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

Archived data pertaining to article: Yung-Chih Chen, Rebecca L. Travers, Jean-Philippe Walhin, Javier T. Gonzalez, Francoise Koumanov, James A. Betts and Dylan Thompson. (2017) **Feeding influences adipose tissue responses to exercise in overweight men [NCT02870075].** *American Journal of physiology-Endocrinology and Metabolism* **In-Press.**

The trial is registered with the ClinicalTrial.gov, available at <https://clinicaltrials.gov> (ref: NCT02870075).

The precise details of the experimental design and protocol has been published as follows: Yung-Chih Chen, Rebecca L. Travers, Jean-Philippe Walhin, Javier T. Gonzalez, Francoise Koumanov, James A. Betts and Dylan Thompson. (2017) Feeding influences adipose tissue responses to exercise in overweight men [NCT02870075]. *American Journal of physiology-Endocrinology and Metabolism.*

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) after a 12 h overnight fasting (baseline, before meal) and at 0 (immediate after meal), 15, 30, 45, 60, 120, 180 and 240 min time-points after breakfast consumption. Glucose, insulin and NEFA measures were obtained throughout the trials as described in the manuscript. IL-6, leptin and adiponectin were measured at baseline, 0, 120, 180 and 240 min time-points. Due to difficulty in cannulating one participant, therefore, all blood parameters were equal to nine. Both adipose gene expression and adipose explants cell culture *ex-vivo* were measured at baseline and 240 min time-points. All blood samples and adipose explants cell culture *ex-vivo* were analysed in duplicate and quadruplicate respectively, as a batch within the same assay/plate, with both intra- and inter-assay/plate coefficients of variation reported below for each parameter. All adipose gene expression were analysed in duplicate and predesigned primers and probes were reported below for each parameter.

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

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).

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).

HIP CIRCUMFERENCE

The measurement hip circumference to the nearest 0.1 cm was undertaken according to World Health Organisation guidelines (2008) *World Health Organisation-Waist Circumference and Waist-Hip Ratio: Report of a WHO Expert Consultation* using a non-stretch tape. Hip circumference was measured horizontally around the widest portion of the buttocks.

WAIST CIRCUMFERENCE

The measurement of waist circumference to the nearest 0.1 cm was undertaken according to World Health Organisation guidelines (2008) *World Health Organisation-Waist Circumference and Waist-Hip Ratio: Report of a WHO Expert Consultation* using a non-stretch tape. Waist circumference was measured at midway point between the lowest rib and the iliac crest at end of normal breath expiration – and the tape was parallel to the floor when passed around the waist.

ADIPOSE TISSUE MASS

Adipose tissue mass (the region between L1-L4) were measured to the nearest 0.1 g using Dual Energy X-ray Absorptiometry (DEXA; Hologic Discovery W) with participants wearing the same minimal clothing as described for BODY MASS (therefore having removed any metal items).

V̇O2MAX

A treadmill speed of 4 km·h-1 and gradient of 8.5% was chosen. Participants exercised at this speed and gradient with the speed increased by 1 km·h-1 after every 3 min stage until volitional fatigue. One minute expired air samples were collected into Douglas bags (Hans Rudolph, MO, USA) was measured in the final minute of each stage and also at the point of volitional fatigue, defined as when the participant indicated that only 1 min remained until fatigue.

RESTING METABOLIC RATE

Energy expenditure under basal conditions was determined via indirect calorimetry from gaseous exchange. Participants rested motionless in a supine position for 30 min whilst breathing through a respiratory valve into Douglas bags. The total volume expired every 5 min as 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. Four Douglas bags were collected from each participant and resting metabolic rate was averaged from the three lowest measurements stable within 100 kcal per day.

EXERCISE INTENSITY

Participants walked on the treadmill at 60% V̇O2max for 60 min and one minute of expired air samples was collected at 5, 20, 40 and 60 min using the equations described by (Jeukendrup & Wallis, 2005) *International journal of sports medicine* **26 Suppl 1,** S28-37.

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%.

INSULIN

Blood serum (serum separation beads treated) concentrations of insulin were assayed via Enzyme-Linked Immuno-Sorbent Assay (ELISA; Mercodia, Uppsala). Intra-assay coefficient of variation < 4%; inter-assay coefficient of variation <5%.

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 and adipose explants cell culture *ex-vivo* concentrations of interleukin-6 were assayed via Enzyme-Linked Immuno-Sorbent Assay (ELISA; R&D Systems, Minneapolis). Intra-assay coefficient of variation <8%; inter-assay coefficient of variation <10%. Adipose interleukin-6 mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK) and predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of interleukin-6 (Hs00985639\_m1).

LEPTIN

Blood serum and adipose explants cell culture *ex-vivo* concentrations of leptin were assayed via Enzyme-Linked Immuno-Sorbent Assay (ELISA; R&D Systems, Minneapolis). Intra-assay coefficient of variation <4%; inter-assay coefficient of variation <6%. Adipose leptin mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK) and predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of leptin (Hs00174877\_m1).

ADIPONECTIN

Blood serum and adipose explants cell culture *ex-vivo* concentrations of adiponectin were assayed via Enzyme-Linked Immuno-Sorbent Assay (ELISA; R&D Systems, Minneapolis). Intra-assay coefficient of variation <5%; inter-assay coefficient of variation <8%. Adipose adiponectin mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK) and predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of adiponectin (Hs00605917\_m1).

AMPK

Adipose AMPK mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK). Predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of AMPK (Hs01562315\_m1 and Hs00178903\_m1 combined). Adipose AMPK protein contents were analysed via Western Blotting analysis using AMPK antibody (Cell Signalling Technology, USA).

GLUT4

Adipose GLUT4 mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK). Predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of GLUT4 (Hs00168966\_m1). Adipose GLUT4 protein content were analysed via Western Blotting analysis using GLUT4 antibody (Millipore).

IRS1

Adipose IRS1 mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK). Predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of IRS1 (Hs00178563\_m1). Adipose IRS1 protein contents were analysed via Western Blotting analysis using IRS1 antibody (Millipore).

IRS2

Adipose IRS2 mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK). Predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of IRS2 (Hs00275843\_s1). Adipose IRS2 protein contents were analysed via Western Blotting analysis using IRS2 antibody (Millipore).

HK2

Adipose HK2 mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK). Predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of HK2 (Hs00606086\_m1).

PI3K-85α

Adipose PI3K-85α mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK). Predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of PI3K-85α (Hs00933163\_m1).

AKT1

Adipose Akt1 mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK). Predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of Akt1 (Hs00178289\_m1).

ATGL

Adipose ATGL mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK). Predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of ATGL (Hs00386101\_m1).

G0S2

Adipose G0S2 mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK). Predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of G0S2 (Hs00605971\_m1).

HSL

Adipose HSL mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK). Predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of HSL (Hs00193510\_m1). Adipose HSL protein content were analysed via Western Blotting analysis using HSL antibody (Cell Signalling Technology, USA).

FAT/CD36

Adipose FAT/CD36 mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK). Predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of FAT/CD36 (Hs00169627\_m1).

CPT1B

Adipose CPT1B mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK). Predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of CPT1B (Hs03046298\_s1).

PDK4

Adipose PDK4 mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK). Predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of PDK4 (Hs00176875\_m1).

Adipose PDK4 protein content were analysed via Western Blotting analysis using PDK4 antibody (ABGENT, San Diego, USA).

FOXO1

Adipose FOXO1 mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK). Predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of FOXO1 (Hs01054576\_m1).

PPARγ

Adipose PPARγ mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK). Predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of PPARγ (Hs01115513\_m1).

SREBP-1c

Adipose SREBP-1c mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK). Predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of SREBP-1c

(Hs01088691\_m1).

MCP1

Adipose MCP1 mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK). Predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of MCP1 (Hs00234140\_m1).

PGC1α

Adipose PGC1α mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK). Predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of PGC1α (Hs01016719\_m1).

INTERLEUKIN-18

Adipose interleukin-18 mRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK). Predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of interleukin-18 (Hs00155517\_m1).

TNF-α

Adipose TNF-αmRNA expression was analysed via Real-time PCR using a StepOneTM (Applied Biosystems, Warrington, UK). Predesigned primers and probes were obtained from Applied Biosystems for the measurement of expression of TNF-α (Hs99999043\_m1).

AKT2

Adipose Akt2 protein contents were analysed via Western Blotting analysis using Akt2 antibody (Millipore).