Absolute Quantification of ATP and Other High Energy Phosphate Compounds in Cat Brain at 9.4T

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INTRODUCTION *In vivo* ^{31}P MRS is unique and can be used to assess the high energy phosphate (HEP) metabolites for studying the ATP metabolism and bioenergetics. However, its utility relies on the accuracy for quantifying the absolute metabolite concentrations, which has been a long-standing challenge in the *in vivo* MRS research field. Although different strategies have been applied for determining the absolute metabolite concentrations noninvasively using *in vivo* MRS, generally, it is believed that the accuracy of such quantification was limited due to the large systematic errors introduced by various calibration techniques. After careful comparison of different calibration approaches reported in the literature [1-3] for absolute quantification of high energy phosphate (HEP) concentrations using *in vivo* ^{31}P MRS, we have come up with a simple, improved experimental design which would eliminate most systematic errors and provide reliable quantification results. This design uses a ^{31}P RF surface coil and an external phantom containing known concentration of ATP to mimic the *in vivo* situation with *identical* sample geometry and loading factor, pulse sequence and acquisition parameters, RF excitation and reception profiles, as well as matched 3D-CSI voxel size and location related to the ^{31}P RF surface coil for both phantom and cat brain measurements.

METHODS All ^{31}P spectra were acquired on a 9.4T/30cm bore Magnex magnet equipped with Varian INOVA consoles with a home-built ^{1}H / ^{31}P RF dual surface coil probe. Six female adolescent cats under gaseous anesthesia (0.9-1.2 % isoflurane in a mixture of 70% nitrous oxide and 30% oxygen) with artificial ventilation were used for the study. We quantified the absolute concentration of ATP in cat brain using a head sized phantom (plastic ball with dia.≈4.5cm) freshly prepared with known ATP concentration ([ATP]=5mM) and proper sample loading ([Na^+]=120mM) matched to the cat head. Other phosphate compounds in the phantom solution were 10mM PCr and 2mM Pi. The same ^{31}P RF surface coil with identical frequency tuning and impedance matching was applied to obtain 3D ^{31}P CSI data for both cat and phantom measurements with fully relaxed ATP signals (TR≥6.5s; i.e., TR≥ 5T_1 of ATP). The identical profile of excitation pulse flip angle (θ) was achieved for cat and phantom measurements through careful RF power calibration using the reference ^{31}P signal of a small glass sphere (dia.≈3mm, contain 1.0M methylphosphonic solution) which was permanently fixed in the ^{31}P coil center. The pulse sequence and other acquisition parameters (total scan number=112 or 224; FOV=4×4×2.5 cm^3; 7×7×5 phase encodes; spectra width =5000Hz; TR=6.5s or 15s etc.) were also identical for both cat and phantom measurements. The ^{31}P spectrum of a central voxel located inside cat brain was extracted from the 3D-CSI cat data and compared with the corresponding phantom data; the voxel size and position relative to the reference sphere were identical for both cat and phantom. These ^{31}P spectra were processed in the same way and analyzed using AMARES time domain spectra fitting algorithm in the JMRUI software package for quantifying the cat brain [ATP]. By comparing the γ-ATP integral (I) measured in phantom and cat brain, we can obtain the absolute [ATP] in brain according to Eq. 1: $[ATP]_{brain} = [ATP]_{phantom} \times I_{brain} \div I_{phantom}$. The results were presented by mean±SD.

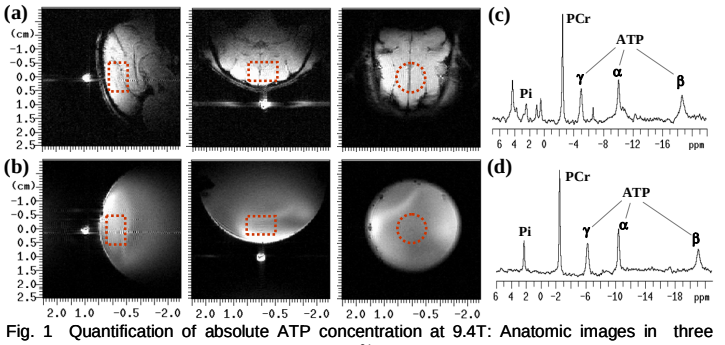

Fig. 1 Quantification of absolute ATP concentration at 9.4T: Anatomic images in three orientations in cat brain (a) and phantom (b); ^{31}P MR spectra (TR=15s) of cat brain (c) and phantom (d) are obtained with identical power from the corresponding voxels outlined in red in (a-b), which have identical position relative to the small sphere at the center of ^{31}P coil. The vertical scale of the spectra are arbitrary, the integral of the γ-ATP are 9523 and 17542 ,respectively, in spectra of cat brain (c) and phantom (d) with 5mM ATP and 120mM sodium.

RESULTS AND DISCUSSION Figure 1 shows the ^{1}H images of a representative cat brain (a) and phantom (b) in three orientations, and the corresponding ^{31}P MR spectra of the brain (c) and phantom (d), respectively, from the voxels outlined in red in (a-b). The selected voxels from the cat and phantom 3D ^{31}P CSI data have identical size and spatial location relative to the small reference sphere (i.e., bright circular spots in Figs. 1a and 1b) fixed at the center of ^{31}P coil. The integral of the ATP signals as shown in Figs. 1c-1d can be obtained from each cat and phantom measurements via spectra fitting, which provided the absolute concentration of ATP in cat brains using Eq. 1.

The ATP quantification results from isoflurane anesthetized cat brain at 9.4T are summarized in Table 1. Several conclusions can be drawn from these results. First, the reproducibility of the measurement is high; this is evident from the small SD (≤ 2.5%) in the $I_{phantom}$ values obtained in four different experimental sessions. Second, the reliability of the measurement is excellent, this is clearly demonstrated through the small SD (≤ 4%) in cerebral γ-ATP concentration, the consistent [ATP] values among γ-, α- and β-ATP contents and the fact that cerebral ATP concentration of 2.8mM based on the γ-ATP quantification in this study (see Table 1) is in general agreement with the literature reported ATP values ranging from 2.5mM to 3.2mM [1, 4-6]. Third, the inter-subject variation of $[ATP]_{brain}$ is very small. In addition, we used another phantom with much lighter sample loading ([Na^+] = 35mM) to quantify $[ATP]_{brain}$. The results indicate that changing coil loading would require a recalibration of the flip angle in order to achieving the same excitation profile, however, the lighter loading led to a better RF coil reception sensitivity, thus an overestimation for $I_{phantom}$ and a systematic underestimation for $[ATP]_{brain}$ as shown in Table 1. Nevertheless, this deviation would not significantly alter the outcomes of the ATP quantification (error ≤ 5%) when [Na^+] is in the range of 35-120mM. Based on the $[\gamma\text{-ATP}]_{brain}$ value obtained herein we calculated other two cerebral phosphate metabolite concentrations under fully relaxed condition (TR=15s, T_1 ≤ 3s for following phosphate metabolites at 9.4T): [PCr]=3.81±0.24 mM and intracellular [Pi]=1.14 ±0.14 mM.

We choose to quantify the absolute concentration of ATP and use it as an internal standard for quantifying other HEP contents in a living brain. This is based on the understanding that the cerebral ATP concentration remains stable across a wide range of physiological condition from the iso-electric (no EEG activity) to the activated brain state, while the cerebral PCr and Pi concentrations could change [7]. This phenomenon could be attributed to the efficient regulation of cerebral ATP metabolism for maintaining a steady ATP level. Therefore, $[ATP]_{brain}$ can serve as a reliable and stable internal reference for calibrating other phosphate concentrations such as [Pi] and [PCr] in the same healthy brain or cross subjects. Moreover, the longitudinal relaxation time (T_1) of ATP is much shorter than other phosphate compounds and it is in the order of ~1sec for both cat brain and phantom solution at 9.4T. Thus, it would take much less sampling time for obtaining fully relaxed ^{31}P ATP signals for quantification purpose.

Table 1. Absolute quantification of ATP concentration in 1% isoflurane anesthetized cat brain using head sized phantoms containing 5mM ATP solution. The results are presented in Mean ± SD.

$I_{phantom}$ (n=4)	ATP-γ	ATP-γ,α	ATP-γ,α,β
Phan A ([Na^+]=120 mM)	17705 ± 410	17210 ± 231	17004 ± 435
Phan B ([Na^+]=35 mM)	17840 ± 231	17602 ± 286	17868 ± 201
$[ATP]_{brain}$ (mM,,n=6)	ATP-γ	ATP-γ,α	ATP-γ,α,β
Phan A ([Na^+]=120 mM)	2.81 ± 0.11	2.75 ± 0.15	2.73 ± 0.22
Phan B ([Na^+]=35 mM)	2.80 ± 0.11	2.69 ± 0.14	2.60 ± 0.20

CONCLUSION The cerebral ATP content is fairly constant under various physiological conditions and among different subjects. Its ^{31}P signal can serve as an internal reference for quantifying the absolute concentrations of other phosphate metabolites if [ATP] in the brain can be determined *in vivo*. The results of cat brain study at 9.4T as presented herein have clearly demonstrated that reliable quantification of cerebral phosphate concentrations can be achieved by using this straightforward experimental design. This quantification approach should be applicable for healthy human brain as well as diseased brain with abnormal ATP concentration.

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