**Info for readme file to accompany *American Journal of Physiology: Endocrinology and Metabolism* data archive:**

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**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. Dual stable isotope tracers ([U-13C] glucose ingestion and [6,6-2H2] glucose infusion) and muscle biopsies were combined to assess plasma glucose kinetics and intramuscular signalling, respectively.

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.

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.

INSULIN CONCENTRATIONS

Blood plasma (EDTA-treated) concentrations of insulin were measured via Enzyme-Linked Immuno-Sorbent Assay (ELISA; Mercodia, Uppsala) according to manufacturer’s instructions.

NEFA CONCENTRATIONS

Blood plasma (EDTA-treated) concentrations of NEFA were assayed via Enzymatic colorimetric assays (Alpha Laboratories Ltd; Hampshire, UK) according to manufacturer’s instructions.

TRIACYLGLYCEROL CONCENTRATIONS

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

LACTATE CONCENTRATIONS

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

INTESTINAL FATTY ACID BINDING PROTEIN (IFAB-P) CONCENTRATIONS

Blood plasma (EDTA-treated) concentrations of insulin were measured via Enzyme-Linked Immuno-Sorbent Assay (ELISA; Cambridge Bioscience Ltd; UK) according to manufacturer’s instructions.

Β- HYDROXYBUTRATE CONCENTRATIONS

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

TIME-AVERAGED AREA AND/OR INCREMENTAL UNDER THE CURVE

The time-averaged area underneath the concentration-time curve (AUC) for the plasma glucose, insulin, lactate and triacylglycerol OGTT responses (0-120 minutes) was calculated via the trapezoid rule. The total AUC was divided by the time over which samples were collected (120 minutes) to provide a time-averaged AUC.

ISI MATSUDA INDEX

The fasted baseline glucose and insulin data were combined with the mean glycemic and insulinemic responses over 2 h during the OGTT and used to calculate the ISI Matsuda Index using the equation: 10000/SQRT((baseline glucose [mg/dL]\*baseline insulin [mIU/L])\*(mean glucose [mg/dL]\*mean insulin [mIU/L])).

WESTERN BLOTTING (AkT, AMPK and ACC ACTIVATION)

Muscle was sampled and analysed for the content and/or activity of proteins involved in glucose metabolism. All samples were collected (~100 mg of wet weight) from the *vastus lateralis* using the Bergstrom biopsy needle technique adapted for suction. The skin surrounding the sampling site was shaved before being cleaned and sterilised with iodine (Videne, EcoLab, UK). A local anaesthetic (2.5 mL of 1 % lidocaine, Hameln Pharmaceuticals Ltd., UK) was injected under the skin using a 27 G needle. After 3-5 min, a second 2.5 mL dose of lidocaine was injected onto the muscle facia (2-5 cm beneath the skin surface) using a 21 G needle. After 5 min, a 3 mm incision at the anterior aspect of the thigh was made with a blade (Swann Morton, size 11), iodine was applied and pressure placed on the incision. Samples were taken using a 5-mm Bergstrom biopsy needle which was vertically inserted into the incision to a depth of ~1 cm beyond the fascia. A vacuum was created using a 100 mL syringe attached to the needle to allow the muscle to be snipped (this was repeated 2-3 times to obtain the sample). After removal of the needle, the incision was sealed with steri-strips and stitching and covered with an adhesive dressing. Samples were extracted from the needle and cleaned in saline with dissection of blood or connective tissue. Samples were placed into ventilated Eppendorf tubes in liquid nitrogen, before long-term storage at -80°C. Samples were powdered and freeze-dried and the protein content was measured.

For western blotting, proteins in a sample were separated via gel electrophoresis. Linear tris-glycine sodium dodecyl sulphate (SDS)-polyacrylamide gels were made from ~15 mL of a resolving gel (which set for 30 min at room temperature) and 4 mL of a stacking gel. Then, an equal amount of protein (40 µg) was loaded per well (50 µL volume), with 8 µL of MW markers (Thermo Fisher Scientific). Proteins were forced through the gel and separated according to their MW by an electrical current (200 V for ~1 h) whilst submerged in an electrophoresis buffer in a method known as polyacrylamide gel electrophoresis (PAGE). Gels were electro-blotted using a semi-dry transfer onto a nitrocellulose membrane (Gelman Sciences, Portsmouth, UK). This procedure uses the electrophoretic mobility of proteins in order to transfer them from the gel onto the membrane which is sandwiched between electrodes with the aid of a conducting solution (SDS-Transfer Buffer). The nitrocellulose membrane and filter papers (Bio-Rad Laboratories; Hercules, California, USA) were cut to an appropriate size and soaked in SDS-transfer buffer, before being placed on a transfer unit (Bio-Rad Laboratories; Hercules, California, USA). An electric current was then created (75 mA) to facilitate the movement of the proteins from the gel to the membrane. Membranes were then washed to remove any transfer buffer, before staining with a 0.1% Ponceau S solution in 3 % Trichloroacetic acid (TCA) and imaging using a chemiluminescent imager (EpiChemi II Darkroom, UVP, Upland, US) to assess transfer efficiency. Then, membranes were washed in Tris-buffered saline and cut according to the MW of the protein of interest and incubated for 30 min in a blocking solution (5 % non-fat dry milk in TBS-T; Marvel, Premier International Foods Ltd, UK) to reduce any nonspecific binding of the detection antibody. This was followed by washing (4 x 5 min) to remove blocking solution. Thereafter, membranes were incubated overnight (~12 h) at 4°C with primary antibodies (diluted ~1:1000 in TBS-T with 1 % BSA) against the protein of interest. Then, membranes were washed with TBS-T (5 x 5 min) and incubated with anti-species IgG horseradish peroxidase-conjugated secondary antibodies (1:4000 dilution), made up in the blocking solution. The primary antibodies are not directly detectable so the secondary antibodies are used to detect the protein of interest and the choice of secondary antibody depends on the animal in which the primary antibody was raised. If the primary antibody was mouse monoclonal, an anti-mouse IgG secondary antibody from a non-mouse host is used with horseradish peroxidase (which reacts with chemiluminescence reagents to enable protein detection). Membranes were visualised using a chemiluminescent imager (EpiChemi II Darkroom, UVP, Upland, USA).

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.

CHO AND FAT UTILIZATION

During rest and any other exercise, Douglas bags were used to collect samples of expired air to enable detection of small changes in substrate utilisation rates. 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 were estimated from V̇O2 and V̇CO2 values via stoichiometric equations (as detailed in the manuscript).

PLASMA GLUCOSE and NON-PLASMA GLUCOSE UTILIZATION

Plasma glucose utilization during exercise was assumed to be equivalent to the plasma glucose rate of disappearance. Muscle glycogen (i.e. non-plasma glucose) utilization during exercise was calculated as total carbohydrate utilization during exercise minus plasma glucose utilization during exercise.