Real-time in vivo quantification of human skeletal muscle metabolism during exercise using hyperpolarised [1-^{13}C]pyruvate reveals training-dependent metabolic adaptation

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Supplementary methods

Flip angle optimisation for 13C MR spectroscopy

All MR spectra were acquired using a 3T GE Premier system (GE Healthcare, Waukesha, WI, USA) equipped with the multinuclear spectroscopy (MNS) research pack Version 3.0 (GE Global Research, Munich, Germany) and a 6-cm diameter transmit-receive surface coil (PulseTeq, Surrey, UK) (Supplementary Figure 4A). The centre frequency (f0) was adjusted manually by using the spectroscopy pre-scan window, and the transmit gain (TG) was selected after carrying out test calibrations on an 8 M 13C urea phantom (Sigma Aldrich, Gillingham, UK) to make sure that the pulse duration, determined by bandwidth and hardware requirements, was at the correct flip angle. To this end, a fiducial vial was placed in the centre of the coil and contained 8 M 13C urea doped with CuSO4 to shorten its T1. The fiducial was used to obtain a reference signal for both transmit power calibration and data analysis (Supplementary Figure 4B).

To assess the 13C transmit and receive B1 sensitivity of the coil, a uniform 16-cm diameter sphere filled with pure polydimethylsiloxane (GE Healthcare, Waukesha, WI) was placed within a coil-loading ring filled with saline solution (0.9% NaCl; Sigma Aldrich, Gillingham, UK)) above or below the coil (Supplementary Figure 4C).

To optimise the flip angle of the coil (measured in the fiducial at the coil centre) for participant fat thickness and the signal decay, a numerical simulation was set up to describe the magnetic field using a finite element method that considered the muscle tissue as a cylindrical slab with uniform and unitary initial magnetisation. The slab was positioned at different distances from the coil to reproduce the effect of different values of fat thickness and the signal progressively depleted during the simulation according to the T1 decay and the local flip angle, which depends on the local value of the B1 field. The signal was integrated across the volume of the slab for various fat thicknesses and flip angles, from 20 s after the start of the scan until its end at 300 s. The integrated value was then used to determine the optimal flip angle for each fat thickness. Scans prior to 20 s were excluded to ensure that the initial spike in MR signal, caused by high flip angles, did not dominate the rest of the scan, thereby maintaining a consistent signal-to-noise ratio throughout the experiment. Supplementary Figure 5A shows the result of the numerical stimulation where the total MR signal (i.e., B1 magnitude) varied with the distance from the centre of the transmit coil. The MR signal was then integrated over the slab volume from 20 to 300 s post scan initiation, and the resulting values were used to determine the flip angle that maximised signal for each simulated tissue thickness. Subsequently, the optimal flip angle values were plotted as a function of subcutaneous fat thickness (in mm) to identify the most suitable flip angle for subsequent hyperpolarised 13C MRS experiments (Supplementary Figure 5B).

Dynamic nuclear polarisation and production of hyperpolarised [1-13C]pyruvate

Sterile fluid paths (SFPs; Phillips-Medisize, Caldwell, Idaho, USA) were filled and assembled under sterile conditions at the University College London (UCL) Radiochemistry GMP facility (London, UK). The SFPs contained 1.47 g of [1-13C]pyruvic acid (Sigma Aldrich, St Louis,

MO, USA) that was mixed with 15 mmol/l of an electron paramagnetic agent (EPA, Syncom, Groningen, Netherlands) in a sealed vial, 38 ml sterile water that was used for dissolution, and 19 ml sterile water with 17.5 ml NaOH/Tris/EDTA (2.4%, 4.03%, and 0.033% w/v respectively, Royal Free Hospital, London, UK), which was used as a buffer for neutralisation. SFPs were stored upright overnight with the vial immersed in dry ice (-78.5 °C). The next day, the SFPs were transported upright using dry ice to the Sir Peter Mansfield imaging centre (University of Nottingham, School of Medicine, UK) where they were stored upright in a dedicated temperature monitored freezer (Forma™ High-Performance, ThermoFisher Scientific, Loughborough, UK) at -20 °C for at least two days prior to use.

On the day of the experiment, two SFPs were loaded into a 5T clinical hyperpolariser (SpinLab system, Research Circle Technology, Niskayuna, NY, USA), which was used for dynamic nuclear polarisation 37. First, the SFPs containing the frozen pyruvate/EPA mix were defrosted in the helium pressurised airlock of the hyperpolariser for one hour. Then the samples were irradiated at 139 GHz at ~ 0.8 K for approximately 3 hours until sufficient polarisation was achieved. Following rapid dissolution, the pyruvic acid was neutralised with buffer, and the EPA was removed by filtration upstream of the receiver vessel. The final injectable product was drawn from the receiver vessel into a 50-ml syringe (Bayer, Indianola, USA) through an additional 0.2-μm sterilising filter (PuroFLo® 65-mm Disc Filter, Saint-Gobain, Gaithersburg, MD, USA).

Rigorous quality control (QC) of the final filtered hyperpolarised sodium [1-13C]pyruvate solution was performed prior to human injection using an integrated QC module, which directly measured pyruvate and residual EPA concentration, pH, temperature, sample polarisation and volume of dissolute. Additional ‘offline’ pH measurement (pH test strips, Sigma Aldrich, Gillingham, UK), pressure bubble point of the sterilisation filter and visual inspection of the product (for visible particulates and appearance) were undertaken prior to release. The 50 ml

injection syringe containing hyperpolarised [1-13C]pyruvate was only released for human injection if the following criteria were met: pH 6.5-8.5, temperature 25.0-37.8 °C, polarisation ≥ 15%, [pyruvate] 220-280 mmol/l, [EPA] ≤ 3.0 μmol/l, appearance: clear, colourless solution with no visible particulate matter and bubble point > 50 psi in water. If these criteria of release were not met, hyperpolarised [1-13C]pyruvate was not injected.

Supplementary Tables

Supplementary Table 1. The time of appearance of ^{13}C-labelling metabolites in the medial gastrocnemius (MG) muscle of trained and untrained participants during plantar flexion exercise at either 40% or 70% Wmax. Data expressed as mean ± SEM and analysed using one-way ANOVA followed by Tukey's multiple comparison test. No statistically significant differences were observed between the groups.

Time of appearance in the MG muscle (s)	Trained 40% Wmax (n=4)	Untrained 40% Wmax (n=3)	Untrained 70% Wmax (n=3)	Overall Subjects (n=10)
[1-^{13}C]pyruvate	23±3	25±4	23±3	24±4
[1-^{13}C]lactate	25±4	30±3	27±3	27±3
[^{13}C]bicarbonate	27±4	33±4	31±2	30±4
[1-^{13}C]alanine	28±4	34±3	29±2	30±3

Supplementary Figure legends

Supplementary Figure 1: Plots of ^{13}C-labelled alanine from injected hyperpolarised [1-^{13}C]pyruvate in the medial gastrocnemius muscle of trained and untrained participants. (A) alanine-to-pyruvate ratio and (B) pyruvate -to-alanine conversion rates (k_{PA}) in the MG muscle of trained (T) and untrained (UT) participants exercising at either 40 or 70% Wmax (N = 3-4 per group). Data expressed as mean ± SEM and analysed using one-way ANOVA followed by Tukey's multiple comparison test. No statistically significant differences were observed between the groups.

Supplementary Figure 2: Correlations between conversion rates of ^{13}C incorporation and PCr rates measured by ^{31}P MRS during plantar flexion exercise. Graphs showing the correlation between A) lactate-to-pyruvate, B) bicarbonate-to-pyruvate, bicarbonate-to-lactate and alanine-to-pyruvate ratios and PCr depletion (k_{PCr}) rates in trained (T) and untrained (UT) participants exercising at either 40 or 70% Wmax, respectively (N=10 in total).

Supplementary Figure 3: Incremental exercise-based MR protocol. (A) Photograph showing the incremental plantar flexion exercise setup using a MR compatible pedal ergometer (Trispect diagnostic pedal, Ergospect GmbH, Austria). Participants laid in a supine position on a Philips MR bed (Philips Medical Systems, Best, the Netherlands) with their dominant limb secured into the Trispect diagnostic pedal and their knee fixed at approximately 30º. (B) Anatomical images of the lower limb of the participant showing the medial gastrocnemius muscle. (C) Graph representing the relationship between muscle strength (force in N) and workload (power in W), which was used to measure 40% or 70% of maximum workload for each participant.

Supplementary Figure 4: Phantom MR spectroscopy. (A) Photograph of the 6-cm diameter transmit-receive surface coil (PulseTeq, Surrey UK), (B) an example graph depicting flip angle

optimisation for our coil, (C) model and experimental B_1 field mapping to assess the ^{13}C transmit and receive B_1 sensitivity of our coil. No signal could be detected in the centre of the map due to the presence of the coil and two phantoms made of a uniform 16-cm diameter sphere filled with pure polydimethylsiloxane were placed within a coil-loading ring filled with saline (GE Healthcare, Waukesha WI) above or below the coil.

Supplementary Figure 5: Numerical simulation for ^{13}C MRS flip angle optimisation. (A) Graph showing the relationship between the total signal acquired between 20 and 300 s over the time course of the scan and the flip angle for different fat thicknesses, with the dashed line connecting all the maxima, (B) graph showing the linear correlation between the values of all maximal flip angles and fat thickness (in mm).