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

Archived data pertaining to article: Betts, J. A., Richardson, J. D., Chowdhury, E., Holman, G., Tsintzas, K. & Thompson, D. (2013) **The causal role of breakfast in energy balance and health: a randomized controlled trial [ISRCTN31521726].** *American Journal of Clinical Nutrition* **In-Press.**

The trial is registered with the International Standard Randomised Controlled Trial Number Register (ISRCTN), available at [www.controlled-trials.com](http://www.controlled-trials.com) (ref: ISRCTN31521726).

The precise details of and justifications for the experimental design and protocol adopted has been published as follows: Betts J. A., Thompson D., Richardson J. D., Chowdhury E. A., Jeans M., Holman G. D. & Tsintzas K. (2011) Bath Breakfast Project (BBP): Examining the role of extended daily fasting in human energy balance and associated health outcomes: study protocol for a randomized controlled trial [ISRCTN31521726] *Trials* 12: 172.

As described in the above protocol, all blood parameters reported in the associated data archive relate to venous samples drawn at 0800 h (± 1 h; hours) in an overnight (10 h) fasted state, except for Interleukin-6 which was measured at 1200 h (± 1 h) with a standardised breakfast 3 h earlier (no substantial variance is expected over this time-course). All samples for a given patient were analysed in duplicate as a batch within the same assay/plate, with both intra- and inter-assay/plate coefficients of variation reported below for each parameter.

Any missing data-points are denoted as ‘NA’ in the 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 GmbH, Birmingham).

BODY MASS

The weight of each patient 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 only for males; lightweight shorts plus lightweight vest-top for females), with the same clothing standardised between baseline and follow-up.

BODY COMPOSITION/PERCENT BODY FAT

Lean and adipose tissue mass were measured to the nearest 0.1 g using Dual Energy X-ray Absorptiometry (DEXA; Hologic Discovery W) with patients wearing the same minimal clothing as described for BODY MASS (therefore having removed any metal items)

WAIST:HIP CIRCUMFERENCE

The distance around the waist and hip were 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 and the widest gluteal girth. The average of three measurements was accepted with agreement <1 cm between them. Due to experimenter error, three patients did not have hip circumference measured.

SAGITTAL ABDOMINAL DIAMETER

The vertical distance from the floor (solid surface) to the top of the abdomen at the level of the iliac crest was measured during gentle expiration with patients supine using a Holtain-Kahn calliper accurate to 0.1 cm (Holtain Ltd., Crymych). This calliper only became available after the baseline measurements of the first four patients enrolled in this trial.

TIME OF WAKING/SLEEPING

Individual patients were provided with a record sheet to keep at their bedside to directly record the times they woke-up and went to sleep. One patient failed to record either the time they woke-up or went to sleep. Wake-up times only are available for two further patients at baseline and/or follow-up, although mean sleep duration has been estimated for these individuals based on the recorded time of the calibration blood sample taken immediately before bed.

DIET COMPOSITION

Individual patients were provided with a food diary and electronic scales 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 mass of each macronutrient ingested each day was then averaged from the 14 days recorded during the 6-week intervention using a database of typical UK foods (CompEat Pro, Nutrition Systems, Banbury) and retained food packaging labels wherever possible. Daily energy intake was then determined from these masses assuming the following energy densities: 4.1 kcal/g non-sugar carbohydrates, 3.85 kcal/g sugars, 8.9 kcal/g fats, 4 kcal/g proteins and 7 kcal/g ethanol/alcohol.

DAILY FEEDING FREQUENCY

In addition to the methods described for DIET COMPOSITION, patients also recorded the time of day at which each food item was consumed. A given eating occasion was then defined as any food items consumed within 45 min of one another, with meals defined as ingestion of >1256 kJ at any given eating occasion and snacks as ingestion of <1256 kJ more than 45 min before or after a meal. Five patients failed to consistently record feeding timings with sufficient accuracy to determine meal/snack patterns according to these stated criteria.

RESTING METABOLIC RATE

Energy expenditure under basal conditions was determined via indirect calorimetry from gaseous exchange. Patients rested motionless in a supine position for 30 minutes whilst breathing through a respiratory valve into Douglas bags. 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). One patient has missing data as the paramagnetic analyser failed to calibrate at baseline. 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. At each time-point, between 4 and 6 Douglas bags were collected from each patient and resting metabolic rate was averaged from the three lowest measurements stable within 100 kcal per day.

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).

PHYSICAL ACTIVITY THERMOGENESIS

The energy expended during physical activity was determined via combined heart-rate/accelerometry (ActiHeart™). To be accepted as a valid measure, at least 4 of every 7 daily physical activity traces had to meet the following criteria for minimum data quality: a maximum of 10 % and 22.5 % of any 24 h heart rate record could be ‘lost’ or ‘recovered’ data, respectively (i.e. where the majority of the energy expenditure estimate would then be dictated by the accelerometer data). Three patients’ data were therefore excluded on this basis. The remaining ‘uncorrected’ data (i.e. inclusive of missing data up to 10 % per day) was normalised up to a 24 h equivalent based upon the average of the measured data to yield the total rate of physical activity thermogenesis averaged across weeks 1 & 6 (n.b. while this correction can confidently be applied to a 24 h period, we cannot confidently know how and when energy would have been expended. The breakdown of physical activity thermogenesis according to intensity and time of day therefore remains uncorrected such that the sum of intensities and durations is slightly below the 24 h total).

BLOOD PRESSURE

Duplicate systolic and diastolic blood pressure measurements were made in a seated position above the antecubital space of the left arm using an automated sphygmomanometer (DINAMAP PRO100V2, GE Healthcare, Fairfield). Due to equipment failure, 13 patients do not have complete blood pressure records at baseline and follow-up. Blood pressure data are not central to our research question, so are not reported in this research paper but are included in the associated data archive. For reference, all patients presented normotensive with mean (SD) systolic 116 (11) mmHg and diastolic 71 (7).

TOTAL CHOLESTEROL

Blood serum concentrations of total cholesterol were assayed using a Daytona RX automated clinical chemistry analyser (Randox, Crumlin). Intra-assay coefficient of variation <4 %; inter-assay coefficient of variation <2 %.

HIGH-DENSITY LIPOPROTEIN (HDL) CHOLESTEROL

Blood serum concentrations of HDL cholesterol were assayed using a Daytona RX automated clinical chemistry analyser (Randox, Crumlin). Intra-assay coefficient of variation <4 %; inter-assay coefficient of variation <3 %.

LOW-DENSITY LIPOPROTEIN (LDL) CHOLESTEROL

Blood serum concentrations of HDL cholesterol were calculated using the Friedwald equation (LDL cholesterol = Total cholesterol - HDL cholesterol - [triacylglycerol/2.2]).

TRIACYLGLYCEROL

Blood serum concentrations of triacylglycerol were assayed using a Daytona RX automated clinical chemistry analyser (Randox, Crumlin). Intra-assay coefficient of variation <4 %; inter-assay coefficient of variation <4 %.

NON-ESTERIFIED FATTY ACIDS (NEFA)

Blood plasma (EDTA-treated) concentrations of NEFA were assayed using a Daytona RX automated clinical chemistry analyser (Randox, Crumlin). Intra-assay coefficient of variation <5 %; inter-assay coefficient of variation <5 %.

INTERLEUKIN-6

Blood serum concentrations of interleukin-6 were assayed via Enzyme-Linked Immuno-Sorbent Assay (ELISA; R&D Systems, Minneapolis). Intra-assay coefficient of variation=5.61 %; inter-assay coefficient of variation=15.76 %. Blood serum samples were not available for one patient at baseline or follow-up. Data were excluded for another patient who reported symptoms of acute infection at follow-up in association with acutely elevated interleukin-6 concentration (15.03 pg/ml).

C-REACTIVE PROTEIN (CRP)

Blood plasma (EDTA-treated) concentrations of CRP were assayed using a Daytona RX automated clinical chemistry analyser (Randox, Crumlin). Intra-assay coefficient of variation <3 %; inter-assay coefficient of variation <5 %. Data were excluded for three patients whose plasma CRP concentrations were acutely elevated at either baseline or follow-up (5- to 59-fold change between measurements), so were not deemed to be reflective of changes in chronic low-grade systemic inflammation.

TRIIODOTHYRONINE (FREE-T3)

Blood serum concentrations of free-T3 were assayed via Enzyme-Linked Immuno-Sorbent Assay (ELISA; Alpco, Salem). Intra-assay coefficient of variation=7.25 %; inter-assay coefficient of variation=15.60 %.

THYROXINE (FREE-T4)

Blood serum concentrations of free-T4 were assayed via Enzyme-Linked Immuno-Sorbent Assay (ELISA; Alpco, Salem). Intra-assay coefficient of variation=4.25 %; inter-assay coefficient of variation=6.32 %.

LEPTIN

Blood serum concentrations of leptin were assayed via Enzyme-Linked Immuno-Sorbent Assay (ELISA; R&D Systems, Minneapolis). Intra-assay coefficient of variation=3.41 %; inter-assay coefficient of variation=6.40 %. Blood serum samples were not collected from one patient at baseline or follow-up. Fasted blood serum samples were not available for patients #6 and #9, for whom 15-min and 60-min post-breakfast time-points were instead analysed, respectively. The same delayed time-points were used for both baseline and follow-up contrasts for these two patients and leptin can be expected to be stable over this time-course in response to feeding.

TOTAL GHRELIN

Blood plasma (EDTA-treated) concentrations of ghrelin were assayed via Enzyme-Linked Immuno-Sorbent Assay (ELISA; Bertin Pharma, Montigny le Bretonneux). Intra-assay coefficient of variation=3.99 %; inter-assay coefficient of variation=7.76 %. Blood plasma samples were not available for four patients at baseline and/or follow-up.

ACYLATED GHRELIN

Blood plasma (EDTA-treated) concentrations of acylated ghrelin were treated with 1 mM PHMB upon collection and assayed via Enzyme-Linked Immuno-Sorbent Assay (ELISA; Bertin Pharma, Montigny le Bretonneux). Intra-assay coefficient of variation=4.19 %; inter-assay coefficient of variation=11.28 %. Blood plasma samples were not available for four patients at baseline and/or follow-up.

PEPTIDE YY (PYY)

Blood plasma (EDTA-treated) concentrations of PYY were assayed via Enzyme-Linked Immuno-Sorbent Assay (ELISA; Millipore, Billerica). Intra-assay coefficient of variation=4.29 %; inter-assay coefficient of variation=11.11 %. Blood plasma samples were not available for four patients at baseline and/or follow-up.

GLUCAGON-LIKE PEPTIDE-1 (GLP-1)

Blood plasma (EDTA-treated) concentrations of GLP-1 were assayed via Enzyme-Linked Immuno-Sorbent Assay (ELISA; Millipore, Billerica). Intra-assay coefficient of variation=4.75 %; inter-assay coefficient of variation=27.00 %. Blood plasma samples were not available for four patients at baseline and/or follow-up.

ADIPONECTIN

Blood serum concentrations of adiponectin were assayed via Enzyme-Linked Immuno-Sorbent Assay (ELISA; R&D Systems, Minneapolis). Intra-assay coefficient of variation=3.95 %; inter-assay coefficient of variation=6.34 %. Blood serum samples were not collected from one patient at baseline or follow-up. Fasted blood serum samples were not available for patients #6 and #9, for whom 15-min and 60-min post-breakfast time-points were instead analysed, respectively. The same delayed time-points were used for both baseline and follow-up contrasts for these two patients and adiponectin can be expected to be stable over this time-course in response to feeding.

GLUCOSE

Blood plasma (EDTA-treated) concentrations of glucose were assayed using a Daytona RX automated clinical chemistry analyser (Randox, Crumlin). Intra-assay coefficient of variation <5 %; inter-assay coefficient of variation <6 %. Fasted blood samples were not acquired from five patients during the oral glucose tolerance test.

INSULIN

Blood plasma (EDTA-treated) concentrations of insulin were assayed via Enzyme-Linked Immuno-Sorbent Assay (ELISA; Mercodia, Uppsala). Intra-assay coefficient of variation=4.70 %; inter-assay coefficient of variation=12.47 %. Fasted blood samples were not acquired from five patients during the oral glucose tolerance test.

HOMA-IR

The fasted glucose and insulin data were used to calculate HOMA-IR using the equation: (fasting insulin [mIU/L]\*fasting glucose [mmol/L])/22.5. Fasted blood samples were not acquired from five patients during the oral glucose tolerance test.

C-ISI MATSUDA INDEX

The fasted glucose and insulin data were combined with the mean glycemic and insulinemic responses over 2 h during the oral glucose tolerance test and used to calculate C-ISI Matsuda Index using the equation: 10000/SQRT((fasting glucose [mg/dL]\*fasting insulin [mIU/L])\*(mean glucose [mg/dL]\*mean insulin [mIU/L])). Fasted blood samples were not acquired from five patients during the oral glucose tolerance test. The cannula also blocked for another patient during the oral glucose tolerance test, so an incomplete data-set is available for calculation of C-ISI Matsuda Index.

ADIPOSE TISSUE GLUCOSE UPTAKE

Adipocytes were isolated from abdominal adipose tissue biopsy by collagenase digestion, with [U-14C]-D-glucose uptake measured at basal, submaximal (50 pmol•l-1) and maximal (20 nmol•l-1) insulin concentrations. The resultant data were expressed as pmol•min-1 relative to lipid mass. Insufficient (i.e. < 200 mg) adipose tissue was acquired from four patients for completion of this assay.

CONTINUOUS GLUCOSE MONITORS

Sub-cutaneous interstitial glucose concentrations were monitored at 5 minute intervals throughout the first and last week of intervention using a portable continuous glucose monitor (iPro, Medtronic, Minneapolis). This monitor did not provide any real-time feedback to patients during the intervention and patients were also blinded to the 4 daily calibrations performed using finger-prick blood samples. Records of daily TIME OF WAKING/SLEEPING were used to separate 24-h glucose profiles into mean, peak and coefficient of variation from waking until 1200 h, from 1200 h until sleep and during sleep. No data are available for one patient who had insufficient abdominal fat for the monitor to be fitted.