

# New approaches to MRS of Cerebral Disorders

Stefan Blüml, Ph.D.

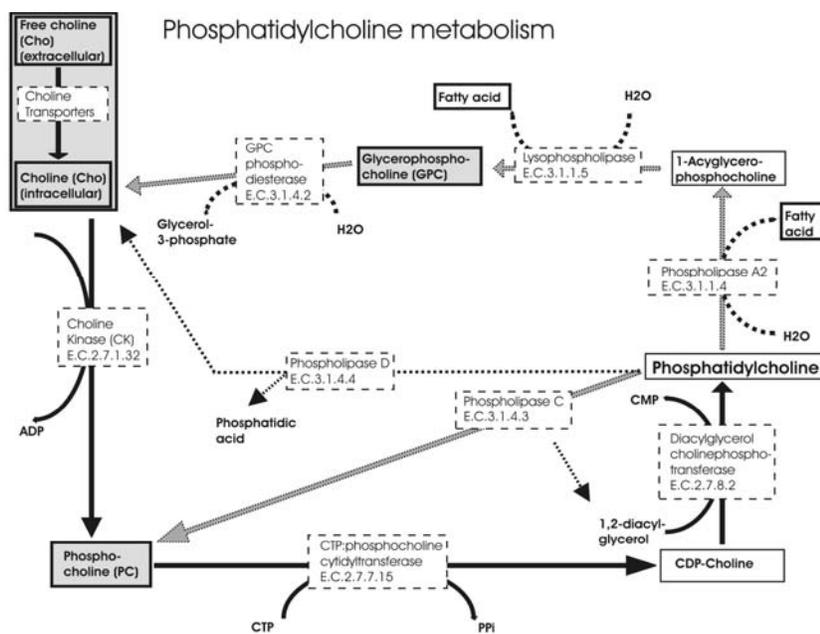
Childrens Hospital Los Angeles, Department of Radiology  
USC Keck School of Medicine

## Phosphorous MR spectroscopy

In the early days of MRS it was believed that  $^{31}\text{P}$  MRS would emerge as the most useful *in vivo* method. The reason was that with  $^{31}\text{P}$  MRS **ATP**, the most widely distributed high-energy compound within the human body - used for all energy requiring processes -, inorganic phosphate (**Pi**), and phosphocreatine (**PCr**) can be observed *in vivo*. Apart from studying energy metabolism,  $^{31}\text{P}$  MRS can also detect phosphorylated metabolites of membrane lipids and can thus be used to study diseases of abnormal membrane metabolism. Phosphorous MRS is by no means a new approach. Indeed a significant number cerebral disorders were studied with  $^{31}\text{P}$  MRS (1-19). However, the emergence of MR imaging as the probably most powerful diagnostic imaging modality and the availability of more sensitive proton spectroscopy diminished the role of  $^{31}\text{P}$  MRS in basic and clinical research. Still, this has not changed the fact that  $^{31}\text{P}$  MRS provides unique information about the status of tissue.  $^{31}\text{P}$  MRS is particularly promising when **proton decoupling**, until now only infrequently utilized, is applied. It is therefore predicted - also because the arrival of more powerful 3T magnets offers the much needed improved sensitivity - that  $^{31}\text{P}$  will re-emerge as an important tool to study cerebral disorders *in vivo*.

## Application: Choline metabolism of tumors

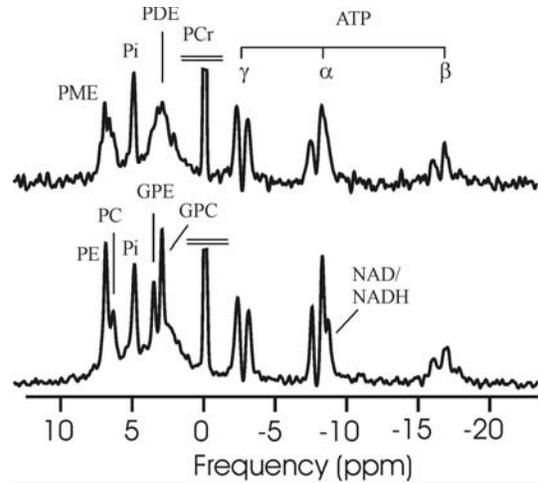
Choline containing metabolites are involved in synthesis and breakdown of phosphatidylcholine, an important membrane phospholipid (**Fig. 1**). Altered levels of choline have been consistently observed in human brain tumors. Indeed, abnormal choline metabolism appears to be a common feature for most forms of cancer including cell lines, animal models of cancer, and various tumors of humans studied *in vivo* (20,21). In particular elevated intracellular phosphocholine (**PC**) pools are associated with increased malignancy.



Adapted from Glunde et al. Cancer Res 2004;64(12):4270-4276.

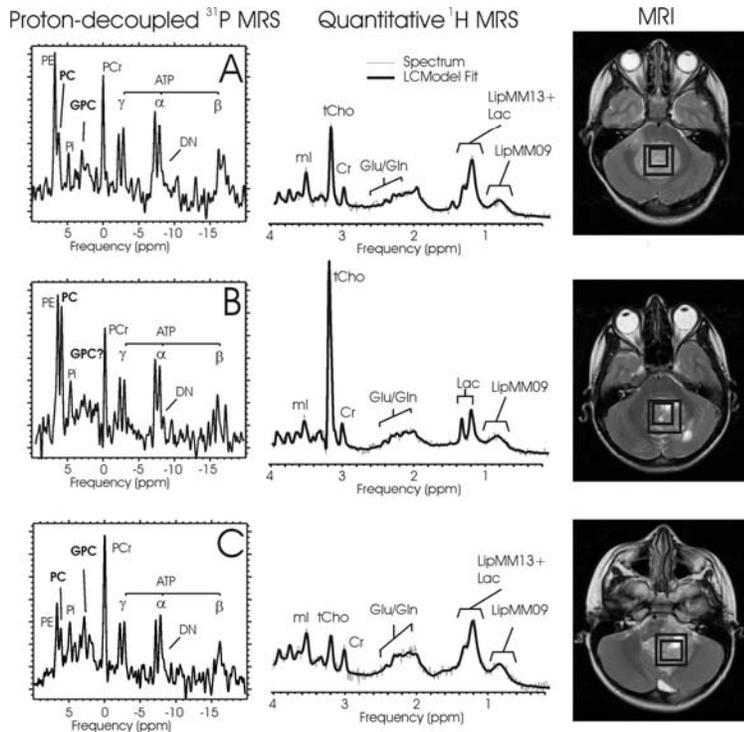
**FIG. 1:** Choline containing metabolites are involved in synthesis and breakdown of phosphatidylcholine an important membrane phospholipid.  $^1\text{H}$  MRS can be used to measure the sum of all choline containing compounds. PC and GPC can be observed with proton-decoupled  $^{31}\text{P}$  MRS.

With  $^1\text{H}$  MRS only the total choline, which comprises PC, glycerophosphocholine (GPC), free choline (fCho), and possibly other choline containing metabolites, can be quantified. For a more detailed study of choline metabolism the contribution of PC and GPC to the total choline peak needs to be determined. This is best accomplished with  $^{31}\text{P}$  MRS. A problem of standard  $^{31}\text{P}$  MRS at clinical field strength is that PC and phosphoethanolamine (PE) and GPC and glycerophosphoethanolamine (GPE) are partially overlapping due to hetero-nuclear J-couplings with protons. **Proton decoupling** is required to collapse the PC, PE, GPC, GPE multiplets into single peaks (22) (**Fig. 2**).



**FIG. 2:**  $^{31}\text{P}$  (upper trace) and proton decoupled  $^{31}\text{P}$  spectra of (normal) brain tissue. PCr peaks are truncated.

### Pediatric brain tumors



**FIG. 3:** Proton-decoupled  $^{31}\text{P}$  MRS,  $^1\text{H}$  MRS, and MRI of cerebellar medulloblastoma (A+B) and, anaplastic ependymoma (C).  $^{31}\text{P}$  spectra are scaled to ATP as an internal reference.  $^1\text{H}$  spectra are scaled to measured absolute concentrations to allow direct comparison with each other. Areas or amplitudes of  $^{31}\text{P}$  and  $^1\text{H}$  spectra can not be directly compared. All spectra were acquired on a clinical 1.5 T scanner. A custom-designed dual tuned head coil was used for clinically indicated MR imaging, proton MRS, and for experimental  $^{31}\text{P}$  MRS. The total examination time was approximately 70 min and  $^1\text{H}$  and  $^{31}\text{P}$  acquisitions were integrated in routine pre-operative work-up of patients. ROIs for  $^1\text{H}$  (small box) and  $^{31}\text{P}$  are marked on MRI, respectively.

$^1\text{H}$  and  $^{31}\text{P}$  spectra and MR images of pediatric patients with **untreated brain tumors** are shown in **Fig. 3**. Proton spectra were acquired with a single voxel PRESS sequence (TE = 35ms). A slice selective spinecho sequence with 2D phase encoding was used for  $^{31}\text{P}$  MRS. Aggressive medulloblastoma have prominent peaks from PE and PC, whereas GPE and GPC are difficult to discern from the noise signal. Significant variations of the metabolic profile in individual tumors of the same pathological type were noted. This is illustrated in the two cases of medulloblastoma shown in **Fig. 3A,B** where PC levels were strikingly different. A high PC/GPC ratio has been

postulated to be a marker for more malignant tumors. To what extent the measurements of PC and GPC (and total choline with  $^1\text{H}$  MRS) are of predictive value in individual patients and can be used for patient stratification needs to be determined by long-term follow-up. A comparison of  $^1\text{H}$  and  $^{31}\text{P}$  spectra also reveals that a significant fraction of choline in tumors is not accounted for by PC+GPC. This is in contrast to normal brain where total choline  $\approx$  1/3 PC+ 2/3 GPC (13,15).

### **Carbon spectroscopy**

The low natural abundance of  $^{13}\text{C}$  (1.1%) and its inherent low sensitivity (1/50 of hydrogen) render *in vivo*  $^{13}\text{C}$  MRS studies in humans very challenging. However, with the infusion of  $^{13}\text{C}$  labeled substrates, such as glucose or acetate, highly specific information about metabolites and metabolic rates can be obtained (23-27). In a typical experiment, e.g. intravenously or orally administered  $^{13}\text{C}$  labeled glucose, enters the blood stream, passes the blood-brain-barrier, and is detected within 1-2 minutes after administration in  $^{13}\text{C}$  spectra. Subsequently glucose is broken down through glycolysis and oxidation in the tricarboxylic acid (TCA)-cycle.  $^{13}\text{C}$  label accumulation can then be detected in all metabolites of sufficient high concentration involved in glucose oxidation. From the time courses of label accumulation important flux rates such as the TCA-cycle activity and glutamine synthesis have been determined. For a more detailed update on methods and progress in  $^{13}\text{C}$  MRS the reader is referred to a recent issue of *NMR in Biomedicine* (28) exclusively dedicated to the application of  $^{13}\text{C}$  MRS to study biological systems and references therein.

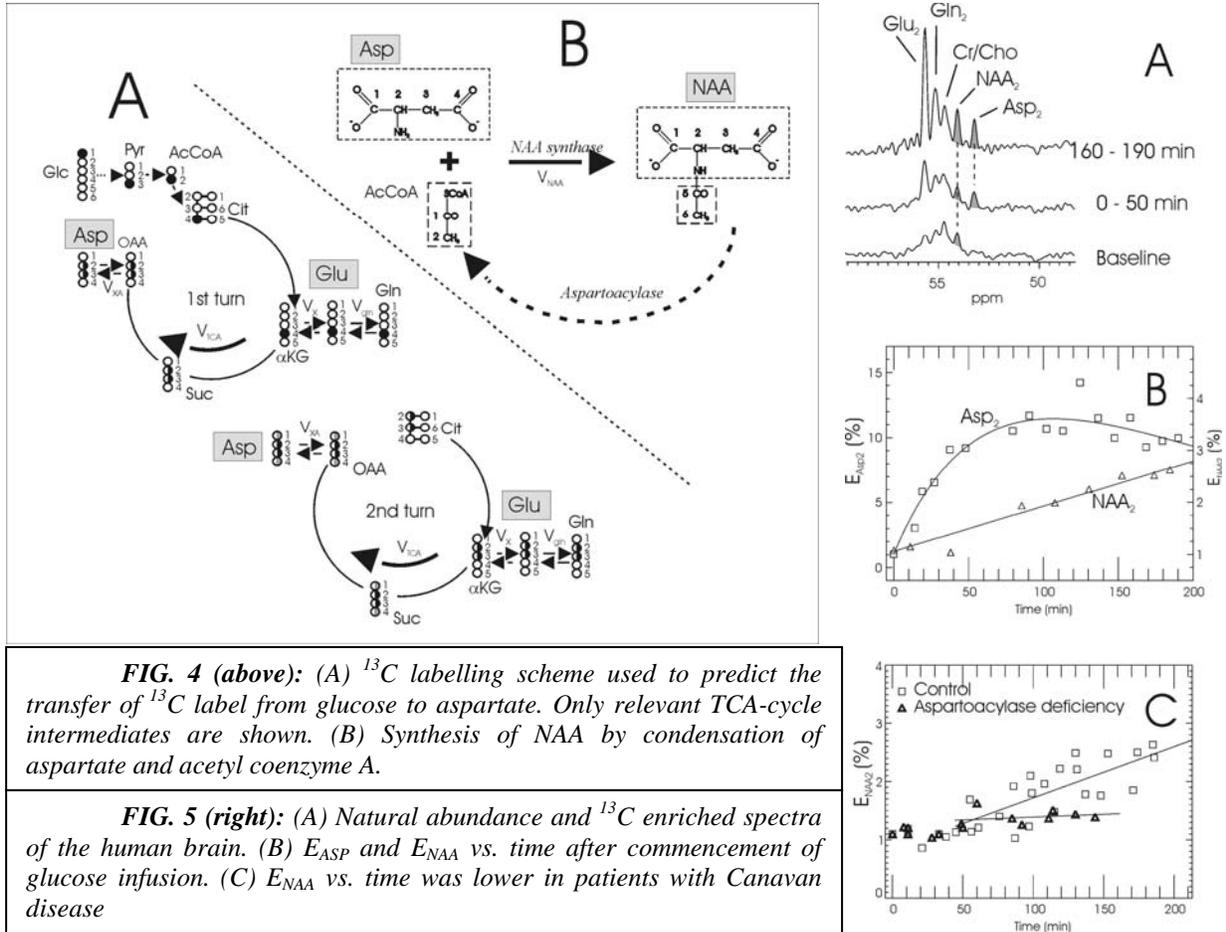
As for  $^{31}\text{P}$  MRS, special hardware is required for  $^{13}\text{C}$  MRS. Presently, even for “niche” manufacturers,  $^{13}\text{C}$  RF coils are too “exotic” and most groups conducting *in vivo*  $^{13}\text{C}$  research have built their own coils. Volume coils are more flexible and have a special advantage when quantitation of cerebral metabolites is the goal. Nevertheless, surface coils have exclusively been used for  $^{13}\text{C}$  MRS since they provide the much-needed extra signal required for *in vivo*  $^{13}\text{C}$  MRS. Because all  $^{13}\text{C}$  methods either apply direct detection with proton-decoupling, polarization transfer, or hetero-nuclear editing, dual-tuned coils ( $^1\text{H}$  and  $^{13}\text{C}$  frequencies) are required. Because of the technical and logistical challenges and the need for large amounts of  $^{13}\text{C}$  labeled substrates (expensive) it is not surprising that there have been only a few patient studies. As of now, *in vivo*  $^{13}\text{C}$  MRS may be more appropriate for studies of small groups of patients. This may change with more support from manufactures towards the implementation of  $^{13}\text{C}$  MRS on clinical systems, a drop of the price of  $^{13}\text{C}$  labeled compounds, and the development of efficient infusion protocols. For some applications oral administration may be appropriate (27,29,30) which would simplify the procedure considerably because one inter-venous (i.v.) infusion line could be eliminated.

### **Application: Direct determination of the NAA-synthesis rate *in vivo* by $^{13}\text{C}$ MRS after 1- $^{13}\text{C}$ glucose infusion**

To illustrate the potential of  $^{13}\text{C}$  MRS to address specific biological questions the determination of the N-acetyl-aspartate (NAA) synthesis rate ( $V_{\text{NAA}}$ ) *in vivo* in human brain is illustrated (27). NAA is an amino acid derivative in vertebrate brain that reaches its highest concentrations in neurons and axons. It is principally synthesized in neurons by energy dependent condensation of aspartate and acetyl coenzyme A, catalysed by the mitochondrial enzyme NAA synthase, and is then exported to the cytosolic compartment. The enzyme to breakdown NAA (N-aspartoacylase, E.C. 35.1.15) is located in oligodendrocytes. Therefore, NAA apparently cycles between neuronal and glial compartments. The role of NAA, and its

regulation *in vivo*, is not well understood (31,32). Hypothetically, disorders of synthesis or breakdown of NAA may contribute to altered steady-state concentrations of NAA. To date, only one definitive disorder of NAA metabolism has been identified, Canavan disease, where N-aspartoacylase deficiency causes increased cerebral NAA concentrations.

For the determination of  $V_{NAA}$ , 1- $^{13}C$  glucose was infused and direct detected  $^{13}C$  MRS with proton decoupling was utilized. As illustrated in **Fig. 4A**, the  $^{13}C$  label from glucose enters the TCA-cycle at citrate and enriches glutamate C<sub>4</sub>, succinate C<sub>2,3</sub> (Suc) and oxaloacetate C<sub>2,3</sub> (OAA) during the first turn. From OAA,  $^{13}C$  label is transferred to aspartate and then on to NAA (**Fig. 4B**).



It is assumed that under steady-state NAA is synthesized from aspartate and catabolized at the same rate. The NAA synthesis rate ( $V_{NAA}$ ) can then be determined from the measured time courses of  $^{13}C$  accumulation in aspartate and NAA (**Fig. 5**) by iteratively resolving the differential equation:

$$d/dt [NAA^*] = E_{Asp}V_{NAA} - E_{NAA}V_{NAA} \text{ (Fig. 6).}$$

- $E_{NAA}$  fractional  $^{13}C$  enrichment of NAA
- $[NAA^*]$  concentration of  $^{13}C$  enriched NAA
- $E_{Asp}$  fractional  $^{13}C$  enrichment of aspartate

The NAA synthesis rate obtained in controls ( $9 \pm 4$  nmol/g/min) with this method is consistent with data obtained from cortex of rat (33-35). The turnover time of NAA from aspartate in controls is approximately 19 hours ( $[NAA] \approx 10$  mmol/kg in controls - determined with  $^1\text{H}$  MRS). In Canavan disease a significant lower rate of NAA synthesis ( $4 \pm 0$  nmol/g/min) was observed which is consistent with what has been estimated from urine excretion of NAA in this disease (36-38). Note,

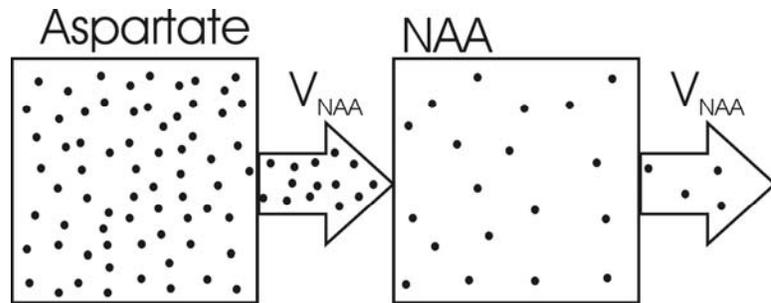
in the absence of breakdown of NAA in the brain, excretion through urine is the main pathway for NAA to leave the system. The turnover time in Canavan disease is approximately 60 h ( $[NAA] \approx 15$  mmol/kg in Canavan disease). An explanation for the reduced NAA synthesis rate in Canavan disease could be feedback inhibition.

## Summary

As illustrated above with two examples, multinuclear spectroscopy can add important information about metabolism of diseased brain. For researchers to take advantage of the potential of  $^{31}\text{P}$  and  $^{13}\text{C}$  MRS to study brain diseases, these modalities need to be integrated on clinical MR systems. Once accomplished, a unique portfolio of methods, MRI, fMRI, DTI, etc, and multinuclear MRS, is available to the clinical and basic researchers. The value of each modality will be amplified and new avenues to study cerebral disorders will be opened.

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**FIG. 6:** At steady-state the total number of synthesized and catabolised NAA molecules is equal. However, the number of  $^{13}\text{C}$  labeled NAA molecules synthesized (per time unit) is given by the fractional enrichment of the precursor Asp ( $E_{\text{Asp}}$ ) times the total flux. The number of NAA molecules broken down (per time unit) is given by the fractional enrichment of NAA ( $E_{\text{NAA}}$ ) times the total flux. There is no further net increase of  $^{13}\text{C}$  labeled NAA once the fractional enrichment of the Asp and NAA pools are equal.

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