In Vivo NMR and MRI

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Typical Physiological NMR Studies

- Energetics $^{31}$P
- Metabolism $^{13}$C, $^1$H
- Ion Homeostasis $^{23}$Na, $^{39}$K, $^{19}$F, $^{31}$P

- Cells
- Tissue
- Organs
Cells

Anchorage independent:
- single cell suspensions, hollow fibers

Anchorage dependent:
- require attachment to an appropriate matrix
  - Microcarriers glass or plastic beads treated to permit cell attachment to the surface-low cell density, high shear forces during superfusion result in cell loss.
  - Macrocarriers porous collagen beads which permit cells to grow on the surface and throughout the inside of the bead-higher cell density, less shear

Hollow Fiber Technology for antibody production

Cells grown on collagen coated microcarrier bead
Perfusion Apparatus

Design considerations

- **Temperature**: superfusion with warmed media, NMR probe variable temperature controller
- **Oxygen**: gassing of perfusion media with O₂ or O₂/CO₂ for normoxia or N₂ or N₂/CO₂ for anoxia studies
- **Nutrient Delivery**: appropriate ionic and substrate concentrations
- **Interface to Magnet**
Organ/Tissue Perfusion

Examples:
Isolated perfused Heart
Isolated perfused Liver
Isolated perfused Kidney
Applications of NMR

Biochemical Pathways in a Heart Cell
Bioenergetics: $^{31}$P NMR

4.7 T
2 g heart
7 min

11.4T
100 mg aorta
2 hrs

Note amount of tissue, cells required for adequate S/N and time for acquisition.
Ion Homeostasis

$^{23}\text{Na}$
High S/N, lots of extracellular sodium, NMR frequency is close to $^{13}\text{C}$

$^{19}\text{F}$ NMR used to indirectly detect Ca, NMR frequency is high, near $^{1}\text{H}$
Metabolism: $^{13}$C NMR Studies

Use $^{13}$C labeled glucose, pyruvate, acetate, etc. “Isotopomer Analysis”

TCA Cycle

- Glucose
- G3P
- DHAP
- Glycerol
- Pyruvate
- Lactate
- Acetyl-CoA
- Acyl-CoA
- citrate
- Fumarate
- succinyl-CoA
- Anaplerotic Substrate
Scheme for Isotopomer Analysis

See website for details:
http://www4.utsouthwestern.edu/rogersnmr/software.htm
Metabonomics (Metabolomics)

Tissue or biofluid sample

$^1$H NMR spectroscopy

Measure the metabolite profile

Treat metabolite profile as statistical ‘object’ for classification purposes

Explore metabolite profile to gain mechanistic insight

- Minimal sample preparation
- Rapid analysis
- Unbiased detector
- Molecular structure

NMR Spectra Data Binned & Normalized (Primary Data Reduction)

PC1

PC2

PC3
Applications of Metabonomics

Classification of toxicity
   (Nephrotoxicity, Hepatotoxicity)

Classification of disease
   (Inborn errors of metabolism, Cancer, Cardiovascular Disease, Diabetes)

Investigation of physiological status
   (Diurnal variation, Hormonal variation, Dietary effects)

Monitoring efficacy of therapeutics
Evaluation of transgenic models
Characterization of natural products
Biomarker Profile for Liver Tumors

Formate*

Succinate*

Acetate*

Lactate

Glycine**

Phosphocholine*

**p<0.01

*p<0.05

Weightings

‘Healthy’ liver

Chemical shift (ppm)
Diagnosis of Errors in Metabolism

$^{1}$H NMR-based metabonomics for the diagnosis of inborn errors of metabolism in urine

Phenylketonuria (phenylalanine)
Maple Syrup Urine Disease (leucine, isoleucine, and valine)
$^1$H-NMR Spectrum of Human Plasma
Total plasma NMR signal varies 30% over time of day (mean of 8 individuals over 24 h)

PCA shows that metabolic profiles separate into 3 classes according to time of day.

- 21% of variation was associated with subjects
- 79% of variation was associated with time of day

Blood lipids represent the major diurnal change

Total plasma signal should be normalized to an added standard
Gender Variations in Plasma

Why is Nestle’s using metabonomics?

Chocolate lovers show different lipid and sugar metabolite profiles
Metabonomics Analysis of CAD

1H-NMR spectra of serum samples from a typical patient with normal arteries (a) and a patient with coronary artery disease (b) were acquired and analyzed by principal components analysis (PCA) and partial least squares-discriminant analysis (PLS-DA). Orthogonal signal correction (OSC) was performed to remove uncorrelated variance components and optimize separation between groups (f).

“…. the availability of a relatively cheap and noninvasive replacement for angiography would revolutionize the provision of healthcare for CHD, allowing both widespread population screening and more efficient targeting of drugs, such as statins.”

Rapid and noninvasive diagnosis of the presence and severity of coronary heart disease using 1H-NMR-based metabonomics
Like the Nestle study, there was clear separation by gender however separation between treated and untreated patients was not as good using the same statistical analysis methods.

“The predictive power of 1H NMR for CAD was substantially higher than predictions based on conventional risk factors however it did not approach the 99% accuracy that would be required of a potential replacement for angiography.”

“The usefulness of the technique….might be as an additional risk assessment of clinically silent disease for noninvasive population screening.”

Proton NMR analysis of plasma is a weak predictor of coronary artery disease
Heide L Kirshenlohr, Julian L Griffin, Sarah C Clarke, Ranyl Rhydwen, Andrew A Grace, Peter M Schofield, Kevin M Brindle & James C Metcalfe
Analysis of CAD in Diabetics


The predictions were correct for only slightly more than 50% of the patients.

<table>
<thead>
<tr>
<th>Table 1. Characteristics of subjects</th>
<th>Cases</th>
<th>Controls</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>95</td>
<td>95</td>
<td>-</td>
</tr>
<tr>
<td>Male/female</td>
<td>75/20</td>
<td>75/20</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60 ± 9</td>
<td>60 ± 9</td>
<td>0.07</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.3 ± 4.6</td>
<td>29.2 ± 4.7</td>
<td>0.90</td>
</tr>
<tr>
<td>Active smoker</td>
<td>16%</td>
<td>19%</td>
<td>0.61</td>
</tr>
<tr>
<td>Percentage of patients with myocardial infarction history</td>
<td>7%</td>
<td>8%</td>
<td>0.35</td>
</tr>
<tr>
<td>Fasting glucose (mM)</td>
<td>9.9 ± 3.1</td>
<td>9.2 ± 2.8</td>
<td>0.12</td>
</tr>
<tr>
<td>Glycated haemoglobin (HbA1c, %)</td>
<td>7.8 ± 1.8</td>
<td>7.7 ± 1.6</td>
<td>0.60</td>
</tr>
<tr>
<td>Total cholesterol (mM)</td>
<td>5.8 ± 1.2</td>
<td>5.6 ± 1.0</td>
<td>0.28</td>
</tr>
<tr>
<td>HDL (mM)</td>
<td>1.2 ± 0.3</td>
<td>1.3 ± 0.4</td>
<td>0.30</td>
</tr>
<tr>
<td>LDL (mM)</td>
<td>3.8 ± 0.9</td>
<td>3.4 ± 0.8</td>
<td>0.26</td>
</tr>
<tr>
<td>Percentage of patients taking cholesterol-lowering drugs</td>
<td>33%</td>
<td>38%</td>
<td>0.66</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>146.5 ± 14.0</td>
<td>143.8 ± 14.2</td>
<td>0.19</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>82.8 ± 7.9</td>
<td>81.9 ± 8.8</td>
<td>0.48</td>
</tr>
<tr>
<td>Hypertension</td>
<td>60%</td>
<td>50%</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Clinical and biological characteristics (mean ± standard deviation) of the type 2 diabetic patients included in the case-control study noted in the placebo arm of the UMBRICA trial, and comparisons between the patients who either presented a fatal/non-fatal acute myocardial infarction or underwent sudden death during the 4-year follow-up, and patients who did not present any such event ($P$-values).

“Our results confirmed that, as suggested by Kirschenlohr et al., the achievable accuracy of the prediction of the NMR plasma results makes the technique unfeasible for clinical use. Kirschenlohr et al. obtained a low prediction accuracy when they compared NMR data analyses to angiographic findings performed at the same time, but, as shown here, it is even worse for the prediction of clinical events from baseline samples in type 2 diabetic patients with high cardiovascular risk.”

NMR-based prediction of cardiovascular risk in diabetes
Ronan Roussel, France Mentré, Nadia Bouchemal, Samy Hadjadj, Michel Lièvre, Gilles Chatellier, Joël Menard, Xavière Panhard, Anne Le Hénaff, Michel Marre & Laurence Le Moyec
Magnetic Resonance Imaging

http://www.cis.rit.edu/htbooks/mri/inside.htm (Online textbook)


MRI - History

Nuclear Magnetic Resonance (NMR)
Felix Block and Edward Purcell
1946: atomic nuclei absorb and re-emit radio frequency energy in an external magnetic field
1952: Nobel prize in physics
1971: NMR Tumor detection (Damadian)

Magnetic Resonance Imaging (MRI)
1973: Lauterbur suggests NMR could be used to form images
1977: clinical MRI scanner patented
1977: Mansfield proposes echo-planar imaging (EPI) to acquire images faster
2003: Nobel Price in Medicine

Functional MRI (fMRI)
1990: Ogawa observes BOLD effect with T2*: blood vessels became more visible as blood oxygen decreased
1991: Belliveau observes first functional images using a contrast agent
1992: Ogawa et al. and Kwong et al. publish first functional images using BOLD signal

Adapted from Jody Culham, http://defiant.ssc.uwo.ca/Jody_web/fmri4dummies.htm
Types of MRI

Functional Imaging

Statistical Map
superimposed on anatomical MRI image
The human or mouse body is primarily fat and water.
Fat and water have many hydrogen atoms.
MRI uses gradients and detects the NMR signal from the hydrogen nuclei in tissue.
In the images above fat is bright and lean tissue is grey.
Lungs and gas pockets in the intestines are dark.
Spin Echo

NMR

180° RF pulse

90° RF pulse

signal

FID

spin echo

MRI

RF

G_s

G_\phi

G_f

S

Echo

TR

TE
Human Studies
Human MRI Systems

MRI Scanner: 1.5 Tesla

“Open” MRI

Mobile MRI
Spin Echo Contrast

Changing TE and TR creates contrast

\[ T_1 \text{ weighted} \]

The T1-weighted sequence uses a short TR and short TE (TR < 1000ms, TE < 30ms).

\[ T_2 \text{ weighted} \]

The T2-weighted sequence uses a long TR and long TE (TR > 2000ms, TE > 80ms).
Spin Echo Contrast

The Proton Density-weighted sequence uses a long TR and short TE (TR > 2000ms, TE < 30ms).

The T2-weighted sequence uses a long TR and long TE (TR > 2000ms, TE > 80ms).
Brain Imaging

CT

Photography

PET

MRI
Anatomical Images
Functional Imaging

Functional images

~2s

fMRI Signal (% change)

ROI Time Course

Time

Condition

Statistical Map superimposed on anatomical MRI image

Condition 1

Condition 2

~ 5 min

Region of interest (ROI)
Body Composition
Animal Studies
Animal MRI Systems
Body Composition in Mice

GLUT4 -/- mice
Lean (14% fat/ water)

GLUT4 +/- mice
Fat (40% fat/ water)
Combined *in vivo* and *Ex vivo* MRI

*in vivo* MRI ex vivo MRI

anesthesia

9.4T magnet

AA

Spine

RV

LV

Chest wall

AR

AA
Spin-echo images of excised aorta as a function of depth in 0.1 mm step. Slices are 0.3 mm thick and were acquired with TR/TE = 4000/20 ms.
Ex vivo MRI Microscopy

T2 maps to study relaxation

ApoE-KO mouse

Control mouse
Ex Vivo Mouse Heart
Ex Vivo Mouse Heart
Ex Vivo Mouse Heart

Different views comparing heart mass and fat mass in three different mouse models
Prevention of right ventricular dilatation in infected mice by selenium therapy

Note the reduction in RV diameter in In-Se2 and Inf-Se4 compared to Inf-Se0.
Animation: *In Vivo* Heart
Reconstructions: *In Vivo* Heart Volumes (mm$^3$)

- ApoE-KO = 262.5
- NOS3-KO = 368.6
- WT = 174.7
GI Tract of the Mouse
GI Tract Animation
GI Tract Tumors in Mice
Reconstructions: GI Tract
Opaque and Transparent Views
Mice with Multiple Tumors
Evaluation of Therapeutics

Before (D-0)
Drug injection x 5 (3ug/g), every second day
0.622 and 1.63 mm³

After (D-7)
PBS control injection X5, every second day
0.792 mm³

0.252 and 1.21 mm³
1.08 and 0.74 mm³
Multimodality Approaches

Combined MRI and micro-PET approach
Use of fiducial markers (yellow arrows)
Image fusion

Micro-PET image of a mouse
Image Fusion

Several views of fused MRI (opaque color) and micro-PET (bright orange, semi-transparent) images of a mouse.

The End