Pulse-acquire ^{31}P MRS allows to measure brain ATP synthesis in humans at 3 Tesla

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^{31}P spectroscopy allows to quantify the unidirectional Pi to ATP flux (V_{ATP}) using the saturation transfer experiment. Potential applications of this approach include the study of brain disease. However, up to now, only one measurement has been reported in the human brain, localized in the visual cortex by a surface coil at 7T [1]. Since numerous cerebral diseases affect the entire parenchyma or large parts of the brain (Alzheimer's disease, Huntington's disease…), the ability to measure the global ATP synthesis rate in the human brain within a reasonable examination time (~30min) on a clinical 3T system would be of great interest. Our purpose has been to demonstrate the ability to perform this measurement. Saturation transfer was combined with a simple pulse-acquire ^{31}P sequence and applied to the upper part of the head. ^{31}P spectra were quantified using time-domain analysis. Whole-brain V_{ATP} was calculated from the data collected in 5 healthy volunteers.

Materials and Methods

NMR System Experiments were performed on a whole-body 3T Bruker system equipped with a quadrature birdcage coil resonating at 50.7MHz (^{31}P frequency). The edge of the cylindrical coil was positioned at the level of the upper lip in order to limit the detection volume to the upper part of the head.

Pulse-Acquire ^{31}P Spectroscopy 1st order shimming was performed manually on the ^{31}P signal, leading to a ~15Hz line width on PCr. Spectra were then collected using a pulse-acquire ^{31}P sequence without localization (100µs 90° hard pulse, TR=7.5s, 28 transients).

Progressive Saturation Transfer Experiment A saturation broad pulse (length t_{sat}) was added just before the pulse-acquire ^{31}P sequence. Spectra were collected for 4 t_{sat}=0.25s, 0.5s, 1s and 1.5s (TR=7.5s, NT=28) with saturation of the γ-ATP frequency, and then of the symmetric frequency relative to Pi for reference measurements. According to literature values at 2T and 4T and to measurements performed in the monkey brain at 3T, the intrinsic longitudinal relaxation time $T1^{int}$ of Pi is expected to be ~2.1s here. Thus TR=7.5s allows full relaxation for Pi. The experiment (~35 minutes including shimming) was performed on 5 healthy volunteers (3 males, 2 females, aged 39±4).

Muscle Contribution to ^{31}P Spectra In order to measure the contribution of skeletal muscle to ^{31}P signal, a ^{1}H RF coil with the same geometry as our ^{31}P coil was used to acquire high resolution 3D GE image for one volunteer. The ^{1}H coil was positioned identically to the ^{31}P coil. The image was manually segmented and total brain and muscle volumes were calculated.

Spectra quantification ^{31}P spectra were quantified using Java-based MR user interface (jMRUI [2]), with an Advanced Method for Spectral Fitting (AMARES) [3]. Thirteen ^{31}P multiplets were included [4]: phosphoetanolamine (PE), phosphoserine (Pser), phosphocholine (PC), the 2,3 diphosphoglycerate doublet (DPG), inorganic phosphate (Pi), glycophosphoethanolamine (GPE), glycophosphocholine (GPC), membrane phospholipids (MP), phosphocreatine (PCr), the γ-ATP doublet, the α-ATP, dinucleotides (DN), and the β-ATP triplet. To get rid of the broad baseline signal, the FIDs were left-shifted 12 points (1.2ms) for quantification. Then the estimated resonances were corrected for amplitude and line widths taking into account the signal of the truncated points.

Determination of ATP synthesis rate For each volunteer and each saturation time, the ratio of Pi magnetization with saturation of γ-ATP over control magnetization $M_S(t_{sat})/M_C(t_{sat})$ was estimated. Then ratios were averaged for the five volunteers. The average curve $M_S(t_{sat})/M_C(t_{sat})$ was fitted to estimate the rate of Pi synthesis k_f, and $T1^{int}$ [1]. In order to stabilize the procedure, $T1^{int}$ was constrained around 2.1s (1.7s<$T1^{int}$<2.5s). Monte Carlo simulation was performed in order to assess fit accuracy.

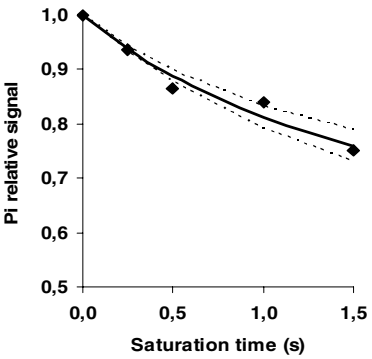

Fig. 2: Pi attenuation (♦) vs. saturation time t_{sat}. The solid line is the best fit to data. The dotted lines represent the lower and upper limits of the fit based on k_f and $T1^{int}$ s.d.

[1] Lei H et al., *PNAS* 100, p.14409 (2003); [2] Naressi A et al., *Comput Biol Med* 31, p.269 (2001); [3] Vanhamme L et al., *JMR* 129, p.35 (1997); [4] Jensen JE et al., *NMRBiomed* 15, p.338 (2002); [5] Lebon V et al., *JCI* 108, p.733 (2001).

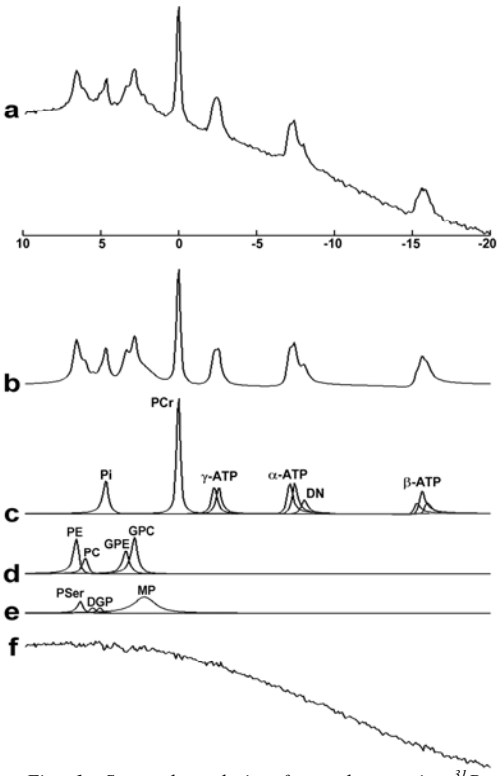

Fig. 1: Spectral analysis of a pulse-acquire ^{31}P spectrum using AMARES. (a) Raw spectrum. (b) Estimation of metabolite global spectrum. (c), (d) and (e) Individual contributions. (f) Residuals.

Results and Discussion

Muscle contribution Image segmentation yielded a 1.43L brain volume and a 0.11L muscle volume. Hence, given the concentrations in brain and muscle [4], muscle contamination resulted in less than ~8% overestimation of brain ATP and Pi.

Pulse-acquire ^{31}P spectra Pulse-acquire ^{31}P spectra exhibit the superimposition of metabolite peaks to a broad baseline (Fig. 1a), which must be eliminated for valuable quantification of metabolites. AMARES analysis is shown in Fig. 1 (b-f). In order to assess the accuracy on Pi quantification, a set of ten spectra was acquired within 1 hour in the same volunteer, the volunteer being removed from the patient table and repositioned in the magnet between each measurement. AMARES analysis of this batch yielded a highly reproducible quantification of Pi with a s.d. of 3%.

Cerebral rate of ATP synthesis in humans k_f and $T1^{int}$ estimations from averaged $M_S(t_{sat})/M_C(t_{sat})$ fitting (Fig. 2) and Monte Carlo simulation yielded k_f=0.27±0.02s^{-1} and $T1^{int}$=2.0±0.4s. The higher accuracy on k_f (s.d.~7%) than on $T1^{int}$ (s.d. ~20%) illustrates the relative insensitivity of k_f estimation to $T1^{int}$ dispersion. Since $V_{ATP} = k_f$ [Pi] only depends on k_f, the higher accuracy on k_f must be regarded as an asset for V_{ATP} determination. In order to estimate Pi absolute concentration, ATP concentration was taken as a reference, since the bias on Pi and ATP quantification due to muscle contribution is identical ($[Pi]_{muscle}/[Pi]_{brain}$~$[ATP]_{muscle}/[ATP]_{brain}$ [4]). Assuming $[ATP]_{brain}$=2mM [4], absolute concentration of Pi could be assessed to 1.2mM, yielding V_{ATP}=17.7±1.3µmol.g^{-1}.min^{-1}.

Whole-brain energy metabolism Reported values of metabolic rates in the human brain based on arterio-venous differences give CMR_{glu}=0.31-0.42µmol.g^{-1}.min^{-1} and $CMRO_2/CMR_{glu}$=5.5. With P/O_2=6 for complete stoichiometry, these numbers lead to an oxidative ATP synthesis rate V_{ATP} of 10-14µmol.g^{-1}.min^{-1}. The slightly higher value of V_{ATP} found here can unlikely be ascribed to muscle contamination, since muscle accounts for less than 8% of the quantified Pi, and k_f in resting muscle including reversible synthesis by GADPH/PGK is ~0.03s^{-1} [5]. Alternatively, a contribution of GADPH/PGK at the glycolytic level in the brain might result in overestimating V_{ATP}, but this contribution has to be much lower than oxidative synthesis. Therefore whole-brain V_{ATP} is dominated by mitochondrial ATP synthesis.

Conclusion

V_{ATP} was measured in the whole human brain (17.7±1.3µmol.g^{-1}.min^{-1}) within a clinical examination time (~30min) at 3T. The present methodology should prove useful to study potential alterations of ATP synthesis during brain disease.