Lecture #18

Brain metabolism during heart/lung bypass surgery
Cardiac Surgery

- Thousands of heart surgeries are performed every day in the United States (~500,000 coronary bypasses/yr).
- Two major advances in medicine made heart surgery possible:
  - The heart-lung machine, which takes over the work of the heart.
  - Body cooling techniques, which allow more time for surgery without causing brain damage.
  - Cooling lets surgeons stop the heart for long periods without damaging the heart tissue.
  - Blood is cooled as it passes through the heart-lung machine.
  - The cooled blood lowers body temperature.

What about the brain?
In this study 261 people (average age 61) having bypass surgery were formally tested to measure their cognitive capacity (i.e. mental ability) at four different times: before surgery, at six weeks, at six months, and at five years after bypass surgery.

Participants were deemed to have significant impairment if they had a 20% decrease in test scores.

The investigators found that 42% of patients had at least a 20% drop in test scores after surgery, and that in many cases the decrease in cognitive capacity persisted for 5 years.

Some basic questions:
What is the optimum temperature?
Should blood flow to the brain continue?
Cerebral mitochondrial dysfunction associated with deep hypothermic circulatory arrest in neonatal swine


OBJECTIVES: Controversy remains regarding the use of deep hypothermic circulatory arrest (DHCA) in neonatal cardiac surgery. Alterations in cerebral mitochondrial bioenergetics are thought to contribute to ischaemia–reperfusion injury in DHCA. The purpose of this study was to compare cerebral mitochondrial bioenergetics for DHCA with deep hypothermic continuous perfusion using a neonatal swine model.

METHODS: Twenty-four piglets (mean weight 3.8 kg) were placed on cardiopulmonary bypass (CPB): 10 underwent 40-min DHCA, following cooling to 18ºC, 10 underwent 40 min DHCA and 10 remained at deep hypothermia for 40 min; animals were subsequently rewarmed to normothermia. 4 remained on normothermic CPB throughout. Fresh brain tissue was harvested while on CPB and assessed for mitochondrial respiration and reactive oxygen species generation. Cerebral microdialysis samples were collected throughout the analysis.

RESULTS: DHCA animals had significantly decreased mitochondrial complex I respiration, maximal oxidative phosphorylation, respiratory control ratio and significantly increased mitochondrial reactive oxygen species (P < 0.05 for all). DHCA animals also had significantly increased cerebral microdialysis indicators of cerebral ischaemia (lactate/pyruvate ratio) and neuronal death (glycerol) during and after rewarming.

CONCLUSIONS: DHCA is associated with disruption of mitochondrial bioenergetics compared with deep hypothermic continuous perfusion. Preserving mitochondrial health may mitigate brain injury in cardiac surgical patients. Further studies are needed to better understand the mechanisms of neurological injury in neonatal cardiac surgery and correlate mitochondrial dysfunction with neurological outcomes.
Single Voxel Spectroscopy
PRESS NFL
TR/TE 2000/35
Total Averages: 64
~3 minutes w/water refs
12 mm x 12 mm x 15 mm
Data Fit with LCModel

Red trace is fit
Black trace is spectroscopy data

Fit quality %SD  Ratios to creatine

Residuals
How did we measure brain temperature?

25 spectra collected at different times
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**Chemical Shift Difference between NAA and Water gives temperature in voxel**

Raw data file names: [link to file names]

Back to 37 C in brain ahead of recorded body temp
Mistake #1: we went to lunch during this time interval and didn’t collect any data.
First 18C in Vivo Brain Spectrum!
Avg of 3 time points
18 C (white)
vs 37 C (red)
Loss of macromolecule (MM) signal at low temperature might be $T_2$ effect (not that interesting but need to confirm it is a $T_2$ effect)

Why would macromolecule $T_2$s shorten but not those for the other metabolites?
Largest metabolic effect of cooling to 18°C appears to be reduction in glutamate and glutamine.

Low Glu and Gln = High NH₄⁺
Gln and Glu can be used as fuel in the TCA cycle...

...but the ammonia has to go somewhere, and ammonia is toxic to the brain.
Let’s look at this data point.
Spectrum collected at end of stopflow

Exam #15257-19  ID=piglet0206@lucas/epperson  02/06/2019 14:01  jpress  TE/TR/NS=35/2000/512  TG/R1/R2=160/13/28  2.2mL  
P29184.7  (Stanford Lucas Center)  

Data of: Radiological Sciences Laboratory, Stanford University School of Medicine


Conc. %ID /Cr+PCh Metabolite
1.619 13%  0.248 GPC
0.106 592%  1.0E-02 Ala
0.000 999%  0.000 Tau
19.301 6%  2.955 Lac
1.624 68%  0.249 Gln
0.785 57%  0.120 Glc
0.653 100%  0.100 GABA
5.439 6%  0.833 Cr
1.091 39%  0.167 Pcr
0.000 999%  0.000 Asc
9.586 4%  1.469 Hi
9.419 4%  1.442 NAA
2.176 47%  0.333 Asp
1.417 28%  0.217 HAAG
1.392 35%  0.213 GSH
0.665 68%  0.102 GSGG
0.465 22%  7.1E-02 Scyline
0.479 48%  7.3E-02 Fch
4.242 32%  0.649 2HG
5.373 18%  0.823 PK
6.503 17%  0.996 Gln
0.000 999%  0.000 CrChz
2.098 5%  0.321 GPC+PCh
10.836 3%  1.659 NAA+NAAG
6.531 4%  1.000 Cr+Pcr
0.126 13%  1.244 Gln+Gln

3.050 100%  0.467 Lip1a
11.601 6%  1.776 Lip13b
3.425 45%  0.524 Lip09
2.164 65%  0.331 HM09
1.057 114%  0.162 Lip20
1.773 10%  0.271 HM20
0.867 91%  0.133 HM12
5.102 35%  0.781 HM14
1.307 57%  0.200 HM17
1.650 20%  2.243 Lip1a+Lip13b
20.620 10%  3.157 HM14+Lip13a+L
5.589 12%  0.856 HM09+Lip09
2.830 62%  0.433 HM20+Lip20

DIAGNOSTICS
2 info's  RFALS1 4
3 info's  RFALS1 11
Doing Water-Scaling
Lactate at low pH (extracellular?)

Pre-Reflow Period
21°C
White
Red

Reflow Period
27-37°C
Green
Purple
Lite Blue
Dark Blue
Yellow

Lactate pH 7

Lactate signal during warm up
37 C after stop flow and recovery (Avg of 3)

Lactate
Glutathione, Glx, ASC

37 C after stop flow (white) vs 37 C baseline (red) Not yet returned to normal.
More lactate created than glucose lost?

Rate of Lactate loss at 37C:
\[ K_{lac} = 60 \text{ min}^{-1}. \]
Estimate you need 3 hours to get back to normal range.
Predict that with high lactate and high $\text{NH}_4^+$ due to reduced Glu & Gln we should see some conversion of lactate to alanine (not usually observed in brain).

Between $\text{NH}_4^+$ scavenging and anaplerosis, could high lactate be an overall advantage to recovery.

**TCA cycle**

- glucose → pyruvate → acetyl$\cdot\text{CoA} → \text{citrate} → \text{OAA} → \text{malate} → \text{fumarate} → \text{succinate} → \text{succinyl$\cdot\text{CoA} → \text{methylmalonyl$\cdot\text{CoA} → \text{propionyl$\cdot\text{CoA} → \text{CO}_2$}
- $\text{GABA} → \text{glutamate} → \text{glutamine}
- $\text{Heptanoate, C5 ketones, Ile, Val}$
Mistake #2: Collect longer post recovery!

Neuronal (NAA) loss in final spectrum collected?
Is myoInositol trending higher after recovery?

Choline stable

Stop Flow
ASC and GSH unstable even in baseline (MR physics/coil problem?)
Spectra are collected as an average of 32 separate 4 second traces.

Temporal Resolution? 4 seconds: sufficient SNR to map any rapid changes in lactate pool. Important for start and end of stopflow.
Other stable isotopes: Deuterium, Nitrogen-15?

- Could pyruvate speed up recovery?
- Would labeled [3-¹³C]pyruvate show anaplerosis during recovery?
- [3-¹³C]pyruvate would also measure LDH activity by isotope exchange with Lactate

Catabolism burns pyruvate as fuel.

Anaplerosis replenishes TCA intermediates

Example spectra

**a:** ¹H NMR spectrum of unlabeled sodium lactate. **b:** ¹H NMR spectrum of sodium [3-¹³C] L-lactate. **c:** ¹³C and ¹H NMR spectra of sodium [U-¹³C] L-lactate. Expansions of the resonance regions are shown above the full spectra to demonstrate the fine structure.
Hi Dan,
Interesting project with lots of metabolic questions, like lactate compartmentation in the brain, glial metabolism, etc. etc. Yes, I would collect blood, for exactly the reason you say. In a nutshell, the problem is whether labeling in the brain originates from one of two possibilities:

\[ \text{[3-13C]} \text{pyruvate} \rightarrow \text{brain} \rightarrow \text{glutamate labeling via anaplerosis or oxidation. (direct metabolism of the pyruvate skeleton)} \]

vs.

\[ \text{[3-13C]} \text{pyruvate} \rightarrow \text{liver} \rightarrow 13C\text{-labeled glucose} \rightarrow \text{brain} \rightarrow \text{glutamate. This would be especially likely if the pigs are fasted.} \]

So, if you collect blood you could do mass spec on the glucose to look at m0, m1, etc., that would tell you if glucose became labeled, but it does not tell you the site which makes it difficult to compare to brain glutamate data. Plasma glucose could be converted to monoacetone glucose which produces very nice 13C NMR spectra, and then you could figure out if the 13C labeling in glutamate is consistent with the 13C labeling in plasma. In other words, if plasma glucose became labeled, did it actually contribute to oxidative metabolism in the brain. Making a decision depends in part on the kinetics. If this is a very short bolus followed immediately by data acquisition, then gluconeogenesis is less likely a factor.

Finally, is this a terminal experiment as the animal warms up? If so, then brain biopsies would be very informative if you could collect high res spectra of the extracts.

Craig Malloy, MD
UTSW Medical Center
Recommendations for next experiment

1. Set total scans (Averages) from 64 to 128 for longer samples between reset of prescan
2. Consult with neuroradiology to factor in sensitive locations with SNR and resolution criteria to select voxel size and location (CSI?)
3. Collect spectra during full duration of study
4. Continue to collect data 1-3 hours after reestablishing 37C
   • Save periodic blood samples. Save brain samples at end?
   • Could potentially measure changes in LDH rates, anaplerosis, and Lactate to Alanine flux.
   • Advantages of bolus vs infusion? What dose?

Engineering challenges: spectroscopic imaging? Improved spectral fitting? Reduce coil vibrations?
Next Lecture: Fast Spin Echo, CPMG and J-coupling