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Project Title: Exercise Effects on Appetite-regulating Hormones and Cardiovascular Risk Factors

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Preliminary measures

Prior to the main trials, participants will visit the human physiology laboratory to undergo screening, familiarisation, anthropometric measurements and a maximal cycling exercise test to determine maximum oxygen uptake ($\dot{V}O_2$ max). Body composition will be assessed using air displacement plethysmography (BodPod; software version 5.2.0, COSMED, Rome, Italy) and waist circumference will be measured in duplicate to the nearest 0.1 cm at the midpoint between the xiphoid process and the iliac crest using a standard anthropometric measuring tape (HaB International Ltd., Southam, UK). Blood pressure will be measured using a digital monitor (Omron M10-IT, Omron Healthcare Co. Ltd., Japan). After familiarisation with the testing equipment, participants will perform an incremental exercise test to volitional exhaustion on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) for the determination of maximum oxygen consumption ($\dot{V}O_2$ max). Participants will cycle at a self-selected pedal rate between 70 to 90 revolutions per minute for 3 min at 80 watts (warm up), followed by increments of 30 watts every 3 minutes until volitional fatigue. Expired air samples will be monitored continuously using an online breath-by-breath gas analysis system (Oxycon Pro, Viasys Healthcare Gmbh, Höchberg, Germany). Heart rate (Polar T31; Polar Electro, Kempele, Finland) and ratings of perceived exertion (RPE) will be also monitored throughout the test. The calculated $\dot{V}O_2$ max will be used to determine the relative intensity of exercise during the main trials.

Main trials

Participants will complete two, 2-day trials (exercise and control) in a random order separated by an interval of at least 1 week. Each trial will require participants to attend the laboratory for 7 hours. On day 1 of the trials, participants will arrive at the human physiology laboratory between 8.00 am and 9.00 am. Upon arrival at the laboratory, a fasting venous blood sample

will be collected for the measurement of appetite-related hormones, inflammatory markers and metabolic profiling. The 7 h trial will commence upon collection of the first blood draw (0 h). After collection of the fasting blood sample, participants will consume a standardised breakfast containing bread, tuna or ham, muffin and orange juice. Another blood sample will be collected 30 minutes after breakfast. Sixty minutes of continuous cycling at 65 % maximum oxygen uptake will be performed using a bicycle ergometer 2 hours after consuming breakfast. Before and after performing the exercise, a blood sample will be collected. Heart rate and RPE will be also monitored during the exercise. One hour after completing the exercise, participants will be given 30 minutes ad libitum access to a buffet meal containing white bread, margarine, butter, mayonnaise, ham, tuna, cheese, chocolate bars, whole fat milk, corn flakes, muesli, granola, orange juice, chocolate muffin, apple, banana, oranges and water. Before and after this meal, a blood sample will be collected. After finishing the meal, participants will rest until the end of the trial (sitting reading, working or watching movies). Another blood sample will be collected before participants will leave the laboratory (7 h). Appetite perceptions will be also assessed at baseline and every 30 min during the trial using validated 100 mm visual analogue scales (VAS). The procedures in the control trial will be identical to the exercise trial except that no exercise will be performed. Blood samples and appetite perceptions will be collected at the same times as in visit 2.

Blood sampling and biochemical analysis

Blood samples will be used to determine metabolic markers, inflammatory markers and appetite hormones, including cell count, glucose, insulin, total cholesterol, HDL-C, LDL-C, TAG, CRP, TNF-α, IL-6, acylated ghrelin, PYY, Leptin and fatty acids. Commercially available enzyme-linked immunosorbent assays (ELISA), colorimetric methods and gas chromatography mass spectrometry (GS-MS) will be used for blood analysis.

Blood samples will be collected into two pre-cooled 4 ml EDTA tubes, one 6 ml EDTA tube and one 6 ml heparin tube on 7 different occasions during the main trials for a total of ~ 140 ml. Blood sampling will be performed by venepuncture and using a cannula inserted into an antecubital vein. These samples will be analysed and immediately disposed of in clinical waste or spun down to serum or plasma and stored at -80°C according to the Human Tissue Act (HTA). All procedures will be carried out by fully trained individuals who are experienced in conducting such protocols. The technical support will always be available and first aiders will be available on site when testing is taking place. All blood sampling will be performed by a trained phlebotomist.

Statistical analysis

All statistical analyses will be conducted using the analytical software SPSS version 23.0 for Windows (SPSS 23.0, IBM Corp, Armonk, NY, USA). Normality of the data will be checked using Shapiro-Wilk tests and not normally distributed variables will be natural log transformed before analysis. Absolute standardised effect sizes (ES) (Cohen's d) will be calculated for each variable and statistical significance will be accepted as $P < 0.05$.