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Dynamic interleaved $^{1}H/^{31}P$ STEAM MRS at 3 Tesla using a pneumatic force-controlled plantar flexion exercise rig

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Abstract

1 Objective—To develop a measurement method for interleaved acquisition of ^{1}H and ^{31}P STEAM localised spectra of exercising human calf muscle.

2 Materials and Methods—A nonmagnetic exercise rig with a pneumatic piston and sensors for force and pedal angle was constructed to enable plantar flexion measured in the 3 Tesla MR scanner, which holds the dual tuned (^{1}H,^{31}P) surface coil used for signal transmission and reception.

3 Results—^{31}P spectra acquired in interleaved mode benefit from higher SNR (factor of 1.34± 0.06 for PCr) compared to standard acquisition due to the Nuclear Overhauser effect (NOE) and substantial PCr/P_i changes during exercise can be observed in ^{31}P spectra. ^{1}H spectral quality is equal to that in single mode experiments and allows Cr2 changes to be monitored.

4 Conclusion—The feasibility of dynamic interleaved localised ^{1}H and ^{31}P spectroscopy during plantar flexion exercise has been demonstrated using a custom-built pneumatic system for muscle activation. This opens the possibility of studying the dynamics of metabolism with multi nuclear MRS in a single run.

Keywords

proton; phosphorus; STEAM; human skeletal muscle; exercise

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Introduction

Proton and phosphorus magnetic resonance spectra can provide complementary information about tissue metabolism. Acquiring two sets of spectra in consecutive experiments is time consuming and may result in different test conditions, e.g. due to fatigue during studies of exercising muscle. Whilst ^{31}P MRS has long been used for investigation of exercising muscle, interest in ^{1}H spectroscopy of muscle tissue has increased during the last few years [1]. As the resonance frequencies of ^{1}H and ^{31}P differ by a factor of 2.47, ^{1}H and ^{31}P spectra can, in principle, be acquired simultaneously without interference, i.e. hydrogen and phosphorus nuclei can be excited by RF pulses on their respective resonance frequencies independently (disregarding heteronuclear coupling effects) and spectra may be acquired synchronously, if hardware permits. When B_0 gradients are used they always act on both nuclei synchronously and simultaneous experiments are no longer fully independent. Interleaving multi-nuclear acquisitions is therfore a more versatile approach than simultaneous acquisition. Here, “interleaved” means nesting ^{1}H and ^{31}P MRS in such way that excitation and acquisition of one nucleus’ spectra is accomplished during the recovery period, T_R, of the other.

Interleaved NMR spectroscopy experiments have been used by various groups for monitoring myoglobin oxygenation and high energy phosphates in exercising human muscle in vivo [2–4]. In these studies localisation of the signal is defined by the sensitive volume of relatively small (d = 2.8−7 cm) surface loop coils. In contrast to previous studies, an interleaved STEAM sequence was developed to acquire gradient-localised ^{31}P and ^{1}H spectra in a single experiment, which has the further benefit of NOE enhancement of the ^{31}P spectra. The main advantage of localised versus non-localised spectroscopy is increased specificity: The signal originates only from a distinct region of interest, e.g. an exercising muscle, excluding surrounding tissue which may be activated to a lesser extent or not at all. In addition, the feasibility of dynamic interleaved localised ^{1}H and ^{31}P spectroscopy during plantar flexion exercise is demonstrated using a novel custom built pneumatic system for muscle activation.

Methods

Sequence design

A pulse sequence for interleaved acquisition of ^{1}H- and ^{31}P spectra with STEAM localisation for both nuclei was implemented on a 3T Bruker Medspec whole-body scanner, utilising MultiScanControl-Tool (Bruker Biospin, Ettlingen, Germany). A standard doubletuned surface coil (d = 10 cm), manufactured by Bruker, was used for RF transmission and reception. A CHESS scheme was used for water suppression. Volume selective shimming was achieved with Bruker’s implementation of FASTMAP [5], selecting a 5×5×5 cm^3 volume of interest (VOI).

In this implementation, all sequence parameters, including size, shape and position of the voxels, can be chosen independently for the respective nuclei. The freedom to select different VOIs does not pose any restrictions (as voxels may also be set to be identical if required) and follows inherently from the necessity to rescale gradients by gyromagnetic

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ratios $\gamma 1_H/\gamma 31_p$ in pulse sequence programming. For large flip angles, as used in a STEAM experiment (α = 90°) optimum signal to noise ratio (SNR) per unit time is obtained for T_R = 1.27 · T_1 [6]. This also applies when using a surface coil, as the pulse angle is adjusted to 90° within the VOI (which is small compared to the coil's dimensions) by seeking the maximum signal achieved with a STEAM acquisition, for each subject. Since longitudinal relaxation times of ^{31}P and ^{1}H metabolites differ significantly, the T_R should preferably be chosen individually for each nucleus. T_1s of ^{31}P metabolites are relatively long, in the range 3 s (α-NTP) to 6 s (PCr) at 3 Tesla [7], compared to the T_1 of ^{1}H metabolites which are typically of the order of 1 s, although they can be as short as 300 ms for lipids [8]. To account for this, the sequence design allows the acquisition of an arbitrary number of ^{1}H spectra between excitations on the ^{31}P channel, as illustrated in Fig. 1. Voxels were positioned in the medial head of m. gastrocnemius (see Fig. 2), because plantar flexion exercise with a straight knee activates primarily the gastrocnemius muscles [9, 10]. The ^{1}H VOI was 2.7 cm^3 and the ^{31}P VOI was 31.4 cm^3 to partly compensate for the 15 times lower ^{31}P sensitivity. The ^{1}H voxel was not chosen to be the same size as the ^{31}P VOI to avoid broad ^{1}H line widths which would render spectra unusable, but both VOIs were positioned in the same muscle. A short echo time T_E = 7.5 ms, T_M = 30 ms, receiver bandwidth BW = 2500 Hz and 1024 data points were chosen for both nuclei.

For calculation of phosphocreatine (PCr), inorganic phosphate (P_i), total creatine (Cr) and pH changes, data were quantified using the AMARES [11] time domain fit routine, as incorporated in the jMRUI software package [12].

Exercise Rig

A non-magnetic rig (Fig. 3) was constructed for plantar flexion exercise with defined constant force during NMR studies. The calf of the extended leg lies on the surface coil which is countersunk in a plywood frame, while the subject pushes a pedal against a pneumatic piston (displacement V = 1.7 l) which generates the force. The lower leg and foot is strapped to the rig and pedal with three belts (white straps in Fig. 3) to minimise motion of the calf. By varying the pneumatic pressure the pedal force can be adjusted arbitrarily, e.g. to match the subject's maximum voluntary contraction force (MVC) – even remotely and during the NMR measurement, which is useful for multi-power (e.g. ramp) exercise protocols. Exercise prameters are recorded continuously by force and angle sensors on the pedal (see Fig. 3 and Results section), an LED bar indicates the pedal angle for subject feedback. Good linearity of force over the pedal's angular range is maintained by a large reservoir air tank and the piston's and pedal's geometric arrangement. A throttle valve controls the pedal's recoil.

To reduce calf motion (a potential source of artifacts) during acquisition subjects were instructed to exercise only during periods without RF excitation and reception, i.e. not to exert force on the pedal during and shortly after gradient noise. Before starting the experiment they were trained in a test run. The optimum T_R for ^{31}P is 8 s. Although optimum SNR for ^{1}H would, in principle, allow shorter repetition times, T_R = 2 s leaves 1.2 s between data acquisition and subsequent ^{1}H excitation; long enough to allow plantar flexion. The most suitable number of ^{1}H acquisitions between consecutive ^{31}P acquisitions

is therefore four. After 3 consecutive plantar flexions and ^{1}H acquisitions, subjects paused once, allowing ^{31}P acquisition without motion. Verbal support was given during the experiment whenever necessary.

Subjects

Healthy subjects (n = 7, 6 male, 1 female), aged 31.5±9.2 years, body mass index 23.6 ± 2.5 kg/m^2 (mean ± SD) were examined. Each subject's maximum test force was estimated using a Biodex Multi-Joint-System 3 Pro (Biodex Medical Systems, Inc., N.Y., USA) dynamometer, one week before NMR examination. Written informed consent to the protocol, which was approved by the local ethics committee, was obtained from all subjects.

Results

The performance of the interleaved ^{1}H/^{31}P STEAM sequence was evaluated using a two-compartment test object [7]. Contamination of the spectra by signals originating from outside the nominal VOI was C = 0 ± 2% under fully relaxed conditions and C = 3 ± 2% for short T_R, i.e. T_R = 1 s ≪ $T1$.

Typical ^{1}H and ^{31}P in vivo spectra of exercising muscle are shown in Fig. 4. After a baseline period of 2 minutes at rest, plantar flexion was executed for 6.5 min at 50% MVC (adjusted individually for each subject via air pressure in the reservoir tank) followed by 15 min of recovery. ^{1}H spectra of resting human calf muscle acquired in a subgroup of n = 4 subjects for in vivo evaluation of the interleaved sequence were equivalent to standard STEAM experiments, whilst SNR in ^{31}P spectra acquired interleaved with ^{1}H spectroscopy was higher by a factor of 1.34 ± 0.06 for PCr and 1.06 ± 0.13 for P_i due to NOE.

The repetition time was 2 s for ^{1}H spectra (left) and T_R = 8 s for ^{31}P spectra (right).

Spectral quality is sufficient to clearly identify peaks of creatine (Cr), choline (TMA) and extra- and intramyocellular lipids (EMCL, IMCL) from ^{1}H spectra and P_i and PCr from ^{31}P spectra. Changes in the EMCL signal in some subjects can be attributed to motion, as tissue with higher EMCL concentration may contaminate the VOI at the onset of exercise despite fixation of the calf and foot and confinement of plantar flexion to periods without NMR acquisitions. The variation of the Cr CH_3 peak area, given as standard error, was however as low as 1.7 %. In ^{31}P spectra substantial PCr/P_i changes, as expected during exercise, can be observed without perceptible deterioration of signals, e.g. by motion or artifacts caused by the pneumatic exercise rig (Figs. 4 and 5). Similar to the total creatine CH_3 signal in ^{1}H spectra, the variation of the sum of PCr and P_i signals was only 2% during the measurement, which indicates that motion did not affect spectral quality. This exercise setup produced a mean (± SD) pH decrease from 7.12 ± 0.11 to 6.94 ± 0.19 and a mean relative PCr depletion of 63 ± 18% after 6.5 min of exercise at 50% MVC (n = 4).

Whilst 6.5 min of moderate exercise is neither expected to deplete IMCL nor alter total creatine concentration, the insert in Fig. 4 shows a drop in peak amplitude of the CH_2 signal of Cr alone during exercise and its recovery post exercise, concomitant with the PCr time

course in ^{31}P spectra (see Fig. 4), which can not be fully explained by variations in line width.

Discussion

Interleaved MRS experiments have been used to investigate metabolism in muscle tissue [2–4] with surface coils and have been demonstrated with a combination of TEM and surface coils for ^{1}H, ^{1}H-{^{13}C}, ^{31}P STEAM and ISIS NMR spectroscopy in the human brain [13, 14]. In this study we present a method for interleaved acquisition of ^{1}H and ^{31}P NMR spectra with STEAM localisation for both nuclei using a dual tuned surface coil and its application to exercising human calf muscle during plantar flexion in a 3 Tesla whole-body MR system. STEAM was chosen because it is a robust single shot sequence that performs better with the surface coil than e.g. PRESS, especially when large voxels are used for ^{31}P spectroscopy (which was also verified on test objects). Localised spectroscopy has the advantage of increased specificity compared to non-localised measuremts. The signal originates only from the region of interest, i.e. one can focus on the muscle which exercises most and exclude surrounding tissue which may be activated to a lesser degree (adjacent muscles) or not at all (skin, fat- or connective tissue). SNR is lower as a consequence of smaller tissue volume contributing to the signal. Our ^{31}P VOIs were typically 30 − 40 cm^3, while the sensitive volume of a 10 cm surface coil is a hemisphere with a volume of ca. 260 cm^3, significantly larger than our VOI. On the other hand, line width and shape, baseline and spectral resolution of multiplets is clearly better in localised spectroscopy.

Note that our ^{31}P spectra were acquired with long T_R = 8 s, to achieve optimum SNR for PCr and P_i the metabolites of major interest during exercise. The intensity of metabolite signals with shorter T_1s (NTP) is therefore reduced compared to short T_R measurements, where in fact PCr and P_i are partially saturated and need significant correction for T_1 relaxation.

Small VOIs are favourable in MRS because line width can generally be reduced by decreasing voxel sizes [15]. This is particularly important for ^{1}H spectra of muscle tissue [16], which are characterised by orientation-dependent splitting of resonances [1]. Characteristic line splitting may become less clear in the accumulation of signal from regions with locally different fibre orientation in a large voxel, as fibre orientation is mostly, but not completely uniform across a muscle. Water suppression is also less efficient for large VOIs, due to the inhomogeneous B_1-field of surface coils. In ^{31}P MR spectroscopy SNR is inherently lower, and a big voxel ($\gtrsim$ 30 cm^3), covering a large fraction of the exercising muscle under investigation seems more appropriate. Studies mapping T_2 of exercising muscle revealed that recruitment does not vary significantly across a single muscle [17]. We can therefore justifiably assume that our interleaved measurements of ^{1}H and ^{31}P metabolites are representative of the whole medial gastrocnemius muscle. Contributions from adjacent muscles, known to be recruited to a highly different extent during exercise [9, 10, 17] can still be largely excluded from the VOI due to the efficient suppression of contamination [7] of the ^{31}P STEAM sequence. If improved time resolution is desired, repetition times may be reduced, with the proviso that exercise be confined to periods without NMR signal excitation or acquisition to minimise motion. Moreover, for metabolites

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with long T_1 at 3 T, such as PCr [7], this decreases SNR per unit time and may introduce complications in quantification due to T_1 weighting and chemical exchange [18] for other metabolites. The quality of ^{1}H spectra acquired in interleaved mode here is equivalent to single mode experiments, while the corresponding ^{31}P spectra benefit from higher SNR compared to standard acquisition due to the Nuclear Overhauser effect (NOE). The extent of NOE enhancement depends on RF power irradiation and is usually accomplished by saturating the water resonance with continuous wave or WALTZ irradiation [19, 20]. In the experiment described here, the NOE is induced by the RF pulses of the ^{1}H STEAM sequence which is interleaved with ^{31}P STEAM acquisitions. Since neither ^{1}H pulse amplitude nor duty cycle were maximised to achieve ^{1}H saturation, but ^{1}H RF pulse power was optimised for ^{1}H STEAM signal amplitude and water suppression, the observed NOE is not the maximum achievable. NOE enhancements between 43% (NTP) and 64% (PCr) in calf muscle have been reported in a 1.5T study [19]. In brain, 25-30% enhancement was achieved for PCr at 1.5 and 7T [20,21], P_i signal was increased by 15.7% in this 7T study. These results match our nonoptimised NOE enhancement factors.

Longitudinal relaxation times of PCr and P_i, in human calf muscle are 6.4 ± 0.2 s and 5.2 ± 1.0 s [7], resulting in saturation factors of 0.71 and 0.78, respectively, for T_R = 8 s. This partly compensates the different NOE factors from 1.34 and 1.06 to calibration factors of 0.96 and 0.83, neglecting any chemical exchange effects.

Whilst substantial changes in PCr and P_i are observed in ^{31}P spectra, the concentration of metabolites in ^{1}H spectra (creatine methyl resonance (Cr3), TMA, IMCL) are not expected to change significantly during 6.5 minutes of exercise with 50% MVC. Intramyocellular lipid depletion in muscle becomes measurable with ^{1}H MRS after prolonged submaximal exercise, e.g. running for several hours [22, 23]. Similar to findings in isometric exercise of tibialis anterior muscle under ischemia [24], a drop in peak amplitude of the methylene group of creatine (Cr2) during exercise and its recovery post exercise, not fully explained by altered line width, can be observed in ^{1}H spectra in this work (see insert in Fig. 4) simultaneously with the PCr time course observed in ^{31}P spectra, which were acquired in the same experiment. During strenuous exercise protocols – and also sporadically at lower exercise intensities in some subjects – a resonance attributed to acetyl carnitine (AcCt) [25] was described in ^{1}H spectra of human muscle. Interleaved acquisition of ^{1}H and ^{31}P spectra can, in principle, be used to simultaneously acquire time courses of AcCt and high energy phosphates during a high workload exercise protocol.

A localised double quantum filter sequence for lactate detection has recently been incorporated into the interleaved sequence [26] and is currently being tested for in vivo use to quantify changes in lactate concentration which will, together with information from ^{31}P spectra acquired simultaneously, be valuable in studying the regulation of glycolysis and cellular acid-base buffering mechanisms.

In summary, we have demonstrated the feasibility of time-resolved localised ^{1}H and ^{31}P spectroscopy of exercising skeletal muscle in a single interleaved experiment. The benefits of this approach are reduced total measurement time and NOE enhancement of ^{31}P spectra. Most significantly it opens the possibility of studying the dynamics of metabolism with

multi nuclear MRS in a single run, e.g. examining PCr depletion and resynthesis with ^{31}P MRS and the concomitant time course of the Cr2 resonance in ^{1}H spectra. The method has been tested on human calf muscle during exercise on a custom-built pneumatic ergometer and shown to be effective.

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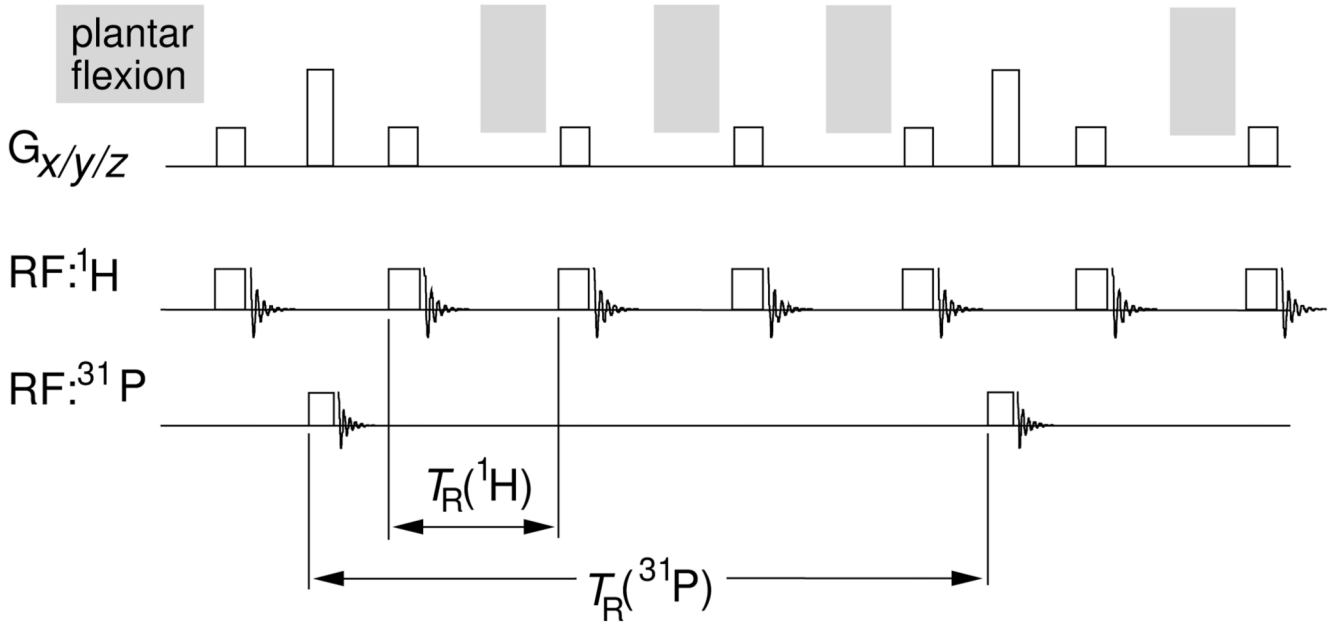

Fig. 1.

Schematic representation of the interleaved ^{1}H and ^{31}P STEAM sequence. Several ^{1}H spectra (four in this example) can be acquired per ^{31}P acquisition T_R to optimise SNR of each spectrum despite the large differences in T_1. Rectangles schematically represent RF and B_0 gradient pulses and periods of plantar flexion.

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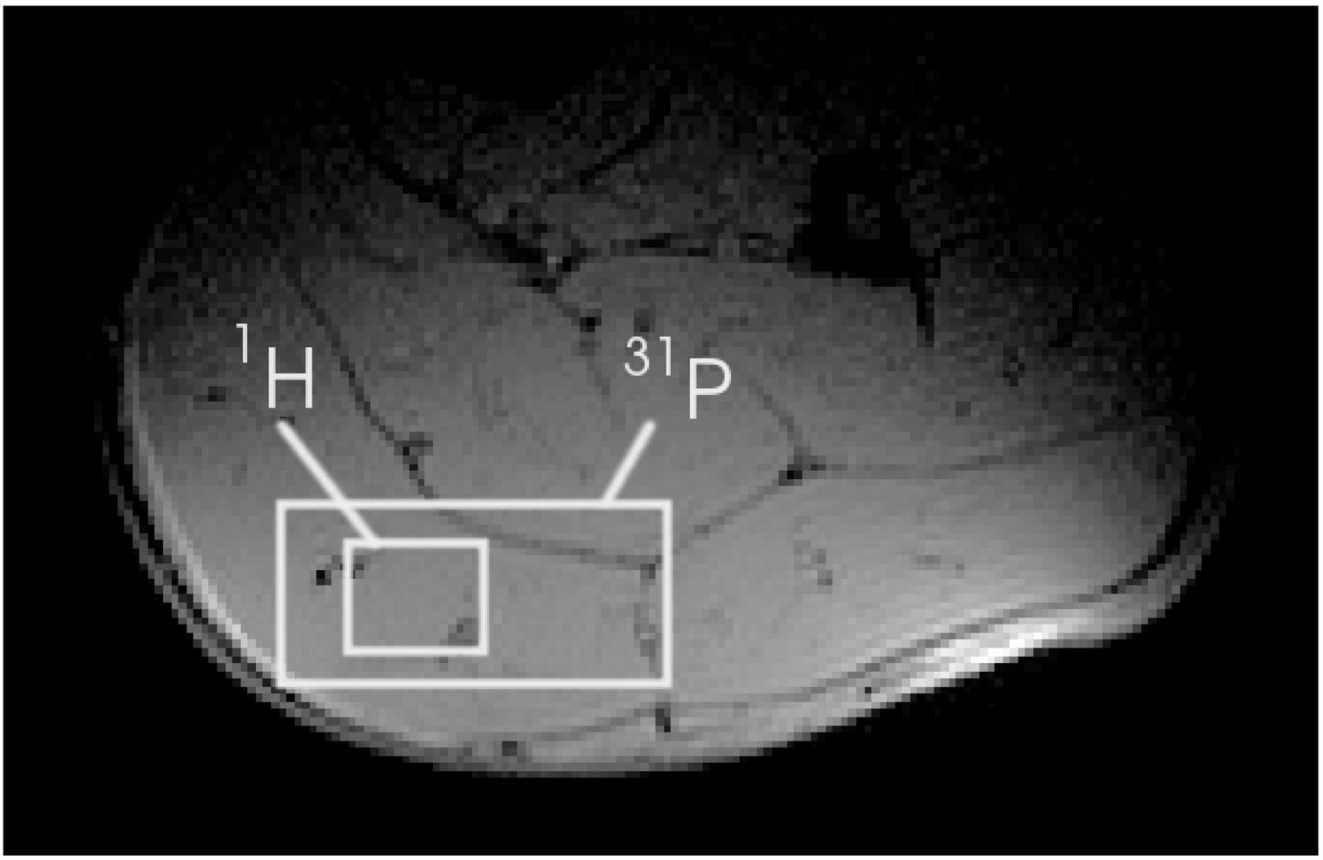

Fig. 2.

Typical position of the volumes of interest for interleaved STEAM spectroscopy in human gastrocnemius muscle. ^{1}H: $V = 2.7$ cm^3, ^{31}P (due to lower sensitivity): $V = 43.1$ cm^3

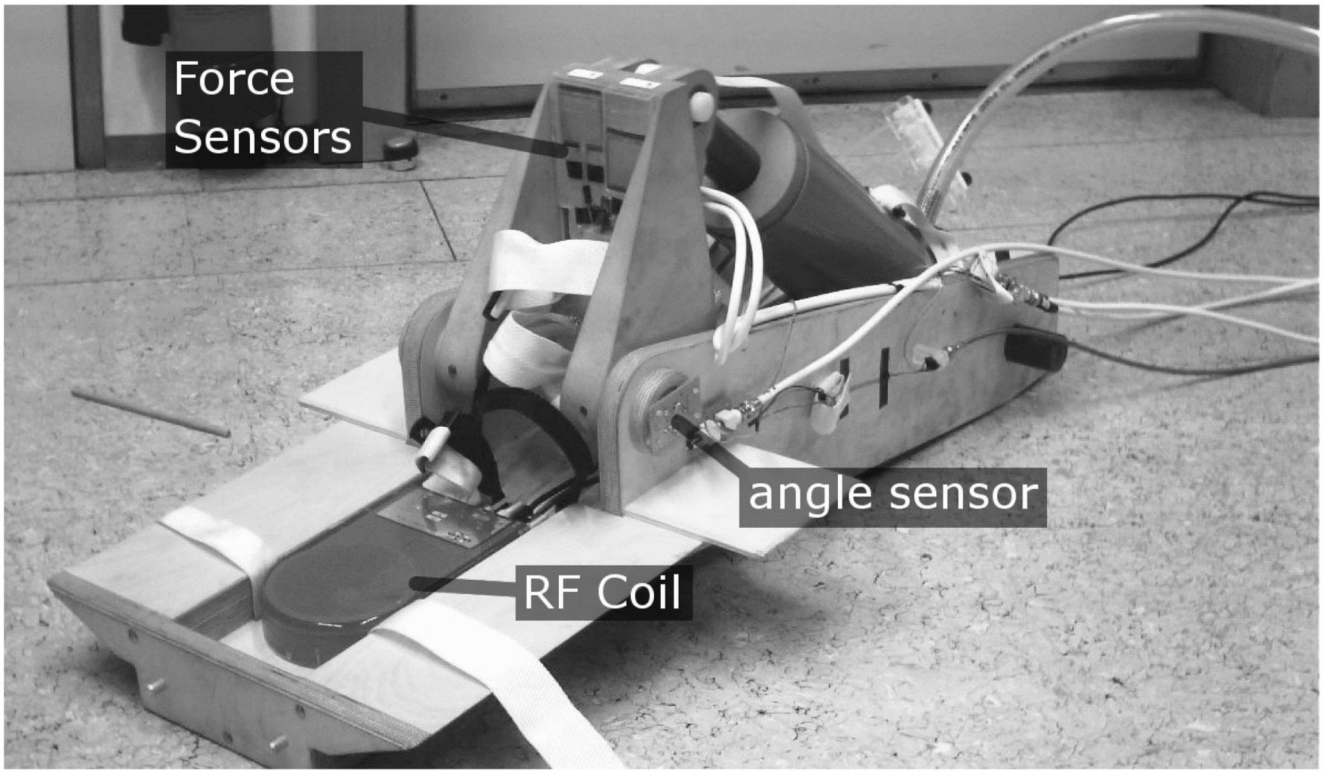

Fig. 3.

Non-magnetic exercise rig with pneumatic piston, RF coil and sensors for monitoring pedal force and angle.

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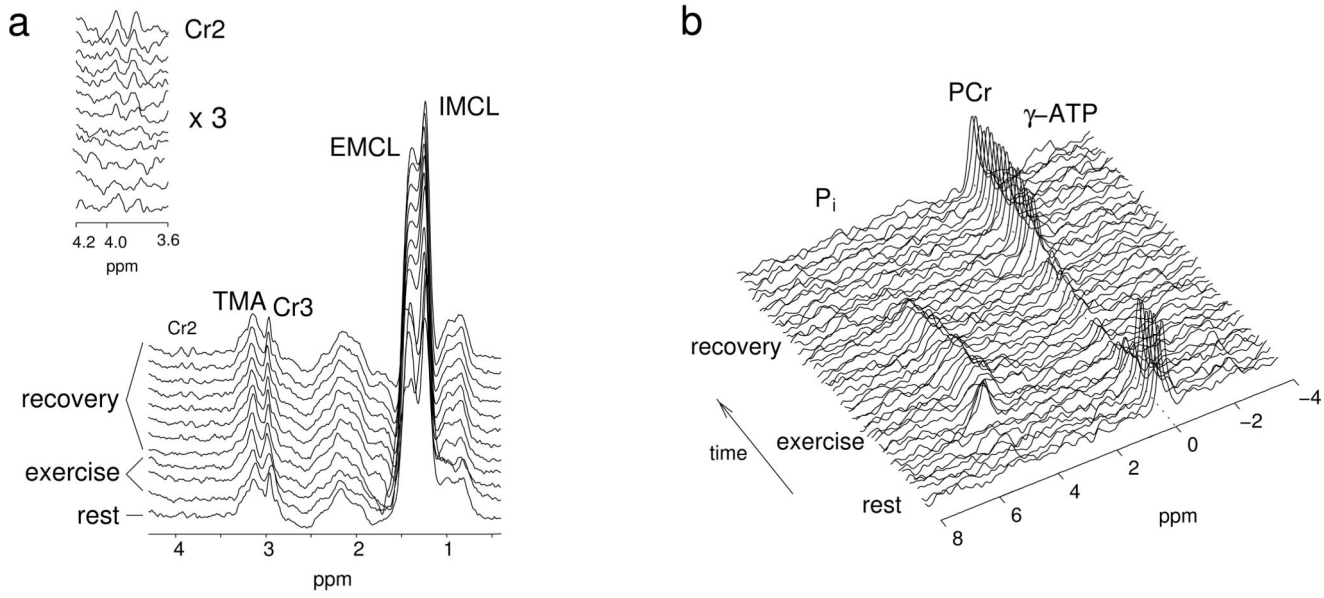

Fig. 4.

^{1}H (left) and ^{31}P (right) STEAM spectra from human gastrocnemius muscle at rest, exercise and recovery, acquired in interleaved mode. ^{1}H spectra (a) were acquired with T_E = 7.5 ms, T_R = 2 s, VOI = 2.7 ml, and averaged to blocks of 64 for display (2'08" per spectrum). The insert shows a magnification (×3) of the Cr2 resonance. ^{31}P spectra (b) were acquired with T_E = 7.5 ms, T_R = 8 s, VOI = 31.4 ml, 2 avgs, yielding a time resolution of 16 s. Relative PCr change: 74 %, pH_{rest} = 7.1, pH_{ex} = 6.7. (Only the first 50 of 96 ^{31}P spectra are shown, for clarity).

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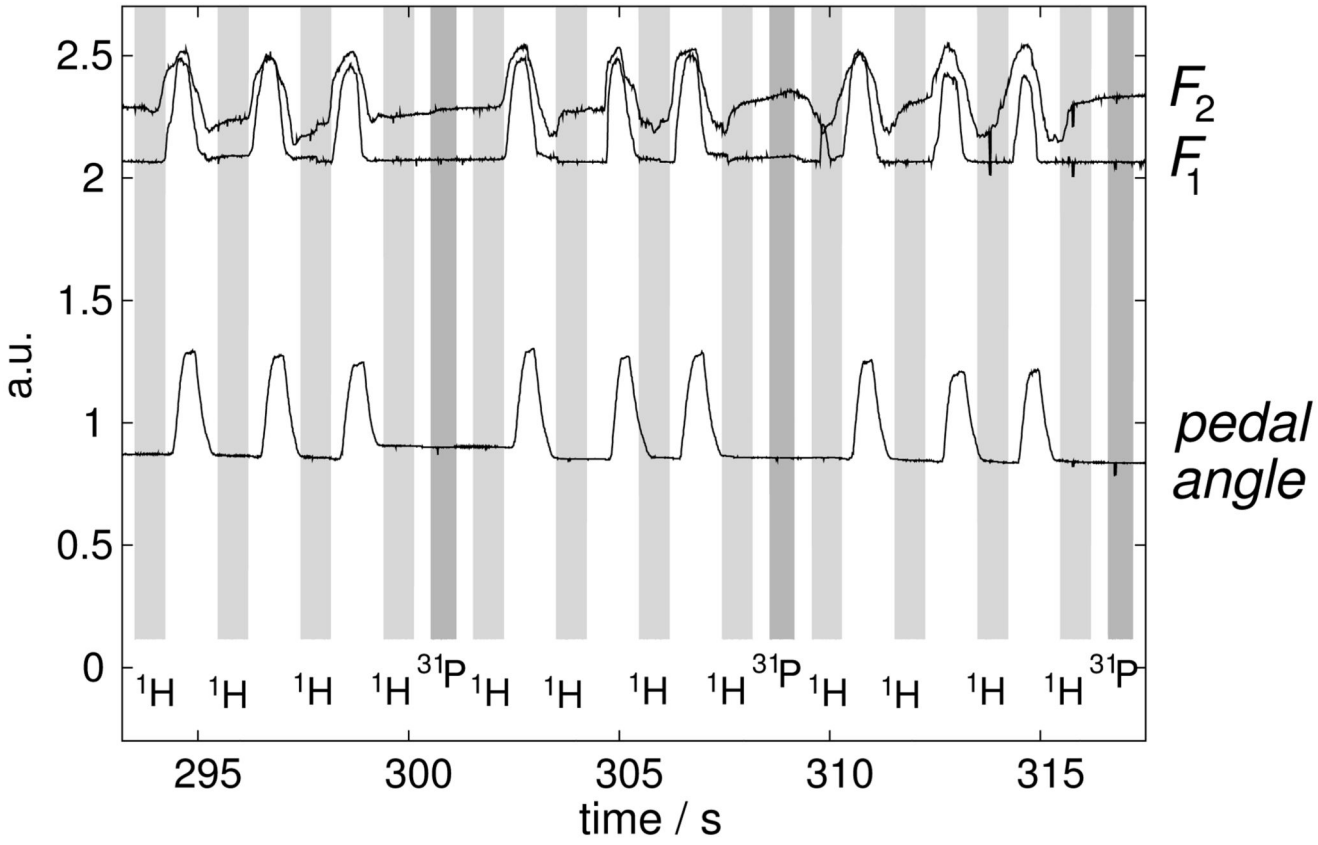

Fig. 5.

Pedal angle and force (F_1, F_2: output of two strain sensors located on the pedal, below the subject's ball of the foot) recorded during an interleaved ^{1}H/^{31}P spectroscopy experiment. Grey bars indicate periods between the first RF pulse and the end of acquisition of the STEAM sequence, as recorded via the NMR scanner's trigger output. (Dark grey: ^{31}P, light grey: ^{1}H) Note that there is no motion during NMR acquisitions in this example.