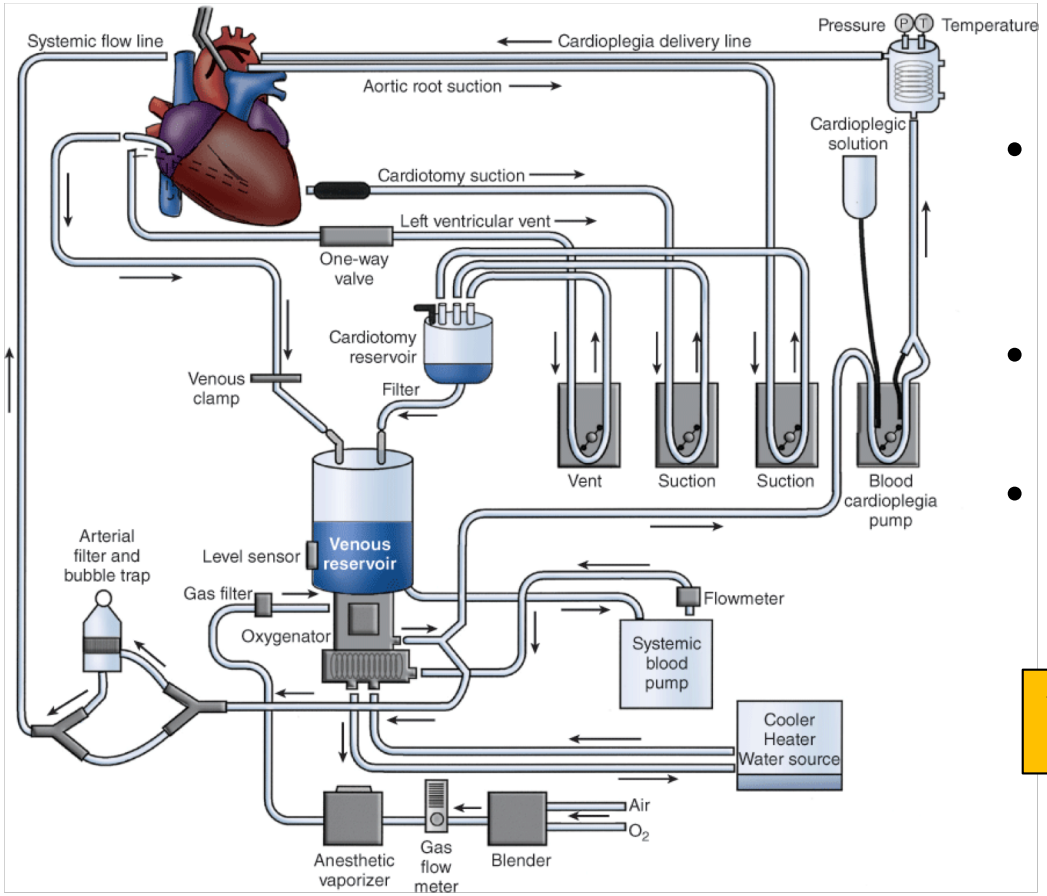
Lecture #18

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 let 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?

Cognitive Impairment After Heart Bypass Surgery

Is "Pump Head" Real, and What Does It Mean?

By Richard N. Fogoros, MD | Medically reviewed by a board-certified physician
Updated March 06, 2019

Morsa Images/Getty Images

- 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?

European Journal of Cardio-Thoracic Surgery 54 (2018) 162–168
doi:10.1093/ejcts/ezx467 Advance Access publication 15 January 2018

ORIGINAL ARTICLE

Cerebral mitochondrial dysfunction associated with deep hypothermic circulatory arrest in neonatal swine†

Constantine D. Mavroudis[a,*], Michael Karlsson[b], Tiffany Ko[c], Marco Hefti[d], Javier I. Gentile[a], Ryan W. Morgan[b], Ross Plyler[b], Kobina G. Mensah-Brown[c], Timothy W. Boorady[c], Richard W. Melchior[e], Tami M. Rosenthal[e], Brandon C. Shade[e], Kellie L. Schiavo[e], Susan C. Nicolson[b], Thomas L. Spray[a], Robert M. Sutton[b], Robert A. Berg[b], Daniel J. Licht[c], J. William Gaynor[a] and Todd J. Kilbaugh[b]

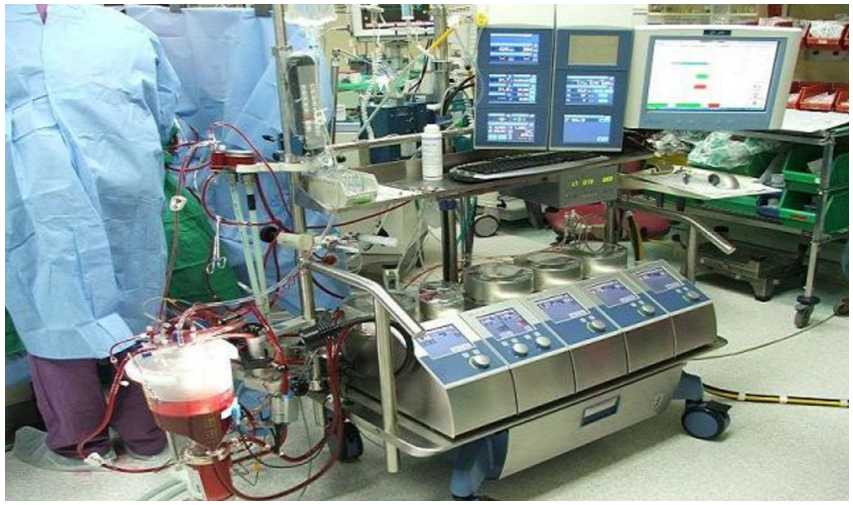
OBJECTIVES: Controversy remains regarding the use of deep hypothermic circulatory arrest (DHCA) in neonatal cardiac surgery. Alterations in cerebral mitochondrial bioenergetics are thought to contribute to ischaemia–reperfusion injury in DHCA. The purpose of this study was to compare cerebral mitochondrial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

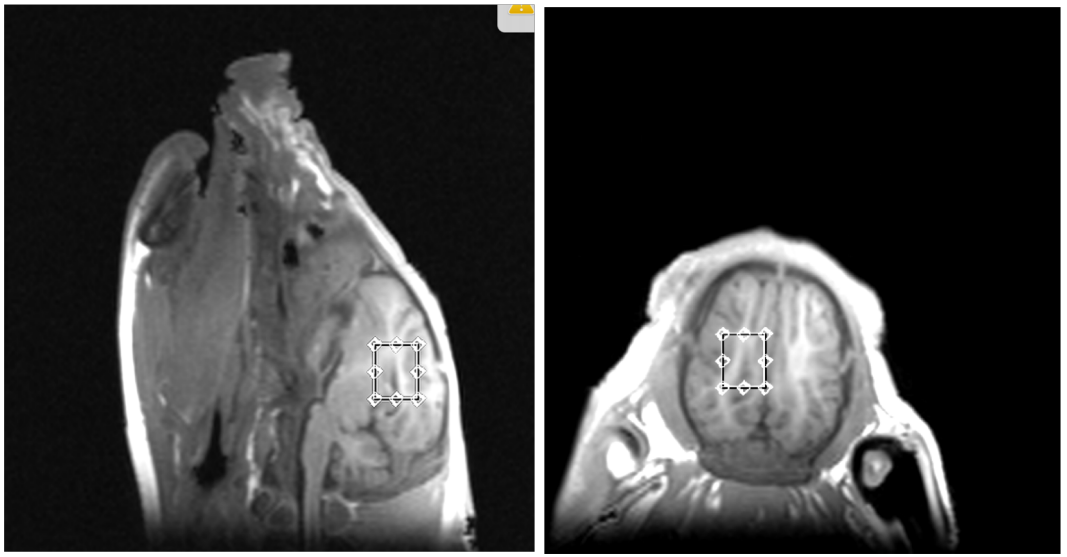
METHODS: Twenty-four piglets (mean weight 3.8 kg) were placed on cardiopulmonary bypass (CPB): 10 underwent 40-min DHCA, following cooling to 18ºC, 10 underwent 40 min DHCA and 10 remained at deep hypothermia for 40 min; animals were subsequently rewarmed to normothermia. 4 remained on normothermic CPB 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.

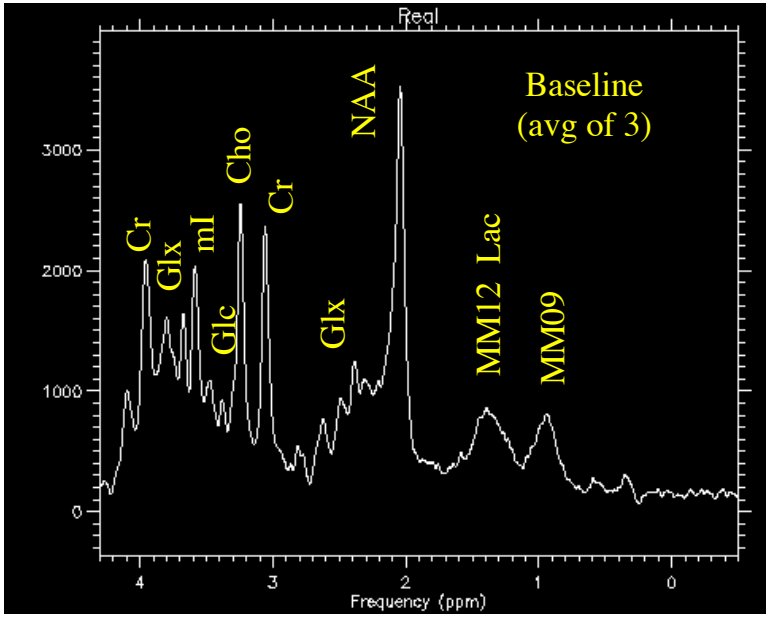
CONCLUSIONS: DHCA is associated with disruption of mitochondrial bioenergetics compared with deep hypothermic continuous perfusion. Preserving mitochondrial health may mitigate brain injury in cardiac surgical patients. Further studies are needed to better understand the mechanisms of neurological injury in neonatal cardiac surgery and correlate mitochondrial dysfunction with neurological outcomes.

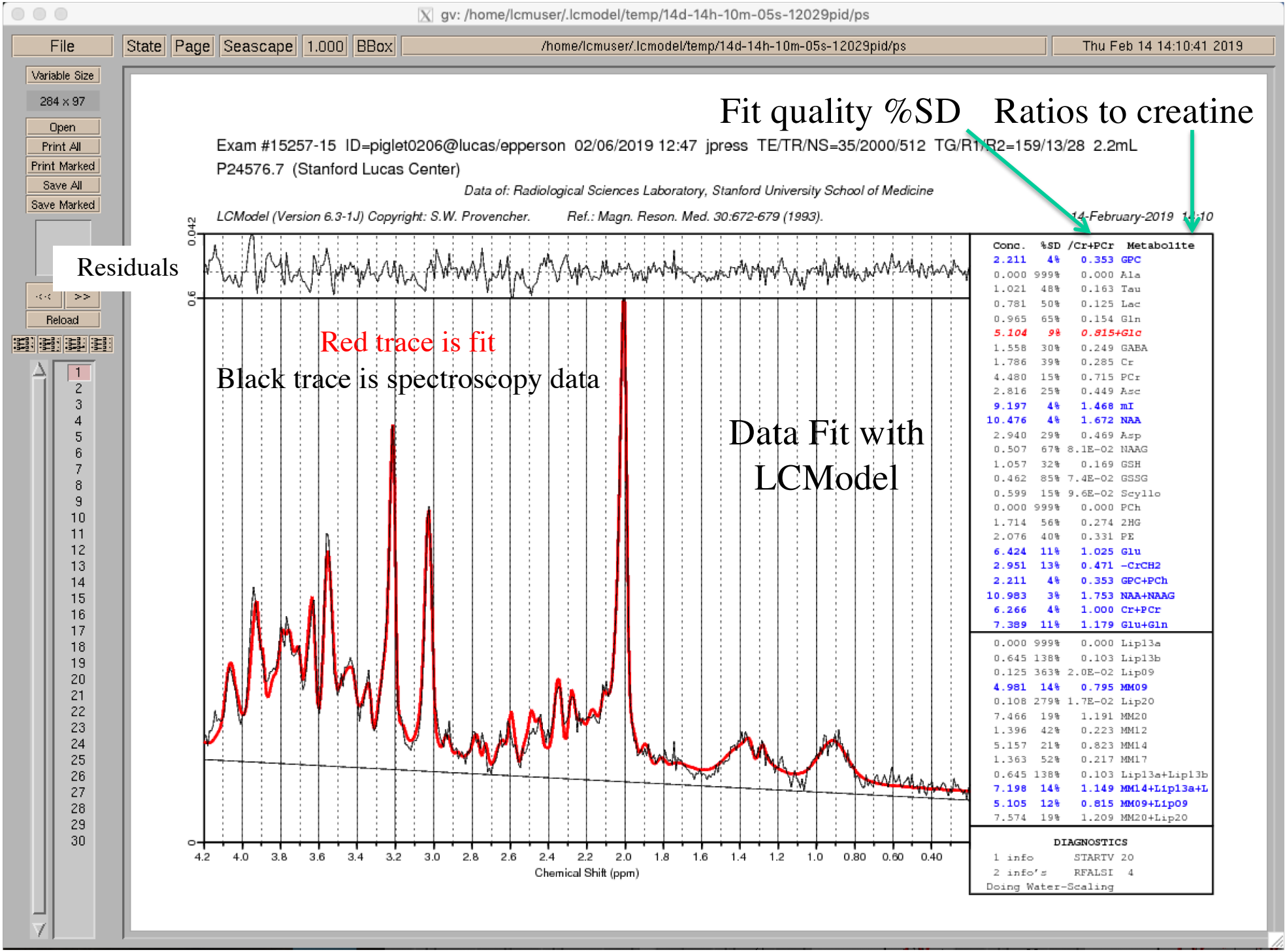
^{1}H MRS Piglet Cardiac Brain Study





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

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Thu Feb 14 14:10:41 2019
Variable Size
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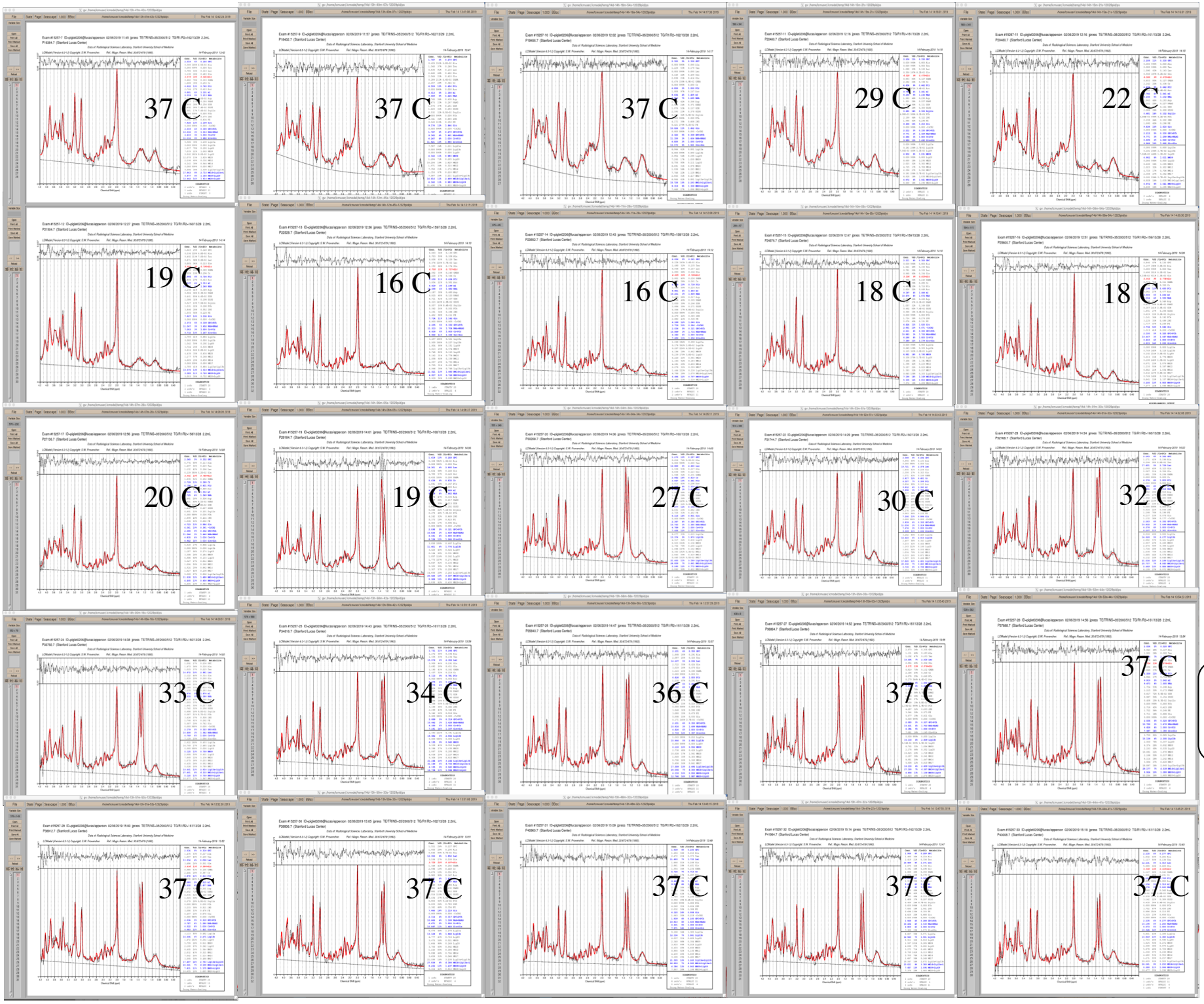
Open
Print All
Print Marked
Save All
Save Marked
Reload
Fit quality %SD
Ratios to creatine
Exam #15257-15 ID=piglet0206@lucas/epperson 02/06/2019 12:47 jpress TE/TR/NS=35/2000/512 TG/R1/R2=159/13/28 2.2mL
P24576.7 (Stanford Lucas Center)
Data of: Radiological Sciences Laboratory, Stanford University School of Medicine
LCModel (Version 6.3-1J) Copyright: S.W. Provencher.
Ref.: Magn. Reson. Med. 30:672-679 (1993).
14-February-2019
Residuals
Red trace is fit
Black trace is spectroscopy data
Data Fit with LCModel
Chemical Shift (ppm)
Conc. %SD /Cr+PCr Metabolite
2.211 4% 0.353 GPC
0.000 999% 0.000 Ala
1.021 48% 0.163 Tau
0.781 50% 0.125 Lac
0.965 65% 0.154 Gln
5.104 9% 0.815+Glc
1.558 30% 0.249 GABA
1.786 39% 0.285 Cr
4.480 15% 0.715 PCr
2.816 25% 0.449 Asc
9.197 4% 1.468 mI
10.476 4% 1.672 NAA
2.940 29% 0.469 Asp
0.507 67% 8.1E-02 NAAG
1.057 32% 0.169 GSH
0.462 85% 7.4E-02 GSSG
0.599 15% 9.6E-02 Scyllo
0.000 999% 0.000 PCh
1.714 56% 0.274 2HG
2.076 40% 0.331 PE
6.424 11% 1.025 Glu
2.951 13% 0.471 -CrCH2
2.211 4% 0.353 GPC+PCh
10.983 3% 1.753 NAA+NAAG
6.266 4% 1.000 Cr+PCr
7.389 11% 1.179 Glu+Gln
0.000 999% 0.000 Lip13a
0.645 138% 0.103 Lip13b
0.125 363% 2.0E-02 Lip09
4.981 14% 0.795 MM09
0.108 279% 1.7E-02 Lip20
7.466 19% 1.191 MM20
1.396 42% 0.223 MM12
5.157 21% 0.823 MM14
1.363 52% 0.217 MM17
0.645 138% 0.103 Lip13a+Lip13b
7.198 14% 1.149 MM14+Lip13a+L
5.105 12% 0.815 MM09+Lip09
7.574 19% 1.209 MM20+Lip20
DIAGNOSTICS
1 info STARTV 20
2 info's RFALSI 4
Doing Water-Scaling



25 spectra collected at different times

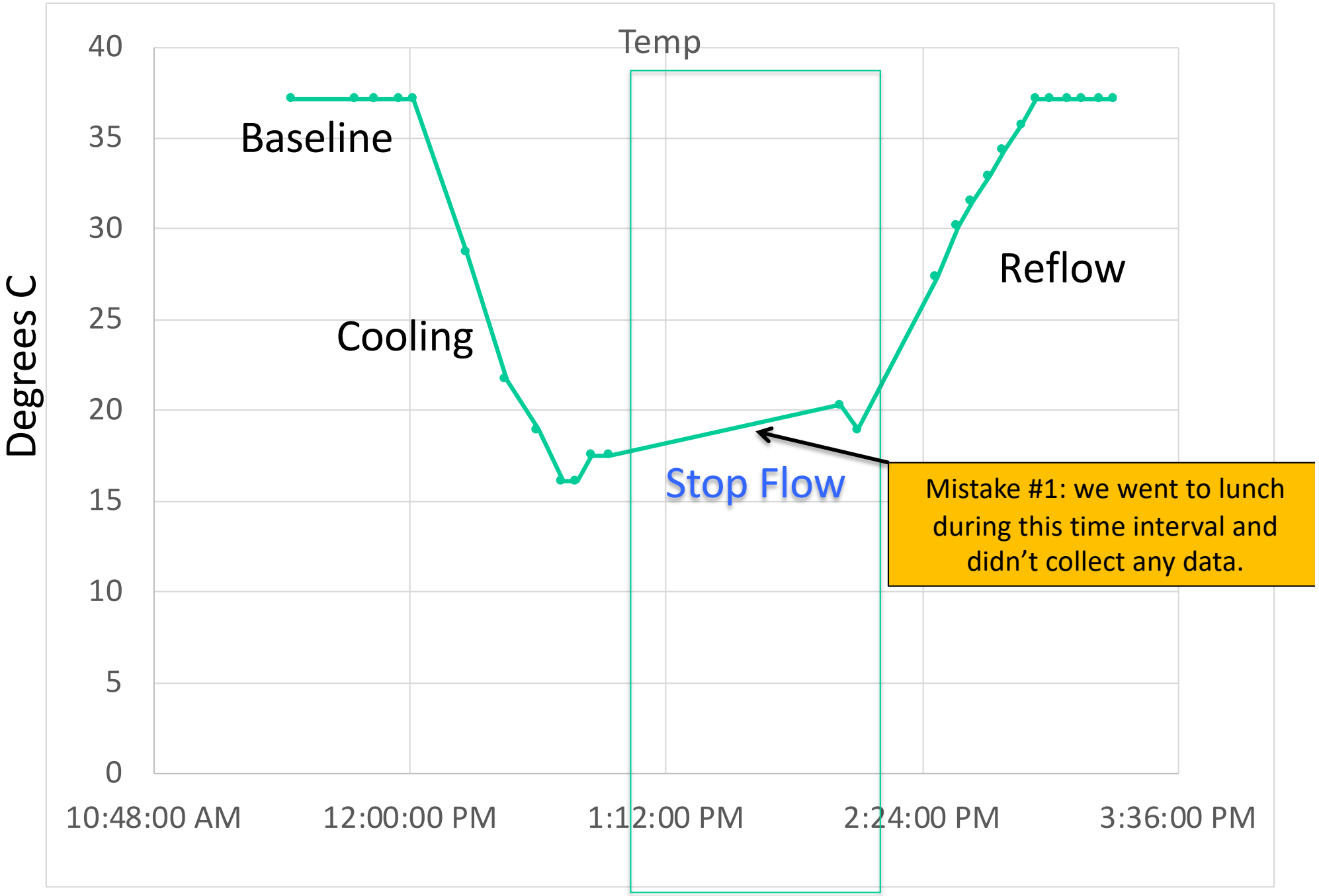
How did we measure brain temperature?

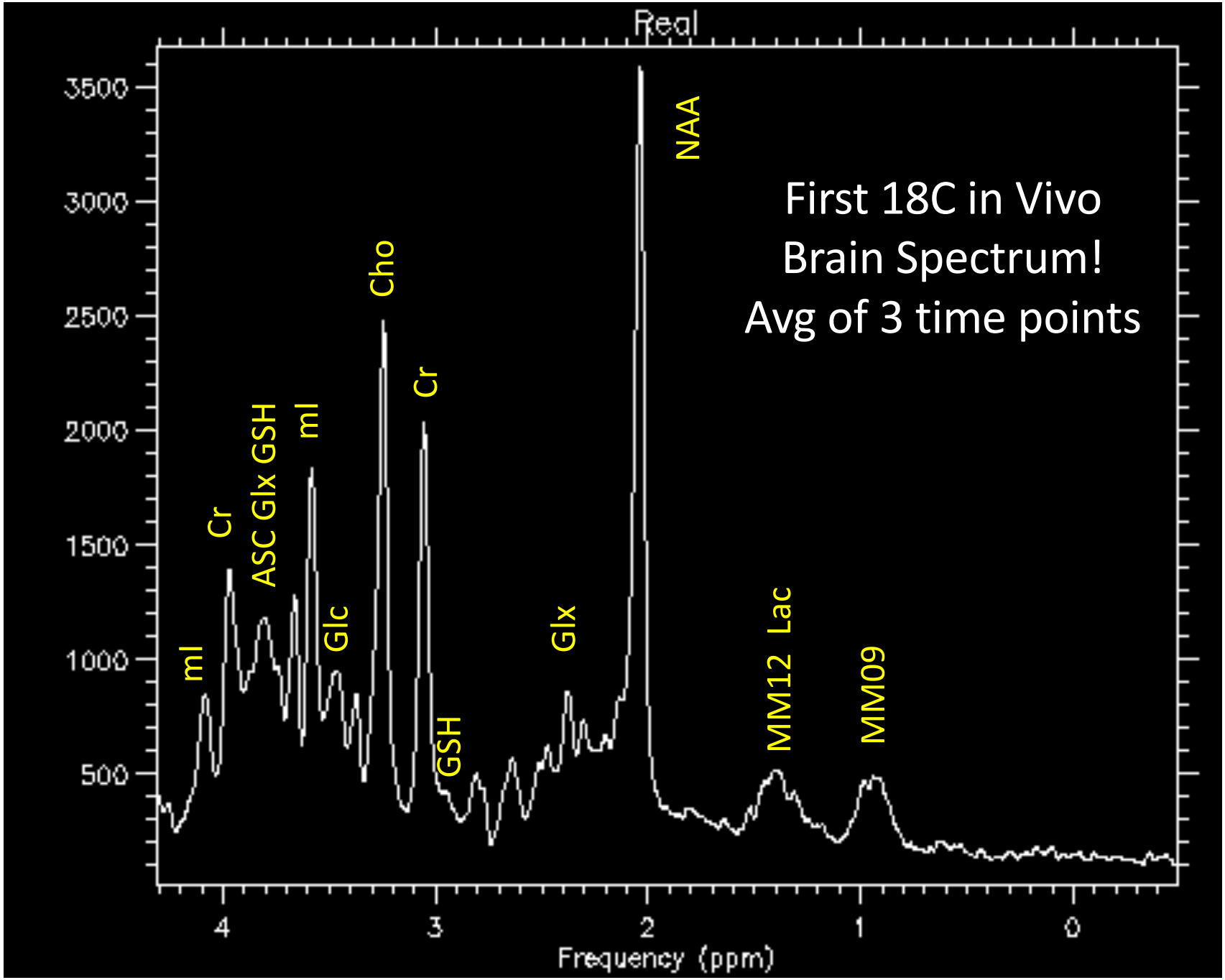
Chemical Shift Difference between NAA and Water gives temperature in voxel

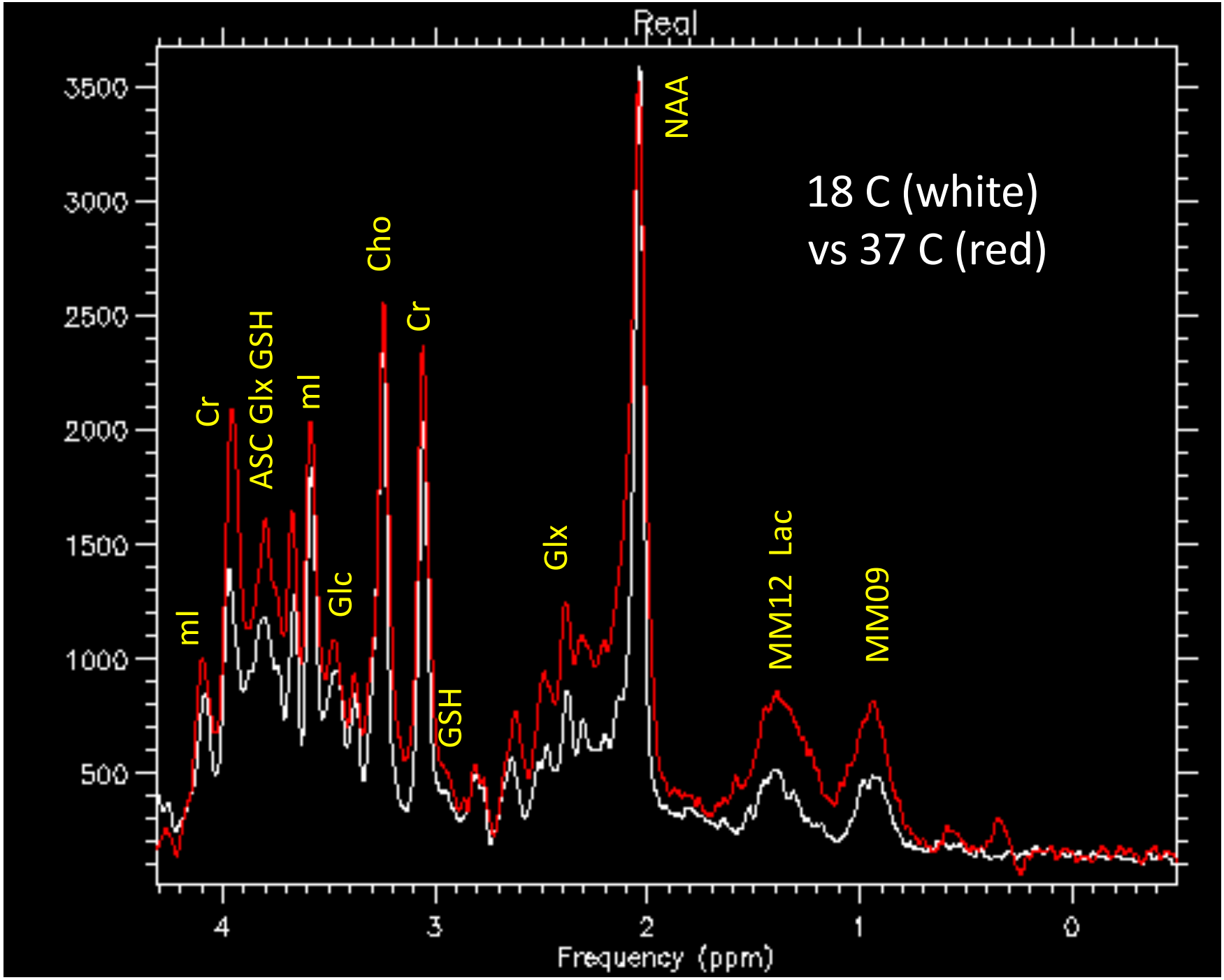
	water	NAA	Delta	Temp	time	Description
P13824.7	4.7009	2.0441	2.6568	37.16	11:27	whole brain
P16384.7	4.7009	2.0441	2.6568	37.16	11:45	baseline
P17408.7	4.7009	2.0441	2.6568	37.16	11:50	contralateral brain
P18432.7	4.7009	2.0441	2.6568	37.16	11:57	baseline
P19456.7	4.7009	2.0441	2.6568	37.16	12:01	baseline
P20480.7	4.7009	1.9546	2.7463	28.75	12:16	34-30C
P21504.7	4.7009	1.8799	2.821	21.73	12:27	25-23C
P22528.7	4.7009	1.8501	2.8508	18.92	12:36	20-18C
P23552.7	4.7009	1.8202	2.8807	16.11	12:43	18C
P24576.7	4.7009	1.8202	2.8807	16.11	12:47	18C
P25600.7	4.7009	1.8352	2.8657	17.52	12:51	18C
P27136.7	4.7009	1.8352	2.8657	17.52	12:56	18C 115
P29184.7	4.7009	1.865	2.8359	20.33	14:01	21C
P30208.7	4.7009	1.8501	2.8508	18.92	14:06	21C
P31744.7	4.7009	1.9396	2.7613	27.34	14:28	24-25C
P32768.7	4.7009	1.9695	2.7314	30.15	14:34	25-26C
P33792.7	4.7009	1.9844	2.7165	31.55	14:38	26-27C
P34816.7	4.7009	1.9994	2.7015	32.96	14:43	27-28C
P35840.7	4.7009	2.0143	2.6866	34.36	14:47	28-29C
P36864.7	4.7009	2.029	2.6719	35.74	14:52	29-30C
P37888.7	4.7009	2.0441	2.6568	37.16	14:56	30-31C
P38912.7	4.7009	2.0441	2.6568	37.16	15:00	31C
P39936.7	4.7009	2.0441	2.6568	37.16	15:05	31.8-32.3C
P40960.7	4.7009	2.0441	2.6568	37.16	15:09	32.3-32.7C
P41984.7	4.7009	2.0441	2.6568	37.16	15:14	32.7-33C
P43008.7	4.7009	2.0441	2.6568	37.16	15:18	33.0-33.3C

Raw data file names

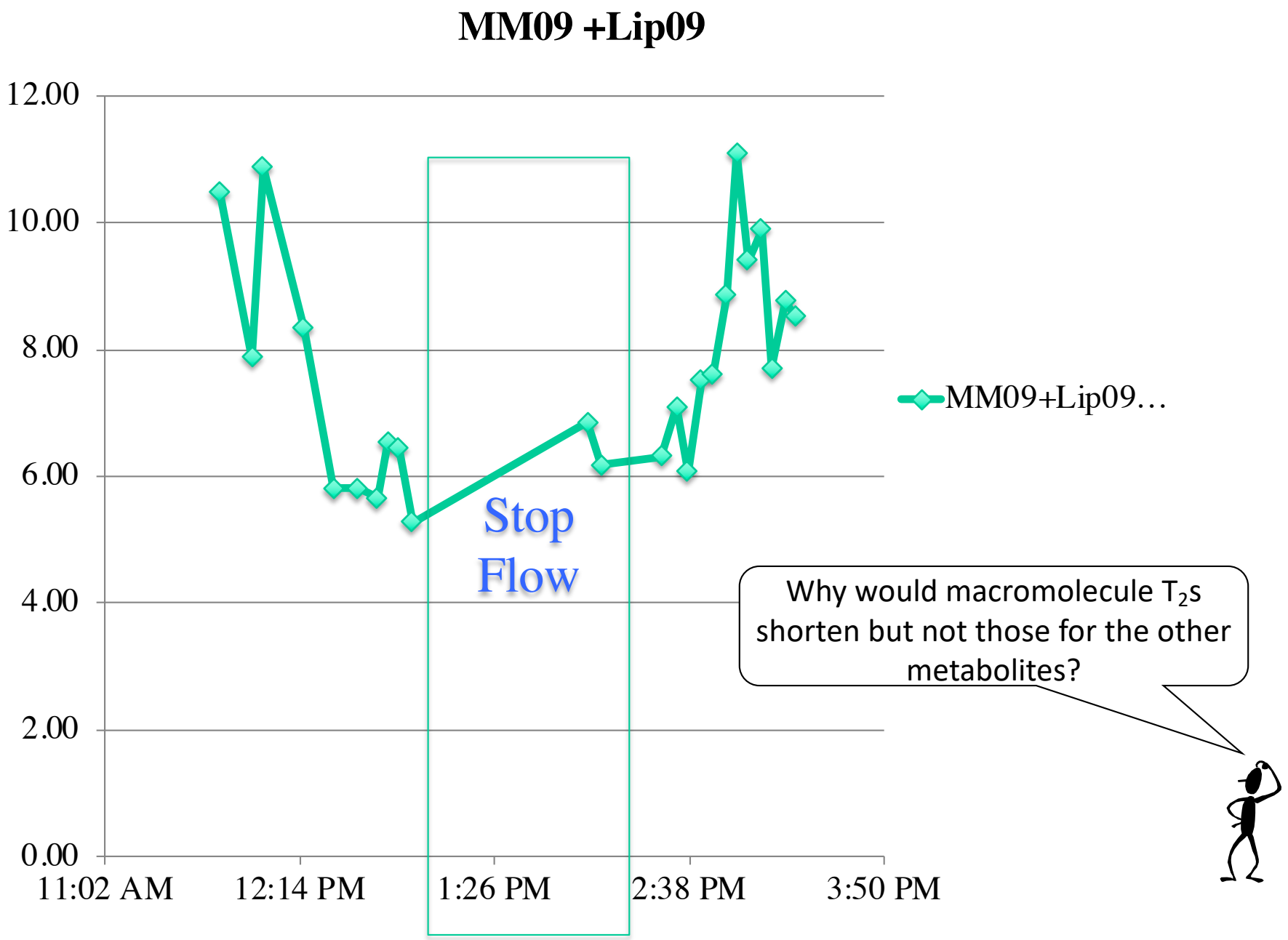
Back to 37 C in brain ahead of recorded body temp


Temp
Degrees C
40
35
30
25
20
15
10
5
0
10:48:00 AM
12:00:00 PM
1:12:00 PM
2:24:00 PM
3:36:00 PM
Baseline
Cooling
Stop Flow
Reflow
Mistake #1: we went to lunch during this time interval and didn't collect any data.

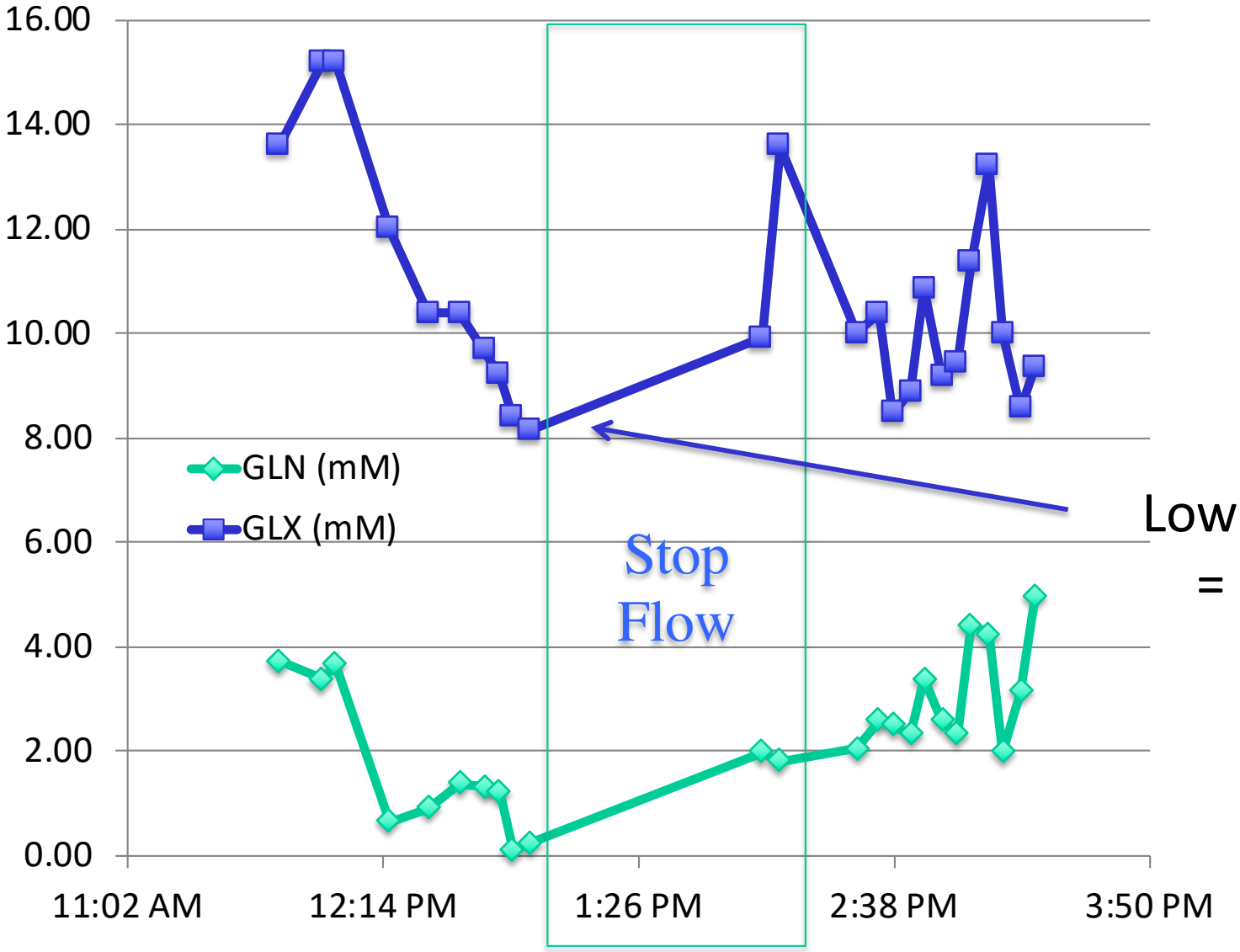

Real
3500
3000
2500
2000
1500
1000
500
4
3
2
1
0
Frequency (ppm)
mI
Cr
ASC Glx GSH
mI
Glc
Cho
Cr
GSH
Glx
NAA
MM12 Lac
MM09
First 18C in Vivo
Brain Spectrum!
Avg of 3 time points


Real
3500
3000
2500
2000
1500
1000
500
4
3
2
1
0
Frequency (ppm)
mI
Cr
ASC Glx GSH
mI
Glc
Cho
Cr
GSH
Glx
NAA
MM12 Lac
MM09
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)

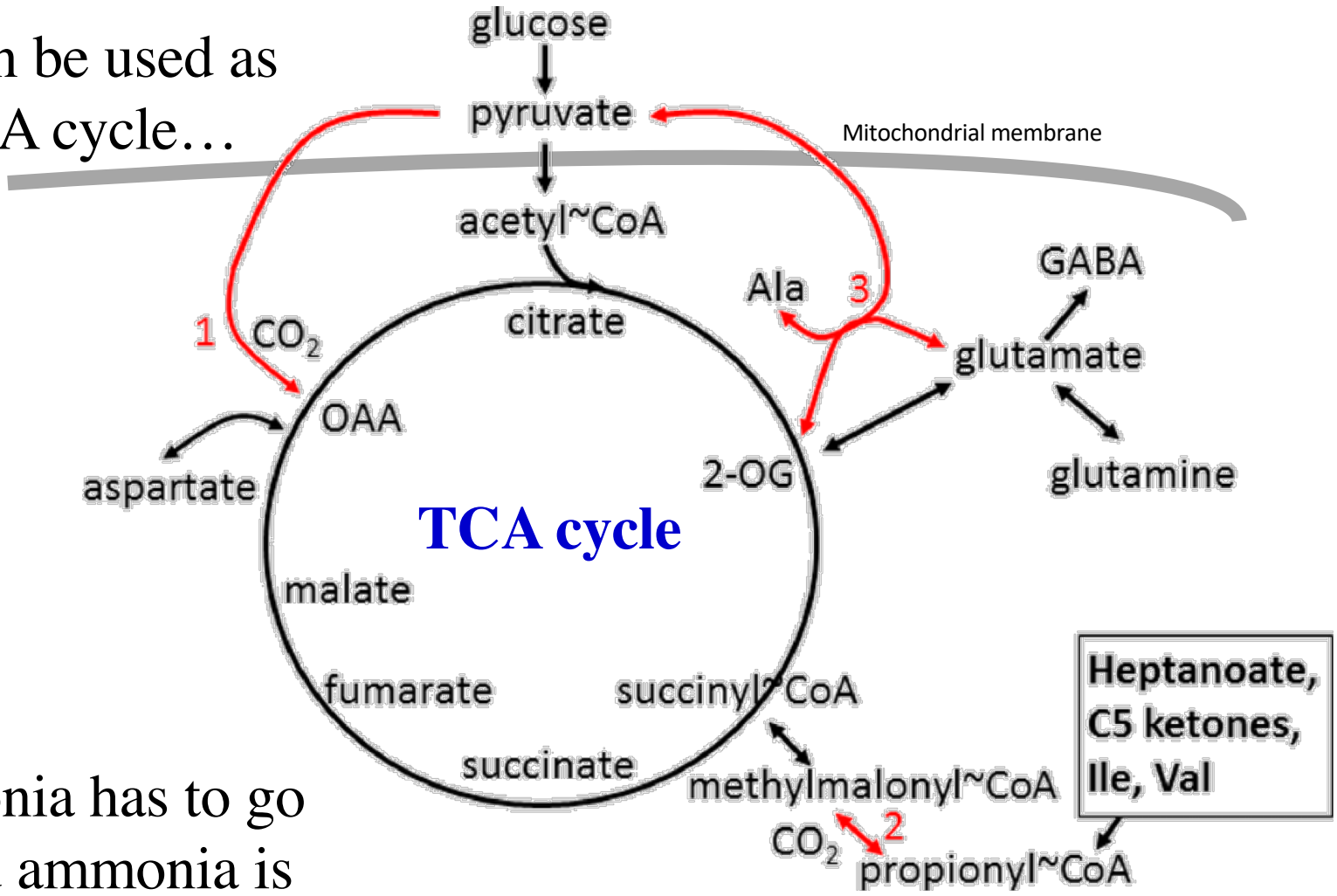


Largest metabolic effect of cooling to 18 C appears to be reduction in glutamate and glutamine

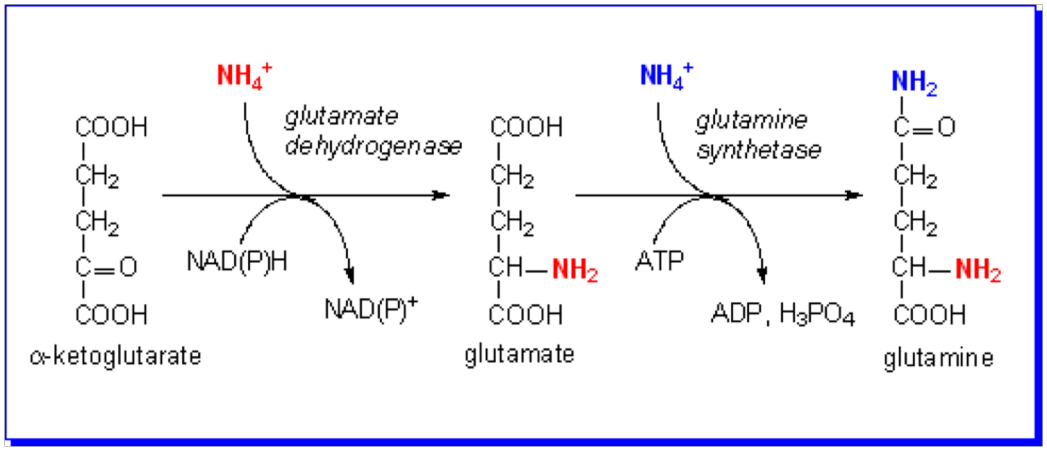


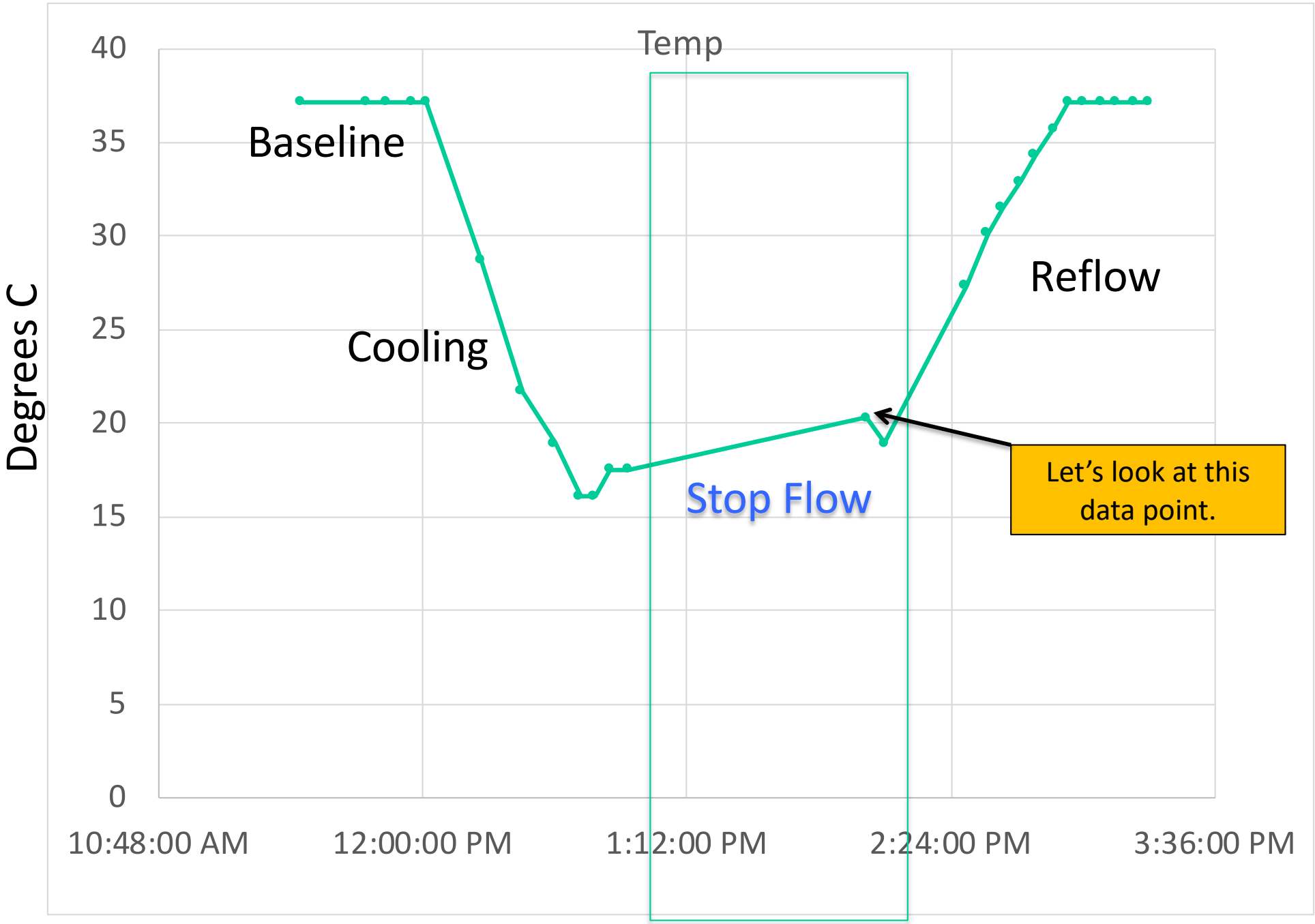
Low Glu and Gln = High NH_4^+

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.




Temp
Degrees C
40
35
30
25
20
15
10
5
0
10:48:00 AM
12:00:00 PM
1:12:00 PM
2:24:00 PM
3:36:00 PM
Baseline
Cooling
Stop Flow
Reflow
Let's look at this data point.

gv: /home/lcmuser/.lcmodel/temp/14d-14h-06m-05s-12029pid/ps

File | State | Page | Seascape | 1.000 | BBox | /home/lcmuser/.lcmodel/temp/14d-14h-06m-05s-12029pid/ps | Thu Feb 14 14:06:37 2019

Variable Size

Open
Print All
Print Marked
Save All
Save Marked

<< >>
Reload

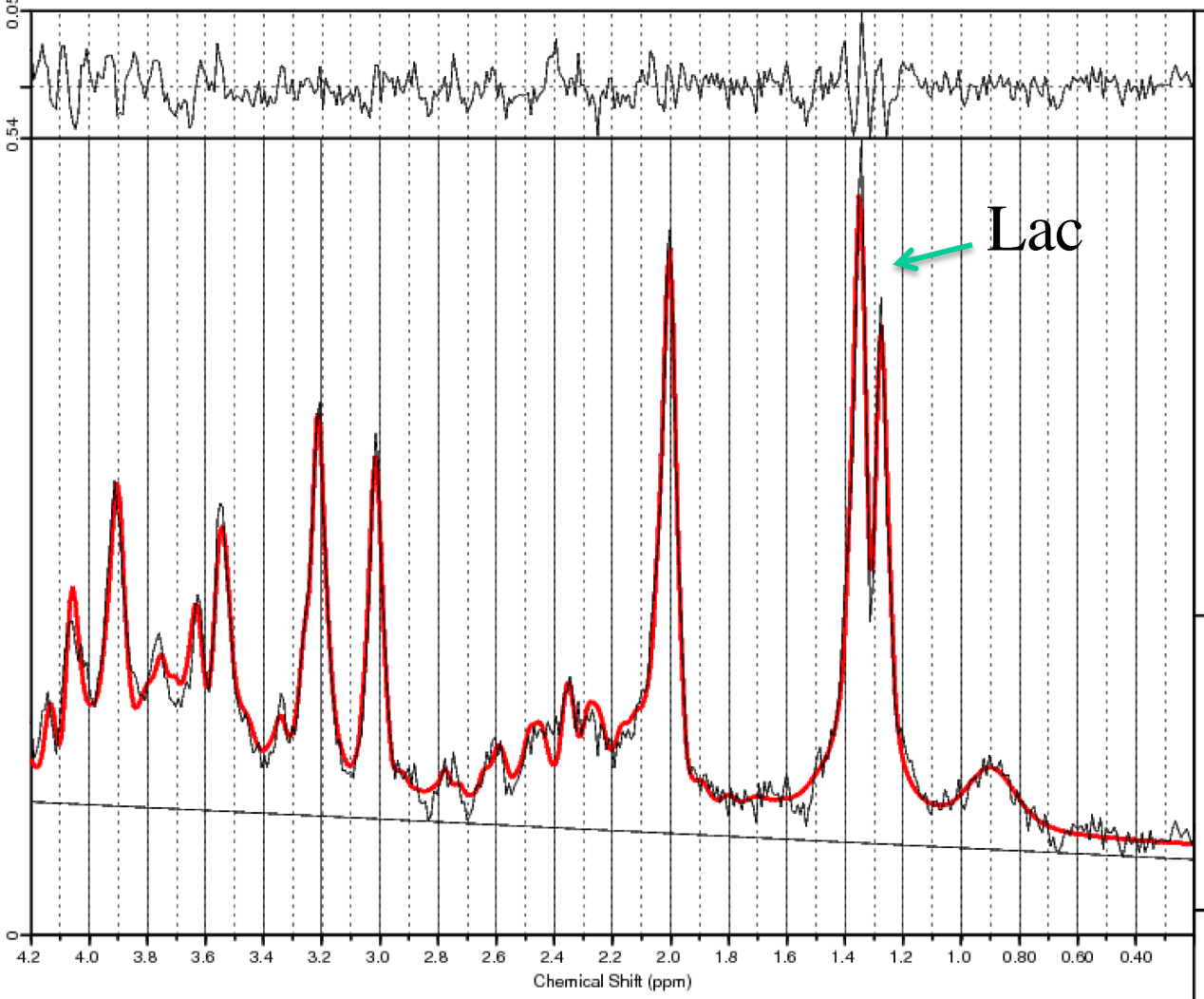
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

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)

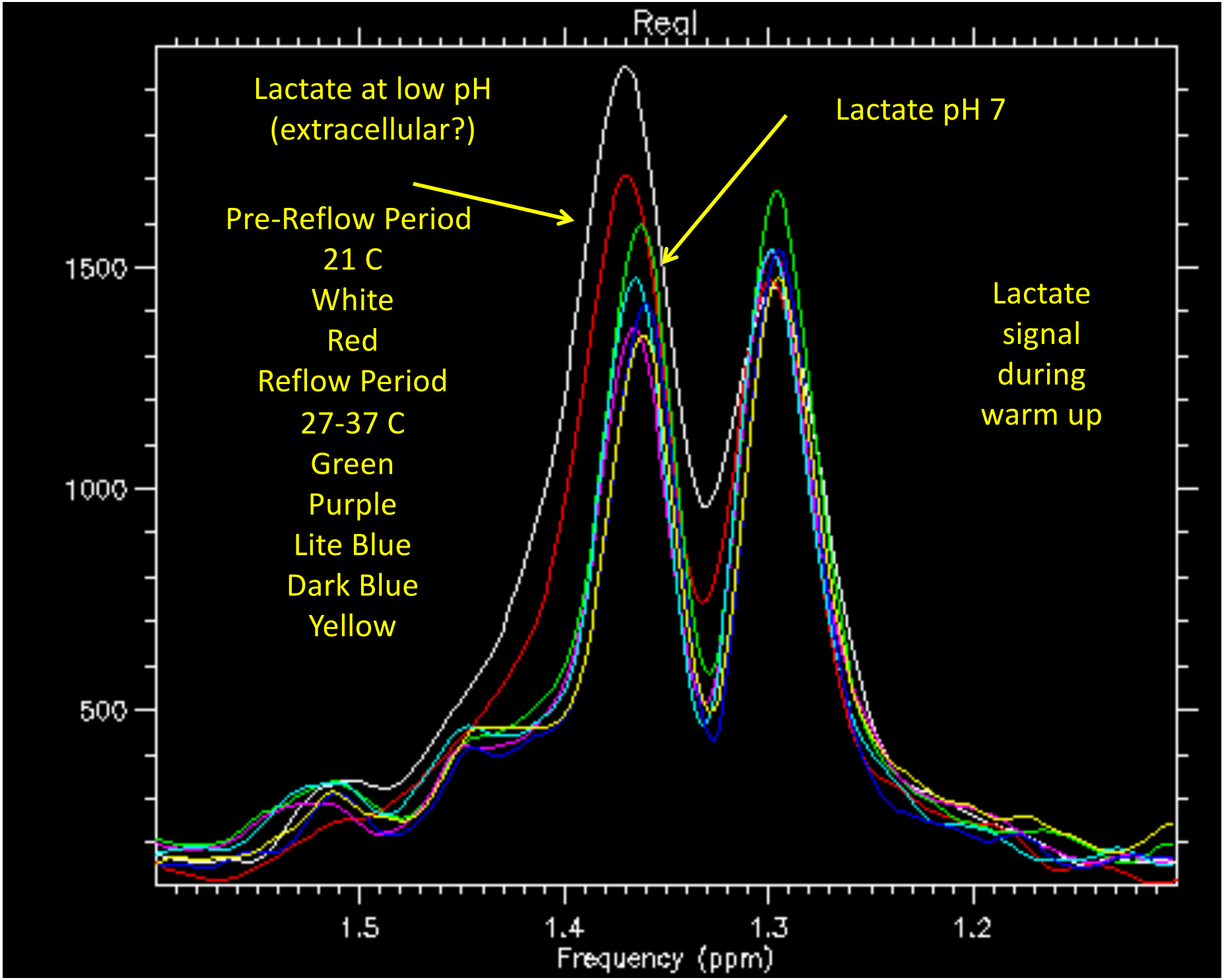
Data of: Radiological Sciences Laboratory, Stanford University School of Medicine

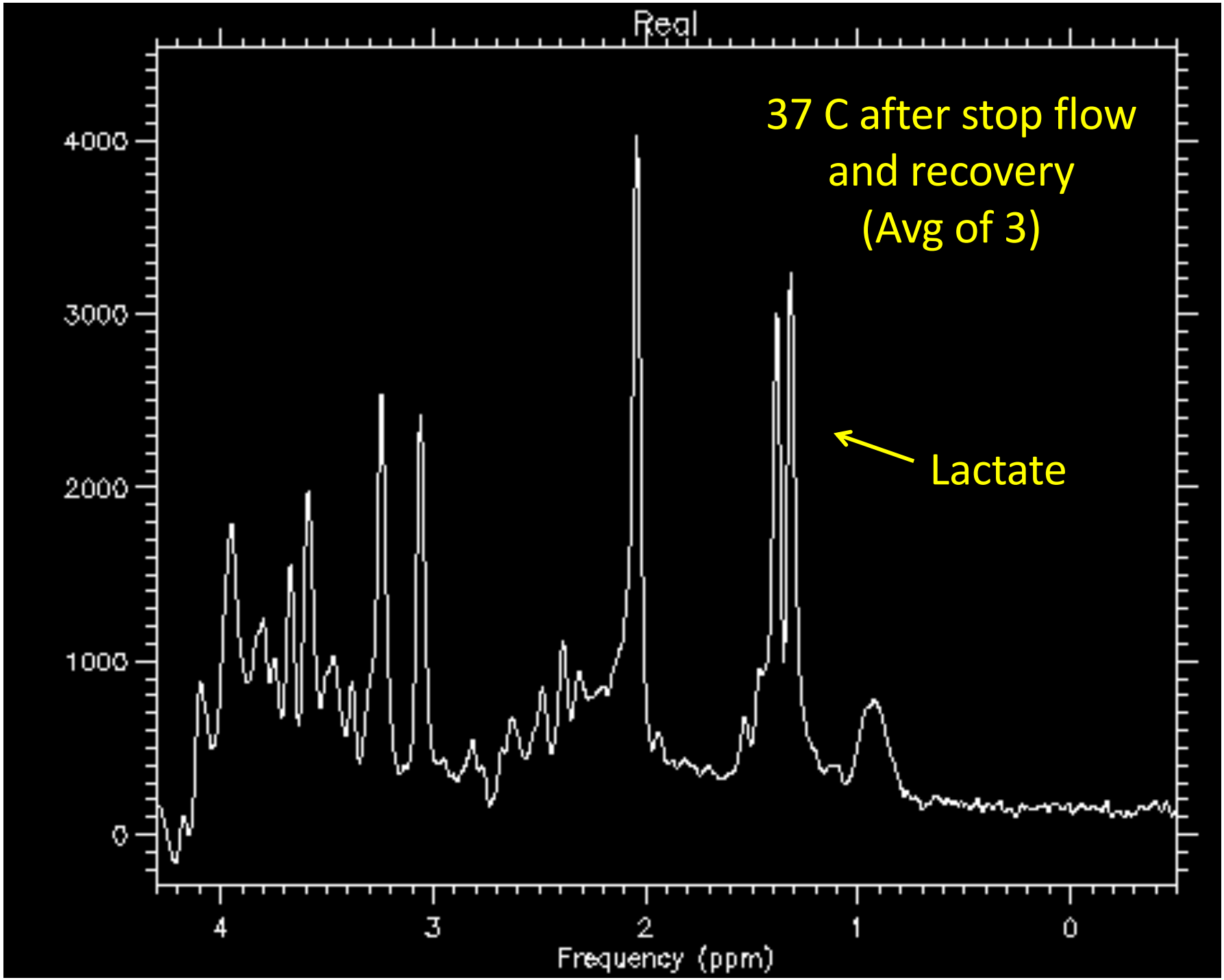
LCModel (Version 6.3-1J) Copyright: S.W. Provencher. *Ref.: Magn. Reson. Med. 30:672-679 (1993).* *14-February-2019 14:06*

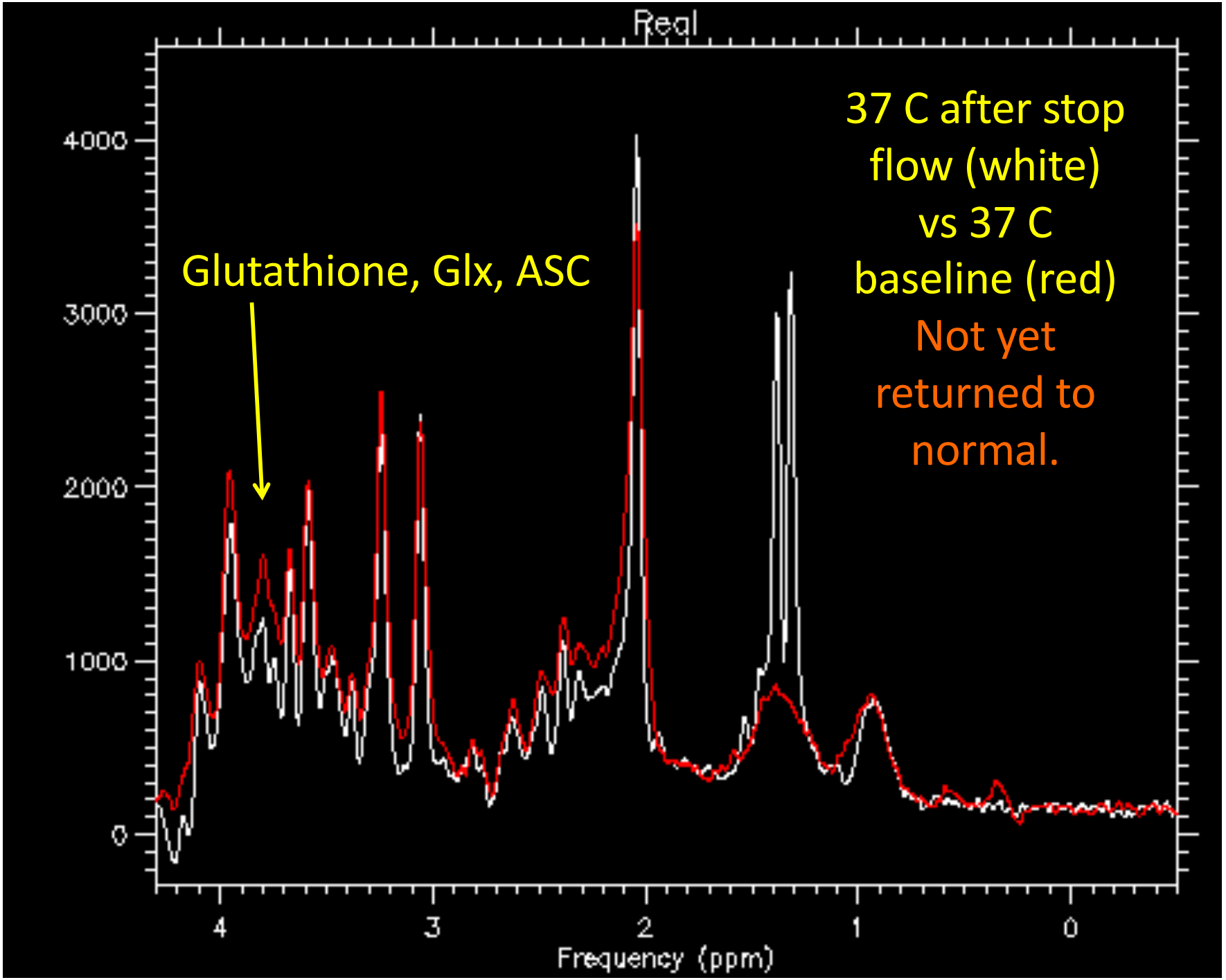


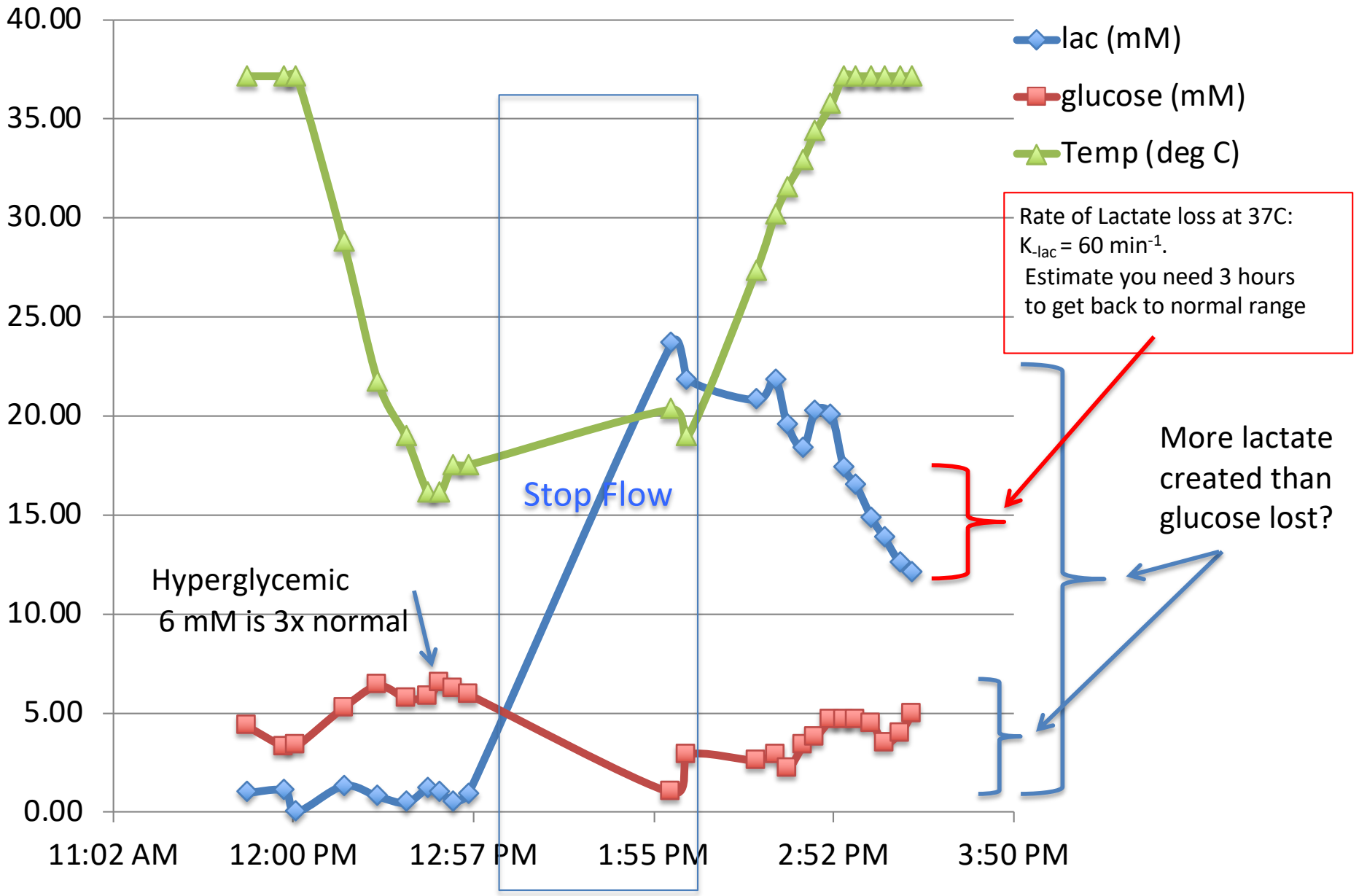
Conc.	%SD	/Cr+PCr	Metabolite
1.619	13%	0.248	GPC
0.106	592%	1.6E-02	Ala
0.000	999%	0.000	Tau
19.301	6%	2.955	Lac
1.624	68%	0.249	Gln
0.785	57%	0.120	Glc
0.653	100%	0.100	GABA
5.439	8%	0.833	Cr
1.091	39%	0.167	PCr
0.000	999%	0.000	Asc
9.596	4%	1.469	mI
9.419	4%	1.442	NAA
2.176	47%	0.333	Asp
1.417	28%	0.217	NAAG
1.392	35%	0.213	GSH
0.665	68%	0.102	GSSG
0.465	22%	7.1E-02	Scyllo
0.479	48%	7.3E-02	PCh
4.242	32%	0.649	2HG
5.373	18%	0.823	PE
6.503	17%	0.996	Glu
0.000	999%	0.000	-CrCH2
2.098	5%	0.321	GPC+PCh
10.836	3%	1.659	NAA+NAAG
6.531	4%	1.000	Cr+PCr
8.126	13%	1.244	Glu+Gln
3.050	100%	0.467	Lip13a
11.601	6%	1.776	Lip13b
3.425	45%	0.524	Lip09
2.164	65%	0.331	MM09
1.057	114%	0.162	Lip20
1.773	107%	0.271	MM20
0.867	91%	0.133	MM12
5.102	35%	0.781	MM14
1.307	57%	0.200	MM17
14.650	20%	2.243	Lip13a+Lip13b
20.620	10%	3.157	MM14+Lip13a+L
5.589	12%	0.856	MM09+Lip09
2.830	62%	0.433	MM20+Lip20

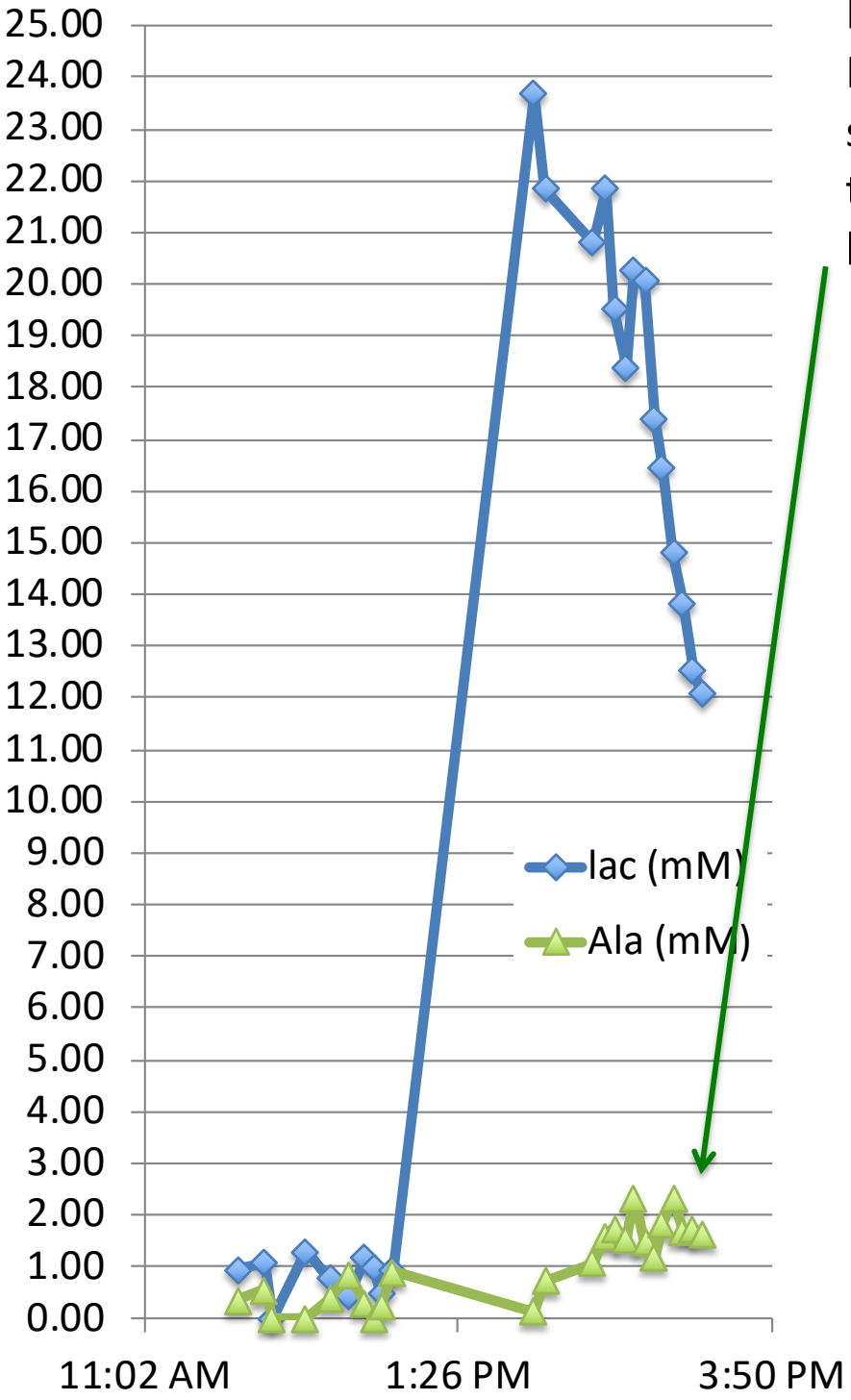
DIAGNOSTICS
2 info's RFALSI 4
3 info's RFALSI 11
Doing Water-Scaling


Real
Lactate at low pH
(extracellular?)
Lactate pH 7
Pre-Reflow Period
21 C
White
Red
Reflow Period
27-37 C
Green
Purple
Lite Blue
Dark Blue
Yellow
Lactate
signal
during
warm up
1500
1000
500
1.5
1.4
1.3
1.2
Frequency (ppm)

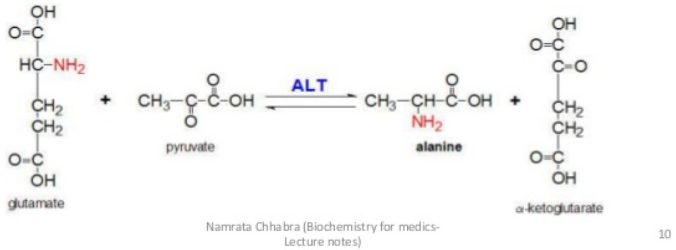

Real
37 C after stop flow
and recovery
(Avg of 3)
Lactate
4000
3000
2000
1000
0
4
3
2
1
0
Frequency (ppm)


Real
4000
3000
2000
1000
0
4
3
2
1
0
Frequency (ppm)
Glutathione, Glx, ASC
37 C after stop flow (white) vs 37 C baseline (red)
Not yet returned to normal.

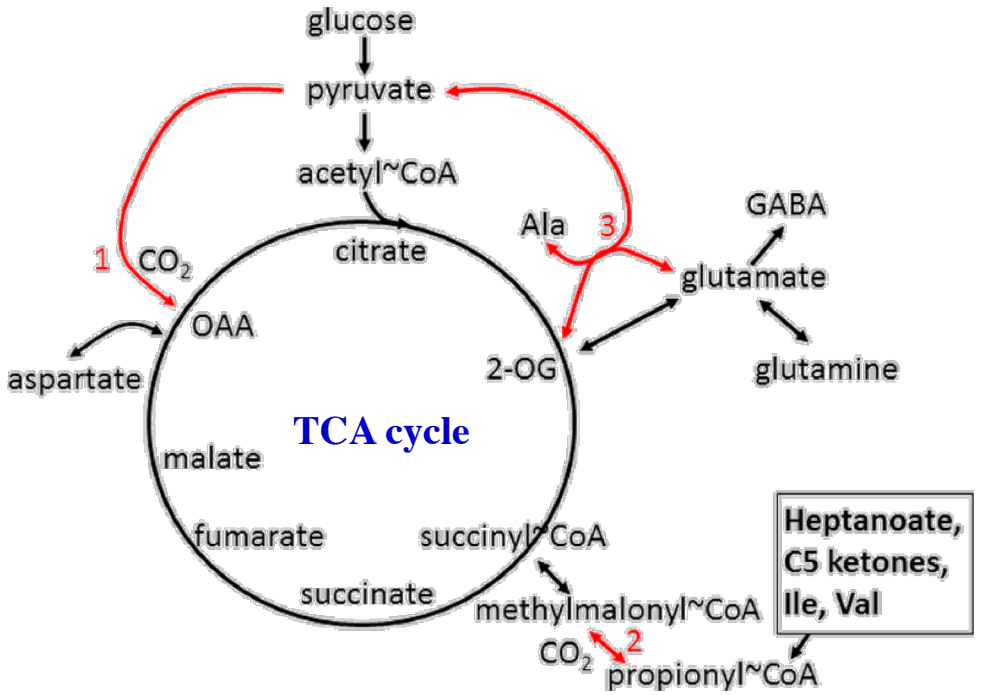

40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
11:02 AM
12:00 PM
12:57 PM
1:55 PM
2:52 PM
3:50 PM
lac (mM)
glucose (mM)
Temp (deg C)
Rate of Lactate loss at 37C:
K-lac = 60 min-1.
Estimate you need 3 hours
to get back to normal range
Stop Flow
Hyperglycemic
6 mM is 3x normal
More lactate
created than
glucose lost?

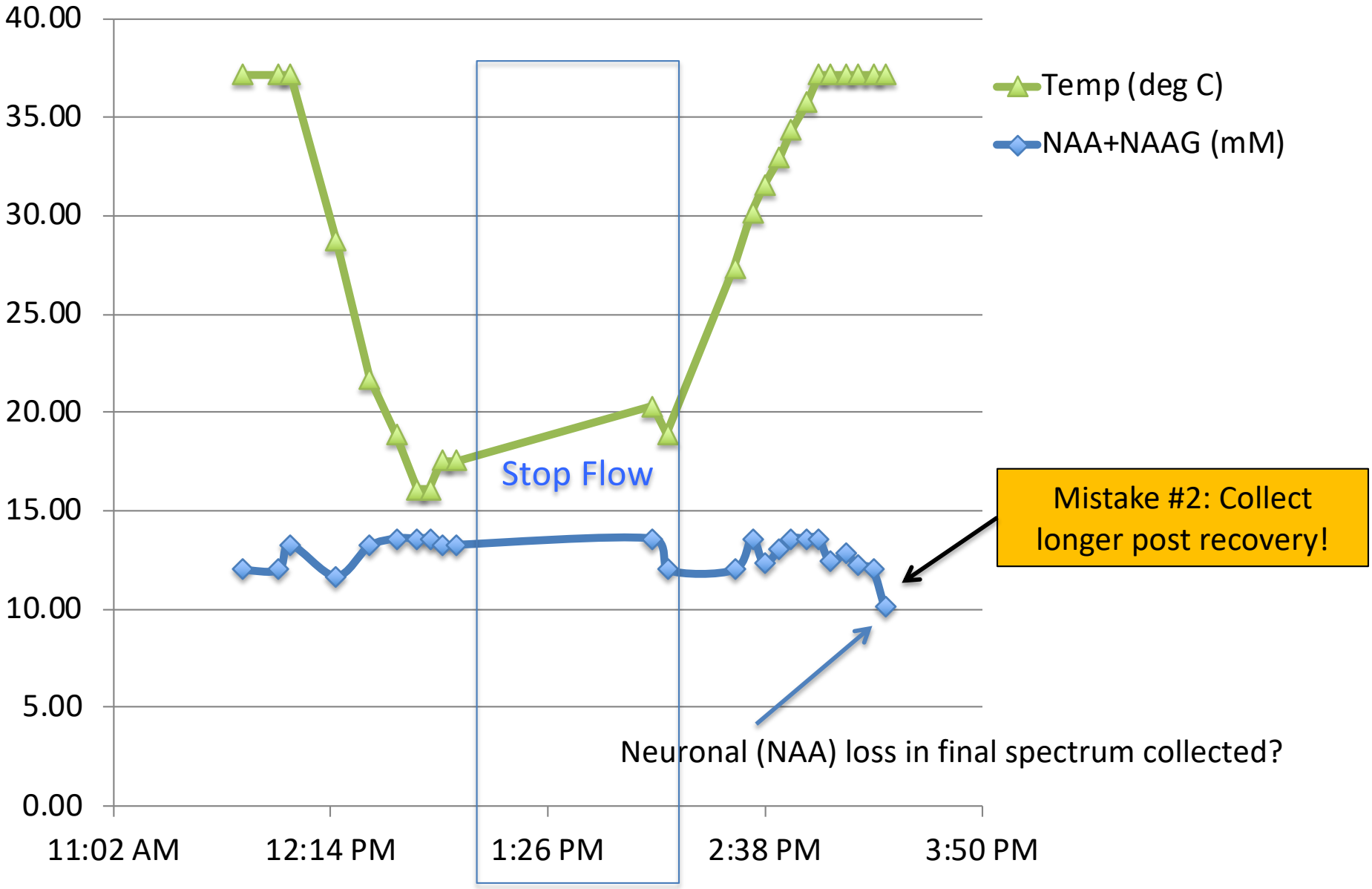


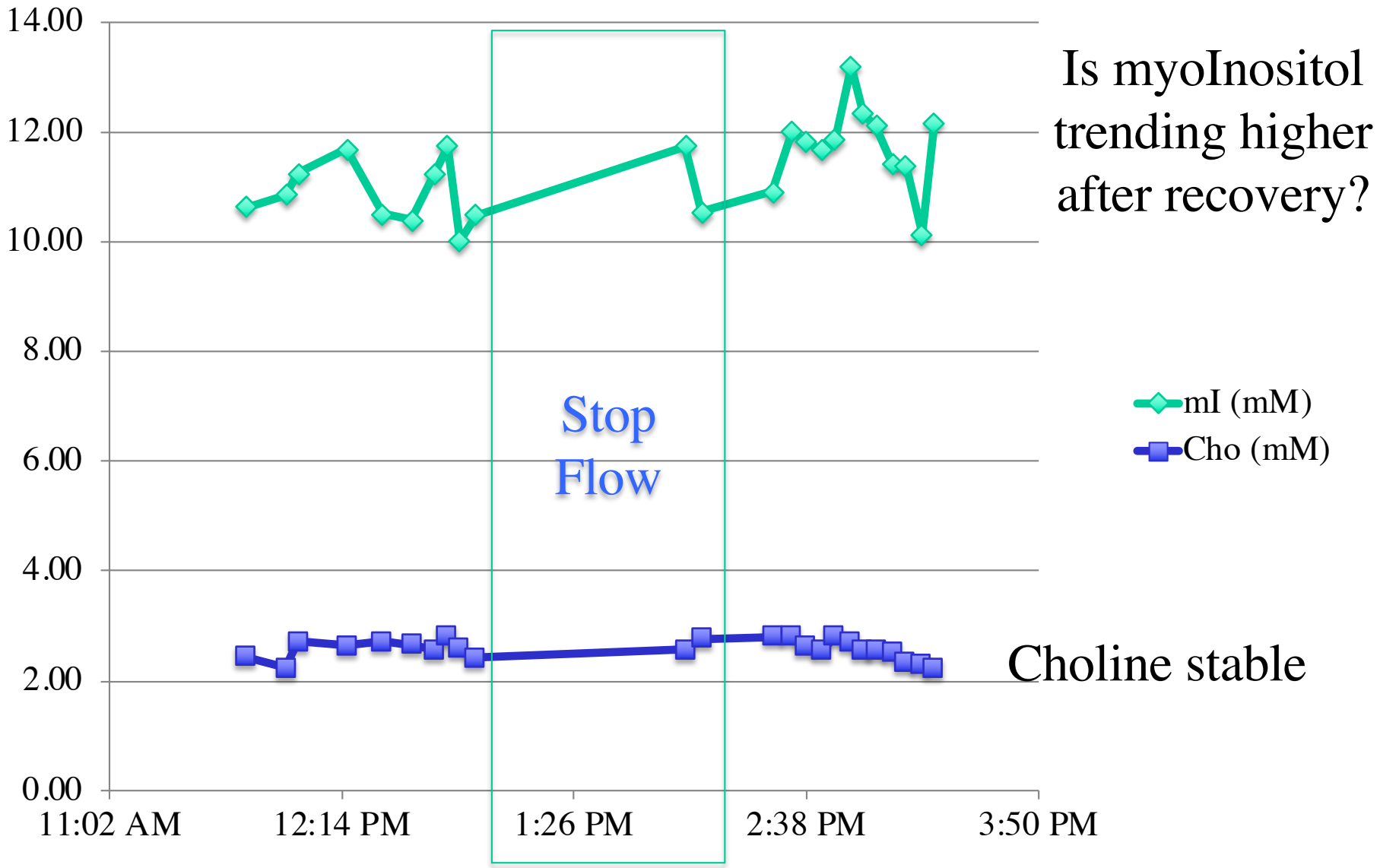
Predict that with high lactate and high NH_4^+ due to reduced Glu & Gln we should see some conversion of lactate to alanine (not usually observed in brain)



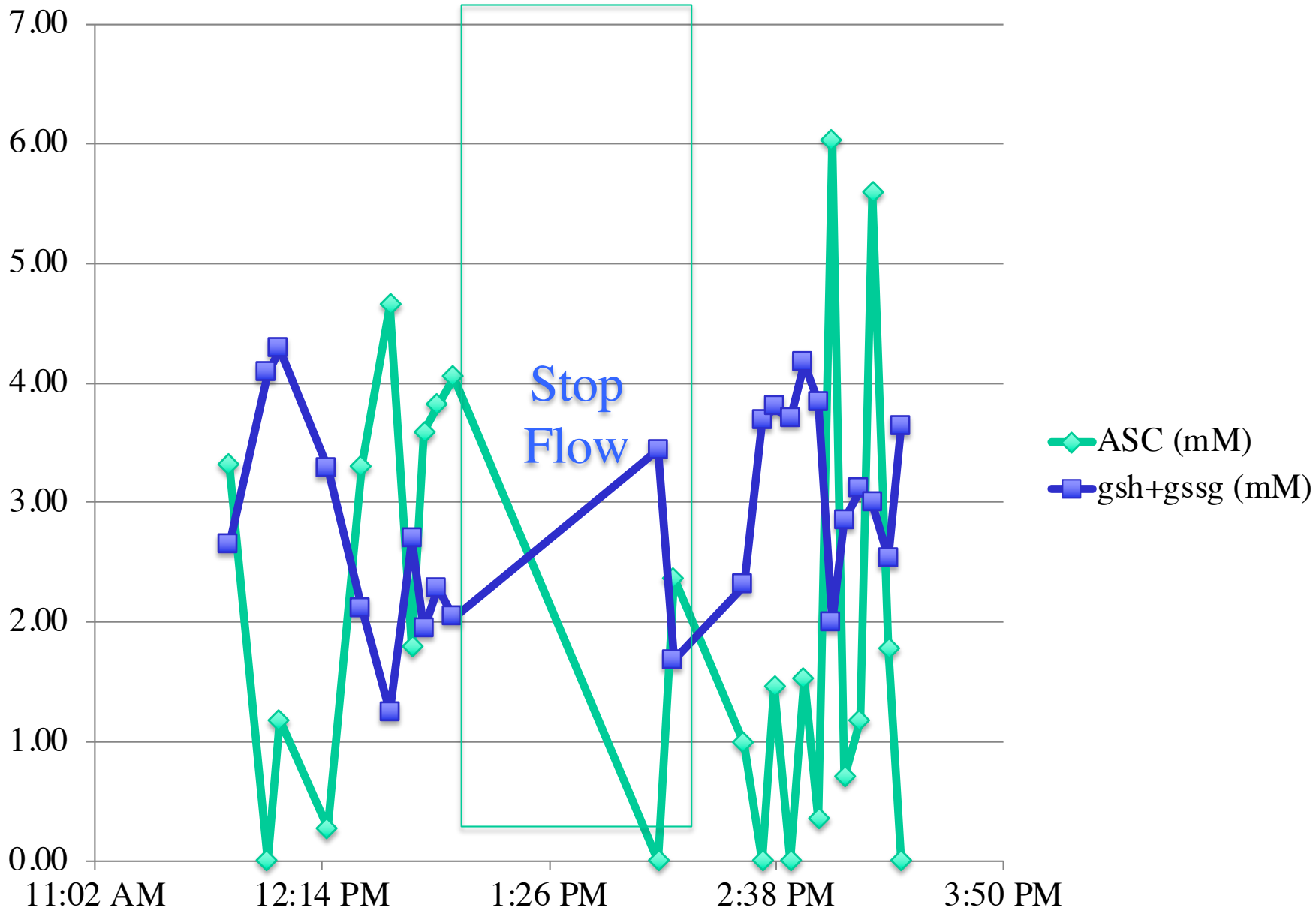
Between NH_4^+ scavenging and anaplerosis, could high lactate be an overall advantage to recovery .



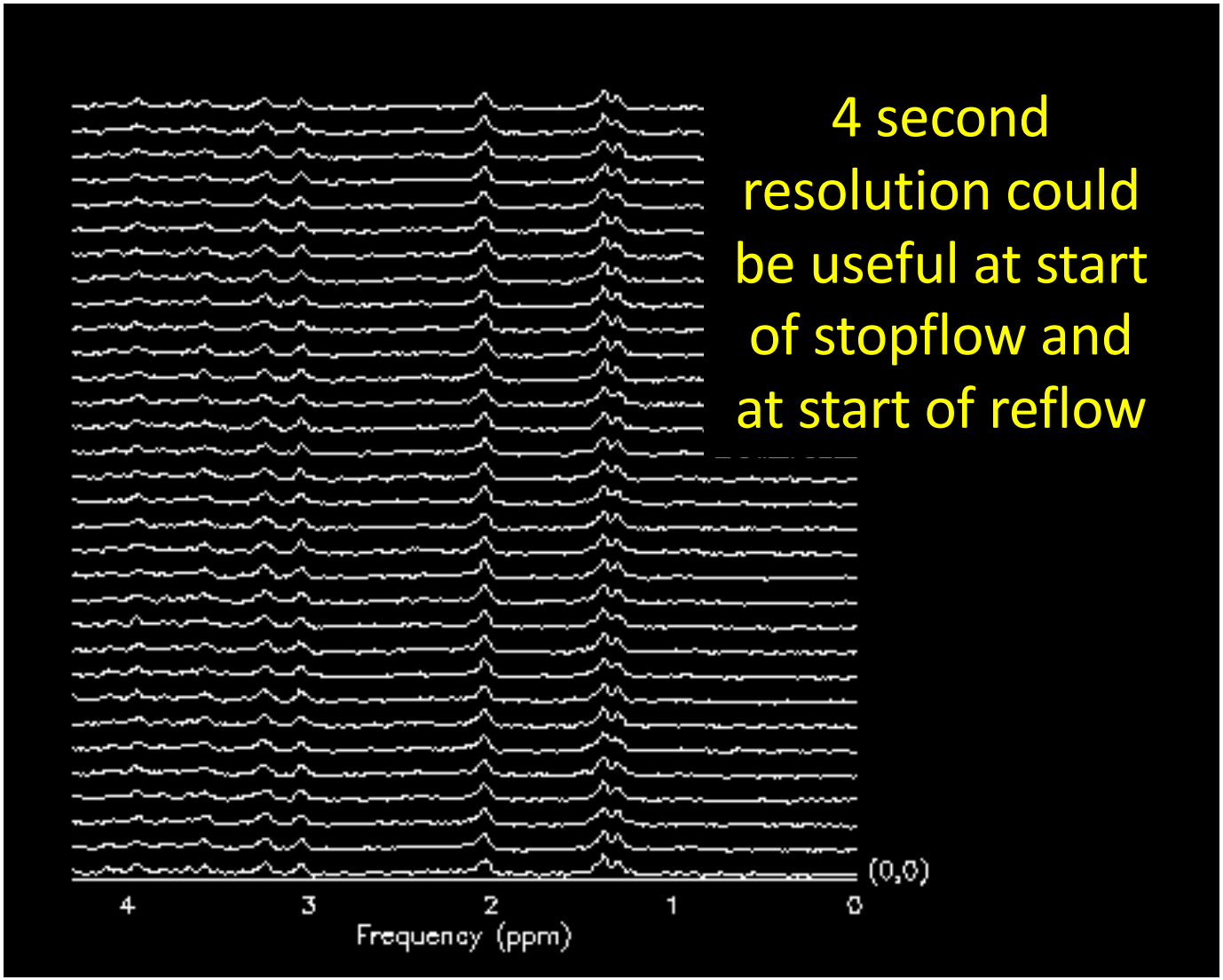

40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
11:02 AM
12:14 PM
1:26 PM
2:38 PM
3:50 PM
Temp (deg C)
NAA+NAAG (mM)
Stop Flow
Mistake #2: Collect longer post recovery!
Neuronal (NAA) loss in final spectrum collected?


14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
11:02 AM
12:14 PM
1:26 PM
2:38 PM
3:50 PM
Stop
Flow
mI (mM)
Cho (mM)
Is myoInositol trending higher after recovery?
Choline stable

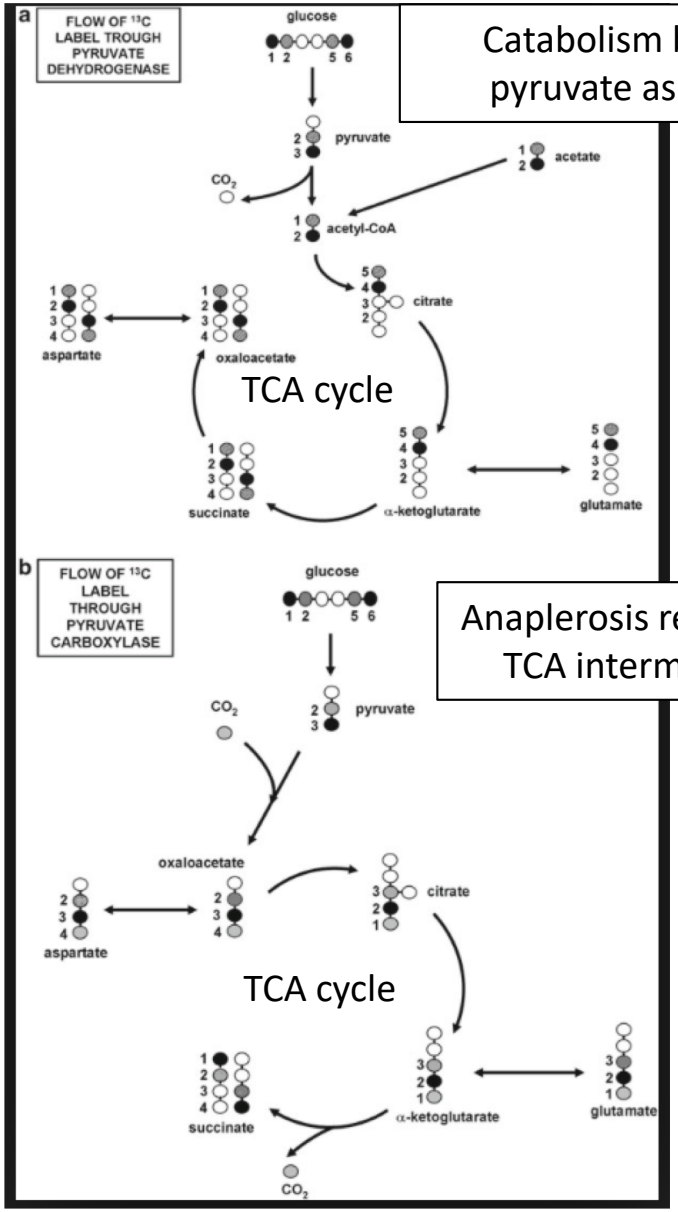
ASC and GSH unstable even in baseline (MR physics/coil problem?)



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.

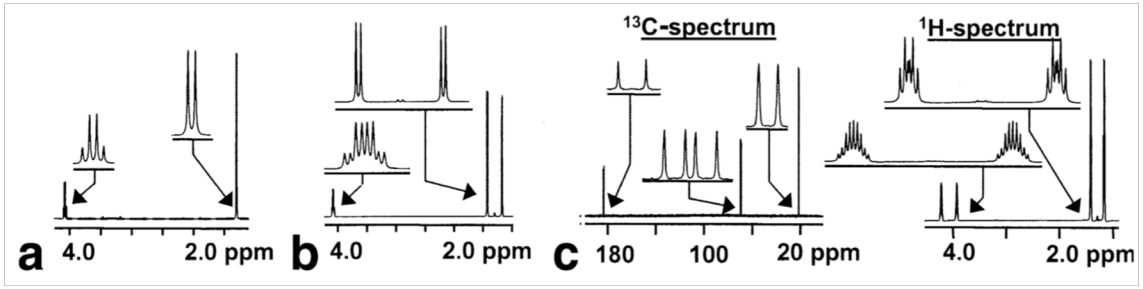


Catabolism burns pyruvate as fuel.

Anaplerosis replenishes TCA intermediates

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

Example spectra



a: 1H NMR spectrum of unlabeled sodium lactate. **b**: ^{1}H NMR spectrum of sodium [3-^{13}C] L-lactate. **c**: ^{13}C and ^{1}H NMR spectra of sodium [U-^{13}C] L-lactate. Expansions of the resonance regions are shown above the full spectra to demonstrate the fine structure.

Other stable isotopes: Deuterium, Nitrogen-15?

Consult an expert in intermediary metabolism?

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:

[3-13C]pyruvate => brain => glutamate labeling via anaplerosis or oxidation. (direct metabolism of the pyruvate skeleton)
vs.
[3-13C]pyruvate => liver => 13C-labeled glucose => brain => 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

Reference goes here

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
5. Explore using stable isotopes in future experiments. E.g. bolus of [3-^{13}C]pyruvate after restart of flow.
   - 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