

Ultra-High Field Measurements of Glycogen, IMCL and Perfusion in Skeletal Muscle in Post-Exercise Recovery: A ^{13}C and ^1H MRS Study

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Background: Muscle glycogen depletion during exercise has been shown to be dependent on muscle fibre type¹ as well as exercise intensity² and duration³. Much less is known about the role of intramyocellular lipid (IMCL) in muscle substrate selection and maintaining performance during exercise. This is particularly difficult in thigh muscles, where reduced alignment of muscle fibres leads to reduced spectral separation of IMCL and extramyocellular lipids (EMCL). It is known that at higher exercise intensities IMCL contributes little to meeting energy demand whereas at lower intensities IMCL may be oxidised to provide energy⁴. In addition to measures of energy stores during exercise, and following recovery, adequate muscle perfusion is vital for maintaining levels of oxygen and nutrients utilized in the muscle during exercise. However, measurement of muscle perfusion in lower limbs is difficult due to low perfusion rates at baseline (~20 ml/100g/min²), susceptibility changes from muscle and bone, and short T₂ relaxation times. This study assesses the feasibility of sequentially monitoring muscle glycogen and IMCL levels, and perfusion, in exercising and non-exercising thigh muscles, prior to and following exercise, by utilizing the higher signal to noise (SNR) and spectral resolution available at 7T.

Methods: Subjects: 5 Healthy, recreationally active, male volunteers (age=27±4years, BMI=23.7±3, VO₂ max=53.9±7.3 ml.kg.min⁻¹) underwent preliminary testing to establish VO₂ max before attending two study visits, separated by at least 1 week. Subjects were overnight fasted and had refrained from alcohol, caffeine and strenuous exercise for 24h and were requested to consume the same quantity and type of food prior to each study visit.

Experimental Protocol: On each visit subjects underwent two baseline scan sessions with the RF coil positioned on the front and the back of the thigh respectively. Measurements were made of IMCL, glycogen, and muscle perfusion. Following the baseline scans, subjects cycled for 1h at either 50% VO₂ max (50.8±1.6%) or 75% VO₂ max (74.9±4.2%) with exercise intensity randomized across the subject's two visits. A post exercise (PE) scan was carried out in the front of the thigh to measure perfusion and glycogen levels before subjects were given a carbohydrate drink (t=20min PE) consisting of a 1litre solution containing 100g of a commercially available glucose polymer. Following ingestion of the drink ^{13}C scans, for measurement of glycogen, were acquired at t=20, 80, and 120mins in the Quadriceps, and t=50 and 100mins in the Hamstring muscle group. Measurements of ^1H IMCL were carried out at t=20 and 80mins in the Vastus Intermedius (VI) muscle and at t=50 and 110mins in the Semitendinosus (ST) muscle. Perfusion measurements were made at t=5, 35, 65 and 95mins interleaving the front and back of thigh muscles.

MR Methods: All MR measurements were acquired on a Philips Achieva 7T system, using a transmit/receive quadrature ^{13}C coil with transmit/receive quadrature ^1H decouple coils. ^{13}C MRS: ^{13}C spectra were acquired using a proton-decoupled pulse acquire sequence with adiabatic pulses and narrowband decoupling (BW=16000Hz, samples=256, TR=1000ms) for measurement of glycogen concentrations. 8 spectra, each with 80 averages, were collected at each time point (total scan time 11mins). ^{13}C spectra were averaged and post-processed using jMRUI. Glycogen/external reference peak areas were determined using in-house software built in Matlab. ^1H MRS: ^1H spectra, for measurement of IMCL, were acquired from the VI and the ST muscles using a STEAM sequence with the following parameters: TE/TM/TR=11/13/8000ms, VOI=18x18x30mm³, samples=4096, BW=4000Hz. 16 water-suppressed averages, and 4 without. Spectra were postprocessed using jMRUI and in-house software built in Matlab. Peak areas were calculated using the AMARES algorithm, fitting to Gaussian lineshapes. Perfusion Measurements: A TurboFLASH (TFL) sequence was used with TE/TR=2/4ms, image matrix 96 x96 with FOV of 160x192mm, slice thickness=6mm, flip angle~20° and a label delay, TI=1.2 s. A 6s repetition time was used per dynamic pair, and 60 dynamic pairs collected. Perfusion weighted difference signals were obtained and ROIs drawn in the ST, and VI muscle.

Results and Discussion: Glycogen (Fig. 2a): Basal levels of glycogen were significantly higher in the quadriceps compared with the hamstring, with ratio = 1.5±0.1 (mean ± standard err, p<0.01). Levels of glycogen decreased significantly in the front of the thigh (exercising muscles) following exercise at both 50% and 75% VO₂ max (p<0.05 and p<0.05 respectively) and were significantly lower when the subjects cycled at 75% VO₂ max compared with 50% VO₂ max. Immediately following the drink, levels of glycogen were not significantly different in the front of the thigh (t=20min). Levels measured at 80 minutes and 120 minutes had returned to baseline levels for the 50% visit, but remained significantly below baseline for the 75% visit. Levels in the back of the thigh were not significantly different from baseline, at any point, but tended to be increased from baseline levels on both visits.

IMCL (Fig 2b): Basal levels of IMCL were not significantly different between the ST and VI muscles and no significant differences were measured at any point following exercise or during recovery. Perfusion (Fig. 2c): Provisional analysis indicates a large increase in muscle perfusion in the front of the thigh immediately following cycling at 75% VO₂ max, with further increases following ingestion of the carbohydrate rich drink. Muscle perfusion levels were still returning to baseline following the final measurement at 65min. In contrast, no significant change was measured in perfusion following cycling at lower intensity, with a small but insignificant increase following the drink. No significant changes were measured in the back of the thigh. Further histogram analysis of the perfusion data will be carried out to resolve vessel contamination of the assessment of muscle perfusion, and the use of true-FISP methods will be assessed (to further improve SNR).

Conclusions: This study has shown that it is possible to measure glycogen, IMCL and perfusion in thigh muscle at 7T during a dynamic study. As expected, levels of glycogen in exercising muscles decreased significantly during exercise, with larger decreases following higher intensity exercise. Carbohydrate re-feeding increased glycogen levels with levels returning to baseline values for moderate exercise intensity (50%). Levels following high intensity exercise did not return back to baseline, probably due to insufficient carbohydrate loading. Levels of glycogen in the non-exercising muscles showed no change following exercise and re-feeding. Although insignificant, levels of IMCL in exercising muscles tended to increase following exercise and re-feeding, with a larger magnitude of change following lower intensity exercise. Increases, and magnitudes of increases in IMCL tended to be mirrored by decreases in IMCL in the non-exercising muscle.

Provisional analysis of perfusion data indicates an increase in muscle perfusion during exercise, with the magnitude scaling with exercise intensity, however further analysis will be carried out to further improve results.

References: 1. Vollestad, N. K. *et al.*, *Acta Physiologica Scandinavica* (1985) **125**(3) pp395. 2. Gollnick, P.D. *et al.* *J. Physiol.* (1974), **241**, pp45. 3. Price *et al.* *J. Appl. Physiol.* (1991), **70** (4), pp1836. 4. Van Loon *et al.* *J. Physiol.* (2001) **536**. 5. Utriainen T, *et al.* *J Clin Invest* **100**: 777–785, 1997

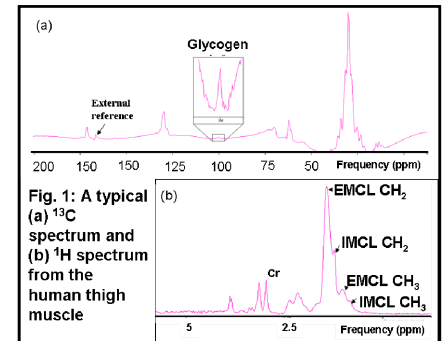


Fig. 1: A typical (a) ^{13}C spectrum and (b) ^1H spectrum from the human thigh muscle

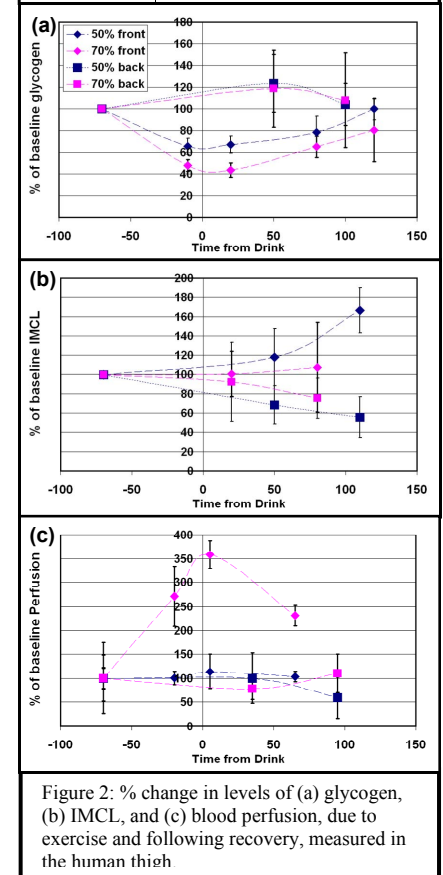


Figure 2: % change in levels of (a) glycogen, (b) IMCL, and (c) blood perfusion, due to exercise and following recovery, measured in the human thigh