In Vivo Nuclear Magnetic Resonance Spectroscopy: A Metabolomics Perspective

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Contents

• How? Hardware and software
• What? Biological information obtained
• Why? Applications in preclinical and clinical settings: ‘In Vivo Metabolomics’ and ‘Imaging Biomarkers’
• Future directions
## Nuclei for *in vivo* MRS

<table>
<thead>
<tr>
<th>Nuclei</th>
<th>Abundance</th>
<th>Relative Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H</td>
<td>99.98%</td>
<td>100</td>
</tr>
<tr>
<td>$^{31}$P</td>
<td>100%</td>
<td>6.6</td>
</tr>
<tr>
<td>$^{13}$C</td>
<td>1.1%</td>
<td>0.016</td>
</tr>
<tr>
<td>$^{19}$F</td>
<td>100%</td>
<td>83</td>
</tr>
<tr>
<td>$^{17}$O</td>
<td>0.04%</td>
<td>0.029</td>
</tr>
</tbody>
</table>
In vivo MRS Hardware

CLINICAL
1.5 – 3 T

HUMAN RESEARCH
4 – 7 - 9.4T

EXPERIMENTAL
9.4 - 11.7 - 16.1T
MR Scanner is a Genuine Hybrid Imager

- Hybrid instrument comprising of multiple imaging modalities
- One modality provides ‘anatomical specificity’, the other ‘physiological/biochemical/pharmacological’ data
- Pioneered by the Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) hybrid

CT – PET  
MRI – PET  
MRI – EEG  
MRI – MEG
What is needed for *in vivo* MRS?

- Excellent $B_0$ homogeneity $>$ focus on RT-shims
- RF (or $B_1$)-homogeneity $>$ focus on RF-coils
- 2 (or more) transmitter channels, one for $^1H$ and the other for X nuclei (broadband)
- Special software, ‘pulse sequences’ or ‘packages’ in clinical scanners
- Analysis software (some of these are free of charge, eg. jMRUI)
Localised MRS

Single Voxel MRS

Multi Voxel MRS = MRSI
Spatially Selective Excitation

\[ \gamma (B_0 + G_z z_0) \]

Laboratory frame frequency \((\omega)\)

\[ \omega = \gamma (B_0 + G_z z) \]

Slice select position

\[ z_0 \]

\[ \Delta \omega \]
Point Resolved Spectroscopy (PRESS)

<table>
<thead>
<tr>
<th>rf</th>
<th>G_z</th>
<th>G_x</th>
<th>G_y</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="90%C2%B0" alt="90°" /></td>
<td><img src="180%C2%B0" alt="180°" /></td>
<td><img src="TE/4" alt="TE/4" /></td>
<td><img src="TE/2" alt="TE/2" /></td>
</tr>
</tbody>
</table>

**SPIN-ECHO**
PRESS Volume Selection

$G_z$

$G_x$

$G_y$

Signal only from this volume
Water Suppression for $^1$H MRS

- Chemical shift selective excitation to suppress water, but not metabolites
- Incorporating a $T_1$ filter
- Suppression by a factor 1:10000 (50000)
- Additional suppression of water signal in time domain
State-of-the-art *in vivo* $^1$H MRS

**Brain $^1$H MRS**
- Alanine
- Aspartate
- Cholines
- Creatine+P-Creatine
- Glucose
- Glutamate
- Gluthatione
- GPC
- myo-Inositol
- Scylo-Inositol
- N-Acetyl aspartate
- NAAG
- Lactate
- Phosphorylcholine
- Phosphorylethanolamine
- Taurine
- Water

*Pfeuffer et al. JMR 141:104 (1999) @9.4T*
State-of-the-art *in vivo* $^1$H MRS art at 1.5, 3 and 7T

**PRESS TE = 30 ms**

**STEAM TE = 6 ms**
Spectral Editing for \textit{in vivo} $^1$H MRS

- To reveal \textquoteleft hidden peaks\textquoteright
- Exploits spin-spin coupling or multiple quantum energy transitions
- Metabolites revealed:
  - Glutamate and glutamine
  - GABA
  - Glutathione (GSH)
  - Ascorbic acid
GABA, Glu & Gln Editing

Editing adds:

- GABA
- Ascorbate
- Glutathione
- Threonine

Gruetter et al. MRM 55: 296, 2006
Spectral analysis

Goal: Quantify metabolite content

Methods for spectral quantification
• Peak integration
• Peak fitting
• Fitting modeled metabolite lines on an experimental spectrum

• Referencing:
  • Internal reference (water)
  • External reference (rf-coil loading considered)
LCModel Analysis of *in vivo* MRS

- Model spectra acquired (or simulated) for metabolites in question
- Experimental spectrum analysed as a *Linear combination of Model spectra of metabolite solutions*

*Provencher S.W. MRM 30:672 (1993)*
‘MRS Biomarkers and Metabolomics’

• The concept introduced in 80’s
• ‘MRS biomarker’: a metabolite that is detected in given cell/tissue type, condition or disease
• ‘Metabolite profile or metabolic phenotype’ were predecessors of ‘Metabolomics’
‘MRS Metabolomics’

Hagberg et al. MRM 34:242, 1995
Tentative Classification of MRS-detectable Biomarkers

• **Pre-biomarkers** (single centre)
  • numerous, need qualification for biomarker status
• **Imaging biomarkers** (multi-centre, safety profile)
  • growing number of biochemicals (e.g. choline-containing compounds, myo-inositol, citrate, $^1$H MRS detected lipids, PME, PDE)
• **Imaging surrogate marker** (“qualified biomarker with established criteria’)
  • specific to a given biochemical event and/or cell type (e.g. ATP, PCr, NAA)
• **Imaging licensed biomarker** (‘approved’)
  • yet to-be-established for MRS-detectable biochemicals
1H MRS in Context of Brain Tumour Classification

TUMOURS IN SITU

TISSUE SPECIMENS

CELLS
### Table 2. Comparative composition of metabolites determined by HPLC analyses in meningioma, neuroblastoma and glioblastoma cells

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Meningioma cell lines (n=6)</th>
<th>Neuroblastoma cell lines (n=3)</th>
<th>Glioblastoma cell lines (n=5)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>10.25±2.07</td>
<td>1.7±0.5</td>
<td>3.6±1.5</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Arginine</td>
<td>6.1±2.3</td>
<td>4.2±0.3</td>
<td>2.7±0.8</td>
<td>0.017</td>
</tr>
<tr>
<td>Asparagine</td>
<td>4.9±1.9</td>
<td>6.5±3.1</td>
<td>2.4±0.6</td>
<td>0.027</td>
</tr>
<tr>
<td>Aspartate</td>
<td>27.0±14.6</td>
<td>13.2±3.9</td>
<td>16.9±11.3</td>
<td>0.230</td>
</tr>
<tr>
<td>GABA</td>
<td>0.6±0.2</td>
<td>2.7±1.4</td>
<td>0.22±0.09</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Glutamine</td>
<td>72.9±32.6</td>
<td>3.8±1.8</td>
<td>44.5±18.9</td>
<td>0.009</td>
</tr>
<tr>
<td>Glutamate</td>
<td>305.5±47.4</td>
<td>101.5±38.3</td>
<td>130.8±11.6</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Hypotaurine</td>
<td>5.0±3.0</td>
<td>6.3±0.8</td>
<td>3.6±2.1</td>
<td>0.188</td>
</tr>
<tr>
<td>NAA</td>
<td>nd^a</td>
<td>2.7±2.3</td>
<td>nd^a</td>
<td>—</td>
</tr>
<tr>
<td>NAAG</td>
<td>4.5±3.9</td>
<td>8.4±5.0</td>
<td>4.8±4.3</td>
<td>0.545</td>
</tr>
<tr>
<td>Serine</td>
<td>28.6±5.9</td>
<td>12.5±3.0</td>
<td>18.2±3.0</td>
<td>0.001**</td>
</tr>
<tr>
<td>Taurine</td>
<td>18.0±2.8</td>
<td>56.1±17.3</td>
<td>32.4±15.7</td>
<td>0.004*</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>10.3±2.1</td>
<td>31.5±13.1</td>
<td>15.2±4.3</td>
<td>0.036</td>
</tr>
</tbody>
</table>

### Table 3. Comparative composition of metabolites quantified from ¹H-NMR spectra of meningioma, neuroblastoma and glioblastoma cell lines

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Meningioma cell lines (n=6)</th>
<th>Neuroblastoma cell lines (n=3)</th>
<th>Glioblastoma cell lines (n=5)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>16.3±2.0</td>
<td>6.7±5.2</td>
<td>5.6±1.2</td>
<td>0.001**</td>
</tr>
<tr>
<td>Cho^b</td>
<td>27.1±5.1</td>
<td>33.9±16.5</td>
<td>13.8±5.4</td>
<td>0.010</td>
</tr>
<tr>
<td>Choline</td>
<td>6.2±4.8</td>
<td>3.5±1.4</td>
<td>2.3±1.3</td>
<td>0.227</td>
</tr>
<tr>
<td>GPC</td>
<td>9.1±4.5</td>
<td>6.8±7.9</td>
<td>6.3±5.2</td>
<td>0.098</td>
</tr>
<tr>
<td>PC</td>
<td>14.0±3.6</td>
<td>23.6±8.7</td>
<td>8.1±2.7</td>
<td>0.004*</td>
</tr>
<tr>
<td>Creatine</td>
<td>7.3±5.8</td>
<td>28.1±4.8</td>
<td>1.3±1.7</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Glutamate</td>
<td>298.4±43.9</td>
<td>79.6±26.8</td>
<td>114.5±7.1</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Glycine</td>
<td>27.5±10.1</td>
<td>15.8±1.1</td>
<td>21.1±3.6</td>
<td>0.123</td>
</tr>
<tr>
<td>Myo-inositol</td>
<td>18.5±14.2</td>
<td>22.3±11.1</td>
<td>nd^a</td>
<td>—</td>
</tr>
<tr>
<td>Succinate</td>
<td>13.1±5.4</td>
<td>10.4±3.0</td>
<td>4.4±0.7</td>
<td>0.011</td>
</tr>
<tr>
<td>Threonine</td>
<td>23.8±5.7</td>
<td>4.8±1.5</td>
<td>7.0±2.4</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>
1H MRS in Classifying Glioma Cell Lines

Pan et al. unpublished
TUMOURS EX VIVO

High-Resolution $^1$H NMR Spectroscopy Studies of Extracts of Human Cerebral Neoplasms

JAMES PEELING*† AND GARNETE SUTHERLAND‡§

*Department of Chemistry, University of Winnipeg, Winnipeg, Canada R3B 2E9; and Departments of †Radiology, ‡Surgery (Neurosurgery), and §Pharmacology and Therapeutics, University of Manitoba, Winnipeg, Canada R3A 1R9

TABLE 3

Metabolite Levels (µmol/100 g tissue ± SEM) in Human Brain Tissue

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Brain (43)*</th>
<th>Malignant astrocytoma (14)</th>
<th>Benign astrocytoma (5)</th>
<th>Meningioma (6)</th>
<th>Metastatic tumor (5)</th>
<th>Schwannoma (1)</th>
<th>Oligodendroglioma (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>26 ± 2</td>
<td>92 ± 13</td>
<td>37 ± 29</td>
<td>127 ± 61</td>
<td>146 ± 61</td>
<td>116</td>
<td>75</td>
</tr>
<tr>
<td>Glutamate</td>
<td>775 ± 35</td>
<td>171 ± 20</td>
<td>129 ± 20</td>
<td>234 ± 62</td>
<td>189 ± 65</td>
<td>144</td>
<td>221</td>
</tr>
<tr>
<td>Glutamine</td>
<td>nq</td>
<td>358 ± 60</td>
<td>216 ± 20</td>
<td>231 ± 63</td>
<td>185 ± 75</td>
<td>95</td>
<td>450</td>
</tr>
<tr>
<td>Succinate</td>
<td>22 ± 1</td>
<td>15 ± 2</td>
<td>9 ± 1</td>
<td>9 ± 2</td>
<td>7 ± 2</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>Creatine</td>
<td>690 ± 40</td>
<td>185 ± 41</td>
<td>194 ± 24</td>
<td>42 ± 19</td>
<td>114 ± 60</td>
<td>17</td>
<td>375</td>
</tr>
<tr>
<td>Cholines</td>
<td>110 ± 5</td>
<td>136 ± 19</td>
<td>101 ± 18</td>
<td>101 ± 26</td>
<td>130 ± 23</td>
<td>143</td>
<td>241</td>
</tr>
<tr>
<td>Glucose</td>
<td>nd</td>
<td>100 ± 18</td>
<td>93 ± 18</td>
<td>103 ± 20</td>
<td>128 ± 38</td>
<td>300</td>
<td>135</td>
</tr>
</tbody>
</table>

* Number of samples analyzed.
† Number of samples in which metabolite was detected.

Note. nq, not quantitated; nd, not detected.
1H MRS in vivo

Noninvasive Differentiation of Tumors with Use of Localized H-1 MR Spectroscopy in Vivo: Initial Experience in Patients with Cerebral Tumors

Bruhn et al. Radiology, 1989
Neural Networks for Brain Lesion MRS

SPECIFICITY 92-100%

Poptani et al. J Clin Oncol 1999

LOW GRADE 73%
HIGH GRADE 98%
ABSCESS 83%
TUBERCULOMA 89%
The method classified correctly 104 of 105 spectra

Conventional technique 71 of 91 tumours

A new approach for analyzing proton magnetic resonance spectroscopic images of brain tumors: nosologic images

Fabien Sabo De Edelnyi, Christophe Rubin, François Esteve, Sylvie Grand, Michel Decorps, Virginie Lefournier, Jean-François Le Bas & Chantal Rémy

1. Unite mixte INSERM-Université Joseph Fourier, U438, LRC CEA, CHU de Grenoble, BP 217, 38043 Grenoble Cedex 9, France
2. Unité IBM and Service de Neuroradiologie, Centre Hospitalier Universitaire de Grenoble, BP 217, 38043 Grenoble Cedex 9, France
Correspondence should be addressed to F.E.; email: F.Esteve@chu-grenoble.fr

<table>
<thead>
<tr>
<th>Histopathology</th>
<th>Group assignment</th>
<th>LGG</th>
<th>HGG</th>
<th>Meta</th>
<th>ME</th>
<th>NE</th>
<th>CSF</th>
<th>HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGG</td>
<td></td>
<td>14</td>
<td>13</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HGG</td>
<td></td>
<td>24</td>
<td>1</td>
<td>19</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meta</td>
<td></td>
<td>10</td>
<td></td>
<td>6</td>
<td>6</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME</td>
<td></td>
<td></td>
<td>8</td>
<td></td>
<td></td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE</td>
<td></td>
<td>9</td>
<td></td>
<td></td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF</td>
<td></td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>HT</td>
<td></td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7</td>
</tr>
</tbody>
</table>

Table 1 Results of the 'leave-one-out' procedure on the training set of examinations.

Left, the histopathological assignment of the 'test' individual; right, corresponding assignment by the analysis. HGG, high-grade glioma; LGG, low-grade glioma; ME, meningioma; Meta, metastasis; NE, necrosis; CSF, cerebrospinal fluid; HT, healthy tissue.

De Edelnyi et al. Nat Med, 2000
Cox Regression performed on all metabolite quantities for 129 brain tumour patients at the Birmingham Children’s Hospital (UK)

- Lip 1.3, Lip 0.9 and MM 0.9 were found to be significant predictors of survival (model significance p=0.0388)

Wilson et al. ISMRM 2009
Independent Component Analysis (ICA) of $^1$H MRSI Data Set

Decomposing tissue in each voxel into three tissue types:

Healthy brain, Invasive Tumour and Necrotising Tumour

Healthy brain: ICA a only
Low grade: ICA b only
High grade: ICA b + c

MRSI of Brain Tumour Patient

**ANALYSIS**

Input Neural Network

Medical Documents

Proposed diagnosis
prognosis
Prostate cancer, Gleeson 3+4, PSA 6.86: MRSI with 1 ml voxels at 3T

- Inherent metabolic heterogeneity by $^1$H MRSI within healthy gland

# ¹H MRSI of Prostate

**Discrimination of prostate cancer from healthy gland**
*(Choline + Creatine)/Citrate by ¹H MRSI (45 patients)*

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Threshold Ratio</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periphery Cancer</td>
<td>&gt;0.41</td>
<td>66%</td>
<td>95%</td>
</tr>
<tr>
<td>Periphery Normal</td>
<td>&lt;0.27</td>
<td>78%</td>
<td>81%</td>
</tr>
<tr>
<td>Central Cancer</td>
<td>&gt;0.41</td>
<td>66%</td>
<td>73%</td>
</tr>
<tr>
<td>Central Normal</td>
<td>&lt;0.33</td>
<td>72%</td>
<td>62%</td>
</tr>
</tbody>
</table>

*Scheenen et al. Radiology 245: 507-16 (2007)*

¹H MRSI showed sensitivity of 95%, specificity of 81% and accuracy of 91% for detecting prostate cancer using ‘Choline and Citrate as biomarkers’

MRS in Treatment Monitoring

- Biochemical information from early cell events in death process (often apoptosis)
- MRS may indicate responders earlier than MRI-detectable changes appear
Induction of apoptosis (p53)

- Condensed chromatin
- Endonucleases

• Acidification
• Externalisation of phosphatidylserine
• Phospholipases
• Cell shrinkage

• Apoptotic bodies
• Phagocytosis

Natural course of apoptosis

- pH$_i$ drop
  - Inhibition of glycolysis
  - Inhibition of phosphatidylcholine synthesis
  - Activation of phospholipases
  - Reduced cell volume

- Cell shrinkage -> Cell kill
- Exogenous cells
\textsuperscript{1}H MRS: Lipids

\textbf{Jurkat cells}
\textbf{Doxorubicin}


\textbf{C6 glioma cells}
\textbf{Cell cycle and lipid droplets}

\textit{Barba et al. Cancer Res 59: 1861 (1999)}
$^1$H NMR spectra of a BT4C during apoptosis *in vivo*

‘Metabolomics’ using MRS Data

PLS model of $^1$H MRS to predict apoptosis

$^1$H MRS Lipids during Gene Therapy: Origin

Liimatainen et al. MRM 59: 1232, 2008
Transmission EM

UNTREATED

GCV-TREATED (day 4)

Droplets: 0.2-2 µm
23±3

43±5 pFOV


Mean characteristic compartment size of ‘lipid bodies’ in C6 glioma of $4.3 \pm 0.7 \ \mu m$ (Lahrech et al. MRM 2001)
Grade 4 glioma following radiation therapy
Responding tissue:
- loss of MRS peaks and appearance of lipids
Recurrence
- Choline (and creatine) peaks reappear

Metabolites During Apoptosis (BT4C glioma)

$[^1]H$ MRS: Cholines in Breast Cancer

Baseline

24 hrs AC X 1

AC X 4

Taxol X 2

$t_{Cho}$ = 4.6
LD = 4.0 cm

$t_{Cho}$ = 3.7
LD = 4.0 cm

$t_{Cho}$ = 0.9
LD = 1.7 cm

$t_{Cho}$ = 4.1
LD = 1.7 cm

$t_{Cho}$ units = mmol/kg$_{water}$
LD = longest diameter

Meisamy et al, Radiology 233:424 2004
$^{1}$H MRS Changes in (tCho) at 24 h
Predicted Clinical Response to AC

Objective Response

No Response

Meisamy et al, Radiology 2004
Characterising Transgenic Animals: $^{31}$P MRS

CK transgenic mouse
$[\text{ADP}]_f$ determined
(Koretsky et al. PNAS 87:3112, 1990)

ODC transgenic mouse
$[\text{Mg}^{2+}]_f$ determined in the brain
(Kauppinen et al. J Neurochem 58:831, 1992)
Characterising Transgenic Animals

Huntington R6/2 mouse

Decrease in NAA

Increase in Cr + PCr, GPC, Glu, Gln, Tau, GSH

‘High throughput’ MR of rodents

MRI of up to 16 mice at the time
MRS not quite yet there

Technical Developments in MR

MRI: spatial resolution should approach that of ‘histology’

Duyn et al. PNAS 104:11796, 2007
Technical Developments in MRS

- Spatial resolution of MRS(I) is an issue
- Hardware: Field strength, B0 + B1
  - shimming, rf-coils
- ‘Some’ unassigned metabolites yet to be discovered in vivo
- Dynamic Nuclear Polarisation (DNP): improving SNR of $^{13}$C, $^{15}$N, (1H?)
Advanced MRSI of A Brain Tumour

7T Scout Image

Tumor ROI

Lac

3T FLAIR

Control ROI


0.8 x 0.8 x 1 cm³
13C MRS and Tumour Drug Treatment

• Hyperpolarised substrates (Ardenkjaer-Larsen et al. PNAS 100:10158, 2003)

Conclusions: MRS and *in vivo* Metabolomics

- ‘Metabolomics’ in its broad sense descends from concepts pioneered by *in vivo* MRS
- MRS: ‘*in vivo* metabolite phenotype’
- Focus currently on data acquisition techniques to improve ‘metabolite coverage and spatial resolution’
- Several disease-oriented applications
- Use of MRS is expanding in clinical centres