**Info for readme file to accompany *Journal of Nutrition* data archive:**

Archived data pertaining to article: Edinburgh, R.M., Hengist, A., Smith, H.A., Travers, R.L., Betts, J.A., Thompson, D., Walhin, J-P., Wallis, G.A., Hamilton, L.D., Stevenson, E.J., Tipton, K.D., Gonzalez, J.T. (2019). Skipping breakfast before exercise creates a more negative 24-h energy balance: A randomized controlled trial in healthy physically active young men. ***Journal of Nutrition***

**Trial Registration:** https://clinicaltrials.gov/ [Number: NCT02258399]

As described in the manuscript, this study was a randomized cross-over design where twelve healthy men completed preliminary testing followed by three trials (separated by > 7 d), namely, breakfast-rest (BR), breakfast-exercise (BE) and overnight fasted-exercise (FE). For all trials participants arrived at the laboratory after a 12 to 14-h overnight fast. In BR, a porridge breakfast was consumed, followed by 3 h of rest, and then a 2-h oral glucose tolerance test (OGTT). In BE, the same breakfast was consumed, before 2 h rest and 60 min of cycling, prior to the OGTT. In FE, breakfast was omitted but the trial otherwise replicated BE. After the OGTT, participants were provided an ad libitum lunch (within-lab) and a researcher-weighed food package, for consumption over the remaining 24-h trial (free-living). Daily energy expenditure was assessed via indirect calorimetry (for within-laboratory components) and heart-rate with accelerometry (free-living after leaving the laboratory).

BODY MASS

The weight of each participant was measured to the nearest 100 g using electronic scales (TANITA Inner Scan Body Composition Monitor-BC453, Tokyo) whilst they wore minimal clothing (lightweight shorts and t-shirt only).

HEIGHT

The height of each participant was measured as the distance from the floor (feet against the wall, barefoot, ankles together) to the top of the head (against the wall, looking ahead) after maximal inspiration and straight legs, to the nearest 0.1 cm using a wall-mounted stadiometer (Holtain Ltd., UK).

BODY MASS INDEX

Body mass index was body mass (kilograms) divided by height (m) squared.

DUAL ENERGY X-RAY ABSORPTIOMETRY (DEXA)

A DEXA scan was completed to quantify fat- and fat free-mass (Discovery, Hologic, Bedford, UK). Participants were positioned centrally on the scanning bed (supine position), with their legs apart and feet turned inwards and arms as wide as possible with their hands in a mid-prone position. This was to ensure that the gap between their arms and trunk was sufficient to allow regions of interest to be easily defined on the analysis software (QDR for Windows, Hologic, UK). Scans were analysed for fat- and fat-free mass (nearest 0.1 kg), the overall percentage body fat and fat mass index (DEXA-derived fat mass [kg] divided by the square of the participant’s height [m]).

MAXIMAL OXYGEN UPTAKE TEST

Participants completed a maximal oxygen uptake test before the first main trial, at a self-selected cadence on an electronically-braked ergometer (Excalibur Sport, Lode®, Groningen, Netherlands). The initial power output was set at 50 W and was increased by 50 W every four minutes, for four stages. Thereafter, the power output was increased by 20 W every minute until volitional exhaustion. Breath-by-breath measurements were recorded using an online gas analysis system (TrueOne2400, ParvoMedics, Sandy, USA). The volume and gas analysers used were calibrated with a 3 L calibration syringe (Hans Rudolph, Kansas City, USA) and known concentrations of a calibration gas (16.04 % O2; 5.06 % CO2) respectively.

PEAK POWER OUTPUT (PPO)

Peak power output was the work rate of the last completed stage, plus the fraction of time in the final non-completed stage, multiplied by the work rate increment.

VO2 PEAK

Peak oxygen uptake (VO2 peak) was taken as the highest average recorded oxygen uptake value over a rolling 30 second period.

ENERGY INTAKE

Prior to participation participants confirmed they had no allergies or aversions to any of the foods provided. The breakfast was 72 g of instant refined oats (Oatso Simple Golden Syrup, Quaker Oats) and 360 mL of semi-skimmed milk (Tesco), providing 431 kcal of energy ([1803 kJ]; 65 g CHO, 11 g FAT, 19 g PRO). Lunch was oats (Everyday Value® Tesco), whole milk (Tesco), maltodextrin, whey protein isolate (both Myprotein, Northwich, UK), olive oil (Tesco), and water, designed to limit the degree of palatability, with the aim of preventing overconsumption (18). The meal provided 150 kcal of energy per 100 g of cooked food ([626 kJ]; 20 g CHO; 5 g FAT; 5 g PRO) and was terminated when participants said that they felt ‘comfortably full’. Remaining food was removed and weighed. The free-living food package comprised: [1] a pasta meal, containing pasta, tomato sauce, cheddar cheese and olive oil (Tesco, prepared by the researchers), providing 151 kcal of energy per 100 g of cooked food ([632 kJ]; 20 g CHO; 6 g FAT; 5 g PRO), [2] four 35 g snack bars (GoAhead®; 367 kcal [1536 kJ] per 100 g; 74 g CHO, 8 g FAT; 3 g PRO) and [3] two 180 mL chocolate milk flavour drinks (Mars® Milk; 63 kcal [264 kJ] per 100 mL; 10 g CHO; 2 g FAT; 3 g PRO). Participants were instructed to eat until they were ‘comfortably full’, not to eat or drink anything not provided by this food package and to bring any remaining food back to the laboratory the following morning. The carbohydrate, fat and protein intake was taken as grams provided (cooked weight) minus grams remaining.

WITHIN-LAB SUBSTRATE OXIDATION and ENERGY EXPENDITURE

Within-lab during rest and exercise, Douglas bags were used to collect samples of expired air to enable detection of small changes in substrate utilisation rates and energy expenditure. The sensor (Mini MP 5200, Servomex Group Ltd., Crowborough, UK) was turned on 60 min prior to testing to allow for the temperature of the O2 and CO2 sensors to stabilise and was then calibrated using a two-point calibration, with concentrations of low (99.998 % Nitrogen, 0 % O2 and CO2) and high (16.04 % O2, 5.06 % CO2) gases (BOC Industrial Gases, Linde AG, Munich, Germany). Participants were provided with a mouthpiece one min before each sample was collected as a stabilisation period. The expired air samples were collected in 200 L Douglas bags (Hans Rudolph, Kansas City, USA) with the mouthpiece connected to a two-way, T shaped non-rebreathing valve via falconia tubing (as detailed previously) and measurements of ambient O2 and CO2 concentrations were recorded. Expired O2 and CO2 concentrations were then measured in a known volume of each sample for two min (or longer if values were not stable after two min) using paramagnetic and infrared transducers within the sensor. The ambient temperature, humidity and barometric pressure were recorded via a weather station (as detailed previously) so that expired air volumes could be corrected to standard temperature and pressure for dry gases. The volume and temperature of expired air samples were measured on a dry gas meter (Harvard Apparatus) and using a digital thermistor, respectively (HI98509 Checktemp 1, Hanna Instruments Ltd, Bedford, UK). The Haldane transformation was applied to calculate inspired gas volumes and to determine V̇O2 and V̇CO2. Rates of lipid and carbohydrate utilisation and energy expenditure were estimated from V̇O2 and V̇CO2 values via stoichiometric equations (as detailed in the manuscript).

FREE-LIVING ENERGY EXPENDITURE

Free-living physical activity energy expenditure was assessed from the time when the participant left the laboratory until 24 h post breakfast consumption (or omission) using an ActiheartTM physical activity monitor which measures accelerometery and heart rate.

CARBOHYDRATE (CHO), FAT and PROTEIN (PRO) BALANCES

Within-lab CHO, FAT and PRO balances were calculated as dietary intake of each macronutrient minus the oxidation of the respective substrate (measured using the Douglas bag technique described previously).

GLUCOSE CONCENTRATIONS

Blood plasma (EDTA-treated) concentrations of glucose were measured using a Daytona RX automated clinical chemistry analyser (Randox, Crumlin) according to manufacturer’s instructions.

DUAL GLUCOSE TRACER METHODS

Glucose tracers ([U13-C]-glucose oral ingestion and an infusion of [6,-6- 2H2]-glucose) were used to assess blood glucose flux. For the OGTT, 73 g of glucose was weighed (81 g of dextrose monohydrate corrected for water content; Myprotein, Northwich, UK). Then, 2 g of [U13-C]-glucose (99 %; Cambridge Isotope Laboratories, MA, USA) was weighed to the nearest 0.01 g with electronic weighing scales (AX124/E Adventurer Analytical Balance, Ohaus, Parsippany, USA) and added with 300 mL of plain water. The infusate and prime were freshly made for each trial. First, a spreadsheet was prepared with desired tracer weights and saline volumes which were calculated from the desired infusion rate. Tracer preperation was completed under a sterlie fume hood and all of the work surfaces, material packaging and equipment were sprayed with 70 % ethanol. The desrired mass of [6,6-2H2]-glucose was weighed (seperately for infusates and primes) to the nearest 0.01 g and the actual mass was recorded. Then, a needle was attached to a 60 mL sterile syringe and used to add the desired volume of saline for the infusate and prime and the actual volume was recorded. A second syringe was used to aspirate the solution for the infusate and prime and was then attached to a 3-way stopcock, a 0.2 micron syringe filter and an intravenous infusion extension line (for the prime, the syringe was only attached to the filter). The spreadsheet was updated with the actual weights recorded for the infusate and the prime so that the required pump rate could be calculated. During trials the infusion rate was administered via an automatic pump (Alaris™ PK Syringe Pump, BD, Wokingham, UK) and the infusate syringe was weighed after each trial to ensure the infusion rate prescribed was accurate.

Plasma [U13C]-glucose and [2H2]-glucose enrichments were determined by gas chromatography-mass spectrometry (GC-MS). First, the glucose was extracted from samples using methanol-chloroform and hydrochloric acid, dried under nitrogen gas and derivatised (to ensure samples were sufficiently volatile and thermally stable for analysis using GC-MS. The glucose derivatives were acquired by using selected ion monitoring at *m*/*z* 519, 521 and 525 for [12-C]-, [6,-62H2]-, and [U13-C]-glucose, respectively and the area underneath each peak was calculated. Corrections were made for the order in which the samples were loaded onto the GC and the plasma enrichments of the isotopes were calculated via standard curves for [13-C]- and [2H2]-glucose and were expressed relative to enrichments for [12-C]-glucose. A baseline sample from every trial was used to account background isotopic plasma enrichments. Plasma glucose flux was assessed using Radziuk’s two-compartment non-steady state model and SAMM II software (SAAM II v2.3, Epilson Group, Charlottesville, VA, USA). The calculations used are detailed within the manuscript.

Plasma glucose utilization during exercise was assumed to be equivalent to the plasma glucose rate of disposal (average of 40 and 50 min samples). Muscle glycogen utilization during exercise was calculated as total carbohydrate utilization minus plasma glucose utilization (average of 40 and 50 min samples) during exercise

FIBROBLAST GROWTH FACTOR 21 (FGF-21) CONCENTRATIONS

Blood plasma (EDTA-treated) concentrations of FGF-21 were measured using a commercially available ELISA kit (BioVendor Research & Diagnostic Products; Karasek, Czech Republic)

LEPTIN CONCENTRATIONS

Blood plasma (EDTA-treated) concentrations of leptin were measured using a commercially available ELISA kit (Mercodia AB, Uppsala, Sweden)

TIME-AVERAGED AREA UNDER THE CURVE

The time-averaged area underneath the concentration-time curve (AUC) for the plasma FGF-21 and leptin responses were calculated via the trapezoid rule. This was divided by the time over which samples were collected to provide a time-averaged AUC.