Hyperpolarization in small animal models

International Training Course 2024

MR Research Centre, Aarhus University

10th October 2024

NVISION

Scope

1	**Importance of animal models**	Different types of small animal models & their importance for research
2	**How to choose your animal model**	What should you consider? What are potential pros and cons?
3	**Small animal handling**	Hygiene, Anaesthesia, Catheters
4	**Application examples**	Oncology, Metabolic disease, Neurology

1.

Understand disease mechanisms & biological processes

2.

Develop and test diagnostic and therapeutic tools

3.

Leverage ethical and practical advantages

1. Understand disease mechanisms & biological processes

- Many small animal models **share genetic, molecular, and physiological similarities** with humans
- Learn about **tissue composition and function** in healthy and different disease states
- Understand **anatomy and morphology** of organs, compartments and subjects
- **Track changes i**n molecules, cells, and structures over time (e.g. disease progression)
- Study genetic or molecular markers that **predict outcomes**

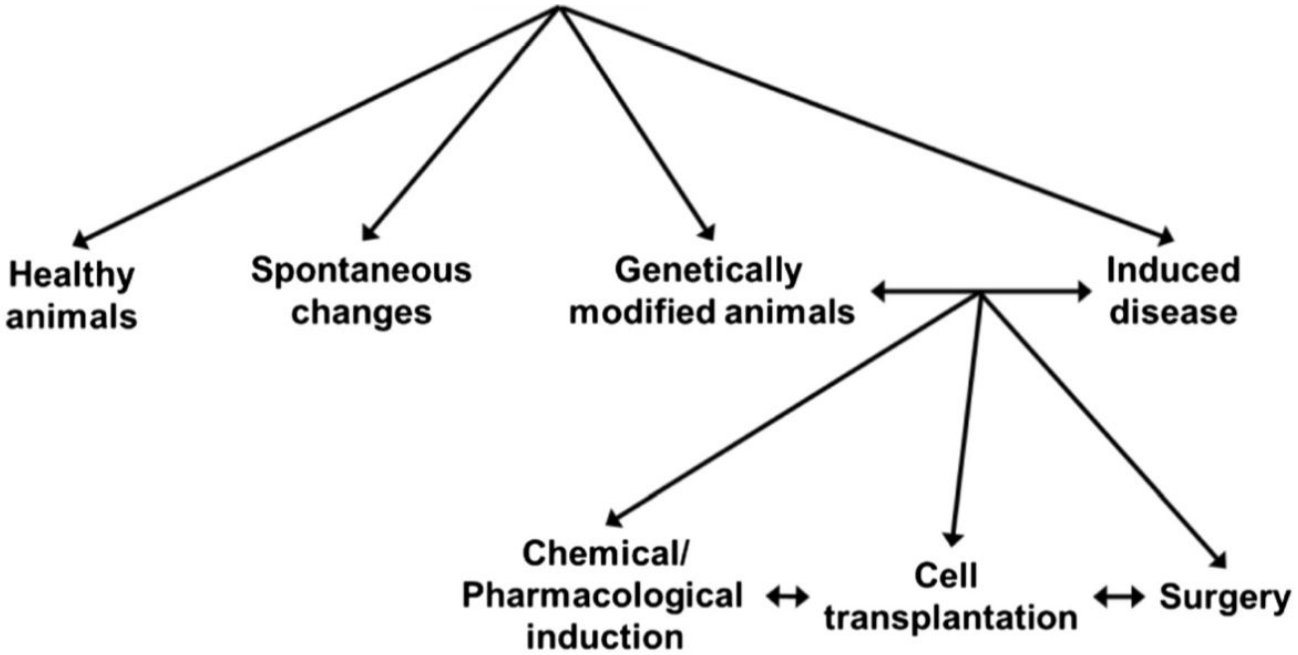
2. Develop and test diagnostic and therapeutic tools

- Evaluate HP **agent distribution and behaviour** within the body
- **Investigate toxicity, safety, and efficacy** of new imaging agents/drugs/viral vectors
- **Verify effectiveness** of treatments **before advancing** to clinical trials
- **Refine HP agents and methods** to improve early detection or treatment personalization
- Evaluate **how diseases respond to specific therapies** using imaging biomarkers
- **Investigate individual variability** in response to treatments

3. Leverage ethical and practical advantages

- Perform **longitudinal imaging studies** over time that could be impractical for humans
- **Conduct invasive procedures for complementary information** (e.g. biopsies) that enhance data for tracking changes in disease progression and response over time
- Ability to have **larger sample sizes** and better statistical power at a fraction of the cost of human trials
- **Test multiple** treatment **strategies** or imaging **methods in parallel**

1.) Types of small animal models



Types of animal models can range from normal health conditions to those with experimentally induced diseases.

Vanhove C. *et al.* **EJNMMI Physics (2015) 2:31**

- What is my hypothesis ? Which disease/ treatment response?
- Which models are commercially available? If not should I make one?
- Which imaging method(s) should I choose?
- How sensitive are my methods?
- Which sample size do I need to achieve significance? (talk to a biostatistician!)
- Should I study both males and females (yes!)?
- Should I do a longitudinal study or a one time-point study?
- Do I need regional information of non-localized data enough?
- How heterogeneous is my region of interest? What resolution do I need?
- Which MR coil type and size should I choose?
- Which correlative ex vivo studies shall I conduct after the imaging experiment ?

2.) PROs & CONs of small animal models

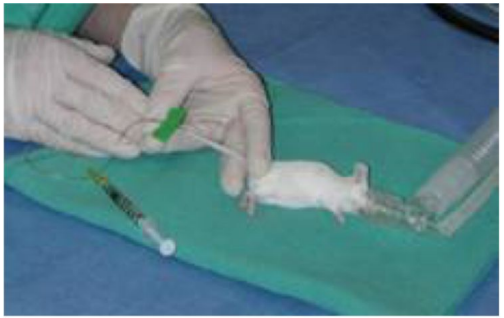
PROs

- low cost of purchase and housing
- possible to breed, combine mutations, develop new models
- wide range of immuno-deficient and humanized strains available
- 99% homology with human genome
  → some similar diseases as humans
- big advances in mouse genomics, vast viability of GEM
- wide range of animal models for human diseases available
- reproductive and nervous system are like those in humans

CONs

- fast breathing = motion artefacts
- small size of vessels and organs = difficult to operate, obtain arterial input function
- small blood volume, limited injection volume
- differences in treatment efficacy and immune responses
- immune reactions and differences in receptor expression in small animals can limit treatment efficacy compared to humans.
- Translational limits → treatments might show significant effects (e.g., tumor reduction) in mice, but may not translate to humans

3.) Handling of small animals in preclinical imaging



All animal imaging studies and related activities must be performed according to established procedures, that are unique in each country

- Tables, floor and **any instruments in contact** with the animals, such as surface coils, **must be cleaned** with disinfectant before and after the imaging

- Animal transportation to and from imaging suites should be performed in an **enclosed escape-proof cage/secure container** that limits exposure between animals and humans and ensures safety of the animal

- Personnel must wear proper **personal protective equipment** (e.g. gowns or scrubs, gloves, masks, safety glasses, and shoe covers) when handling animals

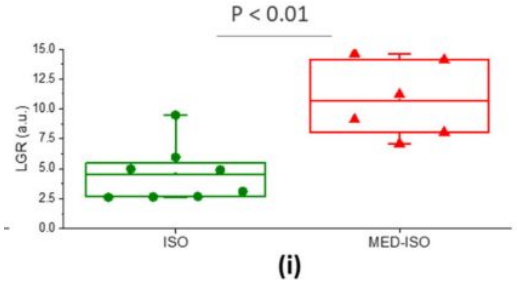
The role of anaesthesia in small animal experiments:

- **Minimizes stress and motion artifacts** during imaging procedures
- Facilitates **surgical and imaging procedures** → ensures the animal remains still and **comfortable** for the duration of invasive interventions or prolonged imaging sessions.
- **Regulates physiological parameters** like heart and respiratory rates → ensure physiological reproducibility of studies
- **Gas anesthesia** is easier for **hyperpolarized metabolic imaging** in small animal models
- Imaging awake animals is possible !

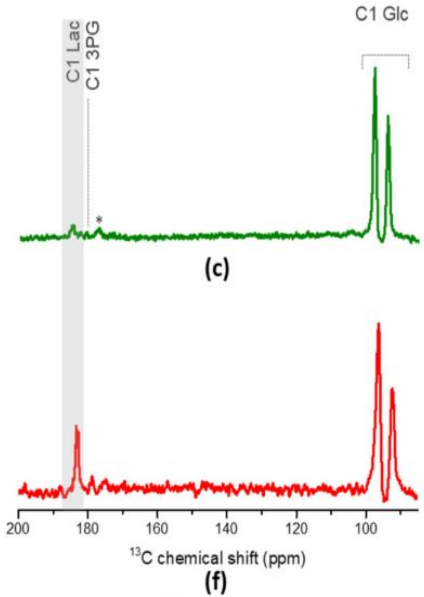
Example study I

Anaesthesia - Different anaesthetics have different haemodynamic effects

Model: male C57BL6/J mice (12 hour-fasted)
HP agent: [2H7, U-13C6]D-Glucose



Lactate-to-Glucose ratio (LGR) was **128%** higher i**n the MED–ISO group** compared to the ISO group



Differences in glycolytic intermediate **3-phosphoglycerate** (3PG, 179.8 ppm) and **C1-Lactate** (183.5 ppm) were identified in the summed **spectra**

Medetomidine (combined with isoflurane), allows for a more stable metabolic state, with less suppression of glycolysis

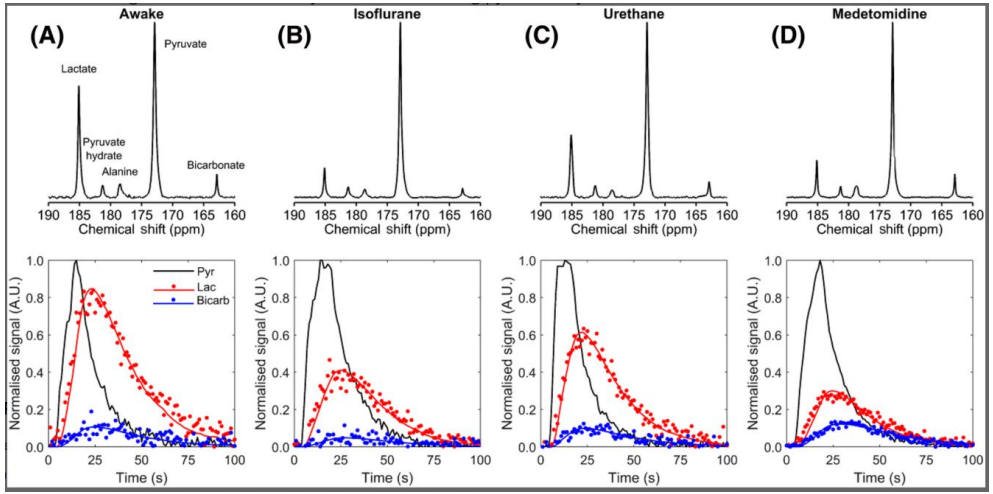
metabolites

Measuring Glycolytic Activity with Hyperpolarized [2H7, U-13C6]D-Glucose in the Naive Mouse Brain under Different Anesthetic Conditions, Flatt et al 2021

Example study II

Anaesthesia - Haemodynamics affect observed pyruvate-to-metabolite labelling rates

Model: female adult Wistar (n=9) and Sprague-Dawley (n=14) rats
HP agent: [2H7, U-13C6]D-Glucose



Significant differences in apparent **lactate-to-pyruvate** and **bicarbonate-to-pyruvate** labelling rates **between different states**, which could be **largely explained by haemodynamic alterations**

Urethane-anaesthetised animals most closely resemble **awake rats** and differ from **isoflurane-anaesthetised** and **medetomidine-sedated animals.**

Haemodynamics impact the observed pyruvate-to-metabolite labelling rates and area-under-time course ratios of referenced to pyruvate.

NMR IN BIOMEDICINE

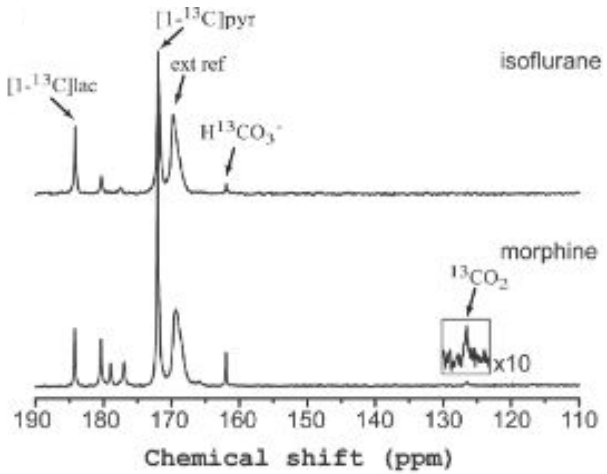
Metabolism of hyperpolarised [1-13C]pyruvate in awake and anaesthetised rat brains, Hyppönen et al 2021

Example study III

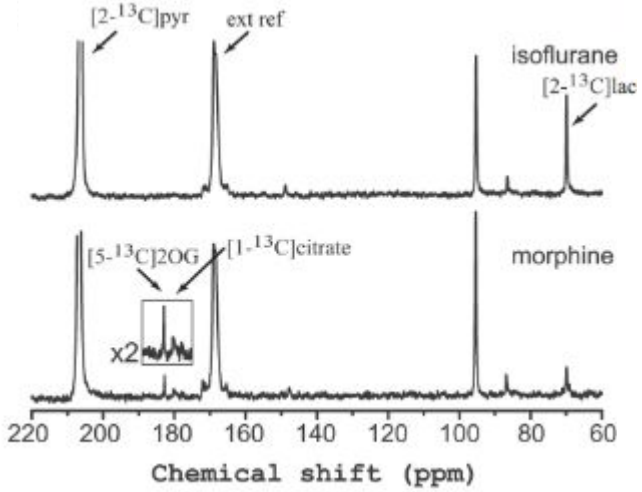
Anaesthesia - Choice of anaesthesia can alter detection of downstream metabolites

Model: male Sprague-Dawley rats
HP agent: [1-13C]pyruvate and [2-13C]pyruvate

Spectra acquired following injection of **[1-^{13}C]pyruvate:**



Spectra acquired following injection of **[2-^{13}C]pyruvate**



Using morphine for sedation greatly improves detection of downstream metabolic products compared with other anaesthetics

NMR IN BIOMEDICINE

Brain metabolism under different anesthetic conditions using hyperpolarized [1-13C]pyruvate and [2-13C]pyruvate, Marjańska et al 2018

The role of catheters in small animal experiments:

- Facilitate **administration of hyperpolarized agents** like [1-13C]pyruvate directly into the bloodstream → critical for precise timing and accurate dosing in HP MRI experiments.
- Enable **repeat injections** without causing added stress to the animal.
- Avoid repeated handling for injections → **reduce animal stress and motion** during imaging → help with minimizing artifacts and improving data quality.

3.) Small animal handling: Catheters

Contents lists available at ScienceDirect

Journal of Magnetic Resonance Open

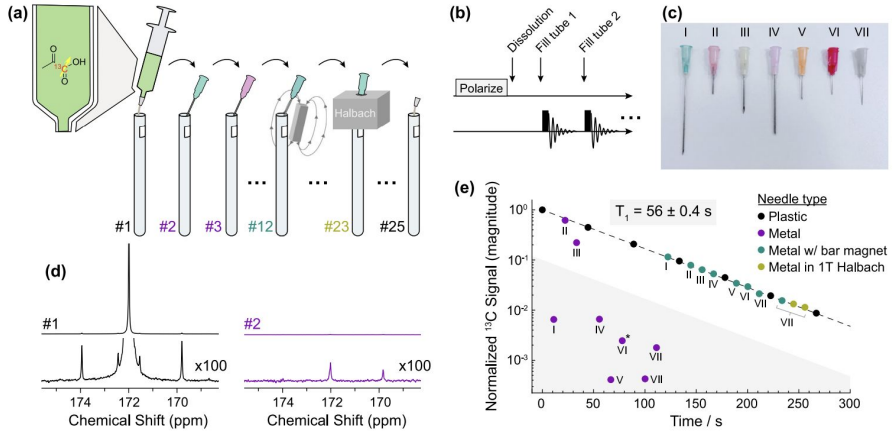
journal homepage: www.sciencedirect.com/journal/journal-of-magnetic-resonance-open

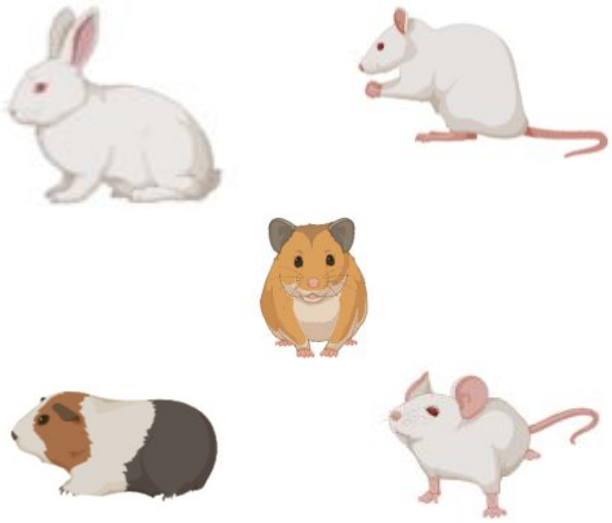
Research article

Polarization losses from the nonadiabatic passage of hyperpolarized solutions through metallic components

James Eills [a], Marc Azagra [a], David Gómez-Cabeza [a], Michael C.D. Tayler [b], Irene Marco-Rius [a,*]

[a] IBEC - Institute for Bioengineering of Catalonia, Barcelona Institute of Science and Technology, 08028 Barcelona, Spain
[b] ICFO - Institut de Ciències Fotòniques, The Barcelona Institute of Science and Technology, 08860 Castelldefels, Barcelona, Spain





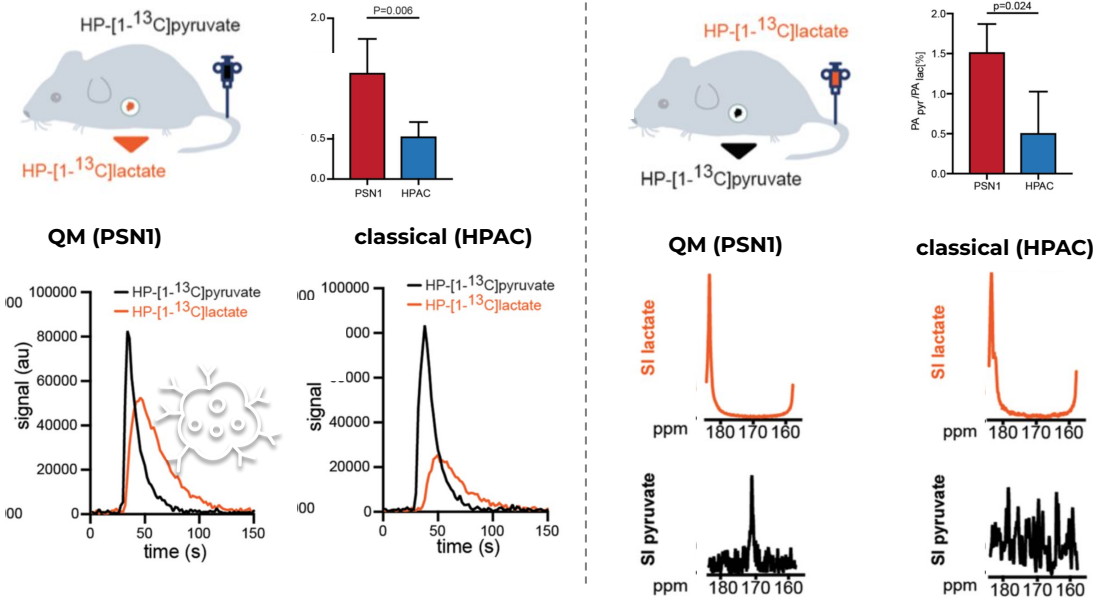
Small animal models

Preclinical applications

- Oncology
- Neurology
- Immunology & Inflammation
- Metabolic diseases
- Cardiology
- Hepatology
- Nephrology
- Aging

Tumor stratification - Pancreatic cancer

Model: Rat xenografts of human cell lines with transcriptionally-defined classical (HPAC) and quasi-mesenchymal (QM, PSN1) subtypes



What are the metabolic differences between molecular tumor subtypes?

Oncology

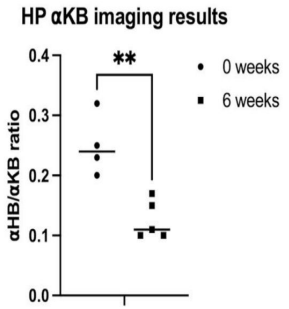
Increased lactate and pyruvate production was observed in aggressive QM subtype in comparison to classical subtype of pancreatic cancer, indicating highly metabolically active tumor

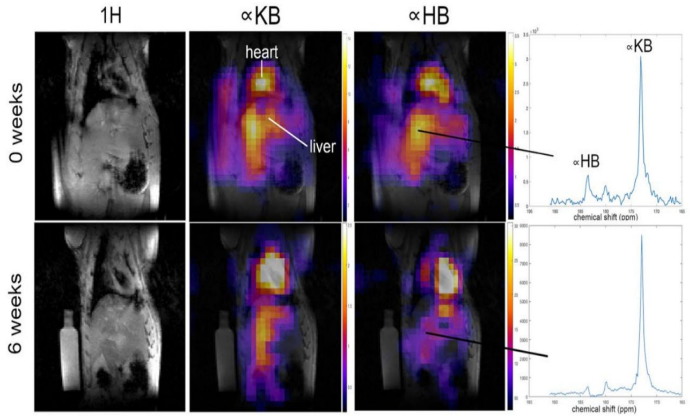
BMC Cancer & Metabolism

Functional noninvasive detection of glycolytic pancreatic ductal adenocarcinoma, Heid et al 2022

Early detection & risk assessment -
Metabolic dysfunction-Associated Steatohepatitis (MASH)

Model: MAFLD in rats, methionine-choline deficient (MCD) diet
HP agent: [1-13C]∝-ketobutyrate (∝ KB), a close molecular analog of pyruvate with modified specificity for LDH isoforms





Fatty liver disease: What are the early metabolic changes?

Metabolic diseases

HP-MRI shows a mean drop of 52% in the apparent metabolic conversion of ∝ KB to ∝ HB in the liver after six weeks of a MCD diet → HP-MRI can be used to detect hepatic metabolic changes in early MASH

SCIENTIFIC REPORTS
nature research

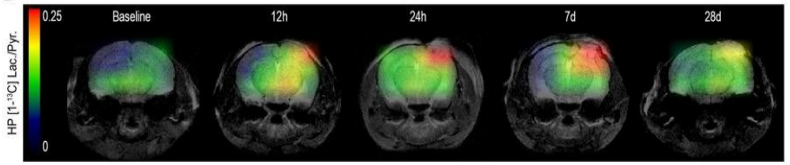
Detection of early-stage NASH using non-invasive hyperpolarized 13C metabolic imaging, Morze et al, 2024

Neurology

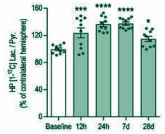
Monitoring inflammation: Transient significant increase of HP [1-13C] lactate-to-pyruvate ratio in mice models, driven by inflammatory response

Controlled cortical impact mouse model (baseline, 12h, 24h, 7d, 28d)

MRI

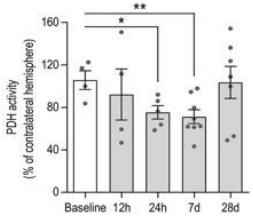


Lac/Pyr

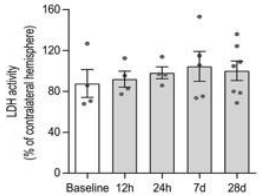


Assays

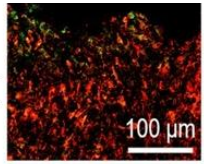
PDH activity



LDH activity



Macrophages



Transient significant increase of HP [13C] lactate-to-pyruvate ratio can be monitored with MRI

Correlated with inflammation in assays

- Increased in CD68 and CD11b macrophage levels
- Decreased PDH activity
- Unchanged LDH activity

UCSF University of California San Francisco

SCIENTIFIC REPORTS nature research

Guglielmetti et al, Scientific Reports (2017

THANK YOU!

Acknowledgement to Dr. Irina Heid for sharing some of her teaching materials for this presentation.

Questions? Comments?

Email myriam.chaumeil@nvision-imaging.com

Web www.nvision-imaging.com

NVISION