation
LOGBOOK070	Microbiological Safety Cabinet Record
LOGBOOK079	Quark RMR User Log
MET0036	Determination of Glucose in serum and plasma
EQP0005	Operation of Thermo – KONELAB 30