Loughborough University

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Influence of High-fat Overfeeding on Circulating Hepatokine Concentrations: A Randomised Crossover Study

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OVEREAT Study Protocol

Objective(s)

The objectives of the present study are as follows:

- To examine the effects of high-fat overfeeding across a 7-day period, on circulating concentrations of the hepatokines LECT2, FGF21 and Fetuin-A.

- To examine the effects of high-fat overfeeding across a 7-day period, on subjective appetite, food preference and circulating concentrations of the appetite regulatory hormones Ghrelin and PYY.

- To observe whether changes in these variables occur alongside impairments in insulin sensitivity and glucose tolerance following seven days of high fat overfeeding.

Design

The present study is a randomised, controlled, crossover design in which 12 recreationally active, healthy males will complete two week-long dietary conditions in a randomised order. The two dietary conditions consist of a high-fat condition and a control condition. In the high-fat condition, participants will consume a diet comprising of 65% of total energy as fat and a total kilocalorie content equal to 150% of their estimated daily requirement. In the

control condition, participants' will consume their habitual diet as confirmed by a three-day food diary both before and during the control condition. The two diets will last seven days each with a three-week washout period separating the two conditions. An oral glucose tolerance test will be performed before and after the two diets to assess glycaemic control and hepatokine and appetite hormone responses will be measured across the seven days.

Methods

Protocol

The participants will firstly undergo a prescreening visit to the laboratory in which written informed consent will be obtained. Further to this, participants will complete a health screen questionnaire, an international physical activity questionnaire and a food preference questionnaire in order to ensure compliance with the eligibility criteria and that the foods provided during the trial will be palatable. Anthropometrical measures will be then be taken including height, weight, waist circumference and body fat percentage (measured via bioelectrical impedance analysis). A finger prick blood sample will also be taken and fasting blood glucose concentrations will be analysed using a Cardiochek device in order to confirm normal fasting blood glucose levels as stated in the eligibility criteria. Prior to completing the prescreening session, participants will be set up with hip-based physical activity monitor (Actigraph) and a thigh-based sedentary behaviour monitor (ActivPAL) and instructed to wear the devices for seven days. Participants will then be provided with an activity diary to record periods where the devices are removed as well as a 3-day food diary (including two

week days and one weekend day) to complete. This will allow for a baseline assessment of the participants' habitual eating and activity habits.

After the completion of the 7-day physical activity and sedentary behaviour monitoring, participants will be randomised to either the high-fat condition or the control condition. Participants will attend the laboratory at 8:00 am for a pre-diet assessment following a 10-hour overnight fast and having refrained from caffeine, exercise and alcohol in the prior 24 hours. Upon arrival, repeated written, informed consent will then be obtained and body weight, body fat percentage and blood pressure will then be measured. Following this, participants will be fitted with a mouth piece and their resting metabolic rate and fat oxidation will be measured via indirect calorimetry using Douglas bags for expired gas collection. Subjective ratings of appetite will then be measured using visual analogue scales and food preference will be assessed using computer-based software (Leeds Food Preference Questionnaire). A cannula will then be inserted into an antecubital vein for blood sampling. A baseline blood sample (0 h) will be collected, followed by the commencement of the oral glucose tolerance test. During this, participants will consume a solution containing 75 g of glucose dissolved in 300 ml of water and further blood samples will be taken at 0.5, 1, 1.5 and 2 h. The cannula will then be removed and the dietary condition will commence upon leaving the laboratory. During the 7-day dietary period, participants will undergo further monitoring of physical activity and sedentary behaviour, while during the control condition; participants will also complete a 3-day food diary in order to confirm maintenance of their habitual diet.

Both one day and three days after commencing the diets, participants will return to the laboratory at 8:00am following a 10-hour overnight fast and having refrained from caffeine,

exercise and alcohol. Further repeat, written informed consent will be and measurements of body weight, blood pressure, subjective appetite and food preference will be obtained. A venous blood sample will then be collected via venepuncture to allow for the assessment of the time-course of changes in fasting circulating hepatokine and appetite hormone concentrations.

Upon completion of the 7-day dietary condition, participants will attend the lab the following morning at 8:00am following a 10-hour overnight fast and having refrained from caffeine, exercise and alcohol. Participants will undergo a post-diet assessment using the same procedures as the pre-diet assessment. A washout period of three weeks will then separate the two dietary conditions.

Diet

During the high-fat dietary condition, all foods will be provided to the participants and will contain an energy content of 50% extra kilocalories above the participants' daily energy requirement and a dietary fat composition of 65%. The participants' daily energy requirement will be estimated using the Mifflin equations and subsequently multiplied by a physical activity correction factor of 1.7 and a thermogenic effect of feeding correction factor of 1.1. The foods provided will be primarily high in saturated fats and participants will be told to consume all foods provided within the study and no other calorie-containing foods or drink. In the event of any leftover food, participants will be told to bring in the food for weighing and subsequent subtraction from the calculated intake. Furthermore, participants will be told to fry foods where applicable and to minimise fat wastage during

the cooking process. During the control condition, participants will be told to consume their habitual diet, which will be confirmed using the 3-day food diaries. In addition to this, participants will be told on both diets to maintain their normal activity habits which will be confirmed by the physical activity and sedentary behaviour monitoring.

Blood Sampling

Blood samples will be collected into pre-chilled 2.7 mL EDTA, 4.9 mL EDTA, 9 mL EDTA, 2.6 mL Heparin and 2.7 mL Fluoride monovettes. All monovettes will be centrifuged at 3500 rpm for 10 min at 4˚C and the resultant plasma supernatant will be aliquoted into 2 mL micro tubes. The 2.7 mL EDTA monovettes will be pre-treated with PHMB, PBS and NaOH and after centrifugation, HCl will be added to prevent the degradation of acylated Ghrelin and the sample will be spun for a further 5 minutes. All samples will then be stored at -80˚C until analysis.