Direct quantification of lactate and high energy phosphates in exercising human skeletal muscle by interleaved localised ^{1}H DQF, ^{1}H- and ^{31}P STEAM

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Introduction Phosphorus MRS has long been used to study cellular energy metabolism of skeletal muscle in vivo. The inability to directly quantify lactate using ^{31}P MRS has necessitated indirect approaches [1] to important issues in the control of glycolysis [2] and cellular acid handling [3, 1]. There are several technical obstacles to quantifying muscular lactate in vivo by ^{1}H MRS. Overlapping strong lipid resonances (at 0.5 - 1.5 ppm) obscure the lactate resonance in this region. Further on, dipolar coupling effects, dependent on the orientation of muscle fibres relative to the static magnetic field B_0, result in reduced lactate visibility. Hence, quantitation of skeletal muscle lactate concentration requires techniques to selectively acquire the lactate resonances while suppressing the strong background signals. Here we describe an interleaved ^{31}P- and ^{1}H MRS method by which lactate accumulation and the accompanying changes in phosphorus metabolites, pH and total creatine concentration can be monitored in calf muscle during plantar flexion exercise and recovery. We show good agreement between directly measured lactate and that measured biochemically and inferred indirectly from ^{31}P MRS data.

Methods A measurement protocol for interleaved acquisition of double-quantum-filtered (DQF) localised MR spectra of lactate and of STEAM localised ^{1}H- and ^{31}P spectra (Fig. 1) during exercise was implemented on a 3 T Bruker Medspec whole-body NMR scanner (Bruker Biospin, Ettlingen, Germany). A standard double-tuned surface coil (d = 10 cm) was used for RF transmission and reception. Healthy subjects (n = 6) performed ischaemic plantar flexion on a custom built ergometer. The VOIs of interleaved loccalised MRS were positioned in the medial gastrocnemius. In vivo lactate quantification was verified using ex vivo porcine gastrocnemius and comparing the results of MRS and biochemical assay. In muscle tissue, in addition to lactate's scalar coupling with J = 7 Hz, dipolar coupling causes an orientation-dependent modulation of the signal acquired with the DQF sequence [5], which is governed by eq. 1. Therefore, and allowing for to lactate's multi-compartmental transverse relaxation, the optimum time ($T_E = \tau_1 + \tau_2$) is shorter than for tissues which do not exhibit dipolar coupling (e.g. brain, where T_E = 135 ms is conventionally used). Absolute quantification used a phantom replacement technique employing test objects filled with lactate solutions (25 and 50 mmol/l), matching the coil load of an in vivo measurement, and using a small vial (filled with water) placed below the surface coil as external reference to estimate coil sensitivity. Absolute lactate concentrations were calculated using knowledge of muscle fibre orientation [6] and the parameters of eq. 1 as given in [5], i.e. compartmentation ratios a, b, T_1 and T_2 relaxation times, together with back-calculation factors for varying voxel sizes, receiver gain and number of averages.

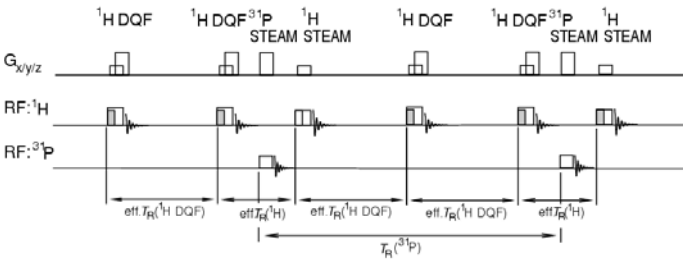

Fig. 1: Schematics of the interleaved localised ^{1}H DQF, ^{31}P STEAM, ^{1}H STEAM sequence

$$S = S_0 \left\{ a \sin(\pi J \tau_1) \sin(\pi J \tau_2) \left(1 + \cos^2(\pi J \tau_1)\right) e^{-\tau_1/T_{21}} e^{-\tau_2/T_{21}} \times \right.$$
$$\left. b \sin(\pi \Delta \tau_1) \sin(\pi \Delta \tau_2) \left(1 + \cos^2(\pi \Delta \tau_1)\right) e^{-\tau_1/T_{2s}} e^{-\tau_2/T_{2s}} \right\}$$

Eq. 1. Signal evolution $S(\tau_1, \tau_2, \varphi)$ in the DQF sequence. $(\Delta = \Delta(\varphi))$.

Results In the validation experiment, lactate concentration was 40 ± 7 mmol/l muscle in porcine gastrocnemius, close to the biochemical measurement of 39 ± 5 mmol/l (n = 21, mean ± SD). Spectra obtained in the triple-interleaved MRS experiments before, during and after ischaemic exercise in healthy subjects are shown in the Fig. 2. During the exercise protocol we could detect a pH decrease of 0.47 ± 0.06 below the resting value of 7.06 ± 0.03, and a PCr decrease to 24 ± 2 % of resting values, as measured from the 31P spectra. This corresponds to an expected [1] lactate concentration of 24 ± 2 mmol/l cell water. Quantitation of this signal yielded lactate a concentration of 24 ± 2 mmol/l. Simultaneously acquired ^{1}H STEAM spectra show no change in the CH_3 group of total creatine resonance but a marked drop in the CH_2 resonance of this compound.

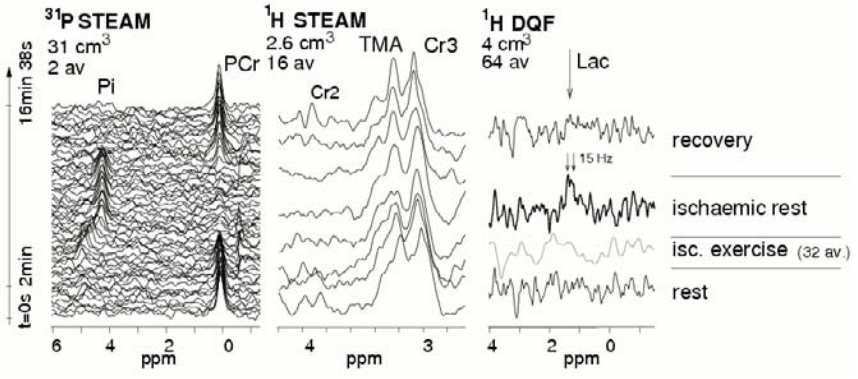

Fig. 2: Spectra of human calf muscle acquired with the interleaved MRS sequence during the exercise protocol

Discussion The method gives a remarkably good fit both to biochemically measured lactate concentration in porcine gastrocnemius and to the lactate concentration in exercising human calf muscle expected on the basis of changes in pH, Pi and PCr. As the latter calculation uses cytosolic buffer capacity based on an indirect analysis of proton handling in ischaemically exercising forearm muscle, the agreement supports both the present method and the analysis of the forearm data [1].

References

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