

# Localized semi-LASER dynamic $^{31}\text{P}$ magnetic resonance spectroscopy of the soleus during and following exercise at 7 T

Georg B. Fiedler<sup>1,2</sup> · Martin Meyerspeer<sup>1,2</sup> · Albrecht I. Schmid<sup>1,2</sup> · Sigrun Goluch<sup>1,2</sup> · Kiril Schewzow<sup>1,2</sup> · Elmar Laistler<sup>1,2</sup> · Arash Mirzahassemi<sup>3,4</sup> · Fabian Niess<sup>1,2,5</sup> · Ewald Unger<sup>1</sup> · Michael Wolzt<sup>6</sup> · Ewald Moser<sup>1,2</sup>

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## Abstract

**Objectives** This study demonstrates the applicability of semi-LASER localized dynamic  $^{31}\text{P}$  MRS to deeper lying areas of the exercising human soleus muscle (SOL). The effect of accurate localization and high temporal resolution on data specificity is investigated.

**Materials and methods** To achieve high signal-to-noise ratio (SNR) at a temporal resolution of 6 s, a custom-built human calf coil array was used at 7T. The kinetics of phosphocreatine (PCr) and intracellular pH were quantified separately in SOL and gastrocnemius medialis (GM) muscle of nine volunteers, during rest, plantar flexion exercise, and recovery.

**Results** The average SNR of PCr at rest was  $64 \pm 15$  in SOL ( $83 \pm 12$  in GM). End exercise PCr depletion in SOL ( $19 \pm 9\%$ ) was far lower than in GM ( $74 \pm 14\%$ ). The pH

in SOL increased rapidly and, in contrast to GM, remained elevated until the end of exercise.

**Conclusion**  $^{31}\text{P}$  MRS in single-shots every 6 s localized in the deeper-lying SOL enabled quantification of PCr recovery times at low depletions and of fast pH changes, like the initial rise. Both high temporal resolution and accurate spatial localization improve specificity of Pi and, thus, pH quantification by avoiding multiple, and potentially indistinguishable sources for changing the Pi peak shape.

**Keywords** Skeletal muscle · Phosphocreatine · In vivo NMR spectroscopy · Soleus muscle · Physical exertion

## Introduction

Phosphorus-31 nuclear magnetic resonance spectroscopy ( $^{31}\text{P}$  MRS) is a well-established, powerful tool for noninvasive measurements of energy metabolism in exercising muscle [1–5]. Particularly, the concentrations and kinetics of phosphorylated creatine (PCr), inorganic phosphate (Pi), and the intracellular pH [6], are accessible, which are directly connected to ATP turnover via the creatine kinase reactions. Numerous dynamic studies have been conducted in the human calf muscle using plantar flexion exercise [2, 4], where the main contributing muscles are gastrocnemius medialis (GM), gastrocnemius lateralis (GL), and soleus (SOL), all of which act on the Achilles tendon. The GM and GL are located superficially and originate from the lower end of the femur [7]. The deeper lying SOL is connected to the tibia and fibula [7], and slightly differs in function. Hence, its activation during plantar flexion is also different from the gastrocnemius muscles, the proportion of recruitment depending, amongst others, on the angle of the knee [8]. Based on the differences of these muscles

Georg B. Fiedler and Martin Meyerspeer are equal contributors.

✉ Martin Meyerspeer  
martin.meyerspeer@meduniwien.ac.at

<sup>1</sup> Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Lazarettgasse 14, 1090 Wien, Austria

<sup>2</sup> MR Centre of Excellence, Medical University of Vienna, Vienna, Austria

<sup>3</sup> Department of Pharmaceutical Chemistry, Semmelweis University, Budapest, Hungary

<sup>4</sup> Research Group of Drugs of Abuse and Doping Agents, Hungarian Academy of Sciences, Budapest, Hungary

<sup>5</sup> Institute of Medical Engineering, Graz University of Technology, Graz, Austria

<sup>6</sup> Department of Clinical Pharmacology, Medical University of Vienna, Vienna, Austria

regarding function and anatomy, PCr and Pi kinetics are also expected to differ [9–14]. Acquiring nonlocalized data from a mixture of muscle tissues implies averaging across their respective metabolite concentrations, exchange rates, and pH. This degrades sensitivity to small metabolic changes, especially when averaging across muscles with different levels of activation, and does not allow assessing specific metabolic properties from muscles with heterogeneous phenotype (e.g., plantar flexor muscles). This has been confirmed recently, comparing standard nonlocalized MRS of the calf with a volume of interest localized in GM, yielding different results [15]. Within each muscle, on the other hand, activation is relatively homogeneous [16], allowing single-voxel spectroscopy to be applied using a large, anatomy-matched volume.

Several localization schemes for  $^{31}\text{P}$  MRS have been used in muscle metabolism studies, the simplest using the limited sensitive volume of a small coil. Further methods include ISIS [9, 17], DRESS [18], and STEAM [19]. Small coil localization and DRESS cannot monitor deeper lying muscles, and STEAM inherently loses half the signal [20]. ISIS needs at least eight scans for 3-D localization, and with a  $T_1$  of PCr of approximately 4 s at 7 T [21]; this results in low temporal resolution. Methods like spectrally selective  $^{31}\text{P}$  MRI or chemical shift imaging (CSI) are rapidly advancing. With these methods, several muscles can be measured simultaneously and potential metabolic heterogeneities within a muscle can be detected; however, they are within their limits of point-spread function, spatial resolution, SNR, and temporal resolution. Parasoglou et al. [22] showed the feasibility of 3-D  $^{31}\text{P}$  MRI to quantify PCr dynamics in the human calf. One serious drawback of this method is that localized pH is not accessible by PCr imaging, which is an important parameter for characterization of aerobic and glycolytic muscle metabolism. CSI, on the other hand, suffers from poor voxel definition and requires far longer acquisition times. While metabolic processes on the order of 20–30 s have been observed using a gated CSI protocol [14], this approach depends on reproducibly reaching a steady-state (via a known dependence on time, e.g., mono-exponential) and imposes restrictions, e.g., acidosis should not be induced. The semi-LASER single-voxel MRS acquisition scheme is applicable to any exercise intensity and metabolic state, also any orientation and size of an individual muscle can be covered by the free positioning of the double oblique voxel in three dimensions, provided there is sufficient  $B_1$ . Further, the contamination by signal from outside of the VOI with the semi-LASER sequence is very low at ca. 1 % [20].

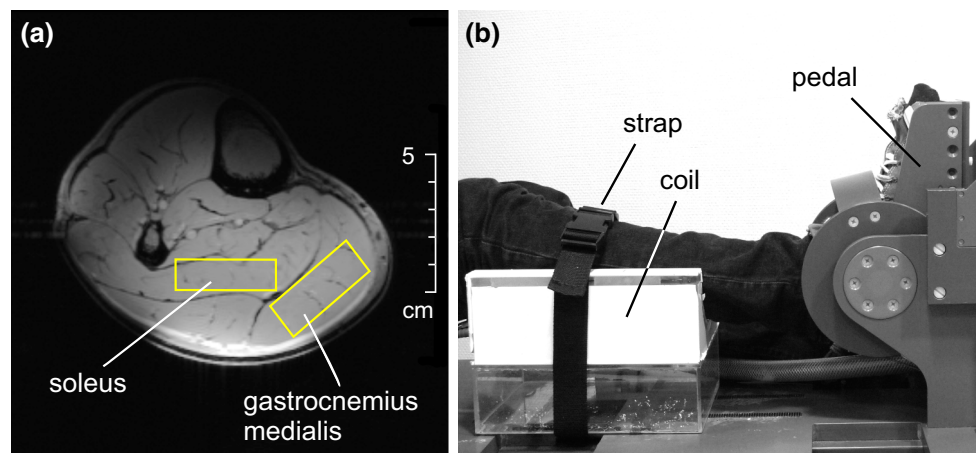
The location of SOL, which is covered by the neighboring gastrocnemius muscles, renders data acquisition more challenging. The aim of this study was to acquire accurately localized  $^{31}\text{P}$  MR spectra from exercising soleus, and

to show that it is possible to follow Pi and, thus, intracellular pH throughout rest, exercise, and recovery with a temporal resolution of 6 s, as well as to quantify the PCr recovery time in this muscle via a monoexponential fit.

To our knowledge, this has so far not been demonstrated for  $^{31}\text{P}$  MRS localized to a deeper muscle area. To achieve this goal, the combined SNR advances from an ultra-high field scanner, a dedicated, multi-channel calf-coil, and an adapted semi-LASER localization scheme were exploited. This extends previous studies (e.g. [15]) by demonstrating that soleus muscle can be monitored under exercise with adequate data quality, comparable to previous results from gastrocnemius.

## Materials and methods

Eleven young healthy subjects (age:  $26 \pm 5$  years, body mass index:  $22 \pm 1 \text{ kg/m}^2$ , four females) were scanned after written informed consent, according to the guidelines of the local ethics committee and the Declaration of Helsinki. Nine subjects completed the full protocol with data acquisition in SOL and GM; two subjects were measured for assessment of reproducibility at low PCr depletions in SOL only. An in-house built  $^1\text{H}/^{31}\text{P}$  transceiver coil array was used in a 7 T whole body MR scanner (Siemens Medical, Erlangen, Germany). The coil consisted of two channels for  $^1\text{H}$  and three channels for  $^{31}\text{P}$  and was shaped to a half cylinder ( $d = 14 \text{ cm}$ ,  $l = 10 \text{ cm}$ ) to accommodate a human calf [23]. A semi-LASER sequence [20, 24] was applied with a repetition time ( $T_R$ ) of 6 s. As semi-LASER is a single-shot sequence, this was also the true temporal resolution of the experiment. The shim and the transmit voltage of the RF pulses were adjusted individually for each subject and voxel position, as described previously [20]. The acquisition of spectra from the deeper-lying SOL requires more transmitting power (at a given pulse duration) to achieve full adiabatic inversion of the spins, compared to the better studied superficial GM. Limited by the available RF transmit power, the inversion pulse duration was typically 4600  $\mu\text{s}$  in SOL (3400  $\mu\text{s}$  for voxels located in GM). The shortest possible echo time ( $T_E$ ) was selected, which was typically 29 ms for SOL (24 ms for GM). To optimize SNR while ensuring specificity to single muscles, volumes of interest (VOI) were positioned double obliquely and chosen to be as large as possible, while fitting entirely within the respective muscle. This was done individually for each subject, using 25 transversal gradient echo MRI slices for voxel planning. The resulting average volumes were  $\text{VOI}_{\text{SOL}} = 45 \pm 9 \text{ cm}^3$  ( $66 \times 44 \times 16 \text{ mm}^3$ ) and  $\text{VOI}_{\text{GM}} = 40 \pm 5 \text{ cm}^3$  ( $63 \times 41 \times 16 \text{ mm}^3$ ). Typical voxel positions can be seen in Fig. 1a. Acquired spectra were processed with the jMRUI software package [25], using the



**Fig. 1** **a** Typical position of the voxels in soleus and gastrocnemius medialis muscle, respectively, for localized  $^{31}\text{P}$  MRS performed during two consecutive plantar flexion exercise bouts. **b** Coil position

and shape, together with a fixation strap, helped to keep the calf in shape even during exercise with the ergometer

AMARES time domain fit algorithm [26]. PCr and Pi signal intensities, representing the area under the peak in the frequency domain, were normalized to baseline PCr concentrations. The pH values were calculated from the chemical shift between Pi and PCr [6], and PCr recovery times  $\tau_{\text{PCr}}$  were fitted mono-exponentially to the PCr recovery time course. Maximum oxidative capacity ( $Q_{\text{max}}$ ) was calculated from  $\tau_{\text{PCr}}$  and the end-exercise values PCr and pH, using the ADP driven model [27, 28], with errors of  $Q_{\text{max}}$  calculated by error propagation. SNR was quantified as the ratio of the PCr peak amplitude to the standard deviation (SD) of noise at around 10 ppm up-field from the PCr frequency (width of noise region: 1/8 of total bandwidth).

The measurement protocol comprised 2 min at rest, during which time baseline data were acquired, with 5 min of submaximal plantar flexion, and 7 min of recovery time. The isotonic exercise on a custom built MR compatible ergometer consisted of two consecutive pedal pushes between each period of MR data acquisition, which was every  $T_{\text{R}} = 6$  seconds, i.e., the effective frequency of the exercise was 0.3 Hz. Maximum voluntary contraction force (MVC) of each subject was measured in the ergometer on the scanner bed, prior to the MRS measurements. The force of the exercises was set to 40 % of MVC, via the pressure in the air piston of the ergometer, and controlled throughout the experiment. The knee was straight and the calf was placed on the form-fitted array coil and fixed in position using a strap across the tibia (see Fig. 1b). Two equivalent exercise bouts were carried out, the first for measuring the time course of localized  $^{31}\text{P}$  MR spectra in GM, and the second for SOL. They were separated by 28 min of inactivity (on average, minimum was 20 min), ensuring that PCr, Pi, and pH values had fully recovered to basal levels at the beginning of the second bout.

Control experiments were performed in four subjects (two from the group performing the full protocol, and two additional subjects) by repeatedly measuring in SOL. The purpose of these measurements was to assess the reproducibility of metabolite quantification and fitting of  $\tau_{\text{PCr}}$  at low PCr depletion in this muscle between subsequent exercise bouts, with a resting phase of 25 min. Day-to-day reproducibility was tested after 1 week in one subject.

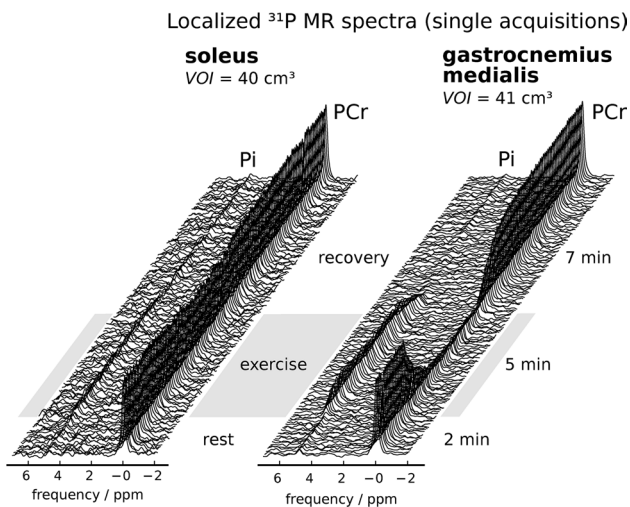
## Results

PCr and Pi were quantified from  $^{31}\text{P}$  spectra acquired in time series with a temporal resolution of 6 s. The average SNR of PCr at rest was  $64 \pm 15$  in SOL, and  $83 \pm 12$  in GM. The mean force applied by the volunteers during exercise, as measured with the sensor on the ergometer pedal, was  $40 \pm 10$  % of MVC in the SOL bout, and  $40 \pm 8$  % of MVC in the GM bout. The mismatch between each individual's forces in the two exercise bouts was  $1.8 \pm 1.3$  % of MVC. All values relate to individual MVC.

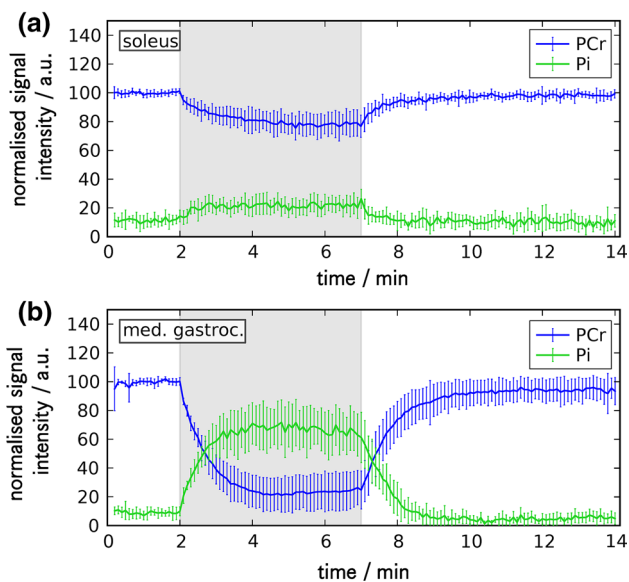
Stack plots of  $^{31}\text{P}$ -spectra from a single subject are shown in Fig. 2. Data are plotted without temporal averaging, representing the acquisition's full time resolution of 6 s.

Time courses of PCr and Pi signal averaged over all nine subjects are shown in Fig. 3. PCr depletion was detected in the soleus muscle of all subjects, and ranged from 6 % to 38 % at the end of exercise. The GM showed far stronger depletion in all subjects, ranging between 49 % and 94 %, which is consistent with [28].

Average end-exercise PCr depletions of SOL and GM can be read from Fig. 3 and are summarized in Table 1. The table also shows the average PCr recovery times  $\tau_{\text{PCr}}$ ,



**Fig. 2** Stack plots of spectra acquired in SOL (*left*) and GM (*right*) during rest, plantar flexion exercise (*gray area*), and recovery of one subject. Single-shot spectra ( $T_R = 6$  s) are shown without averaging. Exponential line broadening of 25 Hz and zero filling ( $8\times$ ) were applied for better visualization



**Fig. 3** PCr and Pi signal in **a** SOL and, for comparison, **b** GM at rest, during exercise (*marked gray*) and during recovery, from two equivalent exercise bouts. Mean  $\pm$  SD of  $n = 9$  subjects

excluding the one subject with only 6 % depletion in SOL, due to insufficient dynamic range for the PCr recovery fit.

Intracellular pH was also quantified at a temporal resolution of 6 s. The time courses of pH in SOL (Fig. 4a) and GM (Fig. 4b), respectively, are shown as the average over all nine subjects. Data points where the SNR of Pi was

below 1.3 for individual subjects were not included. Average pH values at rest, end of exercise, and the post-exercise minimum in SOL and GM are given in Table 1.

In SOL, pH increased immediately at the onset of exercise, rising by  $0.03 \pm 0.01$  within 12 s, see Fig. 4a. The pH remained elevated throughout the exercise, with a maximum of  $7.09 \pm 0.02$ . During recovery, pH returned to resting values within  $23 \pm 8$  s and continued to decrease slightly below resting values by  $-0.016 \pm 0.012$  ( $p < 0.005$  pre vs. post exercise pH), as quantified from four blocks of five averaged spectra at rest and during the last 2 min of the measurement. The pH evolution in SOL was in stark contrast to GM: the initial pH rise in the first 12 s was  $0.07 \pm 0.02$  (see Fig. 4b), and the maximum of  $7.12 \pm 0.01$  was reached after  $23 \pm 8$  s. End-exercise pH in GM varied widely between subjects, ranging from 6.47 to 7.02 and was followed by a post-exercise drop of  $0.15 \pm 0.03$ , before returning towards its resting value.

Two representative individual subjects' datasets are presented in Fig. 5, showing PCr time courses (Fig. 5a, b) with a temporal resolution of 6 s. For better visualization only, the pH is shown in Fig. 5c, d with 12 s time resolution, obtained by averaging two successive spectra at each data point. As for averaged data, points with  $\text{SNR}(\text{Pi}) < 1.3$  were omitted in the individual pH graph.

To investigate possible effects on the measured Pi peak by low spatial resolution or a partial volume effect, retrospective spatial averages of spectra are compared to the original data. Figure 6a, b show spectra localized in SOL and GM, respectively, from unaveraged single-shot acquisitions, after 1 min of exercise. To simulate partial volume effects, Fig. 6c shows the average of the spectra from the two muscles, while Fig. 6d shows a combined spectrum obtained by weighting SOL and GM data by 70:30. All data in Fig. 6 are from the same subject. Depending on the effective time interval of temporal averaging, the width and shape of the Pi peak also varies, corresponding to different derived pH (data not shown).

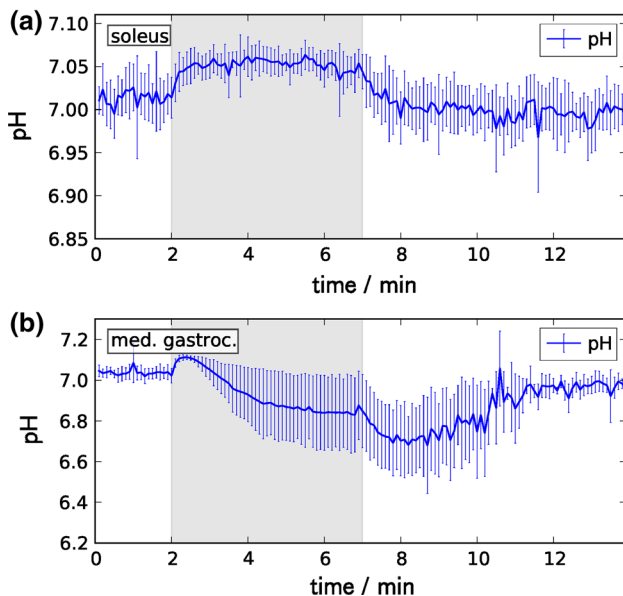
Results of the measurements repeated in the same subjects show good reproducibility of the applied force, which was adjusted to yield relatively low PCr depletion in SOL of 22 % and 26 % (group averages). Also, the end-exercise pH in the neutral to slightly alkaline regime and PCr recovery time of 20 s versus 27 s were reproducible (see Table 2) and not significantly different between bouts. PCr depletion and pH changes were also found to be reproducible in measurements repeated after 1 week.

These measurements also showed a small, significant pH decrease by  $-0.03 \pm 0.02$ , post versus pre-exercise, in all bouts. The pH measured during the 2 min before exercise was not significantly different between bout 1 and 2.

**Table 1** PCr depletion (relative to resting PCr signal), PCr recovery time constant  $\tau_{\text{PCr}}$ , pH values at rest, at end of exercise and at the post exercise minimum and maximum oxidative capacity  $Q_{\text{max}}$  of  $n = 9$  subjects

	Subj.	PCr depletion	$\tau_{\text{PCr}}$	pH <sub>rest</sub>	pH <sub>end ex</sub>	pH <sub>end rcv</sub> (SOL) pH <sub>min</sub> (GM)	$Q_{\text{max}}$ [mM/min]
SOL	1	6 ± 1 %	–	7.04 ± 0.004	7.05 ± 0.01	7.03 ± 0.006	–
	2	38 ± 2 %	54 ± 5 s	6.99 ± 0.014	7.03 ± 0.01	7.00 ± 0.010	23 ± 2
	3	23 ± 2 %	23 ± 4 s	7.03 ± 0.006	7.02 ± 0.02	6.99 ± 0.005	40 ± 7
	4	17 ± 1 %	20 ± 3 s	7.00 ± 0.002	7.02 ± 0.01	6.97 ± 0.014	39 ± 7
	5	14 ± 1 %	63 ± 12 s	7.02 ± 0.001	7.05 ± 0.01	7.00 ± 0.002	10 ± 2
	6	20 ± 3 %	24 ± 7 s	7.02 ± 0.003	7.08 ± 0.01	7.01 ± 0.002	33 ± 10
	7	15 ± 2 %	30 ± 9 s	7.02 ± 0.010	7.07 ± 0.01	7.00 ± 0.018	23 ± 7
	8	11 ± 1 %	77 ± 15 s	7.02 ± 0.006	7.06 ± 0.02	7.00 ± 0.012	8 ± 2
	9	26 ± 1 %	42 ± 5 s	7.02 ± 0.004	7.06 ± 0.01	7.01 ± 0.006	23 ± 2
GM	1	65 ± 1 %	32 ± 1 s	7.03 ± 0.003	7.03 ± 0.01	6.90 ± 0.05	51 ± 1
	2	90 ± 2 %	71 ± 3 s	6.98 ± 0.019	6.47 ± 0.03	6.38 ± 0.06	30 ± 1
	3	94 ± 1 %	68 ± 1 s	7.08 ± 0.003	6.58 ± 0.02	6.46 ± 0.03	30 ± 1
	4	69 ± 1 %	34 ± 1 s	7.01 ± 0.002	6.97 ± 0.02	6.80 ± 0.01	49 ± 1
	5	69 ± 1 %	62 ± 2 s	7.05 ± 0.010	6.78 ± 0.01	6.65 ± 0.10	29 ± 1
	6	76 ± 2 %	44 ± 2 s	7.04 ± 0.005	6.95 ± 0.03	6.83 ± 0.12	41 ± 2
	7	49 ± 1 %	45 ± 1 s	7.04 ± 0.004	7.01 ± 0.01	6.87 ± 0.02	31 ± 1
	8	83 ± 1 %	45 ± 1 s	7.05 ± 0.008	6.87 ± 0.01	6.68 ± 0.01	42 ± 1
	9	73 ± 2 %	37 ± 2 s	7.06 ± 0.007	6.93 ± 0.02	6.75 ± 0.03	48 ± 1
SOL		19 ± 9 %	37 ± 17 s	7.02 ± 0.02	7.06 ± 0.02	7.00 ± 0.01	25 ± 12
GM		74 ± 14 %	49 ± 15 s	7.04 ± 0.03	6.85 ± 0.19	6.70 ± 0.19	39 ± 9

Data were quantified from unaveraged spectra, errors for PCr depletion and  $\tau_{\text{PCr}}$  of individuals are SDs resulting from the fitting algorithm, the uncertainty of  $Q_{\text{max}}$  was derived via error propagation. The pH at the end of exercise and the post exercise minimum in GM were quantified from four unaveraged spectra (SDs over five data points). The resting pH in SOL and GM and the pH during the last 2 min measured in SOL was derived from blocks of five accumulated spectra (SDs over four data points). Group averages represent the mean ± SD of individuals' data

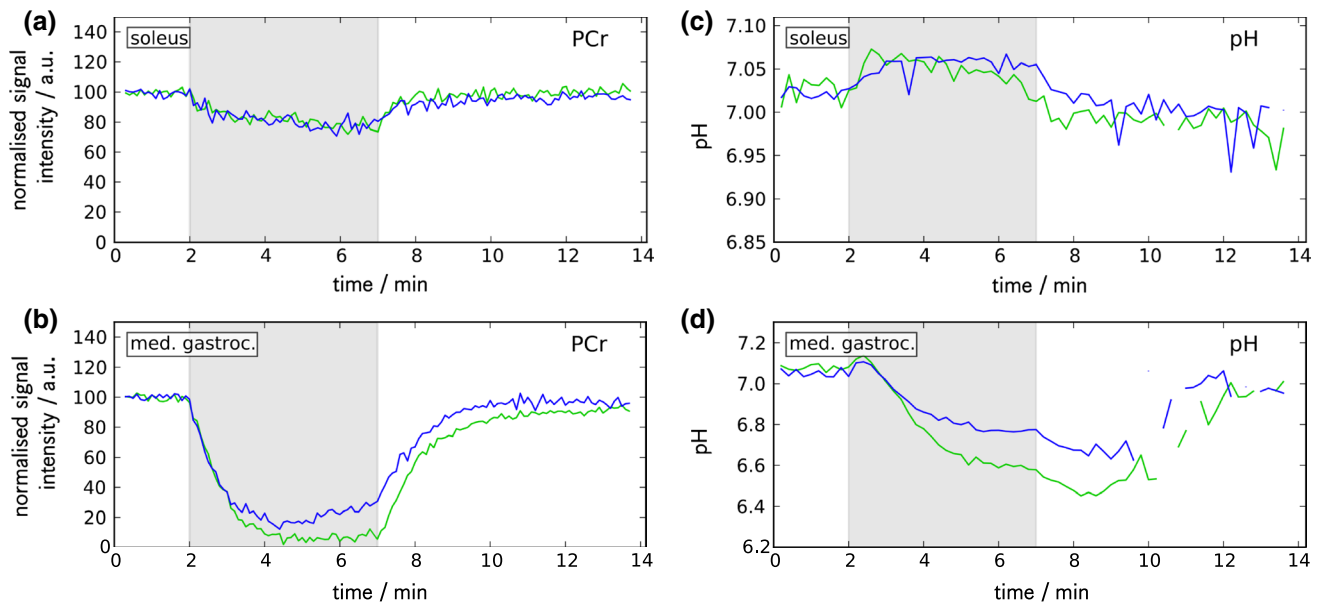


**Fig. 4** Intracellular pH in **a** SOL and **b** GM with a time resolution of 6 s, mean ± SD of all  $n = 9$  subjects. Note the different scale of the two plots

## Discussion

This study successfully shows that quantification of PCr, Pi, and pH is feasible with high specificity, even in the deeper lying human soleus muscle, by combining high temporal resolution of 6 s with spatial selectivity. This was achieved by exploiting the high SNR of  $^{31}\text{P}$  spectra acquired with a semi-LASER sequence, using a custom-built form-fitted array coil [23] on a 7T MR scanner, in combination with a plantar flexion paradigm.

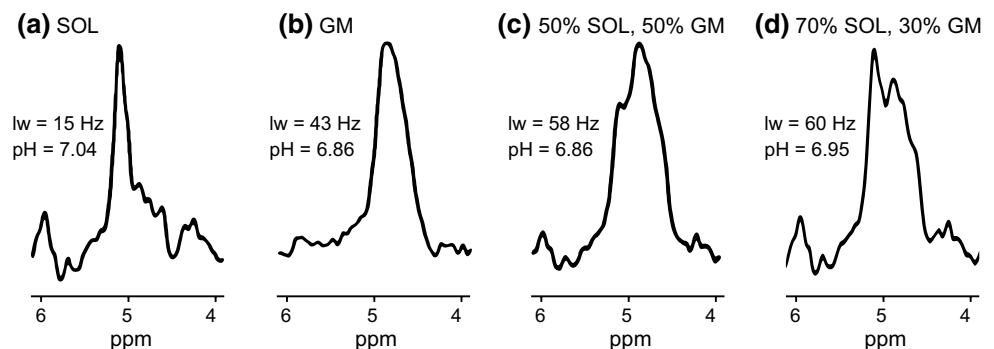
The SOL consistently showed PCr depletion, although at far lower levels than GM. The time courses of pH differed even qualitatively: sharing a similar rise at the beginning of exercise, pH in SOL remained alkaline during the exercise and dropped towards basal levels shortly after the end of exercise. In GM however, pH was declining throughout the exercise, with a further drop post-exercise. This is consistent with ASL data from Schewzow et al. [29], where perfusion in GM increased much more than in SOL in a comparable exercise paradigm. In a previous study [15], simple acquisition of FID versus a localization scheme



**Fig. 5** Time courses of PCr signal and pH evolution of the same two representative subjects. The PCr signal in **a** SOL and **b** GM is shown with 6 s time resolution. The pH evolution shown in **c** for SOL and **d** for GM was derived from spectra after averaging two time points

each (i.e., 12 s time resolution), data points with an SNR of the Pi peak below 1.3 are omitted. Note that the plots showing pH (**c**, **d**) are scaled differently

**Fig. 6** The Pi peaks in spectra from **a** SOL, **b** GM, and averages of the two datasets, **c** 50 % SOL and 50 % GM, **d** 70 % SOL and 30 % GM. All spectra were measured in the same subject, represent the same point in time (after 1 min of exercise), and are scaled to the same amplitude. Linewidth (lw) and the calculated pH are given



was compared, using the same standard single loop coil in both measurements, demonstrating significantly different results from the two methods. The results from the present study indicate that contamination from the lesser activated SOL is a possible source of bias for quantification of PCr, Pi, and consequently pH in unlocalized experiments. This emphasizes the importance of spatial localization in order to provide a more specific interpretation of the muscle under investigation.

Average PCr recovery times  $\tau_{\text{PCr}}$  in SOL were shorter than in GM. Acidification slows down PCr recovery in muscle tissue [30, 31]; therefore, the maximum oxidative capacity  $Q_{\text{max}}$  is a more suitable measure to compare recovery from exercise in muscles with different pH. This parameter was also comparable between SOL and GM, but slightly lower in SOL. Nevertheless, it has to be taken into account that the two muscles were working in a different

metabolic regime, SOL at low activation and alkaline pH working mainly aerobically, and GM at much higher activation and acidification with a high glycolytic contribution. A sound comparison of these muscles would require similar PCr depletion and end-exercise pH values, e.g., via multiple measurements at different exercise intensities, which is beyond the scope of this study.

The reproducibility measurements show good agreement of data acquired from SOL in two bouts on the same day (see Table 2). The stability of mean forces indicates that the subjects were exercising at a comparable level in both bouts, and similar end-exercise values of PCr depletion and pH show that SOL was also activated to nearly the same extent.  $\tau_{\text{PCr}}$  showed a small variation from bout to bout in subjects #6 and #9, with larger variability in subject #11; the differences from bout to bout were not statistically significant. All recovery times in SOL were in the lower range

**Table 2** Repeated measurements in SOL, for each subject with the same exercise intensity

Subject	Bout	PCr depl.	$\tau_{\text{PCr}}$	pH <sub>end</sub>	Force / N
#6	1	48 ± 2 %	27 ± 2 s	6.92 ± 0.03	243 ± 14
	2	34 ± 2 %	34 ± 3 s	6.98 ± 0.02	234 ± 15
#9	1	18 ± 2 %	17 ± 4 s	7.04 ± 0.01	255 ± 22
	2	12 ± 2 %	19 ± 7 s	7.04 ± 0.01	222 ± 16
#10	1	3 ± 2 %	-	7.07 ± 0.01	291 ± 11
	2	4 ± 2 %	-	7.07 ± 0.02	292 ± 10
#11	1	36 ± 1 %	15 ± 2 s	7.06 ± 0.01	285 ± 24
	2	39 ± 2 %	28 ± 3 s	7.06 ± 0.01	283 ± 29
Mean	1	26 ± 20 %	20 ± 6 s	7.02 ± 0.07	267 ± 21
	2	22 ± 17 %	27 ± 7 s	7.04 ± 0.04	256 ± 32
<i>Measurement repeated after 1 week</i>					
#10	1	6 ± 2 %	-	7.10 ± 0.02	284 ± 9
	2	4 ± 4 %	-	7.11 ± 0.03	283 ± 11

Bouts one and two were spaced by 25 min of inactivity. The measurements on subject #10 were repeated after 1 week

of all measurements in this study and, hence, distinct from values typically measured in GM. When testing day-to-day reproducibility over the course of 1 week, PCr depletion was consistently very low and pH was elevated throughout the exercise in SOL on both days and in all exercise bouts, which were performed with the same force (see Table 2, subject #10). Reproducing the same effect of exercise (i.e., equal activation of SOL and, hence, PCr depletion) by repeating the measurements after a longer period of several months was attempted in three subjects and found to be challenging. Possible reasons for larger variability (data not shown) are that the subjects may have distributed the exercise load to individual calf muscles differently, when asked to perform the same exercise after a long time, and that training state and living circumstances can change over longer periods [32], which could influence the measured parameters. Yet, all measurements performed to test reproducibility in SOL resulted in values that corresponded to low metabolic activation, which confirms that the striking differences observed in data from SOL and GM were not caused by repeating the exercise within 25 min, but represent intrinsic differences in the metabolic response of these muscles to the same exercise. This was also consistent with previous studies [15, 33], which indicate that repeating exercise has no severe (if any) influence on PCr recovery kinetics after a sufficiently long phase of inactivity. This applies in particular to SOL, which was activated only modestly.

The main advantage of the semi-LASER sequence is that the size and orientation of the single voxel can be explicitly defined in all three dimensions. Consequently, a large voxel can be selected, anatomically matched to the

muscle, which minimizes partial volume effects. Therefore, the method has high specificity [20], yields good SNR, and, as a single-shot sequence, allows high temporal resolution. Together, this is greatly beneficial for measurements in a superficial muscle like GM. Furthermore, it forms the basis of an inherent virtue of the semi-LASER sequence: the ability to penetrate into deeper lying tissues and afford accurate, localized data. Obviously, in deeper areas localization via a small coil is not applicable, and the location and non-trivial geometry of a muscle like SOL make it particularly inaccessible, even to other localization methods. With a 2-D ISIS-like sequence for instance, as used in [9], placing voxels in SOL without overlap to GM or GL is nearly impossible.

Quantification of fast pH changes requires high temporal resolution, e.g., to follow the immediate pH increase at the onset of exercise. In GM, the average time to maximum pH was 23 s, and some subjects showed a similarly fast increase of pH in SOL. Such dynamics can only be resolved with a temporal resolution of 11 s or better, to avoid undersampling. Also the quantification of the PCr recovery time constant  $\tau_{\text{PCr}}$  greatly benefits from higher temporal resolution. Particularly at low depletions, a sufficient number of data points needs to be acquired while PCr concentration is still significantly below resting values, i.e., the low dynamic range necessitates high time resolution. Aided by the temporal resolution of 6 s, the feasibility of  $\tau_{\text{PCr}}$  quantification at PCr depletions as low as 15 % was demonstrated in this study.

The initial pH rise supports the notion that in the first seconds of exercise PCr breakdown is the dominant mechanism to ATP production, with mitochondrial activity not being elevated beyond resting activity, and with minimal glycolytic contribution. The alkaline pH throughout the exercise in SOL suggests that the ATP need can be met by PCr breakdown and oxidative phosphorylation [27], which is in agreement with the modest PCr depletion rates. A small decrease of post exercise pH below pre-exercise levels in SOL was found within all groups (i.e., with the full exercise protocol and in reproducibility measurements), which can be explained by the proton production associated with oxidative ATP synthesis after aerobic exercise.

The elevated pH in SOL also affirms that the PCr decrease (and Pi increase) is a physiologic effect and not due to a motion artifact or contamination from strongly depleted adjacent muscles, since this would be accompanied by a pH drop or a split Pi peak. Contamination is a form of spatial averaging across two muscles, which can result in artifacts of the apparent Pi peak, regarding position, width, and shape, when the muscles are in different metabolic states. Consequently the resulting value of pH can lie anywhere between the actual pH values of the respective muscles, depending on the volume fraction of

the muscles in the averaged volume (see Fig. 6c, d). This bias of pH can also vary with time, due to the different metabolic evolution of the muscles during exercise. Also temporal averaging can be a source of artifacts, as the Pi peak moves in frequency, when pH changes over time. With low temporal resolution, this is perceived as an increased Pi linewidth, or when the (averaged) acquisition is sufficiently long compared to the pH shift, it even might appear as two components in the broad peak.

To summarize, accurate spatial localization and sufficiently high temporal resolution help to establish a more reliable, precise, and accurate quantification of Pi and, hence pH, specific to a particular muscle.

## Conclusion

Localized  $^{31}\text{P}$  MR spectra were acquired from the deeper lying soleus muscle at high temporal resolution during and after exercise. The time courses of PCr and pH indicate lower activation of SOL than the neighboring GM in this form of exercise. The high temporal resolution is the basis for quantification of fast pH changes, e.g., the initial rise of pH, which can be on the order of 10 s. Furthermore, high temporal resolution is desirable to improve quantification of PCr recovery, and to avoid erroneous Pi peak broadening, by minimizing temporal averaging over shifting metabolite peaks. Altogether, this work illustrates the relevance and effect of high temporal resolution and accurate spatial localization, enabling quantification of fast or subtle metabolic processes, as well as improving specificity in many regards.

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**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical standards** The study has been approved by the local ethics committee and has, therefore, been performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments. All subjects gave informed consent in writing before being included in the study.

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