**Info for readme file day-to-day reliability dataset:**

As described in the corresponding manuscript, this study employed a cross-sectional design where one-hundred and fifteen healthy men and women completed three main Trial days (A, B and C) and habitual lifestyle assessment (physical activity levels and dietary / macronutrient intake). Trial A and Trial B followed matched protocols separated by 7 – 28 days that involved the assessment of anthropometrics, resting metabolic rate, a fasting venous blood sample and an incremental graded exercise test (a FATMAX test) to determine PFO and FATMAX by indirect calorimetry. Trial C was organised 2 – 7 days after Trial B and involved body composition analysis (a whole-body dual energy x-ray absorptiometry (DEXA) scan) and opt-in skeletal muscle and / or adipose tissue biopsies. All trials were completed in an overnight fasted-state. Additionally, in the seven days prior to Trial A, participants recorded a self-weighed diet diary and wore a physical activity monitor for the assessment of habitual energy intake, macronutrient intakes and physical activity levels.

**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. Additionally, an estimate of body fat percentage was also obtained when participants were weighed.

**Body Stature**

Body stature was measured to the nearest 0.1 cm using a wall mounted/attached stadiometer (Holtain Ltd, UK) with participants head positioned in the Frankfort plane and after inhalation of a deep breath.

**Body mass index**

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

**Fat mass index**

Fat mass index was fat mass (kilograms; determined by DEXA) divided by height (m) squared. Participants were classified into four categories (fat deficient, healthy, excess adiposity and obese) as identified by (Kelly, Wilson, & Heymsfield, 2009).

**Waist and hip circumference**

Waist and hip circumference were measured to the nearest 0.1 cm using a non-elastic measuring tape (SECA 201, Hamburg, Germany). Waist circumference was measured at the narrowest point between the 10th Rib and top of the iliac crest at the end of a ‘normal’ expiration. Hip circumference was measured at the point of greatest posterior protuberance of the buttocks. Waist to hip ratio was calculated by dividing waist circumference by hip circumference.

**DEXA scan**

A dual energy x-ray absorptiometry (DEXA) scan was performed to quantify fat mass and fat free mass (FFM). Participants were scanned in light clothing and removed all metal (where possible). Participants were positioned centrally on the scanning bed; in a supine position on their back; with legs apart and feet turned inwards; and arms as wide as possible with hands placed in a mid-prone position such that the maximal gap possible was achieved between the arms and trunk.-AB / LB determination. The DEXA scan was also used to assess body fat distribution. Abdominal (or Android) body fat mass (g), which includes both visceral and subcutaneous fat, was determined by manual placement of three horizontal cut lines: a) an upper limit (neck cut line) set below the inferior edge of the chin (including all soft tissue of shoulders, approximately going through C4/C5 vertebral space); 2) a middle limit set going through T12/L1 vertebral space; and c) a lower limit (pelvic cut line) set going through L1/L2 vertebral space. Abdominal body fat mass was then automatically determined by the Hologic Discovery W QDR APEX Software (Version 4.2 for Microsoft Windows XP) as 20% of the inferior distance between the neck cut line and pelvic (iliac crest) cut line (Stults-Kolehmainen et al., 2013 ). Lower (or Gynoid) body fat mass (g), which is comprised of subcutaneous fat, was automatically determined by the Hologic Discovery W QDR APEX Software (Version 4.2 for Microsoft Windows XP) as 2.0 times the height of the Abdominal region, with the upper boundary set below the pelvic horizontal cut line at a distance of 1.5 times the abdominal region height (Stults-Kolehmainen et al., 2013).

**Incremental graded exercise (FATMAX) test**

Participants completed an incremental graded exercise test (GE) to volitional exhaustion on a mechanically braked cycle ergometer (Monark Peak Bike Ergomedic 894E, Varberg, Sweden). The FATMAX test comprised of: a) four-min stages for the first seven stages and b) two-min stages from the eighth stage onwards. Initial power output was set at ~ 30 or 40 W and increased by ~25 W over the next six stages (excluding the 10 W increment between first and second stage in the 30 W protocol) and by ~50 W from stage seven onwards. Expired gas samples were collected in the final minute of the first seven stages and upon the participant’s signal of one-min remaining before volitional exhaustion.

**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.

**V̇O2peak**

Peak oxygen uptake (V̇O2peak) was measured as the recorded oxygen uptake value of the expired gas sample collected in the final min of the GE test. Participants were categorised into three training classifications (untrained, recreationally trained, highly trained) based on corresponding V̇O2peak thresholds outlined for males and females (De Pauw et al., 2013; Decroix, De Pauw, Foster, & Meeusen, 2016).

**Peak fat oxidation**

Rate of peak fat oxidation during exercise was measured in absolute rates of fat oxidation (g·min-1) and expressed relative to body mass (mg·BM kg·min-1) and/or fat free mass (mg·FFM kg·min-1). This was determined by the measured values approach, the construction of a second order polynomial curve (P2) and the sine model (SIN; (Chenevière, Malatesta, Peters, & Borrani, 2009).).

**FATMAX**

FATMAX reflects the exercise intensity that peak fat oxidation was elicited at and was expressed as a % of V̇O2peak.

**Metabolic measurements**

Expired gas samples were collected into 100-150 L Douglas bags (Cranlea and Hans Rudolph, Birmingham, UK) via a mouthpiece connected to a two-way, T shaped non-rebreathing valve (Model 2700, Hans Rudolph Inc, Kansas City, USA) and falconia tubing (Hans Rudolph Inc, Kansas City, USA). Concentrations of O2 and CO2 were measured in a known volume of each sample via paramagnetic and infrared transducers, respectively (Mini MP 5200, Servomex Group Ltd., Crowborough, East Sussex, UK) and until values were stable. The sensor was calibrated to a two-point low and high calibration of known gas concentrations (Low: 99.998 % Nitrogen, 0 % O2 and CO2; High: Balance nitrogen mix, 20.06 % O2, 8.11 % CO2) (BOC Industrial Gases, Linde AG, Munich, Germany). Concurrent measurements of inspired air composition were made during collection of each expired gas sample to adjust for changes in ambient O2 and CO2 concentrations (Betts and Thompson 2012). The ambient temperature, humidity and barometric pressure were recorded via a weather station (Technoline WS 6730, TechnoTrade Import-Export GmbH, Berlin, Germany). 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 (L·min-1), V̇CO2 (L·min-1), V̇E (L·min-1) and respiratory exchange ratio (RER). Indirect calorimetry was then used to determine energy expenditure (kcal·min-1) and rates of carbohydrate and fat oxidation estimated by stoichiometric equations (Jeukendrup and Wallis, 2005; Frayn 1983) assuming urinary nitrogen excretion was negligible.

**Habitual physical activity levels**

Habitual physical activity levels were assessed by asking participants to wear a physical activity monitor (ActiheartTM, Cambridge Neurotechnology, Papworth, UK) over the 7-days prior to Trial A. A minimum of four days was required to determine habitual physical activity levels. Energy expenditure and heart rate values from rest and the FATMAX test were entered in the ActiheartTM software to derive an individually calibrated model estimate of physical activity energy expenditure (kcal·day-1). Participants were categorised into four physical activity level classifications (sedentary, low active, moderately active, very active) as identified by (Brooks, Butte, Rand, Flatt, & Caballero, 2004).

**Habitual dietary and macronutrient intakes**

Habitual energy and macronutrient intake were assessed by a self-weighed diet diary. Participants were provided with a set of scales (Pro Pocket Scale TOP2KG, Smart Weigh Scales) and asked to keep a written record of their food and fluid intake for at least 4 days in the week preceding Trial A (including at least one weekend day). Diet records were analysed using Nutritics software (Nutritics Ltd., Dublin, Ireland).

**Menstrual cycle status and oral contraceptive use**

The menstrual cycle was divided into two broad phases: the follicular and the luteal phase, which were determined by a progesterone concentration of < and ≥ 5 nmol/L, respectively (Oosthuyse, Bosch, & Jackson, 2005). Participants who were using any form of contraception were categorised into one group: contraceptive use.

**Blood sample and analysis**

A 10 mL whole venous blood sample was obtained from an antecubital vein (BD Vacutainer Safety Lok, BD, USA). Plasma samples were obtained by dispensing blood samples into a ethylenediaminetetraacetic acid-coated tube (K3 EDTA, Sarstedt, Germany) which were immediately centrifuged (1700 g for 15 min at 4°C; Heraeus Biofuge Primo R, Kendro Laboratory Products Plc., UK). Plasma samples were then extracted into 0.5 mL aliquots and immediately frozen at -20°C, before longer-term storage at -80oC for later batch analysis. The plasma samples were analysed for concentrations of various metabolites and hormones according to manufacturer instructions. Total plasma non-esterified fatty acids (NEFA; Cat No: FA115), glucose (Cat No:GL3815), lactate (Cat No: LC3980) and triglycerides (Cat No: TR3823) concentrations were run in singular on a Daytona Rx Series (Randox Laboratories, Crumlin, NI). Total 17β-oestradiol (Elecsys Estradiol III) and progesterone (Progesterone III) concentrations were run in singular on a Cobas 8000 (Modular analytics Cobas e 602, Roche Diagnostics, Rotkreuz, Switzerland). Total plasma insulin concentrations were analysed by an enzyme-linked immunosorbent assay (ELISA) kit in duplicate (Cat No: 900095, Cyrstal Chem, Illinois, USA) with absorption determined by a microplate reader (SPECTROstar Nano, BMG LABTECH, Ortenberg, Germany) at wavelengths specified by the manufacturer.

**Biopsies**

The opt-in skeletal muscle tissue biopsy was obtained from the *vastus lateralis* of the participant’s self-reported dominant leg via the suction-assisted ‘Bergstrom percutaneous needle’ biopsy technique. The adipose tissue biopsy was obtained from the subcutaneous abdominal wall region (approximately 5 cm left lateral from the umbilicus) using the ‘needle aspiration’ technique. Western blotting was performed on both skeletal muscle and adipose tissue samples to semi-quantitatively assess the content of several proteins involved in lipid metabolism. Data not reported in this manuscript.

**Notes:**

One participant did not agree for their completely anonymized forms of data to be archived on the University of Bath’s Research Data Archive system. Therefore, the values obtained will likely differ slightly to those reported in the manuscript (i.e. *n* = minus 1).

When sensitivity analyses performed (as identified below) did not change the interpretation of findings, the data are not reported.

**Study protocol deviances**

**Overnight fast time**

The majority of participants arrived to the lab on each occasion after a 10 – 13 h overnight fast (*n* = 84 out of 99) of which *n* = 82 (out of 84) self-reported to replicate (+/- 1 h) this fasting time before subsequent trials. In *n* = 5 of these participants, the actual length of self-reported overnight fast time was not recorded in either Trial A or Trial B, but was verbally confirmed. Of the 15 participants who were out of the requested overnight fast timeframe on at least one of the trials (*n* = 1 < 10 h, and *n* = 14 between 13 – 16 ½ h), *n* = 7 replicated their fast time (+/- 1 h) upon request from OCS. Sensitivity analysis was performed on participants who were ≥ 14 ½ h self-reported fasting time on either or both Trial A and Trial B. However, due to no systematic differences in PFO (Frandsen *et al.*, 2019) or substrate utilisation (Montain *et al.*, 1991) between an approximate 10 – 14 ½ h overnight fast and 8 – 12 h fast, respectively, participants within these time frames were kept in for all analyses. The participants who had an overnight fast time of ≥ 14 ½ h were:

* IDs 7, 32, 65, 71, 87, 99

**Trial A and B not between 7 and 28 days:**

* ID 91 = 4 days
* IDs 3, 4, 5, 28, 50, 96, 97, 11 = 6 days
* IDs 38, 83 = 29 days

**Trial B and C not between 2 – 7 days:**

* IDs 54, 78, 100, 103, 113 = 1 day
* ID 77 = 8 days
* ID 22 = 9 days
* IDs 9, 23, 48 = 10 days
* ID 7, 87 = 11 days
* IDs 60, 61, 73 = 14 days
* ID 33 = 21 days

These deviances in study timeframes (between Trial A and Trial B, and Trial B and Trial C) were due to logistical reasons (e.g. annual leave), availability of participant, lab or researcher and / or attempts to control for estimated menstrual cycle phase. Sensitivity analysis was not performed on these participants.

**Resting metabolic gas data**

* IDs 4 and 90: no data for Trial A due to hyperventilation
* ID 22: no data for Trial B hyperventilation
* IDs 54, 56, 68: Trial A data derived from one Douglas bag
* IDs 4, 35, 78, 99: Trial B data derived from one Douglas bag

Resting metabolic gas data was obtained from only one Douglas bag due to time constraints in lab availability and / or on the participant, in n = 6 trials, RMR was estimated from the collection and analysis of only one 5-min resting expired gas sample (after the collection of at least 3 x 5-min expired gas samples). In all but one participant, these estimates of RMR were all <100 kcal from estimated RMR using the Schofield equation (Schofield, 1985) which in addition to an average of RMR from Trial A and Trial B calculated these RMR estimates were included for all descriptive and data analysis. Sensitivity analysis was not performed on these participants.

When the assessment of RMR was highly variable for a participant within a trial (e.g. agreements outside +/- 100 kcal of averaged 24-hr resting energy expenditure), RMR estimates for that trial occasionally included gas samples outside this level of agreement. This was evident for *n* = 24 out of 597 expired gas sample collections (<5%; range: 1 to 97 kcal >100 kcal·day-1). These values were included due to conceptually an average of estimates provides a more accurate assessment of a value, where a clear physiological reason to exclude such bags [e.g. RER > 1 or a known issue in the collection or analysis (leak in a bag, contamination with atmospheric air)] was not apparent.

**Exercise metabolic gas data**

* ID 39: no substrate oxidation data from Trial A and Trial B due to participant hyperventilating during the graded exercise tests
* ID 67: no exercise data from Trial B due to fainting during the blood sample
* IDs 82, 103: V̇O2peak on Trial A and/or Trial B estimated by Astrand-Rhyming nomogram (Astrand and Ryhming, 1954). This was due to technical issues on the gas analysis machine, or participant terminating the test prior to exhaustion (due to dizziness). No sensitivity analysis was performed.

**Habitual dietary intake data**

* ID 100 estimate of habitual dietary intake derived from two days

All participants self-reported that they replicated the diet as closely as possible in the two days prior to Trial A and Trial B. Twenty-three participants noted a discrepancy in food item(s), food weight or restaurant *vs* homemade version. Given the self-report nature of the dietary standardisation and the variation / fluctuation in the nutritional content of food and drink items (e.g. from seasonal variability, tolerance limits of nutrient values, food preparation, cooking and serving techniques), sensitivity analysis was not performed. Additionally, one participant only recorded two days of dietary analysis and was excluded from relevant analyses.

**Habitual physical activity data**

* IDs 1, 3, 11, 18, 38, 53: estimates of habitual physical activity levels derived from three days
* IDs 20, 39: estimates of habitual physical activity levels were obtained from a slightly different data analysis technique due to an issue with the actiheartTM monitor

Data on the confirmation of physical activity standardisation prior to Trial A and Trial B (i.e. the prior 48 hrs) via objective assessment was only available for *n* = 36 out of 99 participants. The missing data for remaining participants was due to recording issues with the actiheartTM device or collected data not meeting validity criteria as identified above. Of these *n* = 36 participants, only *n* = 7 participants did not perform any vigorous physical activity in the two days preceding Trial A and Trial B, where total energy expenditure and/or estimated time spent in activity thresholds (<10% variance) across the two days prior to Trial A and Trial B were not matched in any of these *n* = 7 participants. Therefore, the planned sub-group analysis on the objective confirmation of physical activity standardisation was not able to be performed.

**Mathematical data analysis approaches to determine PFO and FATMAX**

Mathematical data modelling techniques (P2 and SIN) were unable to be applied to determine PFO and FATMAX on a number of participants for the following reasons:

* Less than four observed fat oxidation points [*n* = 18 trials (Trial A, *n* = 9; Trial B, *n* = 9]
* A plateau or minimal/no decline in rates of fat oxidation during the FATMAX test [*n* = 16 trials (Trial A, *n* = 9; Trial B, *n* = 7]
* No estimates of fat oxidation rates obtained in *n* = 1 participant in either Trial A or Trial B due to hyperventilation
* No rates of fat oxidation obtained for *n* = 1 participant on Trial B due to fainting

For the participants whose resting rates of fat oxidation were not able to be estimated in one of the trials, the resting rate of fat oxidation from the alternative trial was used to facilitate these modelling techniques. Additionally, for *n* = 16 participants, modelling was only able to be conducted on either Trial A (*n* = 9) or Trial B (*n* = 7), and for *n* = 9 participants modelling was not possible for either Trial A or Trial B.

Therefore, the number of participants with a complete dataset in which PFO and FATMAX were estimated for both Trial A and Trial B through the measured values approach, P2 and SIN was *n* = 72.

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