**Info for readme file to accompany *AAAS* data archive**

Archived data pertaining to article: Templeman I., Smith H. A., Chowdhury E. A., Chen Y. C., Carroll H. A., Johnson-Bonson D., Hengist A., Smith R., Creighton J., Clayton D. J., Varley I., Karagounis L. G., Wilhelmsen A., Tsintzas K., Reeves S., Walhin J. P., Gonzalez J. T., Thompson D. & Betts J.A. (2021) **Interactive effects of fasting & energy restriction on energy balance & metabolic health: a randomized controlled trial in lean adults.**  *Science Translational Medicine.*

The trial is registered and available to view at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (ref: NCT02498002).

The precise details of and justifications for the experimental design and protocol adopted has been published as follows: Templeman, I., Thompson D., Gonzalez, J.T., Walhin, J.P., Reeves, S., Rogers, P.J., Brunstrom, J.M., Karagounis, L.G., Tsintzas K., Betts, J.A. (2018) Intermittent fasting, energy balance and associated health outcomes in adults: study protocol for a randomised controlled trial. *Trials* 19 (1): 86. doi: 10.1186/s13063-018-2451-8

As described in the above cited protocol, all blood parameters reported in the associated data archive relate to venous samples drawn during the preliminary visit (Baseline), in a ~10 h overnight fasted state (Pre and Post) and at regular intervals following a standardised breakfast and lunch both before and after the intervention. Both intra- and inter-assay/plate coefficients of variation reported below for each parameter.

Any missing data-points are denoted as ‘NA’ in the accompanying data-set.

HEIGHT

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 was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Seca Stadiometer, Germany).

BODY MASS

The weight of each participant was measured to the nearest 0.1 kg using a sliding balance scale (Weylux 424, UK) whilst they wore minimal clothing (lightweight shorts only for males; lightweight shorts plus lightweight vest-top for females), with the same clothing standardised between baseline and follow-up.

WAIST CIRCUMFERENCE

The distance around the waist was measured with a metallic (non-elastic) tape-measure based on WHO guidelines for anatomical landmarks (i.e. the mid-point from lowest rib to top of iliac crest). The average of three measurements was accepted with agreement <1 cm between them.

BODY COMPOSITION/PERCENT BODY FAT

Lean and adipose tissue mass and were measured using Dual Energy X-ray Absorptiometry (QDR Discovery W, Hologic; MA, USA). Before scans participants voided and wore the same minimal clothing as described for BODY MASS (therefore having removed any metal items and shoes).

BONE MINDERAL DENSITY/ BONE MINERAL CONTENT

Participant’s bone mineral content and bone mineral density were measured using Dual Energy X-ray Absorptiometry (QDR Discovery W, Hologic; MA, USA). Before scans participants voided and wore the same minimal clothing as described for BODY MASS (therefore having removed any metal items and shoes).

DIET COMPOSITION

Individual participants were provided with a food diary and electronic scales (Pocket Pro 2000, Smart Weigh; NY, USA) to directly weigh and record the mass of all food and drink consumed at the time of eating (i.e. not retrospective), along with a detailed description of the food. The weighed records were analysed (NutriticsTM version 5.031; Ireland) to determine energy and macronutrient intake.

RESTING METABOLIC RATE/SUBSTRATE OXIDATION

Energy expenditure under basal conditions was determined via indirect calorimetry from gaseous exchange. Participants rested motionless in a supine position for 20-minutes and then three consecutive 5-minute samples were measured. The total volume expired every 5 minutes was measured using a dry gas meter (Harvard Apparatus, UK) and the relative gas fractions of oxygen and carbon dioxide measured using paramagnetic and infra-red analysers, respectively (Servomex 1440, UK). Metabolic substrate utilization and energy expenditure were then determined from rates of oxygen uptake and carbon dioxide production using the equations described by Frayn (1983) *Journal of Applied Physiology* **55** (2): 628-634. Resting metabolic rate was recorded by using the values from two or more samples from each participant that were stable within 100 kcal per day and taken as an arithmetic average.

DIET-INDUCED THERMOGENESIS

The rate of daily diet-induced thermogenesis under free-living conditions was determined as the product of the mass of each macronutrient ingested (determined as described for DIET COMPOSITION) and the following established constants for the thermogenic effect of each macronutrient: carbohydrate 8 %, fat 2 %, protein 21 % and ethanol/alcohol 15 % (the latter balancing the likely differing contributions of the ADH and MEOS systems for ethanol metabolism).

TIME OF WAKING/SLEEPING

Individual participant’s daily physical activity traces were used to identify when physical movements commenced and ceased at the beginning and end of each waking phase to determine the times at which participants rose in the morning and went to bed at night. Data for two participants were not recorded at either the time they woke-up or went to sleep for both the baseline and intervention measurement.

PHYSICAL ACTIVITY THERMOGENESIS

The energy expended during physical activity was determined via combined heart rate/accelerometry (ActiHeart™,Cambridge Neurotechnology; Cambridge, UK). Individual participant’s physical activity monitors were calibrated using a submaximal treadmill protocol which involved four 3-minute intervals of incremental treadmill locomotion with concurrent measurements of both heart rate and energy expenditure (indirect calorimetry of expired gas samples). This was used to produce a hear rate-physical activity intensity regression equation which estimates were based upon.

TOTAL CHOLESTEROL

Blood plasma concentrations of total cholesterol were assayed using an automated analyser (RX Daytona; Randox Laboratories, Northern Ireland) and commercially available reagents (Randox Laboratories, Northern Ireland). Intra-assay coefficient of variation 4.0 ± 0.9 %; inter-assay coefficient of variation 3.8 ± 1.1 %.

HIGH-DENSITY LIPOPROTEIN (HDL) CHOLESTEROL

Blood plasma concentrations of HDL cholesterol were assayed using an automated analyser (RX Daytona; Randox Laboratories, Northern Ireland) and commercially available reagents (Randox Laboratories, Northern Ireland). Intra-assay coefficient of variation 4. 2 ± 1.8 %; inter-assay coefficient of variation 5.3 ± 0.4 %.

LOW-DENSITY LIPOPROTEIN (LDL) CHOLESTEROL

Blood plasma concentrations of LDL cholesterol were assayed using an automated analyser (RX Daytona; Randox Laboratories, Northern Ireland) and commercially available reagents (Randox Laboratories, Northern Ireland). Intra-assay coefficient of variation 2.1 ± 0.5 %; inter-assay coefficient of variation 2.3 ± 0.5 %. Blood samples were not available for participant #12 at baseline.

LEPTIN

Blood plasma (EDTA-treated) concentrations of leptin were assayed via commercially available ELISAs (Mercodia, Sweden). Intra-assay coefficient of variation 3.2 ± 0.2 %; inter-assay coefficient of variation 4.4 ± 1.0 %.

ADIPONECTIN

Blood plasma (EDTA-treated) concentrations of adiponectin were assayed using an automated analyser (RX Daytona; Randox Laboratories, Northern Ireland) and commercially available reagents (Randox Laboratories, Northern Ireland). Intra-assay coefficient of variation 3.0%; inter-assay coefficient of variation 3.1%.

HOMA2-IR

Homeostatic model of insulin resistance-2 (HOMA2-IR; Levy, Matthews and Hermans, 1998) was calculated using the University of Oxford HOMA2 calculator (downloaded from: <https://www.dtu.ox.ac.uk/homacalculator/download.php>). Blood samples were not available for five participants at baseline.

GLUCOSE

Blood plasma (EDTA-treated) concentrations of glucose were assayed using an automated analyser (RX Daytona; Randox Laboratories, Northern Ireland) and commercially available reagents (Randox Laboratories, Northern Ireland). Intra-assay coefficient of variation 3.0 ± 0.7 %; inter-assay coefficient of variation 3.3 ± 0.3 %.

INSULIN

Blood plasma (EDTA-treated) concentrations of insulin were assayed via commercially available ELISAs (Mercodia, Sweden). Intra-assay coefficient of variation 5.5 ± 7.4 %; inter-assay coefficient of variation 10.6 ± 6.1 %. Baseline blood samples were not acquired from five participants during the oral glucose tolerance test.

NON-ESTERIFIED FATTY ACIDS (NEFA)

Blood plasma (EDTA-treated) concentrations of NEFA were assayed using an automated analyser (RX Daytona; Randox Laboratories, Northern Ireland) and commercially available reagents (Randox Laboratories, Northern Ireland). Intra-assay coefficient of variation 5.6 ± 2.0 %; inter-assay coefficient of variation 8.8 ± 3.9 %. Blood samples were not available from two participants at baseline.

GLYCEROL

Blood plasma (EDTA-treated) concentrations of glycerol were assayed using an automated analyser (RX Daytona; Randox Laboratories, Northern Ireland) and commercially available reagents (Randox Laboratories, Northern Ireland). Intra-assay coefficient of variation 12.4 ± 5.0 %; inter-assay coefficient of variation 17.7 ± 6.1 %. Blood samples were not available from two participants at baseline. One blood sample was not available at baseline.

TRIACYLGLYCEROL

Blood plasma concentrations of triacylglycerol were assayed using an automated analyser (RX Daytona; Randox Laboratories, Northern Ireland) and commercially available reagents (Randox Laboratories, Northern Ireland). Intra-assay coefficient of variation 4.4 ± 1.9 %; inter-assay coefficient of variation 4.9 ± 2.2 %.

PEPTIDE YY (PYY)

Blood plasma (EDTA-treated) concentrations of PYY were assayed via commercially available ELISAs (Merck-Millipore, United States). Intra-assay coefficient of variation 17.3 ± 21.1 %; inter-assay coefficient of variation 18.6 ± 12.1 %. Blood plasma samples were not available for twelve participants. Blood plasma samples were not available for participant #15 Post 0 minutes; participant #33 Pre 0 minutes and Post: 0, 60 and 120 minutes. Blood plasma samples were not available for participant #32 Pre 0 and 180 minutes, for participant #34 Pre 0 and 60 minutes and participant #55 Post 0 minutes.

ACYLATED GHRELIN

1 mL of whole blood (EDTA-treated) concentrations of acylated ghrelin were treated with 50 μL of a p-hydroxymercuribenzoic acid solution (prepared as 100 mM concentrate solution in potassium phosphate buffer containing 1.2 % 10 m-NaOH). Samples were assayed using commercially available ELISAs (Merck-Millipore, United States). Intra-assay coefficient of variation 16.7 ± 32.0 %; inter-assay coefficient of variation 27.7 ± 20.9 %. Blood samples were not available for six participants. Blood samples were not available for participant #56 Pre 0 and 60 minutes. Blood samples were not available for participant #9 Post 60 minutes and participant #43 Pre: 0, 60, 120, 180 and 270 minutes.

C-TERMINAL TELOPEPTIDE of TYPE I COLLAGEN (CTX)

Blood Plasma (EDTA-treated) concentrations of CTX were assayed using commercially available ELISAs (Immunodiagnostic Systems, UK). Intra-assay coefficient of variation=5.9%; inter-assay coefficient of variation=10.5%. Blood samples were not available for six participants.

URINE OSMOLALITY/ SPECIFIC GRAVITY

Urine samples were analysed for specific gravity via refractometry (SUR-NE Clinical, Japan) and Osmolality by the freezing-point depression method (Micro Osmometer 3300; Advanced Instruments, USA).

PROTEIN OXIDATION

Individual participants total urine output was collected during the 3-hour postprandial period after meal 1 to correct rates of energy and substrate metabolism.

**ACETYL-COA CARBOXYLASE 1** (ACACA)

Subcutaneous ACACA (Hs01046047\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

ACADM ACYL-COA DEHYDROGENASE MEDIUM CHAIN (ACADM)

Subcutaneous ACADM (Hs00936580\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

ADIPONECTIN (ADIPOQ)

Subcutaneous ADIPOQ (Hs00605917\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

AKT SERINE/THREONINE KINASE 2 (AKT2)

Subcutaneous AKT2 (Hs00609846\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

**ANGIOPOIETIN-LIKE 4 (**ANGPTL4**)**

Subcutaneous ANGPTL4 (Hs01101127\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

APELIN (APLN)

Subcutaneous APLN (Hs00175572\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

C/EBP- ALPHA (CEBPA)

Subcutaneous CEBPA (Hs00269972\_s1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

C/EBP- BETA (CEBPB)

Subcutaneous CEBPB (Hs00942496\_s1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

CELL DEATH INDUCING DFFA LIKE EFFECTOR A (CIDEA)

Subcutaneous CIDEA (Hs00154455\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

CELL DEATH INDUCING DFFA LIKE EFFECTOR C (CIDEC)

Subcutaneous CIDEC (Hs01032998\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

CIRCADIAN LOCOMOTOR OUTPUT CYCLES KAPUT (CLOCK)

Subcutaneous CLOCK (Hs00231857\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

CARNITINE PALMITOYLTRANSFERASE 1A (CPT1A)

Subcutaneous CPT1A (Hs00912671\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

CAMP RESPONSIVE ELEMENT BINDING PROTEIN 1 (CREB1)

Subcutaneous CREB1 (Hs00231713\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

CRYPTOCHROME CIRCADIAN REGULATOR 1 (CRY1)

Subcutaneous CRY1 (Hs00172734\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

FATTY ACID BINDING PROTEIN 4 (FABP4)

Subcutaneous FABP4 (Hs01086177\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

FATTY ACID SYNTHASE (FASN)

Subcutaneous FASN (Hs00188012\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

HYDROXYACYL-COA DEHYDROGENASE TRIFUNCTIONAL MULTIENZYME COMPLEX SUBUNIT BETA (HADHB)

Subcutaneous HADHB (Hs01027270\_g1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

INSULIN LIKE GROWTH FACTOR 1 RECEPTOR (IGF1R)

Subcutaneous IGF1R (Hs00609566\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

INTERLEUKIN 6 (IL6)

Subcutaneous IL6 (Hs00985639\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

INSULIN RECEPTOR SUBSTRATE 1 (IRS1)

Subcutaneous IRS1 (Hs00178563\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

INSULIN RECEPTOR SUBSTRATE 2(IRS2)

Subcutaneous IRS2 (Hs00275843\_s1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

LEPTIN (LEP)

Subcutaneous LEP (Hs00174877\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

LIPASE E, HORMONE SENSITIVE TYPE (LIPE)

Subcutaneous LIPE (Hs00193510\_m10) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

LIPOPROTEIN LIPASE (LPL)

Subcutaneous LPL (Hs01012571\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

MLX-INTERACTING PROTEIN-LIKE (MLXIPL)

Subcutaneous MLXIPL (Hs00263027\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

NICOTINAMIDE PHOSPHORIBOSYL TRANSFERASE (NAMPT)

Subcutaneous NAMPT (Hs00237184\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

NEURONAL PAS DOMAIN PROTEIN 2 (NPAS2)

Subcutaneous NPAS2 (Hs00231212\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

POLY [ADP-RIBOSE] POLYMERASE 1 (PARP1)

Subcutaneous PARP1 (Hs00242302\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

PYRUVATE DEHYDROGENASE KINASE 4 (PDK4)

Subcutaneous PDK4 (Hs01037712\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

PERIOD CIRCADIAN REGULATOR 1 (PER1)

Subcutaneous PER1 (Hs00242988\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

PHOSPHATIDYLINOSITOL 3-KINASE REGULATORY SUBUNIT ALPHA (PIK3R1)

Subcutaneous PIK3R1 (Hs00381459\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

PERILIPIN 2 (PLIN2)

Subcutaneous PLIN2 (Hs00605340\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

PATATIN LIKE PHOSPHOLIPASE DOMAIN CONTAINING 2 (PNPLA2)

Subcutaneous PNPLA2 (Hs00386101\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

PATATIN LIKE PHOSPHOLIPASE DOMAIN CONTAINING 3 (PNPLA3)

Subcutaneous PNPLA3 (Hs00228747\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR GAMMA (PPARG)

Subcutaneous PPARG (Hs01115513\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

PPARG COACTIVATOR 1 ALPHA (PPARGC1A)

Subcutaneous PPARGC1A (Hs00173304\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

PROTEIN KINASE AMP-ACTIVATED CATALYTIC SUBUNIT ALPHA 1 (PRKAA1)

Subcutaneous PRKAA1 (Hs01562315\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

PROTEIN KINASE AMP-ACTIVATED CATALYTIC SUBUNIT ALPHA 2 (PRKAA2)

Subcutaneous PRKAA2 (Hs00178903\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

SIRTUIN-1 (SIRT1)

Subcutaneous SIRT1 (Hs01009006\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

SIRTUIN-3 (SIRT3)

Subcutaneous SIRT3 (Hs00953477\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

SOLUTE CARRIER FAMILY 2 MEMBER 4 (SLC2A4)

Subcutaneous SLC2A4 (Hs00168966\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

STEROL REGULATORY ELEMENT-BINDING TRANSCRIPTION FACTOR 1 (SREBF1)

Subcutaneous SREBF1 (Hs01088691\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

TUMOR NECROSIS FACTOR (TNF)

Subcutaneous TNF (Hs99999043\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.

UNCOUPLING PROTEIN 2 (UCP2)

Subcutaneous UCP2 (Hs01075227\_m1) were assayed using Applied Biosystems, run on a real-time PCR system (7900HT, Applied Biosystems). Subcutaneous adipose tissue biopsies were not available for 18 participants.