World Journal of Radiology

Online Submissions: http://www.wjgnet.com/1949-8470office
wjr@wjgnet.com
doi:10.4329/wjr.v3.i4.105

World J Radiol 2011 April 28; 3(4): 105-113
ISSN 1949-8470 (online)

BRIEF ARTICLE

Applications of multi-nuclear magnetic resonance spectroscopy at 7T

Mary C Stephenson, Frances Gunner, Antonio Napolitano, Paul L Greenhaff, Ian A MacDonald, Nadeem Saeed, William Vennart, Susan T Francis, Peter G Morris

Mary C Stephenson, Susan T Francis, Peter G Morris, The Sir Peter Mansfield Magnetic Resonance Centre, School of Physics and Astronomy, University of Nottingham, Nottingham NG7 2RD, United Kingdom
Frances Gunner, Paul L Greenhaff, Ian A MacDonald, School of Biomedical Sciences, University of Nottingham Medical School, Nottingham NG7 2UH, United Kingdom
Antonio Napolitano, Academic Radiology, University of Nottingham, Nottingham NG7 2UH, United Kingdom
Nadeem Saeed, William Vennart, Molecular Medicine, Pfizer, Sandwich, Kent CT13 9NJ, United Kingdom
Author contributions: Stephenson MC, Gunner F, Morris PG, Francis ST, Saeed N, MacDonald IA, Greenhaff PL and Vennart W designed the research; Stephenson MC and Gunner F performed the research; Stephenson MC analyzed the data; Napolitano A contributed new analytic tools; Stephenson MC and Morris PG wrote the paper.
Supported by The Haldane-Spearman Consortium for PhD funding for Dr. Gunner F, Swecarb AB for provision of the carbohydrate drink, and Pfizer for funding the 1H Repeatability work. Dr.Stephenson M was supported by the University of Nottingham's Mansfield Fellowship scheme. 7T work was supported by grant G9900259 from the Medical Research Council; Pfizer and grant G0901321 from the Medical Research Council; The 7 T MR Scanner in Nottingham, was funded by a Joint Infrastructure Fund Grant from the Wellcome Trust UK
Correspondence to: Mary C Stephenson, PhD, The Sir Peter Mansfield Magnetic Resonance Centre, School of Physics and Astronomy, University of Nottingham, Nottingham NG7 2RD, United Kingdom. mary.stephenson@nottingham.ac.uk
Telephone: +44-115-9566881 Fax: +44-115-9515166
Received: October 22, 2010 Revised: April 2, 2011
Accepted: April 9, 2011
Published online: April 28, 2011

Abstract

AIM: To discuss the advantages of ultra-high field (7T) for ^{1}H and ^{13}C magnetic resonance spectroscopy (MRS) studies of metabolism.

METHODS: Measurements of brain metabolites were made at both 3 and 7T using ^{1}H MRS. Measurements of glycogen and lipids in muscle were measured using ^{13}C and ^{1}H MRS respectively.

RESULTS: In the brain, increased signal-to-noise ratio (SNR) and dispersion allows spectral separation of the amino-acids glutamate, glutamine and γ-aminobutyric acid (GABA), without the need for sophisticated editing sequences. Improved quantification of these metabolites is demonstrated at 7T relative to 3T. SNR was 36% higher, and measurement repeatability (% coefficients of variation) was 4%, 10% and 10% at 7T, *vs* 8%, 29% and 21% at 3T for glutamate, glutamine and GABA respectively. Measurements at 7T were used to compare metabolite levels in the anterior cingulate cortex (ACC) and insula. Creatine and glutamate levels were found to be significantly higher in the insula compared to the ACC ($P < 0.05$). In muscle, the increased SNR and spectral resolution at 7T enables interleaved studies of glycogen (^{13}C) and intra-myocellular lipid (IMCL) and extra-myocellular lipid (EMCL) (^{1}H) following exercise and re-feeding. Glycogen levels were significantly decreased following exercise (-28% at 50% VO_2 max; -58% at 75% VO_2 max). Interestingly, levels of glycogen in the hamstrings followed those in the quadriceps, despite reduce exercise loading. No changes in IMCL and EMCL were found in the study.

CONCLUSION: The demonstrated improvements in brain and muscle MRS measurements at 7T will increase the potential for use in investigating human metabolism and changes due to pathologies.

Key words: Magnetic resonance spectroscopy; ^{13}C; ^{1}H; 7 Tesla; Glutamate; Glutamine; γ-aminobutyric acid

Peer reviewer: Kubilay Aydin, MD, Professor, Istanbul University, Istanbul Faculty of Medicine, Department of Radiology, Neuroradiology Division Capa, Istanbul, Turkey

Stephenson MC, Gunner F, Napolitano A, Greenhaff PL, MacDonald IA, Saeed N, Vennart W, Francis ST, Morris PG. Applications of multi-nuclear magnetic resonance spectroscopy at 7T. *World J Radiol* 2011; 3(4): 105-113 Available from: URL: http://www.wjgnet.com/1949-8470/full/v3/i4/105.htm DOI: http://dx.doi.org/10.4329/wjr.v3.i4.105

INTRODUCTION

Magnetic resonance spectroscopy (MRS) is a versatile technique which can be used for measurement of metabolite levels, studies of bioenergetics, and measurement of chemical reaction rates without the need for invasive procedures such as biopsy. Whilst magnetic resonance imaging has quickly become one of the most widely used clinical tools, progress in MRS has been much slower. MRS has the potential to become a vital tool for aiding the understanding of changes due to pathology in specific regions of the body, as well as for clinical diagnosis and treatment monitoring. Improvements in hardware, which have allowed higher field spectrometers to be developed, provide increased sensitivity and spectral resolution. Many studies have demonstrated these improvements with increasing field[1-10], however the extent has been variable, with increases in signal-to-noise ratio (SNR) of 20% to 46% reported between 1.5 and 3T[1,4-6] and 80% from 1.5T to 4T[2]. This paper compares SNR and measurement reproducibility for ^{1}H and ^{13}C MRS measurements in the human brain and skeletal muscle, and discusses applications of ^{1}H and ^{13}C MRS for studying human metabolism, utilizing the increased sensitivity and spectral resolution at 7T.

Improved ^{1}H MRS reproducibility of glutamate, glutamine and γ-aminobutyric acid measurements in the human brain at 7T

Levels of metabolites, measurable in the human brain with ^{1}H MRS, are important in understanding changes involved in neurological[11,12] and psychiatric diseases[13-17], and potential therapies[18-19]. Studies at low field strength (≤ 1.5T) tend to concentrate on measurement of N-acetyl aspartate (NAA), Creatine (Cr) and Choline (Cho). Measurement of glutamate (Glu), glutamine (Gln) and γ-aminobutyric acid (GABA) is difficult at low field strength due to their overlapping resonances with each other, and with those of other molecules such as myo-Inositol (mI) and NAA. Thus, at low field, the concentrations of Glu and Gln are often combined as Glx = Glu + Gln. This could mask relative changes in Glu and Gln, such as might be expected if the rate of the glutamate/glutamine cycle is altered. Many different methods have been suggested for individual measurement of Glu, Gln and GABA, including constant time point resolved spectroscopy[20], chemical shift selective filters[21], 2D J-resolved spectroscopy[22] and MEGA-editing sequences[23,24]. However, these techniques are often time consuming, or may result in the loss of other metabolite signals which may be of interest. At higher fields, increasing spectral resolution enables metabolites to be accurately quantified without the need for sophisticated editing, and various sequences, optimized to give maximum separation, have been proposed[25-29]. Little work has been done to compare optimized sequences, or to establish levels of reproducibility based on different sequences. The aim of this study was to compare the ^{1}H MRS reproducibility of measurements of Glu, Gln and GABA at 3 and 7T using both a short TE STEAM sequence (TE/TM = 16/17 ms for optimum SNR) and a long TE STEAM sequence (TE/TM = 74/68 ms, shown to give pseudo-singlets for these metabolites[29]). Levels of variation in neurotransmitter concentrations over a week were then assessed in the anterior cingulate cortex (ACC) and insula (Ins) using the sequence which provided the most reproducible results.

Ultra-high field studies of skeletal muscle energy stores

Glycogen, intra-myocellular lipid (IMCL) and extra-myocellular lipid (EMCL) are the major sources of energy in human skeletal muscle[30] and can be measured *in vivo* using ^{13}C[31-33] and ^{1}H MRS respectively[34-40]. Studies of energy stores in skeletal muscle (or hepatic tissue) can provide much information on utilization during exercise or during postprandial replenishment[41-45], and are important for understanding diseases where glucose or lipid metabolism is thought to be perturbed. Due to the low natural abundance and low relative sensitivity of the ^{13}C nucleus, natural abundance ^{13}C MRS acquisition times tend to be long. Increased signal, available at 7T, allows for shorter acquisition times, which can be used to achieve better temporal resolution for dynamic studies. Shorter acquisition times for dynamic studies allow ^{13}C MRS measurements of glycogen to be made sequentially with ^{1}H MRS measurements of lipid stores thus allowing both of the major sources of energy to be observed on a reasonable timescale. The separation of IMCL and EMCL peaks in ^{1}H MR spectra is determined by the orientation of the muscle fibres in the magnetic field[40]. For well aligned fibres, orientated with the static magnetic field, the resonances from EMCL shift approximately 0.2 ppm from their respective IMCL resonances. Thus, at higher field, increased spectral resolution should provide more accurate quantitation as well as enabling separation of peaks in muscles with reduced alignment, for example the quadriceps and hamstrings in the human thigh.

Previous studies of energy stores have shown that muscle glycogen depletion during exercise is dependent on muscle fibre type[46] as well as exercise intensity[47] and duration[48]. Much less is known about the role of IMCL in muscle substrate selection and maintaining performance during exercise, although it is suggested that at higher exercise intensities IMCL contributes little to meeting energy demand, whereas at lower intensities IMCL may be oxidised to provide energy[49]. Here, a study was performed to assess the feasibility of sequential monitoring muscle glycogen and IMCL levels, in thigh muscles, prior to and following exercise, by utilizing the higher SNR and spectral resolution available at 7T.

MATERIALS AND METHODS

Ethical permission was obtained from the University of

Nottingham Medical School Ethics Committee and all subjects provided informed written consent before participation in the study. All measurements were performed on the Philips Achieva 3T and 7T systems at the Sir Peter Mansfield Magnetic Resonance Centre, Nottingham.

^{1}H reproducibility study

3T scans were acquired using an 8-channel SENSE head coil with transmission on the Q-Body coil. 7T scans were acquired on a 16-channel SENSE head, with transmission on a head volume coil.

Sequence reproducibility: Twelve healthy male subjects (age = 28 ± 11 years) attended two scan visits, 8 ± 2 d apart. On each visit subjects were scanned for 1h in each scanner, the protocol consisted of 3 survey images (to allow voxel positioning within the ACC) and 3 ^{1}H MRS acquisitions. Subjects were asked to reposition their head between repeats. For each spectral acquisition a 1 mm isotropic anatomical T_1 weighted Turbo-field Echo (TFE) image was acquired with TE/TR = 3.8/8.3 ms. This image was used to estimate the tissue percentage within the voxel to allow correction of metabolite concentrations since metabolites (with the exception of Gln and lactate) are present in much lower concentrations in the cerebrospinal fluid (CSF) compartment (levels of Gln are given without correction).

3T spectra were acquired with a bandwidth (BW) = 3000 Hz, and the number of points (No. samples) = 2048. 7T spectra were acquired with BW = 4000 Hz, No. samples = 2048. At both 3T and 7T the "short TE" STimulated Echo Acquisition Mode (STEAM) sequence was acquired with TE/TM/TR = 16/17/2000 ms, and the "long TE" sequence with TE/TM/TR = 74/68/2000 ms. The volume of interest (VOI) = 20 mm × 18 mm × 25 mm was placed in the ACC. Spectra for metabolite analysis consisted of 288 water-suppressed averages. Reference spectra consisted of 18 averages without water suppression.

Regional and longitudinal variation: 12 healthy male subjects (age = 30 ± 5 years) were scanned twice 7 ± 0 d apart. On visit 1, three repeat spectra (7T short TE) were acquired from the insula (VOI = 40 mm × 12 mm × 18 mm), to assess single session repeatability, and one spectrum acquired from the ACC. On visit 2, one spectrum was acquired from the ACC and one from the insula.

Post-processing: All spectra were processed in jMRUI. The water suppressed spectra were summed in jMRUI before analysis using LCModel and sequence specific basis-datasets based on 10 metabolites: N-acetyl aspartate (NAA), Creatine (Cr), Choline (Cho), Glu, Gln, GABA, Myo-Inositol (mI), Aspartate (Asp), Taurine (Tau) and Guanidinoacetate (Gua). Cramer-Rao lower bounds (CRLB) > 100% were eliminated from averages. Metabolite concentrations from LCModel were then corrected for tissue concentrations (by dividing by the tissue fraction). Metabolite concentrations are given in arbitrary units and no correction has been made for relaxation effects. Estimated standard deviations (%SDs) were taken directly from LCModel and average values were calculated across all subjects. Coefficients of variation [%CV = (SD/mean) × 100] were calculated across the three repeat measures in a single visit in ACC and insula cortex. Longitudinal variation [%LV = (SD/mean) × 100] was calculated from repeat measures over a week. SNR measurements were calculated from post-processed spectra using an in-house Matlab script [SNR = peak height/(1.96 × RMS_{noise})]. Significance was calculated using a Wilcoxon signed ranks test in SPSS 17 (SPSS for Windows, Chicago Ill, USA).

3T vs 7T comparisons of muscle glycogen and IMCL measurements

Subjects: Four healthy subjects (age 18-30 years) were scanned for ^{1}H MRS measurement of lipid levels in muscle on both the 3 and 7T scanners. 3T ^{1}H IMCL scans were acquired using the Q-Body coil for signal transmission and reception. 7T ^{1}H IMCL scans were acquired using a transmit/receive quadrature ^{1}H coil (with inbuilt ^{13}C quadrature coil), supplied by Philips (Cleveland, Ohio, USA). Spectra were acquired from the soleus muscle using a PRESS sequence with TE/TR = 40/7000 ms and the following parameters: VOI = 30 mm × 30 mm × 50 mm, with 16 water-suppressed averages. Reference spectra consisted of 16 acquisitions without water suppression. At 3T BW = 2000 Hz, No. samples = 1024, and at 7T BW = 4000 Hz, No. samples = 2048. To assess the repeatability of measurements, three measurements were made in a single subject.

For measurement of glycogen SNRs, spectra were acquired from a phantom containing 250 mol/L oyster glycogen. 3T ^{13}C glycogen measurements were acquired using a transmit/receive 13cm diameter ^{13}C coil with quadrature ^{1}H decouple coils (PulseTeq Ltd, Gloucestershire, UK). 7T glycogen measurements were acquired using a transmit/receive ^{13}C quadrature coil with quadrature ^{1}H decouple coils. Spectra were acquired using a pulse-acquire sequence with optimized adiabatic pulses and narrowband decoupling (3T BW = 8000 Hz, No. samples = 256; 7T BW = 16 000 Hz, No. samples = 256). Eight spectra, each with 80 averages, were collected at each time point (total scan time 11 min) before signal averaging in jMRUI.

^{1}H and ^{13}C MRS of muscle energy stores

Subjects: Six healthy, recreationally active, male volunteers (age = 26 ± 1.5 years, body mass index = 23.7 ± 0.9 kg/m^2, VO_2 max = 53.4 ± 2.7 mL/kg per minute) underwent preliminary testing to establish VO_2 max, before attending two study visits, separated by at least 1 wk. Subjects were overnight fasted and had refrained from alcohol, caffeine and strenuous exercise for 24 h and were requested to consume the same quantity and type of food prior to each study visit.

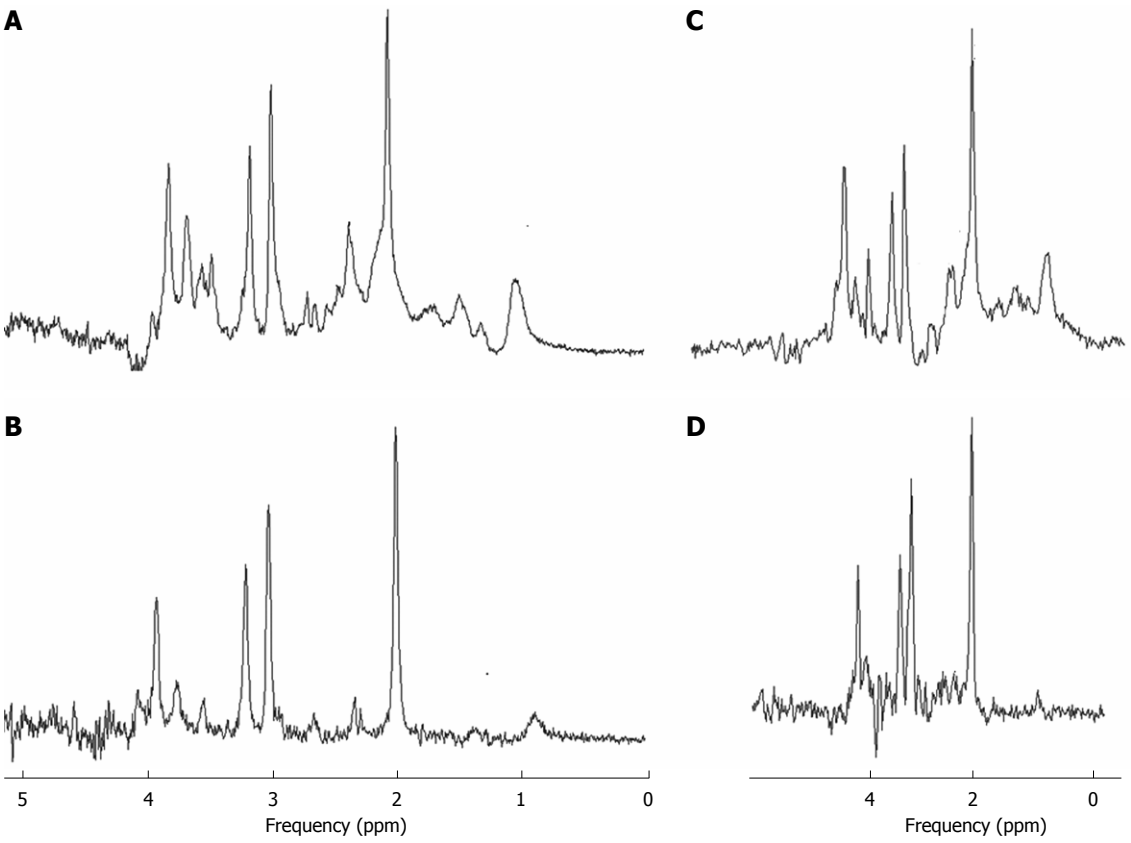

Figure 1 Example spectra acquired from subject 1 using 7T short TE (A), 7T long TE (B), 3T short TE (C) and 3T long TE (D) sequence.

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 and glycogen. Following the baseline scans, subjects cycled for 1h at either 50% VO_2 max (50.8% ± 0.7%) or 75% VO_2 max (74.9% ± 1.9%) with exercise intensity randomized across the subject's two visits. A post exercise (PE) scan was carried out on the front of the thigh to measure glycogen levels before subjects were given a carbohydrate drink (t = 20 min PE) consisting of a 1 litre solution containing 100 g 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 120 min in the quadriceps, and t = 50 and 100 min in the hamstring muscle group. Measurements of ^{1}H IMCL were carried out at t = 20 and 80 min in the vastus intermedius (VI) muscle and at t = 50 and 110 min in the semitendinosus (ST) muscle.

^{13}C MRS: ^{13}C spectra were acquired using a proton-decoupled pulse acquire sequence with adiabatic pulses and narrowband decoupling (BW = 16 000 Hz, No. samples = 256, TR = 1000 ms) for measurement of glycogen concentrations. Eight spectra, each with 80 averages, were collected at each time point (total scan time 11 min). ^{13}C spectra were post-processed by signal averaging and 50 Hz Lorentzian line broadening added before a phase correction was applied using jMRUI. Glycogen/external reference peak areas were determined using in-house software built in Matlab.

^{1}H MRS: ^{1}H MR spectra, for measurement of IMCL and EMCL, were acquired from the VI and the ST muscles using a STEAM sequence with the following parameters: TE/TM/TR = 11/13/8000 ms, VOI = 18 mm × 18 mm × 30 mm, No. samples = 4096, BW = 4000 Hz. Sixteen water-suppressed averages, and 4 reference spectra were acquired. Spectra were post-processed by realigning and phase correcting using jMRUI. Peak areas were calculated using the AMARES algorithm[50], fitting to Gaussian lineshapes. Values were converted to absolute levels as described by Szczepaniak *et al*[51], using T_2 values measured at 7T[52].

RESULTS

^{1}H reproducibility study

Sequence optimization: Example spectra, acquired in the ACC for a single subject, are shown in Figure 1. Average ACC SNR values, calculated for each sequence from the unfiltered NAA peak at 2.008 ppm, were highest for the 7T short TE sequence (SNR = 69 ± 7), which was significantly better than the 3T short TE sequence (SNR = 51 ± 6, $P < 0.002$). Similarly the 7T long TE sequence produced significantly higher SNR values than the 3T long TE sequence (SNR = 37 ± 9 *vs* 27 ± 6, $P = 0.006$).

The mean estimated error in metabolite quantifica-

Table 1 Mean Cramer-Rao lower bounds (SD) from LCModel averaged across all subjects

	NAA	Glu	Gln	mI	GABA	Cr	Cho
7T short	2 (0)	2 (0)	6 (1)	5 (1)	9 (2)	3 (2)	2 (0)
3T short	3 (1)	8 (2)	24 (8)	6 (1)	24 (10)	12 (3)	7 (7)
7T long	2 (1)	8 (1)	28 (13)	10 (3)	26 (9)	2 (0)	2 (0)
3T long	3 (1)	16 (5)	40 (15)	13 (6)	50 (20)	12 (5)	5 (1)

NAA: N-acetyl aspartate; Glu: Glutamate; Gln: Glutamine; mI: Myo-Inositol; GABA: γ-aminobutyric acid; Cr: Creatine; Cho: Choline.

Table 2 Mean % coefficients of variation (SD) averaged across all subjects

	NAA	Glu	Gln	mI	GABA	Cr	Cho
Uncorrected							
7T short	3 (2)	4 (2)	10 (6)	9 (3)	10 (6)	3 (2)	5 (4)
3T short	5 (3)	8 (6)	29 (11)	8 (4)	21 (14)	10 (4)	16 (16)
7T long	6 (6)	10 (6)	29 (19)	19 (10)	16 (8)	7 (6)	8 (6)
3T long	6 (6)	16 (9)	32 (30)	22 (10)	36 (25)	22 (13)	8 (7)
Tissue corrected							
7T short	4 (3)	5 (2)	10 (5)	9 (4)	10 (6)	4 (2)	6 (3)
3T short	6 (4)	8 (6)	29 (12)	8 (5)	22 (15)	10 (5)	17 (16)
7T long	6 (6)	10 (7)	29 (19)	20 (10)	15 (6)	6 (6)	8 (6)
3T long	7 (6)	16 (10)	32 (30)	23 (10)	38 (25)	22 (14)	9 (7)

NAA: N-acetyl aspartate; Glu: Glutamate; Gln: Glutamine; mI: Myo-Inositol; GABA: γ-aminobutyric acid; Cr: Creatine; Cho: Choline.

Table 3 Mean Cramer-Rao lower bounds (SD) from LCModel averaged across all subjects

	NAA	Glu	Gln	mI	GABA	Cr	Cho
CRLB ACC	2 (0)	2 (0)	6 (1)	5 (1)	9 (2)	3 (2)	2 (0)
CRLB Ins	3 (1)	3 (1)	9 (3)	7 (1)	11 (3)	2 (0)	2 (1)

CRLB: Cramer-Rao lower bounds; ACC: Anterior cingulate cortex; NAA: N-acetyl aspartate; Glu: Glutamate; Gln: Glutamine; mI: Myo-Inositol; GABA: γ-aminobutyric acid; Cr: Creatine; Cho: Choline.

tion, the Cramer-Rao lower bounds (CRLB), from LCModel analysis are shown in Table 1. CRLBs for Glu, Gln and GABA were lowest for the 7T short TE sequence, as expected from the SNR values. CRLB values for the 3T short TE and 7T long TE sequence were similar, despite much increased SNR for the 3T short spectra. CRLBs were highest for the 3T long TE sequence. The signals from Gln and GABA were not measurable (CRLB > 100%) in one spectrum using the 7T long TE sequence, and GABA was not found in 9 spectra using the 3T long TE sequence.

The intra-subject coefficients of variation for repeat measures of ACC metabolite levels are shown in Table 2. Values are given both uncorrected (direct from LCModel) and following correction for the voxel tissue fraction.

Regional and longitudinal variation

Spectral SNRs, averaged across all subjects, were significantly higher in the ACC than in the insula cortex (ACC SNR = 63 ± 10, insula SNR = 36 ± 11, $P = 0.002$) despite similar VOIs (9.00 mL *vs* 8.64 mL respectively) and similar average tissue fractions (0.94 ± 0.2 and 0.94 ± 0.1, calculated from 1 mm isotropic images)(Tables 3-5).

Table 4 Mean % coefficients of variation (SD) and % longitudinal variation (SD) averaged across all subjects

	NAA	Glu	Gln	mI	GABA	Cr	Cho
%CV							
ACC	4 (3)	5 (2)	10 (5)	9 (4)	10 (6)	4 (2)	6 (3)
Ins	6 (6)	8 (6)	12 (9)	10 (6)	21 (11)	7 (7)	6 (4)
%LV							
ACC	6 (3)	8 (7)	11 (9)	13 (13)	16 (13)	8 (9)	9 (10)
Ins	6 (5)	8 (10)	18 (18)	18 (11)	20 (24)	6 (6)	6 (6)

CV: Coefficients of variation; LV: Longitudinal variation; ACC: Anterior cingulate cortex; NAA: N-acetyl aspartate; Glu: Glutamate; Gln: Glutamine; mI: Myo-Inositol; GABA: γ-aminobutyric acid; Cr: Creatine; Cho: Choline.

Table 5 Mean (SD) metabolite levels (AU)

	NAA	Glu	Gln	mI	GABA	Cr	Cho
ACC	6.3 (0.7)	11.0 (1.4)	2.3 (0.4)	3.8 (0.3)	1.7 (0.4)	6.1 (0.6)	1.6 (0.2)
Ins	7.1 (0.6)	12.1 (1.3)	2.5 (0.5)	3.8 (0.5)	1.9 (0.4)	6.5 (0.4)	1.7 (0.2)

ACC: Anterior cingulate cortex; NAA: N-acetyl aspartate; Glu: Glutamate; Gln: Glutamine; mI: Myo-Inositol; GABA: γ-aminobutyric acid; Cr: Creatine; Cho: Choline.

3T vs 7T comparisons of muscle glycogen and IMCL measurements: measurements of glycogen and lipid

The SNR for the C1 peak of glycogen at 100.4 ppm (measured using ^{13}C MRS) was increased by 60% at 7T compared with the 3T values (11 *vs* 7) for the same number of acquisitions. Using ^{1}H MRS, SNRs (measured for the water peak) at 7T were 90% higher than values measured at 3T. %CVs for measurement of EMCL levels at 7T were much lower compared with the 3T measurements (6% *vs* 20% respectively). Similarly, repeat measurement of IMCL levels showed improved repeatability at 7T compared with 3T (2% *vs* 6%).

^{1}H and ^{13}C MRS of muscle energy stores

Basal glycogen levels were not significantly altered between each subject's visits. Similarly there were no basal differences in glycogen levels between the 50% VO_2 max visit and the 75% VO_2 max visit. Basal glycogen concentrations in the quadriceps tended to be higher than in the hamstrings, although this did not reach significance (front = 204 ± 56 mmol/L, back = 171 ± 49 mmol/L, $P = 0.2$).

Levels of glycogen (Figure 2) decreased significantly in the quadriceps following exercise ($t = 10$ min) at both 50% and 75% VO_2 max (-28% ± 20% and -52% ± 10%, $P < 0.05$) and were significantly lower when the subjects cycled at 75% VO_2 max compared with 50% VO_2 max ($P < 0.05$). Levels remained significantly below baseline levels at 20 and

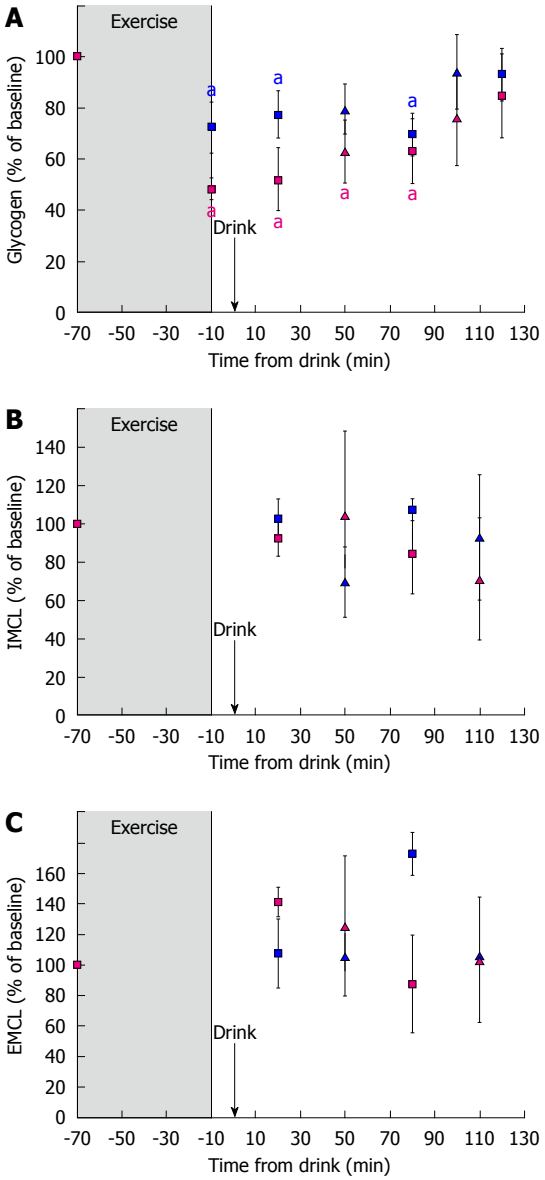

Figure 2 Percentage changes in glycogen (A), intra-myocellular lipid (B) and extra-myocellular lipid (C) levels due to exercise and following recovery. Values are mean ± SE. Squares represent measurements in the front of thigh, triangles represent measurements in the back of thigh. Points shown in blue and pink indicate exercise at 50% and 75% VO_2 max respectively ($^aP < 0.05$). IMCL: Intra-myocellular lipid; EMCL: Extra-myocellular lipid.

80 min following the drink (-23% ± 21% and -30% ± 19% respectively following cycling at 50% VO_2 max and -48% ± 28% and -37% ± 29% respectively at 75% VO_2 max). By 2 h after ingestion of the carbohydrate rich drink, glycogen levels in the front of the thigh were recovering towards baseline level (-7% ± 23% and -15% ± 37% following cycling at 50% VO_2 max and 75% VO_2 max respectively).

Post-exercise concentrations of glycogen in the hamstrings were not measured until 50 min after ingestion of the drink. Despite this, glycogen levels were still significantly below baseline level following exercise at 75% VO_2 max (-37% ± 28%) but had recovered towards baseline by 100 min (-24% ± 19%). Measurements of glycogen were not significantly different from baseline levels in the back of the thigh following exercise at 50% VO_2 max. As expected, mean glycogen concentrations were consistently lower in both the quadriceps and hamstrings following exercise at 75% compared with exercise at 50%.

IMCL and EMCL content

Basal IMCL content in the VI was not significantly different from levels in the ST muscle (0.4% ± 0.2% *vs* 0.3% ± 0.1%). No significant differences in IMCL were measured at any time point following exercise and re-feeding. Levels of EMCL were significantly larger in the ST compared with the VI (2.2% ± 0.3% *vs* 0.8% ± 0.3%, $P < 0.05$). No changes in EMCL levels were observed following exercise and re-feeding.

DISCUSSION

^1H reproducibility study

Increases in SNR from 3 to 7T are approximately 35% and 37% for the short TE and long TE sequence, respectively. Previous studies have reported various levels of increase in SNR with increasing field; however it is likely the 7T sequence would suffer from increased T_2 relaxation effects at the same TE, as well as increased saturation of signal due to longer T_1 relaxation values. Due to these relaxation effects, the 3T short TE sequence produced significantly higher SNR values than the 7T long TE sequence ($P = 0.002$).

As shown in Table 2, CVs for Glu, Gln and GABA from repeat measures are much lower for the 7T short TE sequence than for the 3T short TE sequence. It is possible this is in part due to reduced SNR; however, %CVs for GABA, using the 7T long TE sequence, are lower than those measured using the 3T short TE sequence despite the reduced SNR. This improvement in quantification is likely due to increased spectral resolution, as previously shown by Tkác *et al*[10].

Regional and longitudinal variation

Differences in SNR values, measured in the ACC and insula are likely due to increased field inhomogeneities for the long, thin VOI used in the insula (linewidths were measured to be approximately 15% wider in the insula compared with the ACC, $P = 0.05$), and poorer water suppression.

In spite of the much reduced SNR levels in the insula, CRLBs (Table 3) are only slightly increased. This is in agreement with single session CVs which, with the exception of GABA, are only slightly larger in the insula compared with the ACC. The reduced ability to accurately measure GABA is likely due to decreased spectral resolution as a consequence of the increased linewidths in the insula since the measured concentrations of GABA in the insula are similar to those measured in the ACC (Table 5).

%LVs tend to be larger than %CVs for all metabolites in the ACC (Table 4). This implies biological variation over a week, greater than the reproducibility of the measurements. %LVs for Gln and GABA were also larger

than %CVs in the insula. In contrast, %LVs for NAA, Cr, Cho, and Glu in the insula were not larger than %CVs. It is possible that there is some biological variation occurring in these levels which is masked by decreased single session repeatability in the insula.

Metabolite concentrations from the ACC and insula showed levels of Glu and Cr were significantly higher in the insula compared with the ACC ($P = 0.05$ and $P = 0.02$ respectively). No other differences in metabolites levels were found.

3T vs 7T comparisons of muscle glycogen and IMCL measurements: measurements of glycogen and lipid

Assuming that signal increases linearly with the number of averages (N_{ave}) while noise increases with $\sqrt{N_{ave}}$, obtaining the same SNR as measured for the C1 glycogen peak at 7T would take approximately 2.5 times longer at 3T. Utilizing this increase in signal strength at 7T allows either increased temporal resolution or improved measurement accuracy.

Improved measurement repeatability at 7T is likely due to the increase in spectral separation of IMCL and EMCL at 7T compared to 3T. However, repeatability of lipid measurements (particularly EMCL) in muscle is extremely susceptible to voxel repositioning errors. The voxels used for these measurements are quite large and so there are limited positions in which the voxel can be placed whilst avoiding adipose lipids and bone (particularly at 7T where chemical shifts between fat and water are increased). This may make repositioning between repeat measurements less variable and therefore improve measurement repeatability.

^{1}H and ^{13}C MRS of muscle energy stores

As expected, levels of glycogen in exercising muscles decreased significantly during exercise, with larger decreases following higher intensity exercise. At 2 h, levels of glycogen were returned to baseline levels indicating replenishment of glycogen stores due to carbohydrate refeeding. Interestingly, levels of glycogen in the hamstrings followed those in the quadriceps, despite the expected reduced exercise load.

No changes were measured in levels of IMCL due to exercise. If there are changes, they are likely to be small, and poor measurement repeatability (due to large spatial variation in levels of IMCL[40]) may mask these changes. It is thought that EMCL turnover is slow in contrast to IMCL, so EMCL levels would not be expected to change significantly over the timescale observed.

Increased spectral resolution at 7T allows improved ^{1}H MRS measurement of Glu, Gln and GABA concentrations which are thought to be perturbed in many neurodegenerative disorders and psychiatric diseases. Quantification is further improved by increases in sensitivity with increasing field strength. Using a short TE STEAM sequence, Glu, Gln and GABA were measured repeatedly in the ACC with coefficients of variation of 5%, 10% and 10% respectively within 15 min. Measurements made 1 wk apart showed increased variability indicating biological change in excess of single session reproducibility levels.

Increased sensitivity and spectral resolution available at 7T allows dynamic changes in glycogen and lipid levels in skeletal muscles to be observed with increasing temporal resolution. Measurements following exercise and re-feeding show the expected[45-50,53,54] decrease in glycogen levels in muscle, with a larger decrease in levels for increased exercise intensity. Levels of lipid were not significantly altered despite cycling for 1 h at 50% and 75% VO_2 max.

COMMENTS

Background

Magnetic resonance spectroscopy (MRS) has the potential to become a vital tool to aid the understanding of changes due to pathology in specific regions of the body, as well as for clinical diagnosis and treatment monitoring. Since signal increases with magnetic field strength, the use of ultra-high field (7T) scanners allows increased potential for measuring metabolite concentrations more accurately as well as allowing measurement of low concentration metabolites not seen at lower field.

Research frontiers

Levels of metabolites, particularly neurotransmitters glutamate and γ-aminobutyric acid (GABA), as well as glutamine, are thought to be important in understanding changes involved in neurological and psychiatric diseases. This study shows increased reproducibility for ^{1}H MRS measurement of glutamate, glutamine and GABA at 7T compared with 3T. In addition, measurement of energy stores [glycogen and intra-myocellular lipid (IMCL)] in skeletal muscle using ^{13}C and ^{1}H MRS respectively, are shown to be improved at 7T compared to 3T. This study utilizes the increased signal to noise to improve temporal resolution for subsequent measurements of IMCL and glycogen, and shows that higher intensity exercise (70% VO_2 max *vs* 50% VO_2 max) increases utilization of glycogen. No change in IMCL levels were measured due to exercise.

Innovations and breakthroughs

Little work has been done to compare optimized sequences and to establish levels of reproducibility based on different sequences for measurement of glutamate, glutamine and GABA. This paper contains single session repeatability for various proposed sequences at 3 and 7T, as well as measuring levels of biological variation over time. This paper also shows improved measurement of glycogen and IMCL at 7T, and is one of the first papers to sequentially measure dynamic changes in IMCL and glycogen levels at 7T.

Applications

Measurement of metabolite levels are important in understanding changes involved in neurological and psychiatric diseases, as well as for monitoring potential therapies. More accurate measurements will allow smaller changes to be measured which may provide new information for treatments.

Peer review

The current paper discusses the advantages of ultra-high field MR spectroscopy. It is a very well designed exceptional study.

REFERENCES

1 **Barker PB**, Hearshen DO, Boska MD. Single-voxel proton MRS of the human brain at 1.5T and 3.0T. *Magn Reson Med* 2001; **45**: 765-769

2 **Bartha R**, Drost DJ, Menon RS, Williamson PC. Comparison of the quantification precision of human short echo time (1)H spectroscopy at 1.5 and 4.0 Tesla. *Magn Reson Med* 2000; **44**: 185-192

3 **Dydak U**, Schär M. MR spectroscopy and spectroscopic imaging: comparing 3.0 T versus 1.5 T. *Neuroimaging Clin N Am* 2006; **16**: 269-283, x

4 **Gonen O**, Gruber S, Li BS, Mlynárik V, Moser E. Multivoxel 3D proton spectroscopy in the brain at 1.5 versus 3.0 T:

signal-to-noise ratio and resolution comparison. *AJNR Am J Neuroradiol* 2001; **22**: 1727-1731
5 **Inglese M**, Spindler M, Babb JS, Sunenshine P, Law M, Gonen O. Field, coil, and echo-time influence on sensitivity and reproducibility of brain proton MR spectroscopy. *AJNR Am J Neuroradiol* 2006; **27**: 684-688
6 **Kantarci K**, Reynolds G, Petersen RC, Boeve BF, Knopman DS, Edland SD, Smith GE, Ivnik RJ, Tangalos EG, Jack CR Jr. Proton MR spectroscopy in mild cognitive impairment and Alzheimer disease: comparison of 1.5 and 3 T. *AJNR Am J Neuroradiol* 2003; **24**: 843-849
7 **Otazo R**, Mueller B, Ugurbil K, Wald L, Posse S. Signal-to-noise ratio and spectral linewidth improvements between 1.5 and 7 Tesla in proton echo-planar spectroscopic imaging. *Magn Reson Med* 2006; **56**: 1200-1210
8 **Posse S**, Otazo R, Caprihan A, Bustillo J, Chen H, Henry PG, Marjanska M, Gasparovic C, Zuo C, Magnotta V, Mueller B, Mullins P, Renshaw P, Ugurbil K, Lim KO, Alger JR. Proton echo-planar spectroscopic imaging of J-coupled resonances in human brain at 3 and 4 Tesla. *Magn Reson Med* 2007; **58**: 236-244
9 **Srinivasan R**, Vigneron D, Sailasuta N, Hurd R, Nelson S. A comparative study of myo-inositol quantification using LCmodel at 1.5 T and 3.0 T with 3 D 1H proton spectroscopic imaging of the human brain. *Magn Reson Imaging* 2004; **22**: 523-528
10 **Tkác I**, Oz G, Adriany G, Uğurbil K, Gruetter R. In vivo 1H NMR spectroscopy of the human brain at high magnetic fields: metabolite quantification at 4T vs. 7T. *Magn Reson Med* 2009; **62**: 868-879
11 **Antuono PG**, Jones JL, Wang Y, Li SJ. Decreased glutamate + glutamine in Alzheimer's disease detected in vivo with (1)H-MRS at 0.5 T. *Neurology* 2001; **56**: 737-742
12 **Kantarci K**, Jack CR Jr, Xu YC, Campeau NG, O'Brien PC, Smith GE, Ivnik RJ, Boeve BF, Kokmen E, Tangalos EG, Petersen RC. Regional metabolic patterns in mild cognitive impairment and Alzheimer's disease: A 1H MRS study. *Neurology* 2000; **55**: 210-217
13 **Moore CM**, Breeze JL, Gruber SA, Babb SM, Frederick BB, Villafuerte RA, Stoll AL, Hennen J, Yurgelun-Todd DA, Cohen BM, Renshaw PF. Choline, myo-inositol and mood in bipolar disorder: a proton magnetic resonance spectroscopic imaging study of the anterior cingulate cortex. *Bipolar Disord* 2000; **2**: 207-216
14 **Rosenberg DR**, Macmaster FP, Mirza Y, Smith JM, Easter PC, Banerjee SP, Bhandari R, Boyd C, Lynch M, Rose M, Ivey J, Villafuerte RA, Moore GJ, Renshaw P. Reduced anterior cingulate glutamate in pediatric major depression: a magnetic resonance spectroscopy study. *Biol Psychiatry* 2005; **58**: 700-704
15 **Auer DP**, Pütz B, Kraft E, Lipinski B, Schill J, Holsboer F. Reduced glutamate in the anterior cingulate cortex in depression: an in vivo proton magnetic resonance spectroscopy study. *Biol Psychiatry* 2000; **47**: 305-313
16 **Auer DP**, Wilke M, Grabner A, Heidenreich JO, Bronisch T, Wetter TC. Reduced NAA in the thalamus and altered membrane and glial metabolism in schizophrenic patients detected by 1H-MRS and tissue segmentation. *Schizophr Res* 2001; **52**: 87-99
17 **Hoerst M**, Weber-Fahr W, Tunc-Skarka N, Ruf M, Bohus M, Schmahl C, Ende G. Correlation of glutamate levels in the anterior cingulate cortex with self-reported impulsivity in patients with borderline personality disorder and healthy controls. *Arch Gen Psychiatry* 2010; **67**: 946-954
18 **Carrey N**, MacMaster FP, Fogel J, Sparkes S, Waschbusch D, Sullivan S, Schmidt M. Metabolite changes resulting from treatment in children with ADHD: a 1H-MRS study. *Clin Neuropharmacol* 2003; **26**: 218-221
19 **Pollack MH**, Jensen JE, Simon NM, Kaufman RE, Renshaw PF. High-field MRS study of GABA, glutamate and glutamine in social anxiety disorder: response to treatment with levetiracetam. *Prog Neuropsychopharmacol Biol Psychiatry* 2008; **32**: 739-743
20 **Mayer D**, Spielman DM. Detection of glutamate in the human brain at 3 T using optimized constant time point resolved spectroscopy. *Magn Reson Med* 2005; **54**: 439-442
21 **Schulte RF**, Trabesinger AH, Boesiger P. Chemical-shift-selective filter for the in vivo detection of J-coupled metabolites at 3T. *Magn Reson Med* 2005; **53**: 275-281
22 **Ryner LN**, Sorenson JA, Thomas MA. Localized 2D J-resolved 1H MR spectroscopy: strong coupling effects in vitro and in vivo. *Magn Reson Imaging* 1995; **13**: 853-869
23 **Mescher M**, Merkle H, Kirsch J, Garwood M, Gruetter R. Simultaneous in vivo spectral editing and water suppression. *NMR Biomed* 1998; **11**: 266-272
24 **Terpstra M**, Ugurbil K, Gruetter R. Direct in vivo measurement of human cerebral GABA concentration using MEGA-editing at 7 Tesla. *Magn Reson Med* 2002; **47**: 1009-1012
25 **Hu J**, Yang S, Xuan Y, Jiang Q, Yang Y, Haacke EM. Simultaneous detection of resolved glutamate, glutamine, and gamma-aminobutyric acid at 4 T. *J Magn Reson* 2007; **185**: 204-213
26 **Tkác I**, Starcuk Z, Choi IY, Gruetter R. In vivo 1H NMR spectroscopy of rat brain at 1 ms echo time. *Magn Reson Med* 1999; **41**: 649-656
27 **Thompson RB**, Allen PS. Sources of variability in the response of coupled spins to the PRESS sequence and their potential impact on metabolite quantification. *Magn Reson Med* 1999; **41**: 1162-1169
28 **Thompson RB**, Allen PS. Response of metabolites with coupled spins to the STEAM sequence. *Magn Reson Med* 2001; **45**: 955-965
29 **Yang S**, Hu J, Kou Z, Yang Y. Spectral simplification for resolved glutamate and glutamine measurement using a standard STEAM sequence with optimized timing parameters at 3, 4, 4.7, 7, and 9.4T. *Magn Reson Med* 2008; **59**: 236-244
30 **Campbell I**. Intermediary metabolism. *Anaesth Intens Care Med* 2004; **5**: 141-143
31 **Taylor R**, Price TB, Rothman DL, Shulman RG, Shulman GI. Validation of 13C NMR measurement of human skeletal muscle glycogen by direct biochemical assay of needle biopsy samples. *Magn Reson Med* 1992; **27**: 13-20
32 **Stevens AN**, Iles RA, Morris PG, Griffiths JR. Detection of glycogen in a glycogen storage disease by 13C nuclear magnetic resonance. *FEBS Lett* 1982; **150**: 489-493
33 **Gruetter R**, Magnusson I, Rothman DL, Avison MJ, Shulman RG, Shulman GI. Validation of 13C NMR measurements of liver glycogen in vivo. *Magn Reson Med* 1994; **31**: 583-588
34 **Thomsen C**, Becker U, Winkler K, Christoffersen P, Jensen M, Henriksen O. Quantification of liver fat using magnetic resonance spectroscopy. *Magn Reson Imaging* 1994; **12**: 487-495
35 **Longo R**, Pollesello P, Ricci C, Masutti F, Kvam BJ, Bercich L, Crocè LS, Grigolato P, Paoletti S, de Bernard B. Proton MR spectroscopy in quantitative in vivo determination of fat content in human liver steatosis. *J Magn Reson Imaging* 1995; **5**: 281-285
36 **Garbow JR**, Lin X, Sakata N, Chen Z, Koh D, Schonfeld G. In vivo MRS measurement of liver lipid levels in mice. *J Lipid Res* 2004; **45**: 1364-1371
37 **Tarasów E**, Siergiejczyk L, Panasiuk A, Kubas B, Dzienis W, Prokopowicz D, Walecki J. MR proton spectroscopy in liver examinations of healthy individuals in vivo. *Med Sci Monit* 2002; **8**: MT36-MT40
38 **Szczepaniak LS**, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, Hobbs HH, Dobbins RL. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 2005; **288**: E462-E468
39 **Schick F**, Eismann B, Jung WI, Bongers H, Bunse M, Lutz O. Comparison of localized proton NMR signals of skeletal

muscle and fat tissue in vivo: two lipid compartments in muscle tissue. *Magn Reson Med* 1993; **29**: 158-167

40 **Boesch C**, Slotboom J, Hoppeler H, Kreis R. In vivo determination of intra-myocellular lipids in human muscle by means of localized 1H-MR-spectroscopy. *Magn Reson Med* 1997; **37**: 484-493

41 **Taylor R**, Magnusson I, Rothman DL, Cline GW, Caumo A, Cobelli C, Shulman GI. Direct assessment of liver glycogen storage by 13C nuclear magnetic resonance spectroscopy and regulation of glucose homeostasis after a mixed meal in normal subjects. *J Clin Invest* 1996; **97**: 126-132

42 **Moriarty KT**, Mclntyre DGO, Bingham K, Coxon R, Glover PM, Greenhaff PL, Macdonald IA, Bachelard HS, Morris PG. Glycogen resynthesis in liver and muscle after exercise: measurement of the rate of resynthesis by 13C magnetic resonance spectroscopy. *Magma Magn Reson Mater Phys Biol Med* 1994; **2**: 429-432

43 **Casey A**, Mann R, Banister K, Fox J, Morris PG, Macdonald IA, Greenhaff PL. Effect of carbohydrate ingestion on glycogen resynthesis in human liver and skeletal muscle, measured by (13)C MRS. *Am J Physiol Endocrinol Metab* 2000; **278**: E65-E75

44 **Franklin RM**, Ploutz-Snyder LL, Szeverenyi NM, Kanaley JA. The effects of an acute resistance exercise bout on IMCL content in obese younger and older women. *Med Sci Sport Exer* 2010; **42**: 1

45 **Awad S**, Stephenson MC, Placidi E, Marciani L, Constantin-Teodosiu D, Gowland PA, Spiller RC, Fearon KC, Morris PG, Macdonald IA, Lobo DN. The effects of fasting and refeeding with a 'metabolic preconditioning' drink on substrate reserves and mononuclear cell mitochondrial function. *Clin Nutr* 2010; **29**: 538-544

46 **Vøllestad NK**, Blom PC. Effect of varying exercise intensity on glycogen depletion in human muscle fibres. *Acta Physiol Scand* 1985; **125**: 395-405

47 **Gollnick PD**, Piehl K, Saubert CW 4th, Armstrong RB, Saltin B. Diet, exercise, and glycogen changes in human muscle fibers. *J Appl Physiol* 1972; **33**: 421-425

48 **Price TB**, Rothman DL, Avison MJ, Buonamico P, Shulman RG. 13C-NMR measurements of muscle glycogen during low-intensity exercise. *J Appl Physiol* 1991; **70**: 1836-1844

49 **van Loon LJ**, Greenhaff PL, Constantin-Teodosiu D, Saris WH, Wagenmakers AJ. The effects of increasing exercise intensity on muscle fuel utilisation in humans. *J Physiol* 2001; **536**: 295-304

50 **Vanhamme L**, van den Boogaart A, Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *J Magn Reson* 1997; **129**: 35-43

51 **Szczepaniak LS**, Babcock EE, Schick F, Dobbins RL, Garg A, Burns DK, McGarry JD, Stein DT. Measurement of intracellular triglyceride stores by H spectroscopy: validation in vivo. *Am J Physiol* 1999; **276**: E977-E989

52 **Ren J**, Sherry AD, Malloy CR. 1H MRS of intramyocellular lipids in soleus muscle at 7 T: spectral simplification by using long echo times without water suppression. *Magn Reson Med* 2010; **64**: 662-671

53 **Krssak M**, Petersen KF, Bergeron R, Price T, Laurent D, Rothman DL, Roden M, Shulman GI. Intramuscular glycogen and intramyocellular lipid utilization during prolonged exercise and recovery in man: a 13C and 1H nuclear magnetic resonance spectroscopy study. *J Clin Endocrinol Metab* 2000; **85**: 748-754

54 **Price TB**, Rothman DL, Taylor R, Avison MJ, Shulman GI, Shulman RG. Human muscle glycogen resynthesis after exercise: insulin-dependent and -independent phases. *J Appl Physiol* 1994; **76**: 104-111

S- Editor Cheng JX **L- Editor** O'Neill M **E- Editor** Zheng XM