

Analysis of Intermediary Metabolites

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MetBioNet April 2009

Intermediary Metabolites

- Glucose
- Lactate
- NEFA's
- 3-OH Butyrate

- Pyruvate
- Acetoacetate

Overview

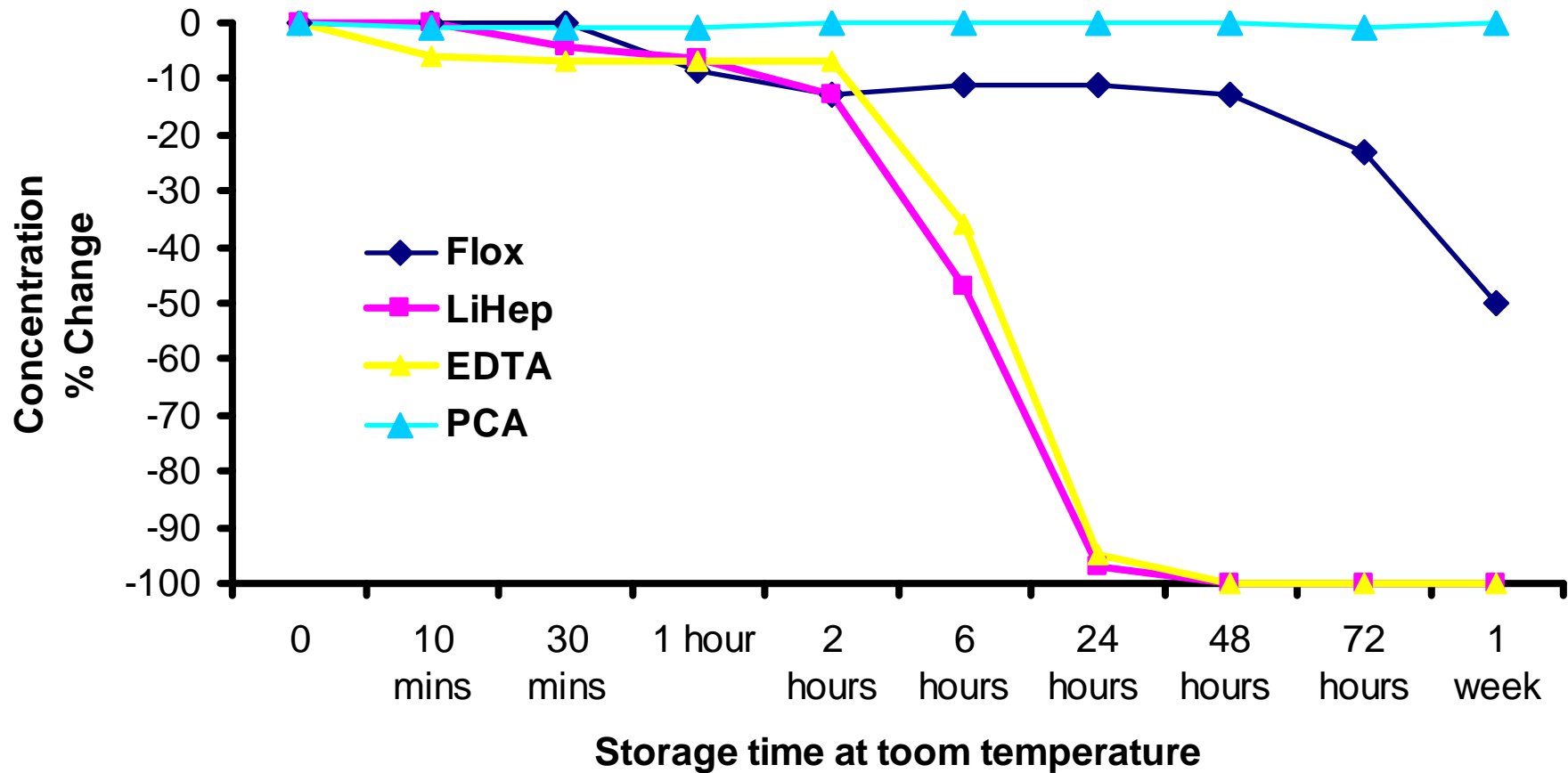
- Specimen collection and handling
- Sample stability
- Methodology – interferences & performance

Glucose

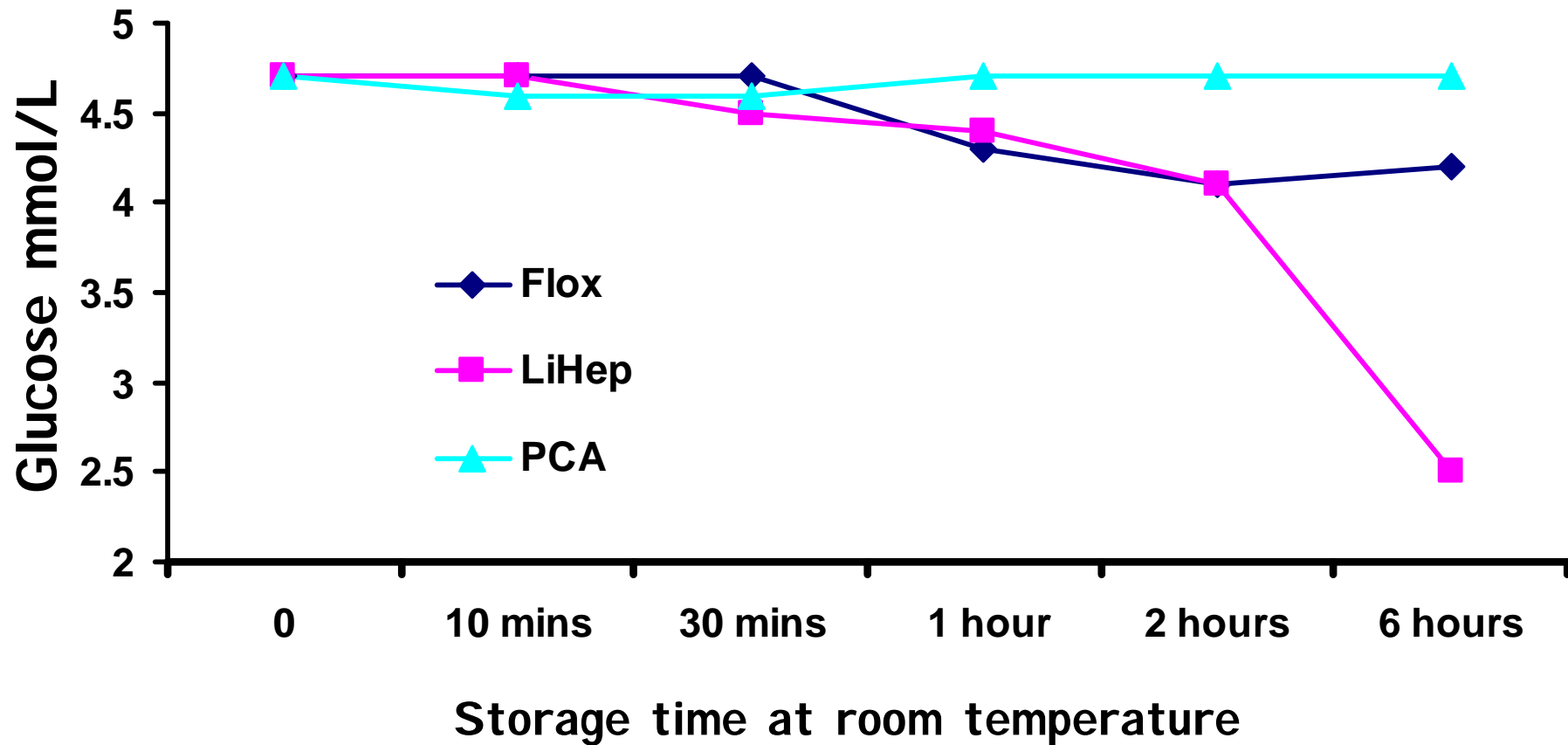
Glucose – sample collection & handling

- Continued glycolysis *in vitro* – decrease glucose ~7%/hour in un-separated blood
- Glycolytic inhibitors (Flox/FLHep)
- PCA, TCA – denatures & precipitates proteins
- Manufacturers recommend serum samples!

Effect of storage and specimen type on plasma glucose concentrations

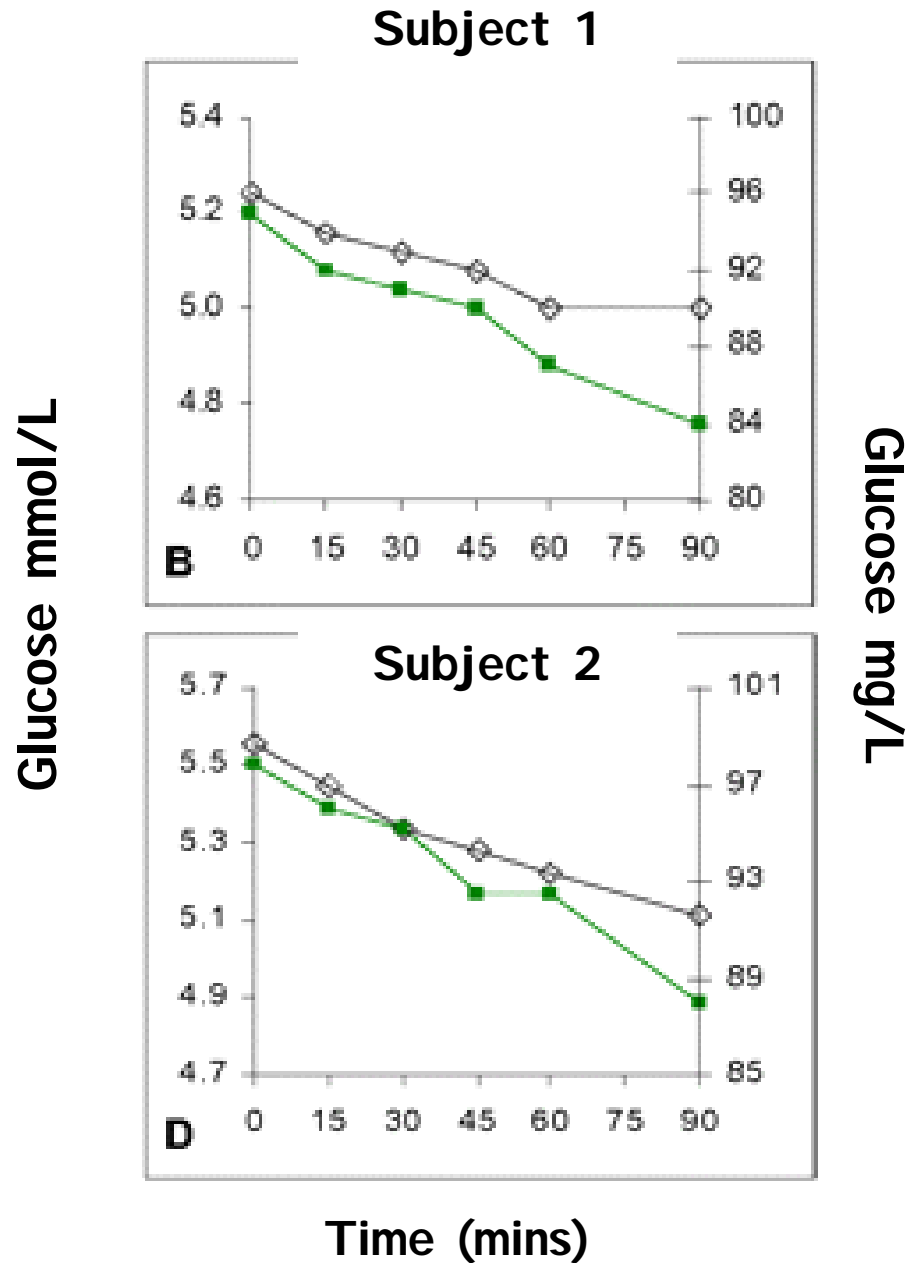


Effect of storage and specimen type on plasma glucose concentrations

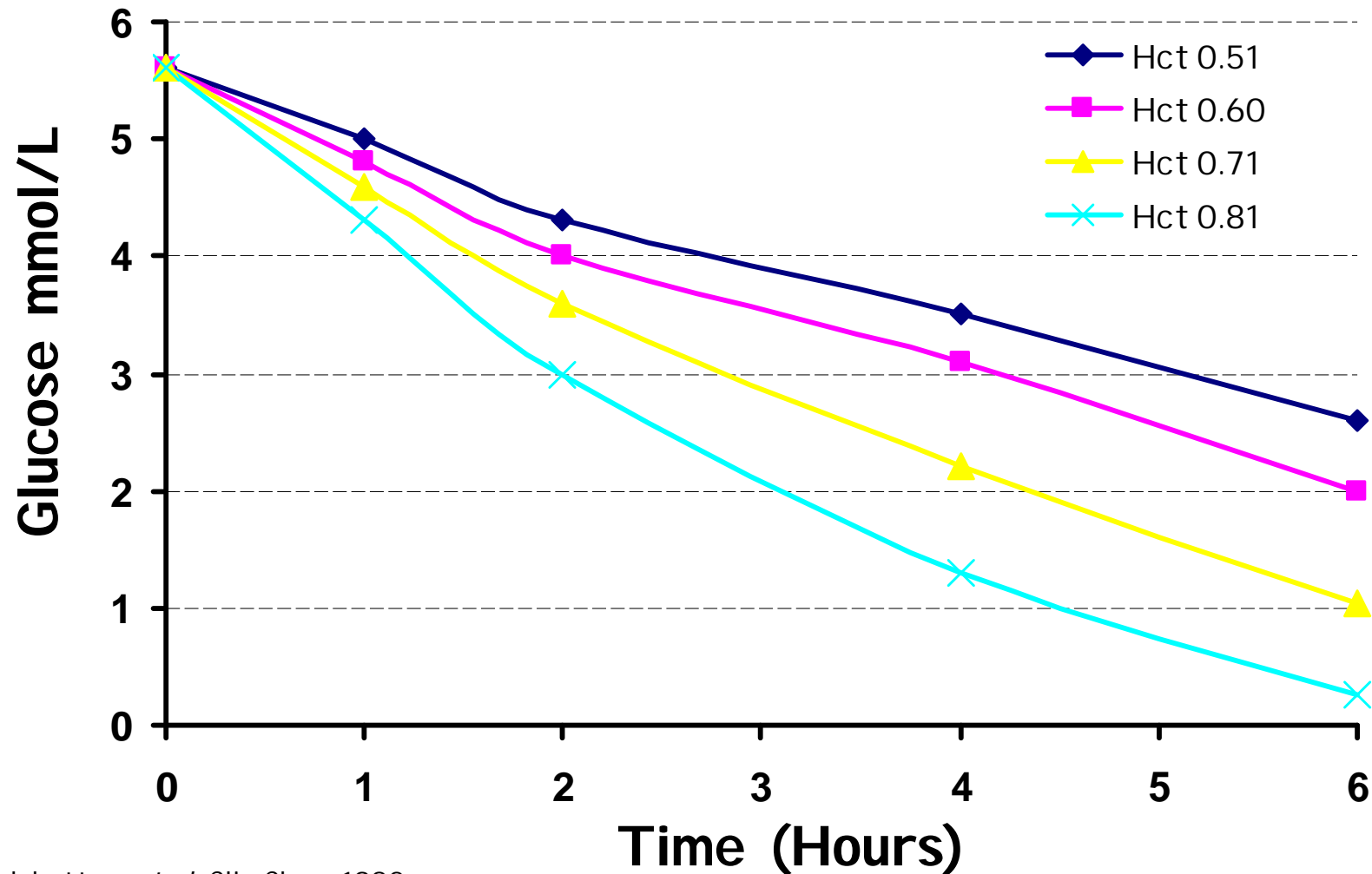


Changes in plasma glucose concentrations with time

- ◇ Flox plasma
- LiHep plasma



Effect of increasing haematocrit and time on blood glucose

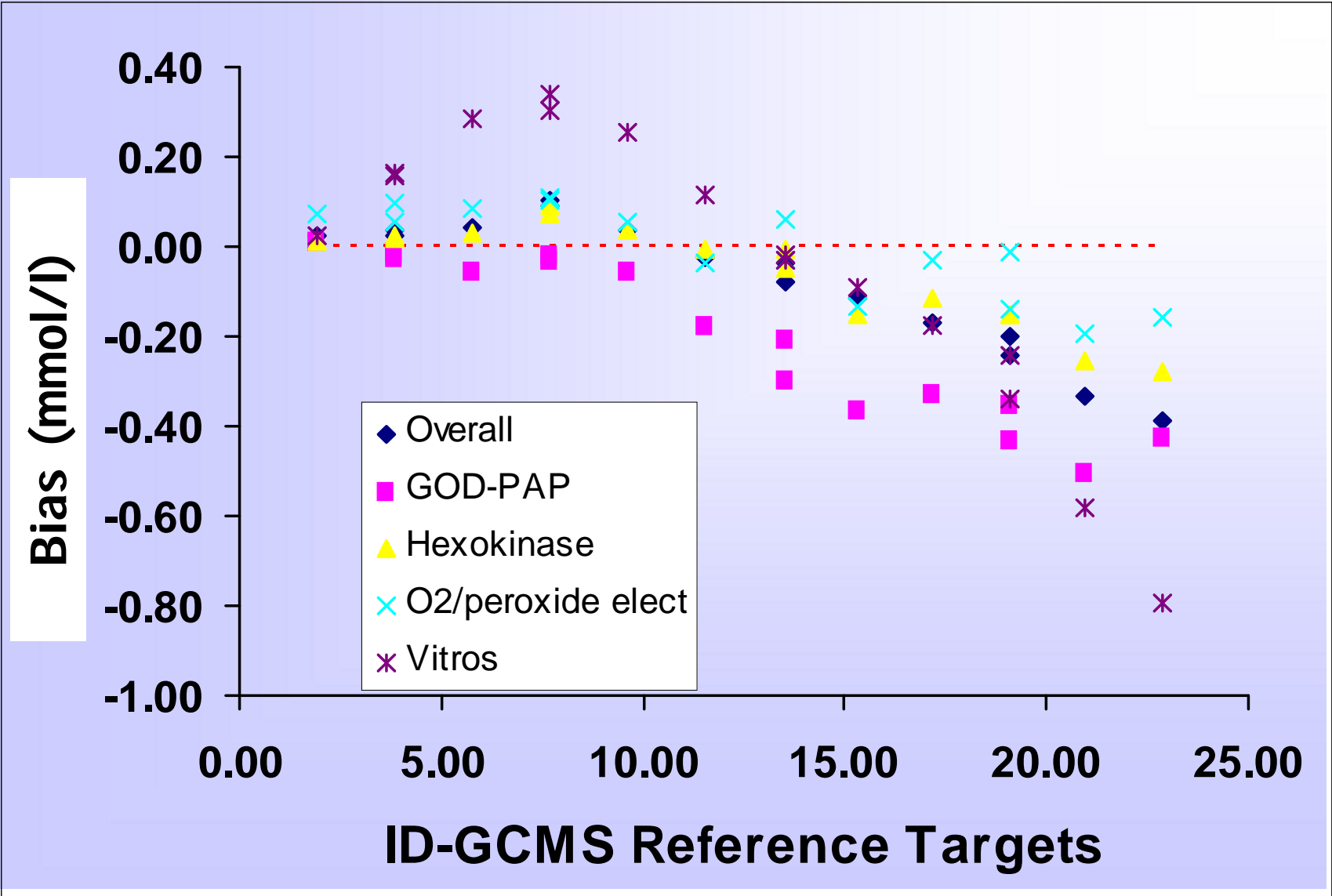


Glucose - Methodology

- Hexokinase
- Glucose oxidase
- Glucose dehydrogenase

- Pyrroloquinoline quinone glucose dehydrogenase
- PQQ glucose POCT systems – NOT glucose specific

WEQAS Glucose – Method Bias



Glucose - Interpretation

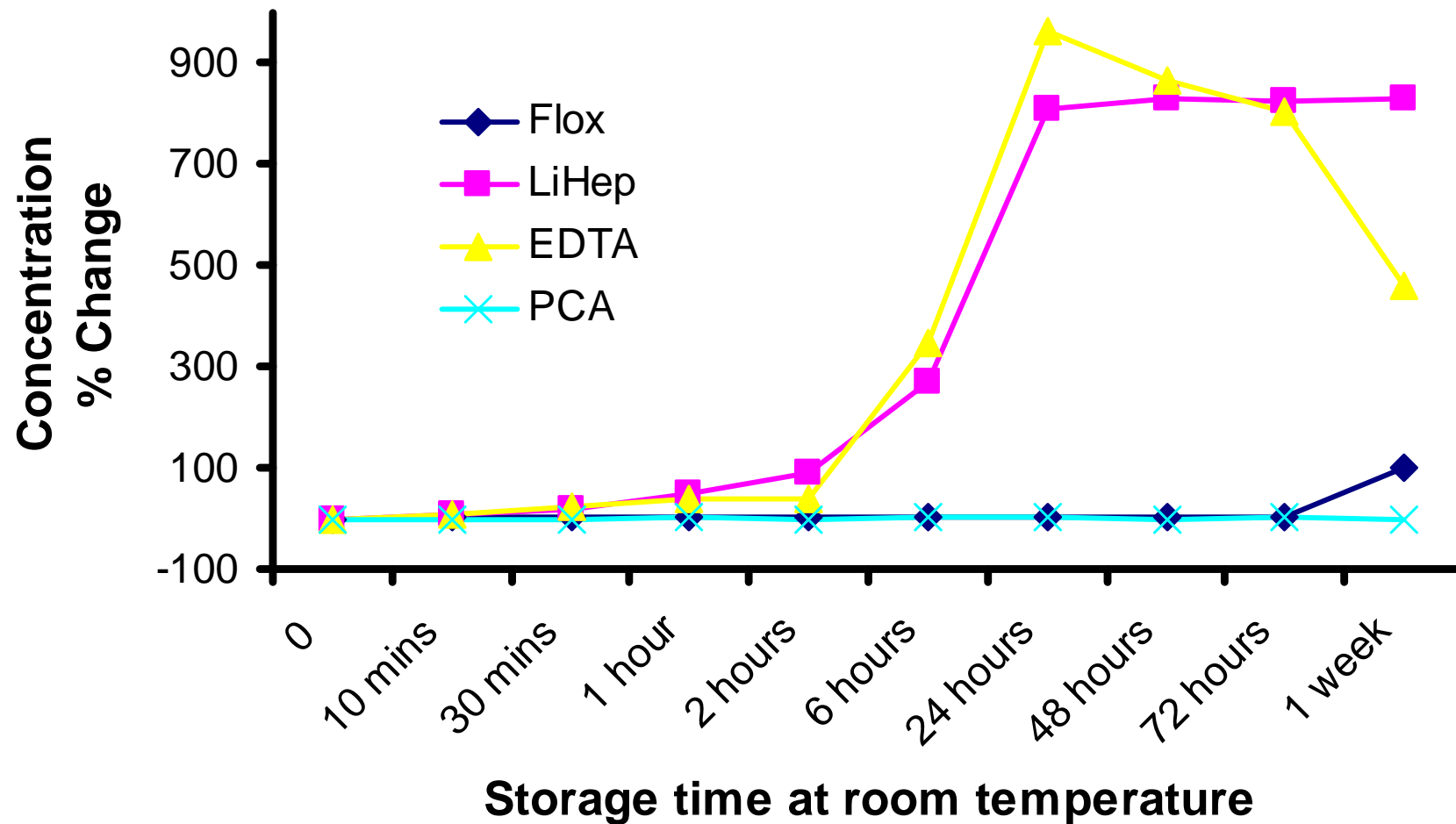
- Pre-analytical factors have greater influence on blood glucose result than assay bias and imprecision
- Threshold glucose for investigation?

Lactate

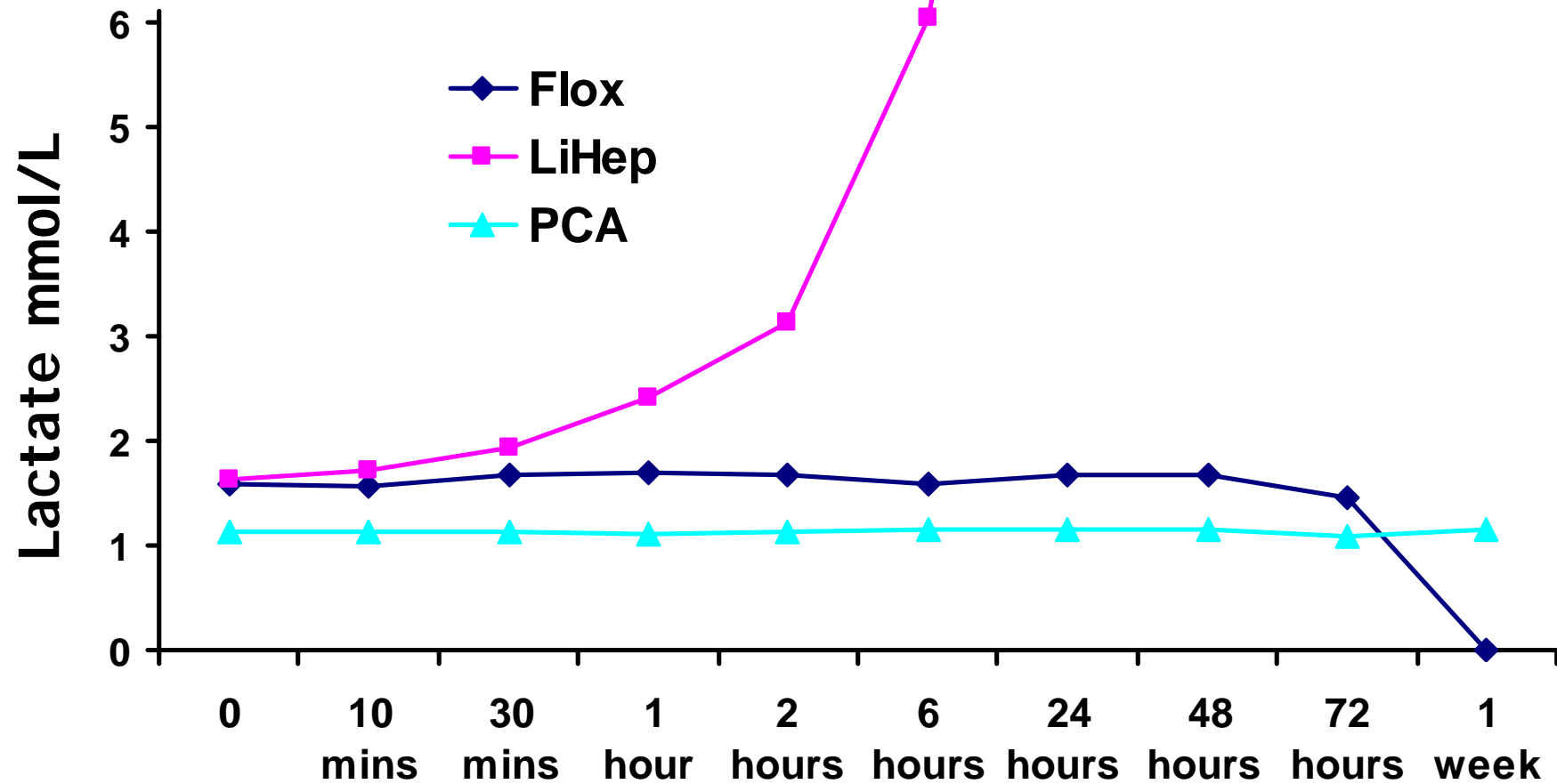
Lactate – specimen collection & sample handling

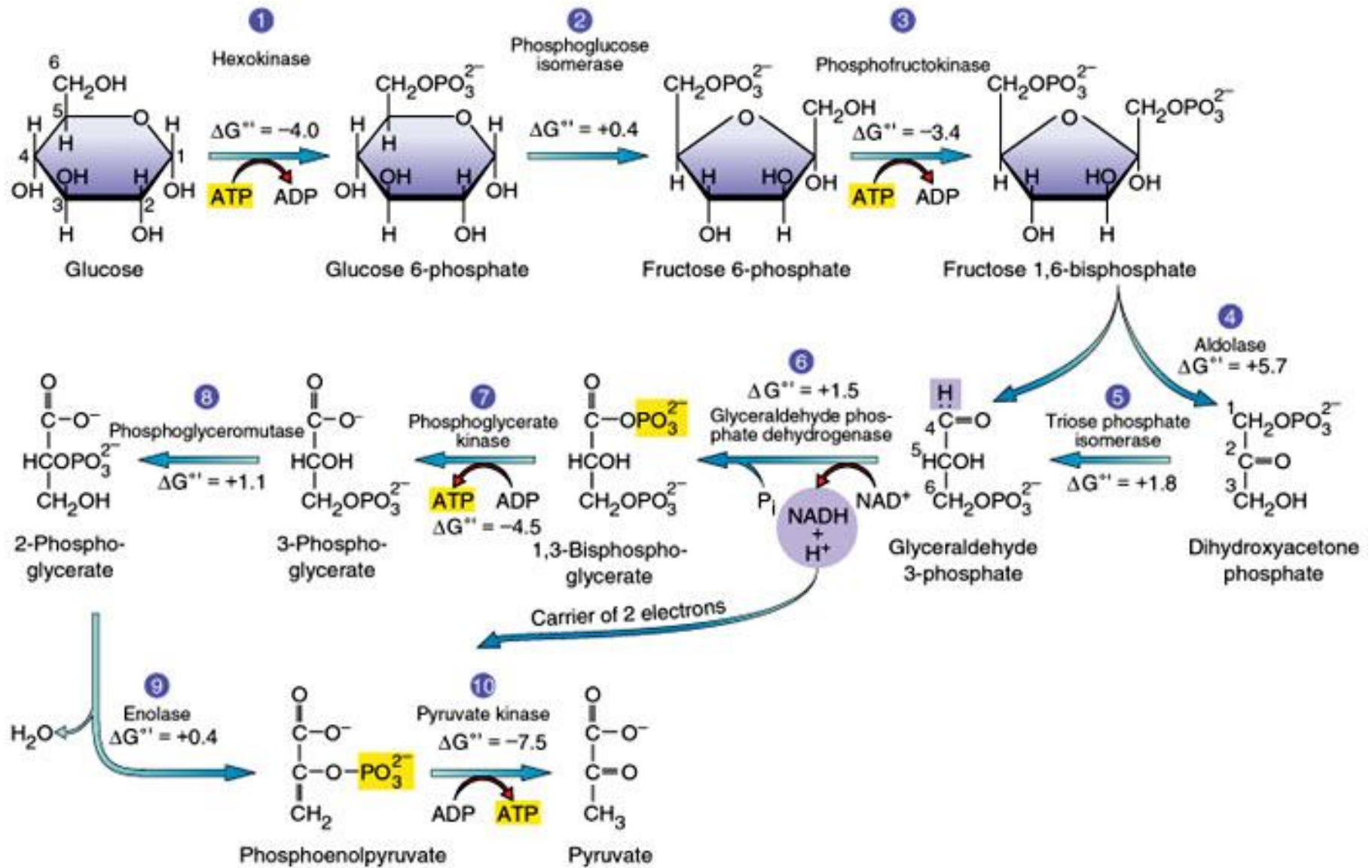
- RBC's metabolise glucose to lactate
- Glycolytic inhibitors
- Patient should be rested and avoid hand clenching
- Venous stasis – no effect
- Indwelling catheters

Effect of storage and specimen type on plasma lactate concentrations



Effect of specimen type on plasma lactate concentrations



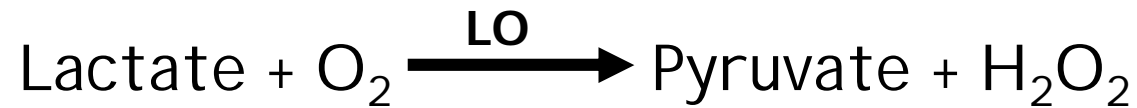
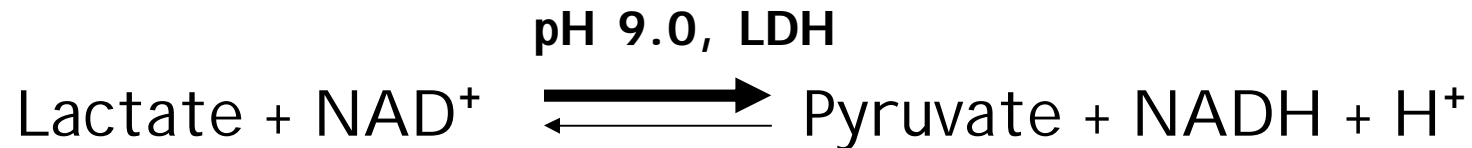


Effect of storage temperature on blood lactate concentrations

Time	0	5	10	15	20	25	30
Ice (n=26)	3.83 (1.1)	3.86 (1.12)	3.88 (1.12)	3.88 (1.10)	3.89 (1.10)	3.9 (1.07)	3.89 (1.07)
RT (n=26)	4.29 (2.07)	4.37* (2.08)	4.42* (2.10)	4.5* (2.10)	4.54* (2.10)	4.6* (2.10)	4.64* (2.06)

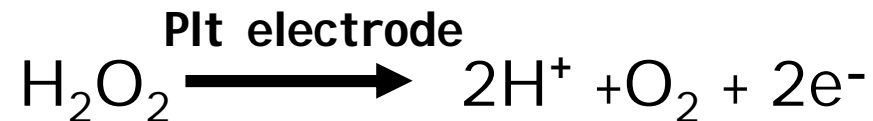
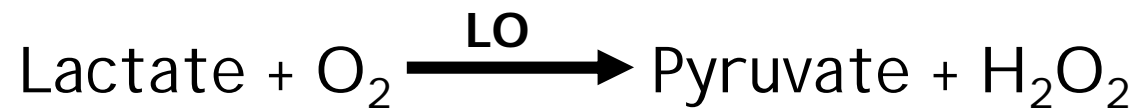
Lactate - Methodology

- **Mainline chemistry analysers**



Lactate - Methodology

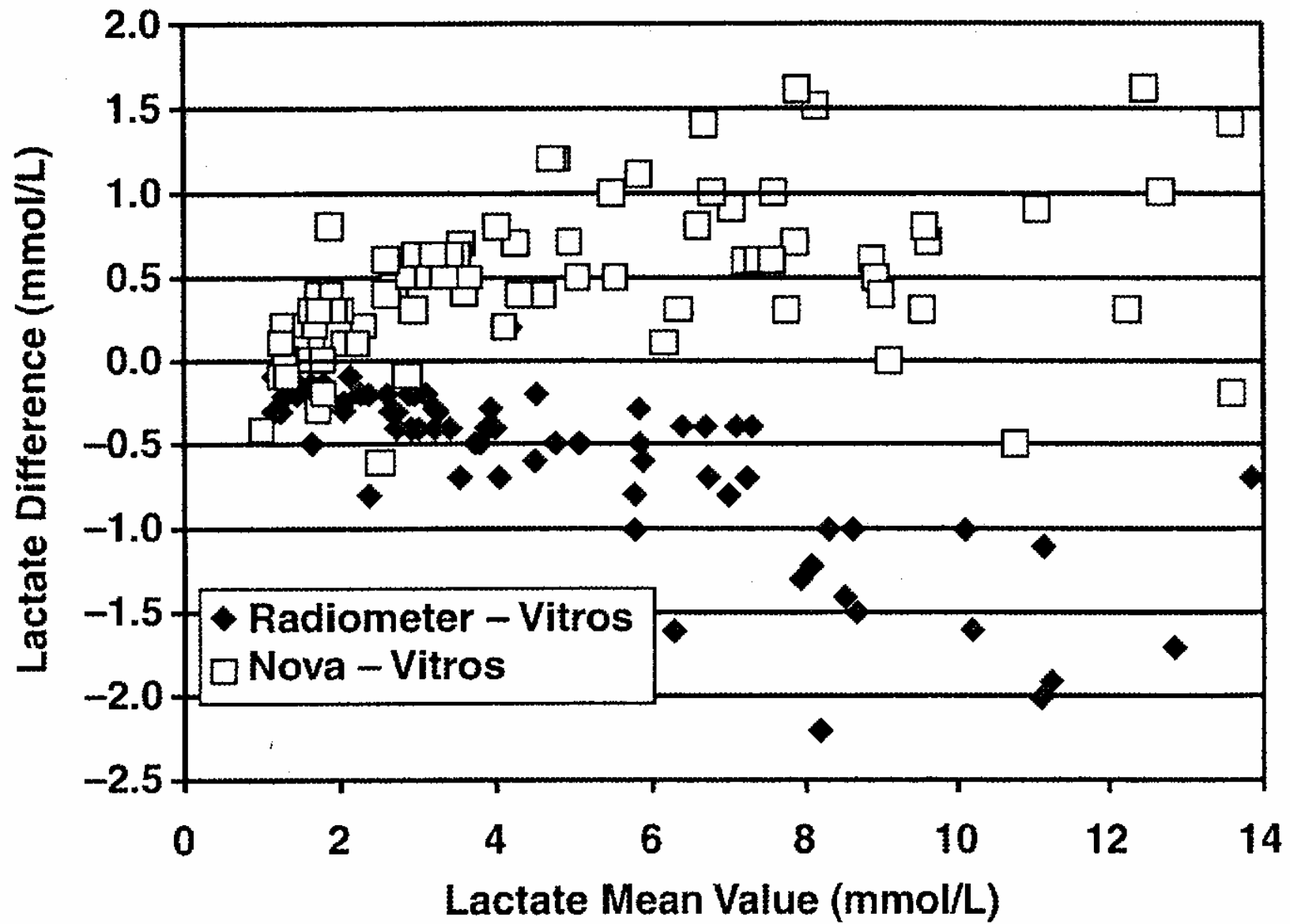
- **Blood gas analysers**



- Whole blood heparinised tubes
- Analysis within 20 minutes of collection

WEQAS Lactate EQA scheme (Feb 2009)

All methods n=680 returns	Mean mMol/L	1.44	13.34	7.05
	SD	0.17	1.46	0.78
	CV%	11.8	10.9	11.1
POCT ABL systems n=216	Mean mMol/L	1.35	12.25	6.44
	SD	0.07	0.73	0.33
	CV%	5.2	5.9	5.1
Enzymatic methods n=35	Mean mMol/L	1.47	13.22	7.05
	SD	0.11	1.0	0.92
	CV%	11.8	10.9	13.1



Lactate - Interpretation

- Interpretation often difficult
- Plasma vs whole blood & Blood vs CSF
- Comparison of lactate between POCT and mainline lab analysers?

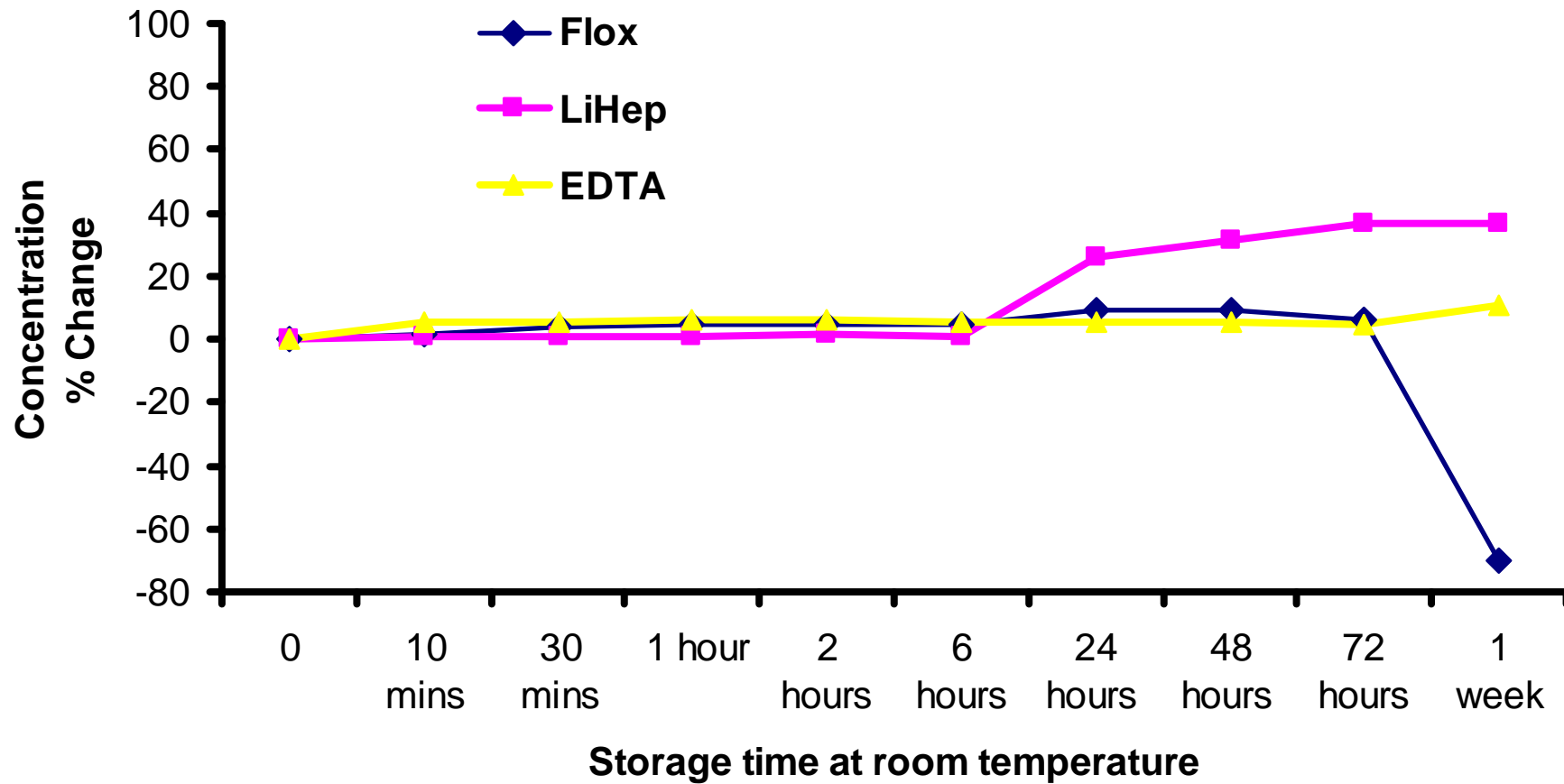
- Age related reference intervals
- Within subject variation ~30%
- Threshold value for investigation?

NEFA's

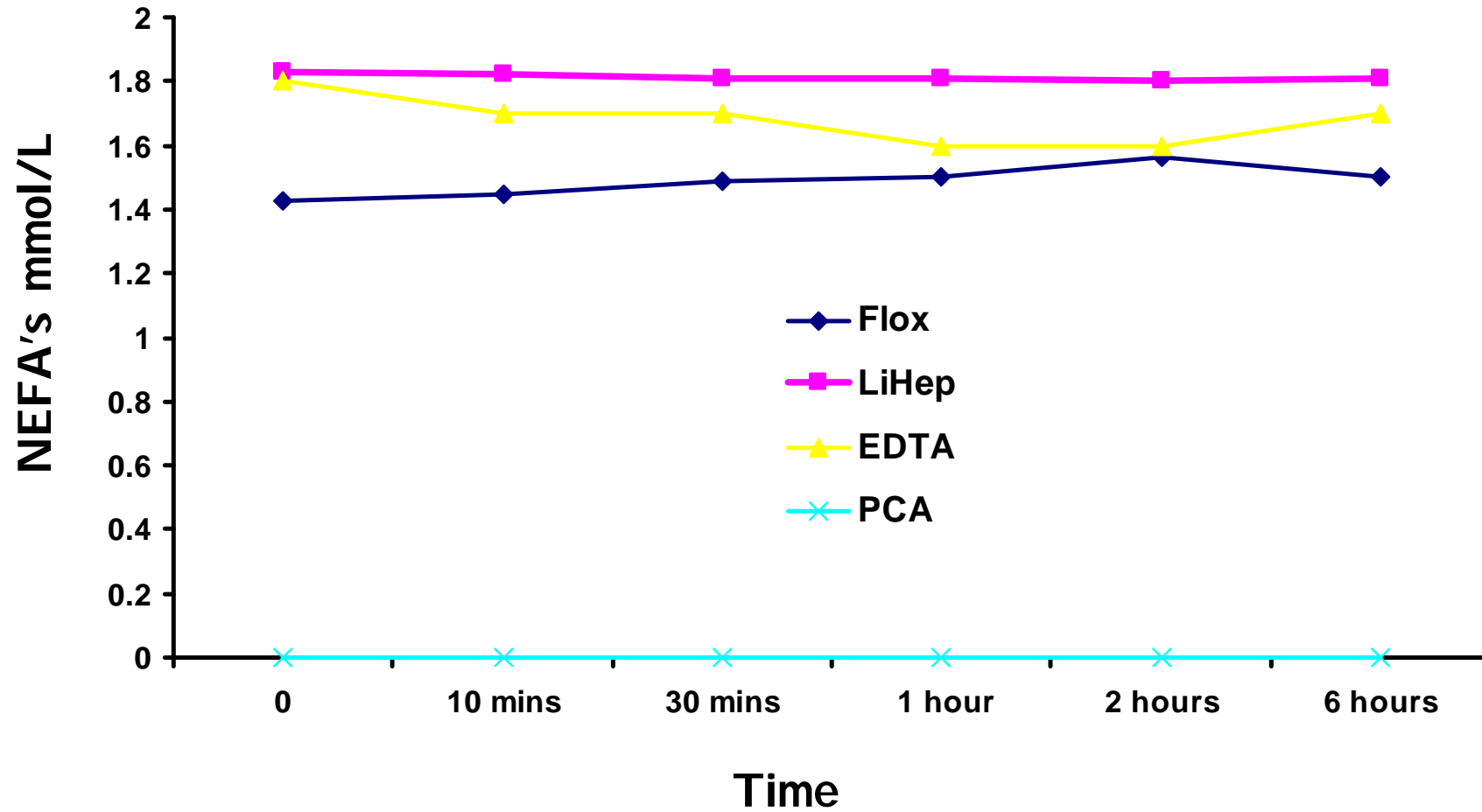
NEFA's - sample handling

- Stability - contradictory data
- Manufacturers recommend:
 - EDTA or serum samples NOT heparin
 - Immediate analysis

Effect of storage and specimen type on plasma NEFA concentrations



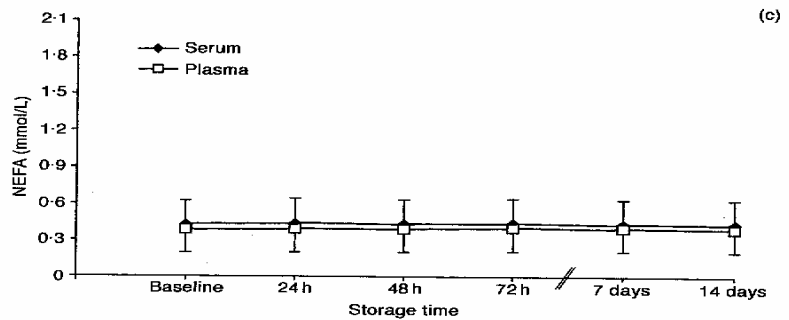
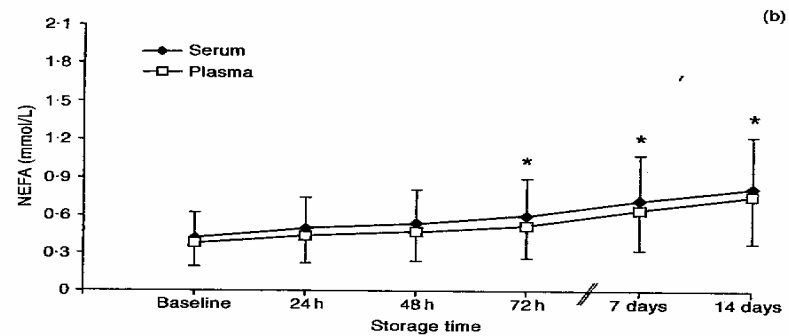
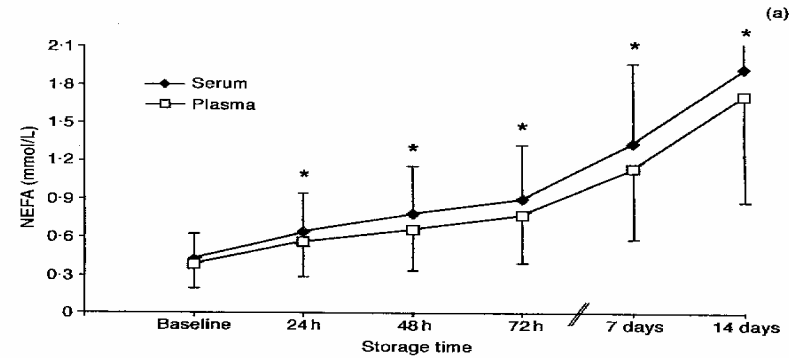
Effect of specimen type on plasma NEFA concentrations



Effect of storage temperature on NEFA's in serum and EDTA plasma

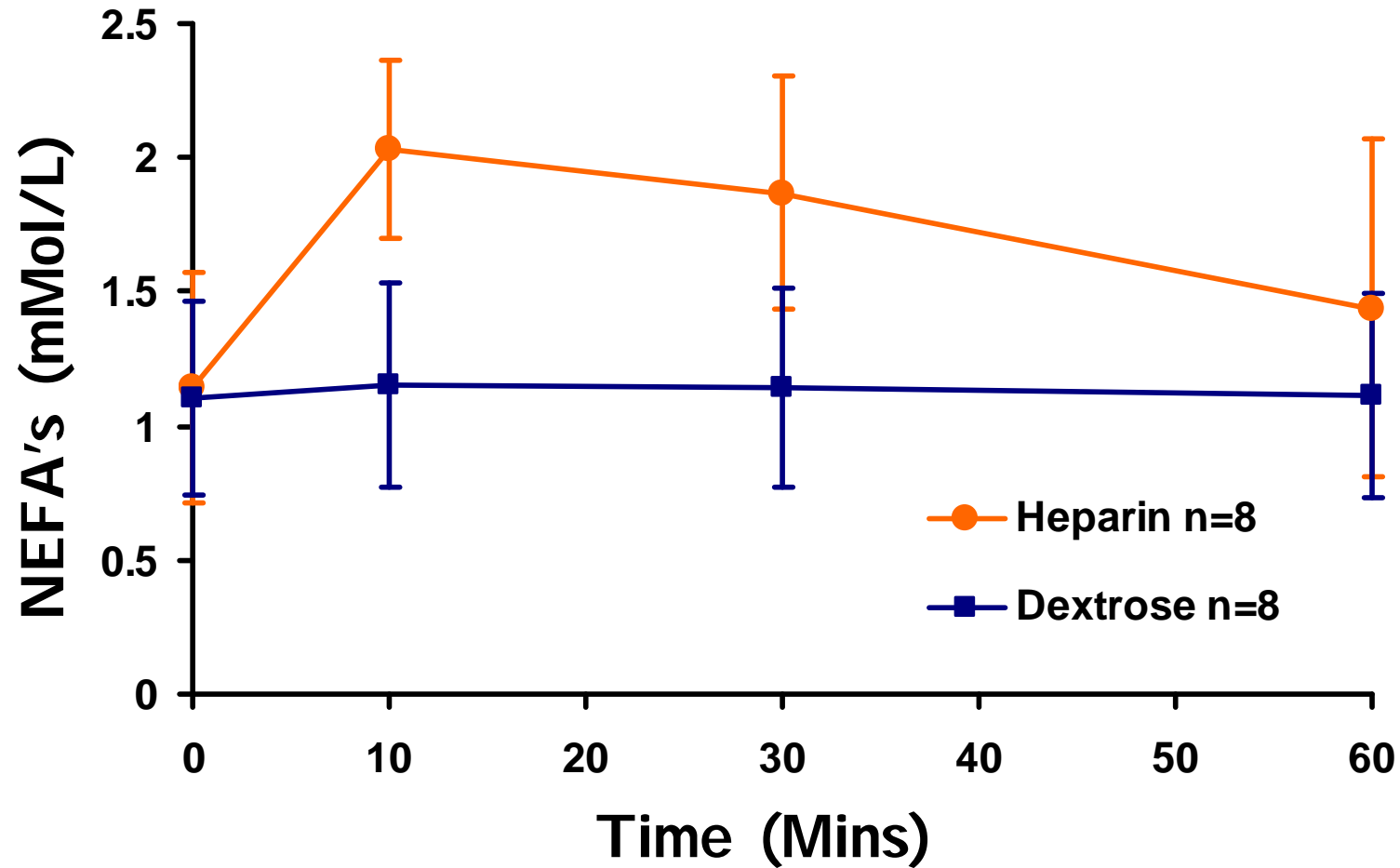
254 Menendez et al.

NEFA's mmol/L



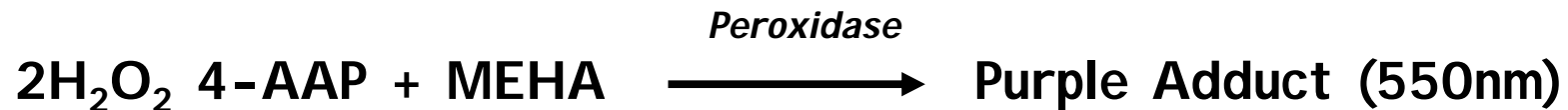
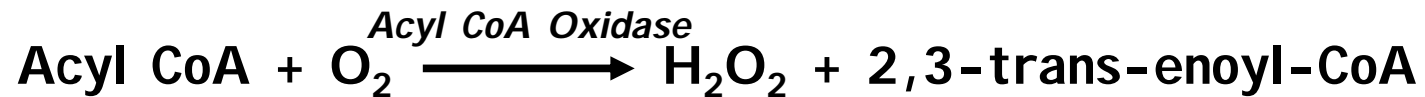
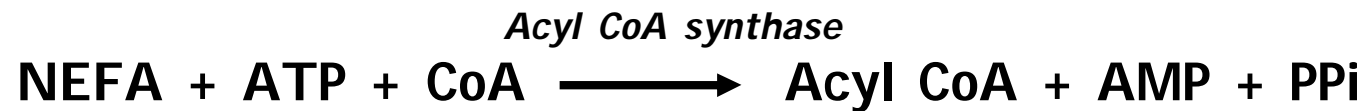
Storage time

Effect of therapeutic heparin administration on plasma NEFA's



NEFA's – Methodology (1)

- Wako, Randox & Biovision Kits



NEFA's – Methodology (2)

- Wako – linearity 4mmol/L
- Freeze thaw analysis?
- Wako vs Randox
 - Randox – 15% positive bias and problems with reagent stability
- QC Material – Randox & Wako
- No EQA scheme

NEFA's - Interpretation

- Separated within 6hrs and stored -20°C
- Heparin
- Catecholamines
- Haemolysis
- Bilirubin
- Assay precision ~6%

- Use of reference intervals?

3 - Hydroxybutyrate

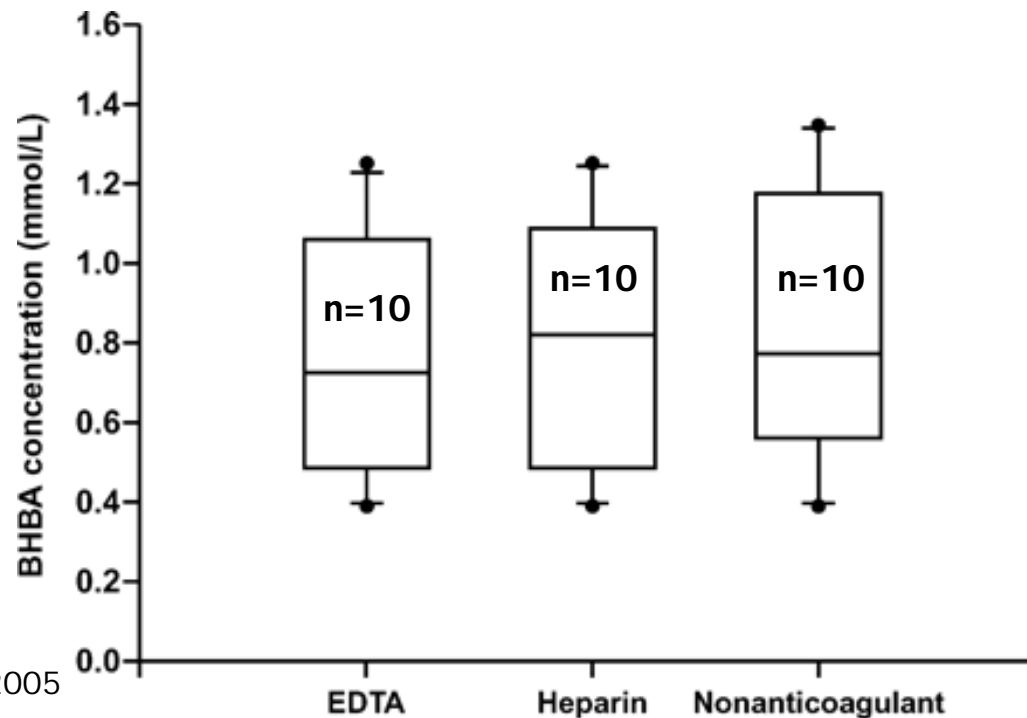
3-OH Butyrate – sample handling

- Stable in whole blood at room temp
~48 hours
- Plasma & PCA extracts stable long
term at -20°C
- Freeze-thaw cycles no effect

Effect of storage and specimen type on plasma 3-OH Butyrate concentrations

Moat & Bonham 1995
- Unpublished
Observations

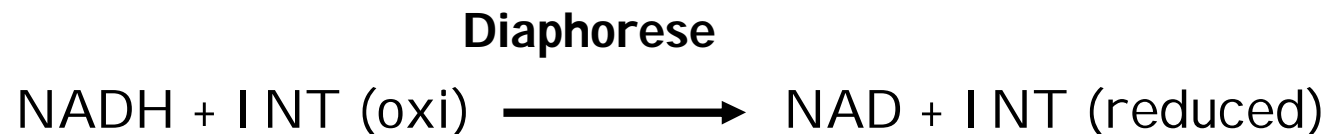
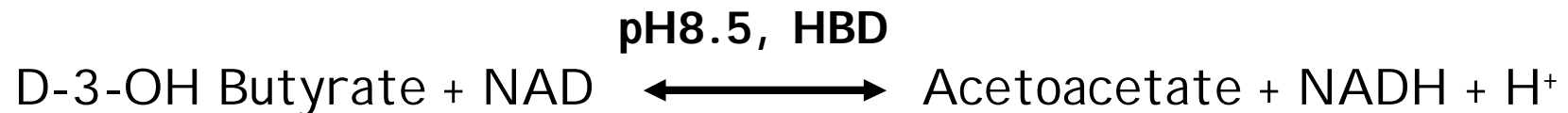
	Flox	LiHep	EDTA	PCA
BL	2.02	2.13	2.08	1.93
6 Hours	2.28	2.20	2.14	1.90
24 Hours	2.25	2.15	2.12	1.88



Stokol & Nydam 2005

3-OH Butyrate - Methodology

- Randox, Wako, Thermo Kits & in house assays



- POCT devices

3-OH Butyrate - Methodology

- D-3-OH-Butyrate standards
- Assay linearity up to 10mmol/L
- Good reagent stability (Randox)
- Unaffected by haemolysis, increased lactate & LDH (Randox)
- QC material - Randox

- Randox vs Wako kits?
- EQA schemes – Randox, ERNDIM & NEQAS

RANDOX 3-OH Butyrate EQA scheme

Sample Number	Number of labs	Mean 3-OHBut concentration mmol/L	SD	CV%
1	28	0.51	0.029	5.7
2	29	1.20	0.055	4.6
3	30	0.30	0.024	8.1
4	26	0.95	0.036	3.8
5	28	2.46	0.133	5.4
6	29	0.32	0.018	5.7
7	28	0.32	0.016	5.0
8	27	1.20	0.041	3.4
9	29	0.81	0.030	3.7
10	27	2.45	0.113	4.6
11	29	0.32	0.022	6.9
12	25	0.50	0.033	6.6

3-OH Butyrate - Interpretation

- Stable analyte
- Robust assay (analytical CV~5%)
- 3-OH-But should NOT be interpreted in isolation
- Use of reference intervals?

Summary

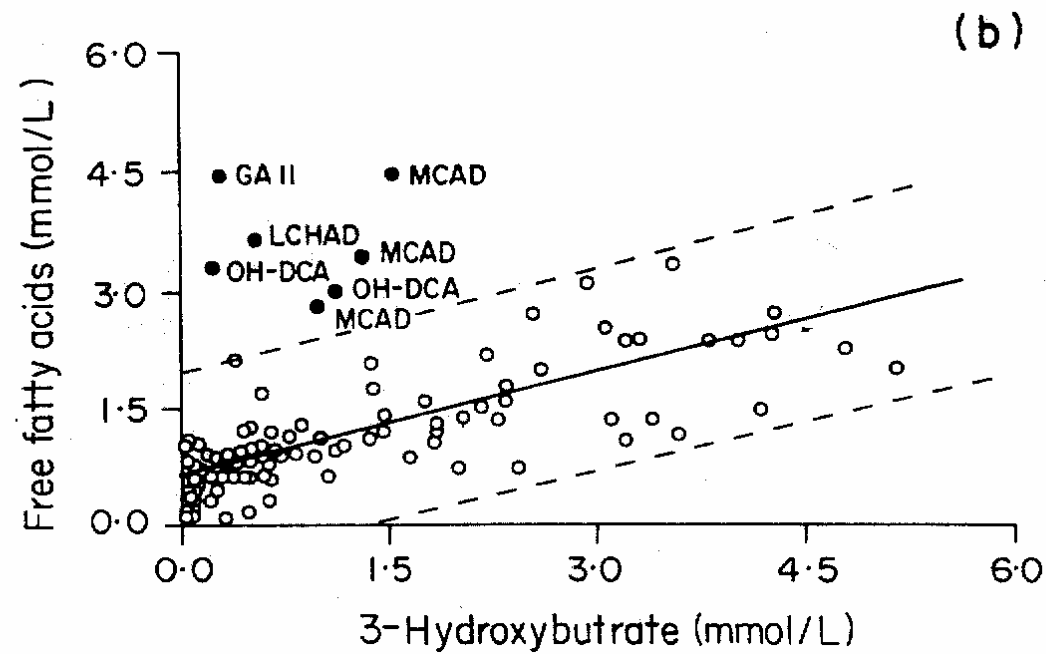
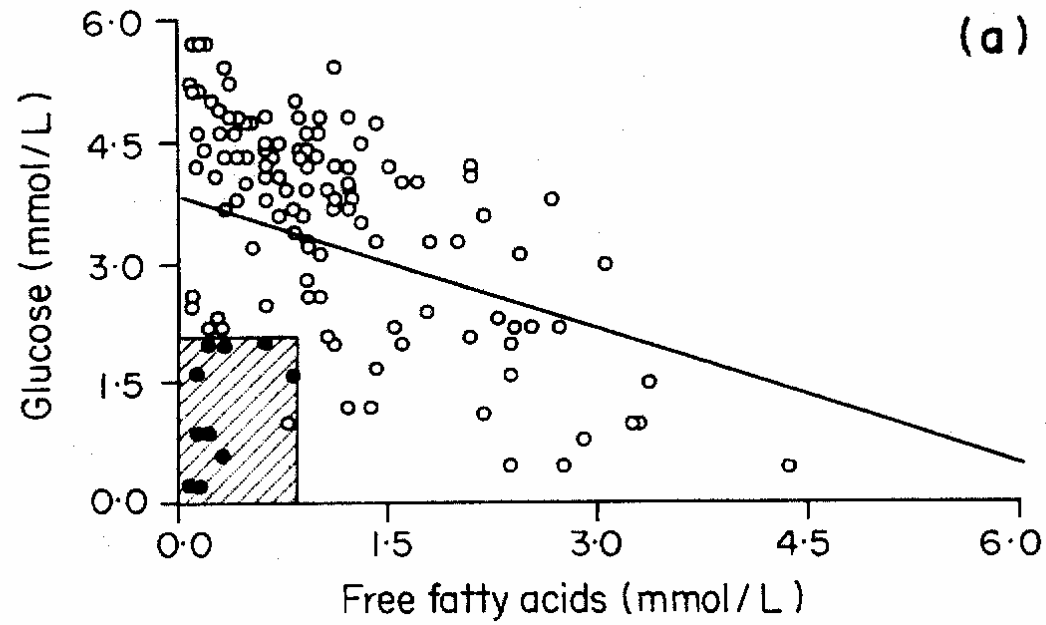
- I M's useful analytical tools
- Simple commercially available assays
- Lack of pre-analytical/sample collection guidelines

Discussion

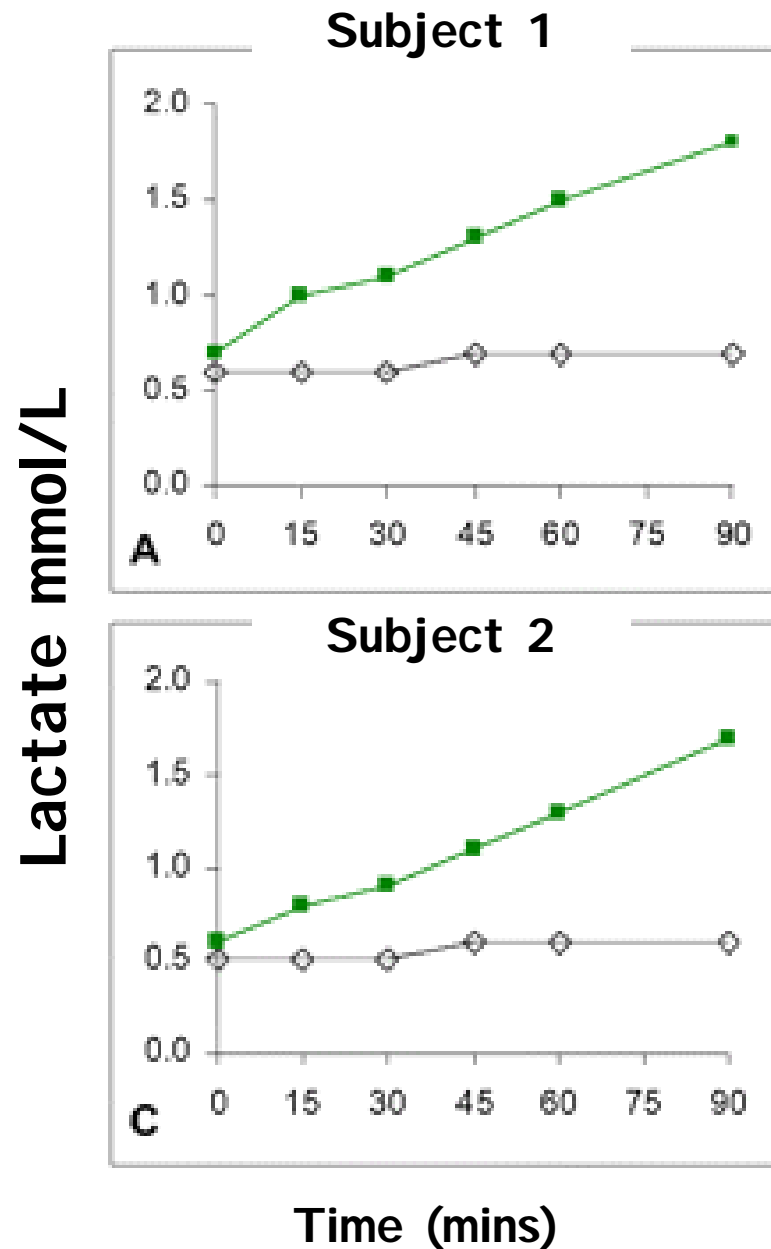
- Further studies:
 - effect of anticoagulants and storage on I M's and to assess any concentration dependent effects
 - Freeze thaw stability studies
- Validation of kits/assays in use
- EQA scheme for NEFA's
- MetBioNet Guidelines for the analysis of I M's

Acknowledgements

- Metabolic Section - Sheffield Children's Hospital
- Special Chemistry - UHW



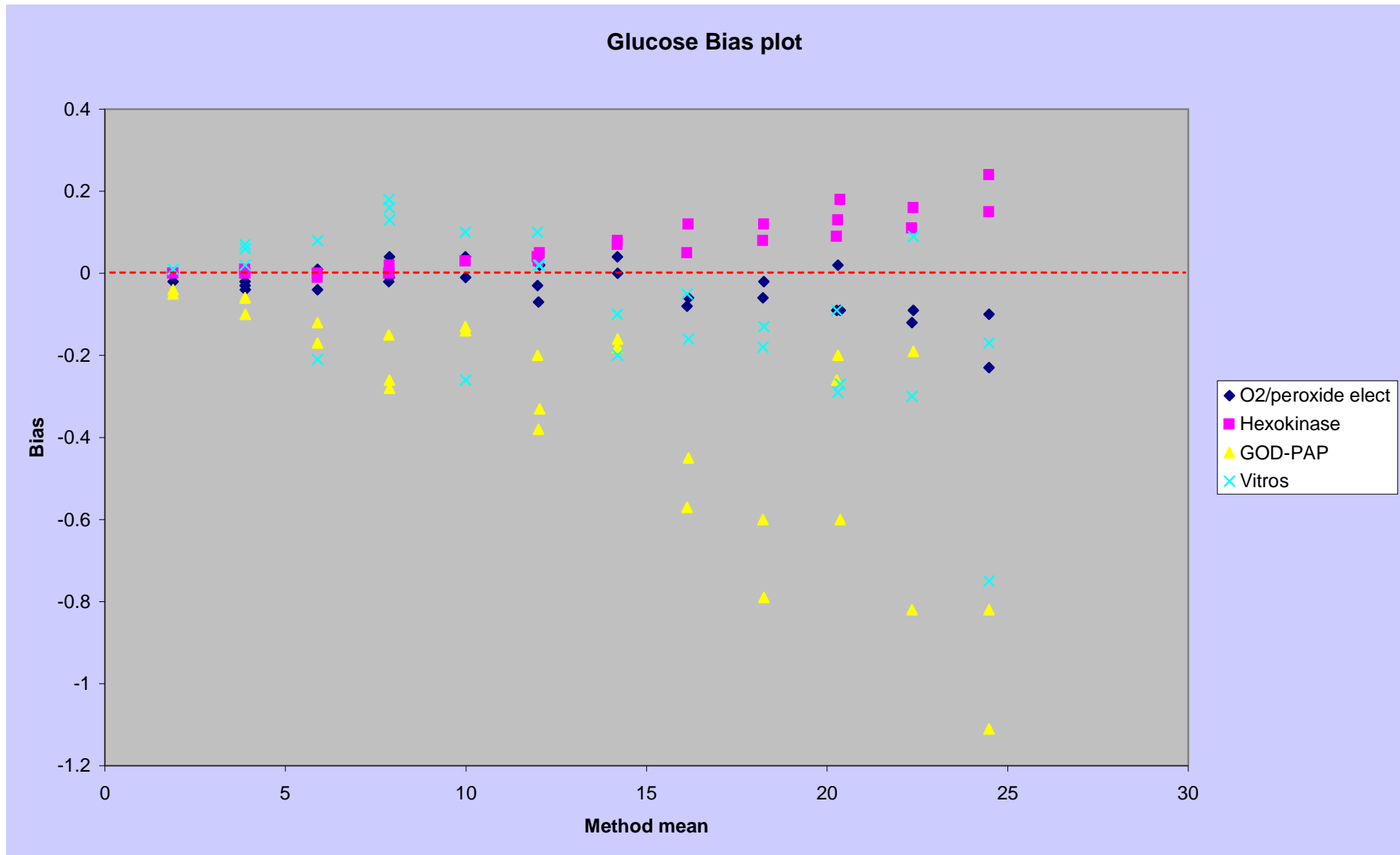
Changes in plasma Lactate concentrations with time



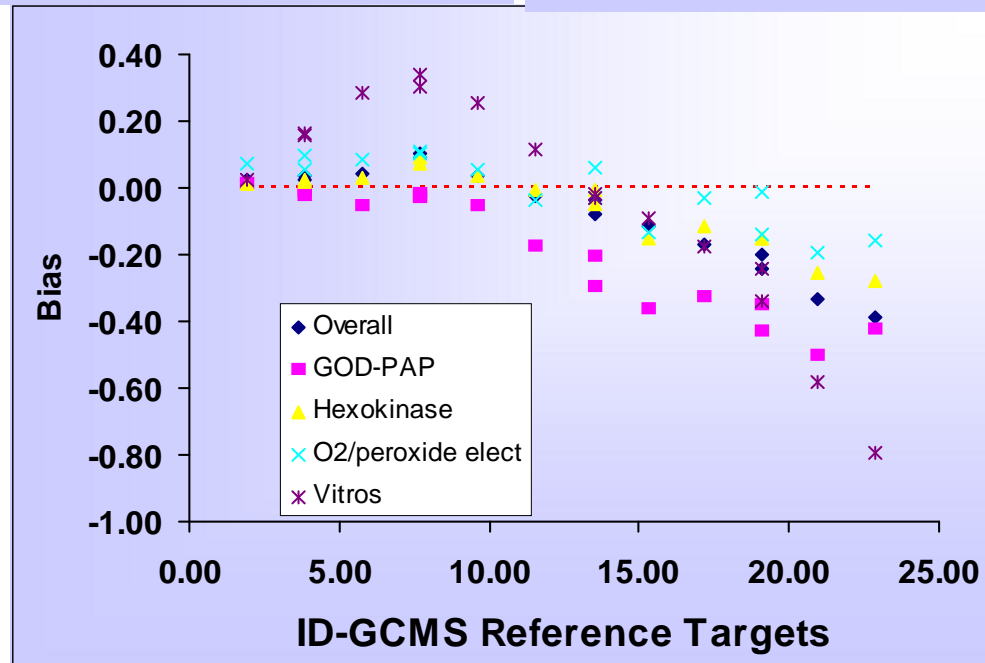
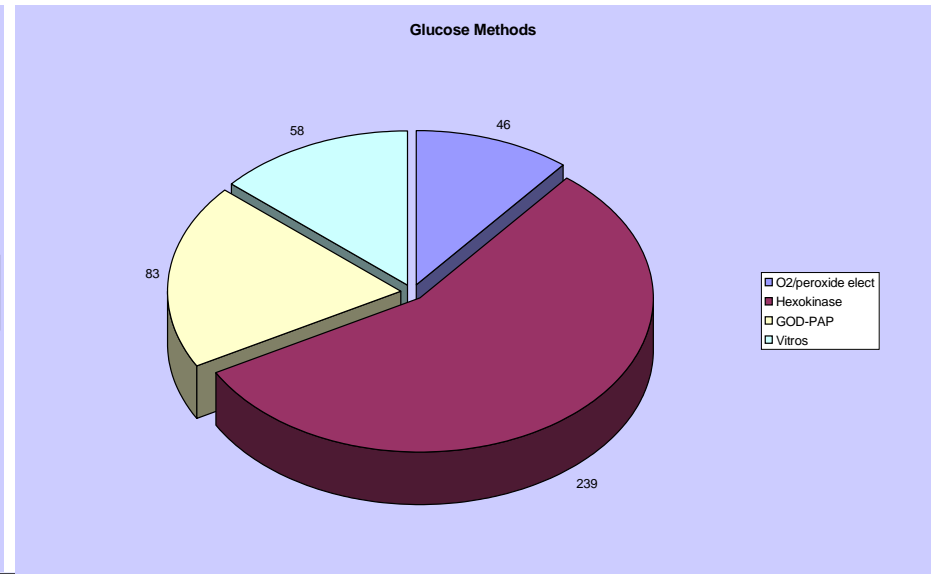
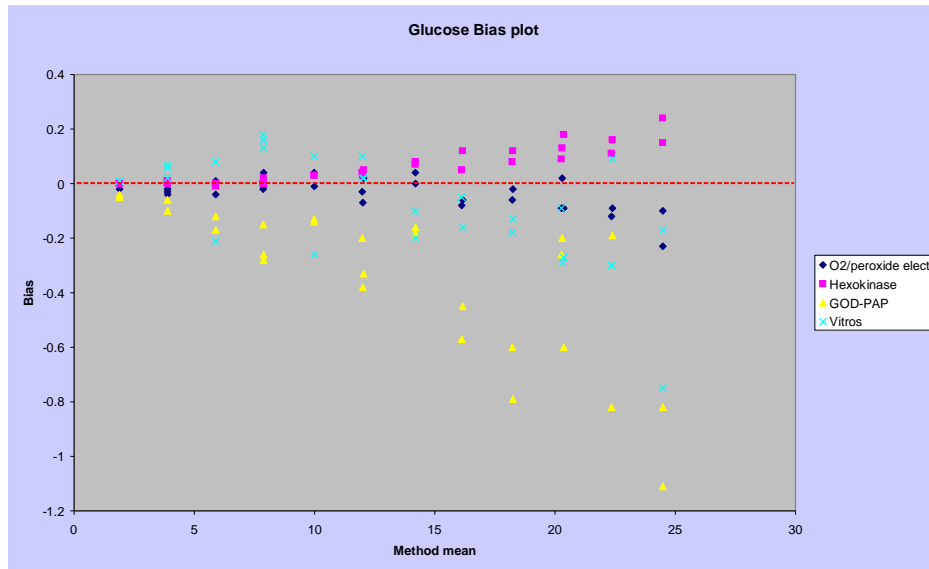
◇ Flox plasma

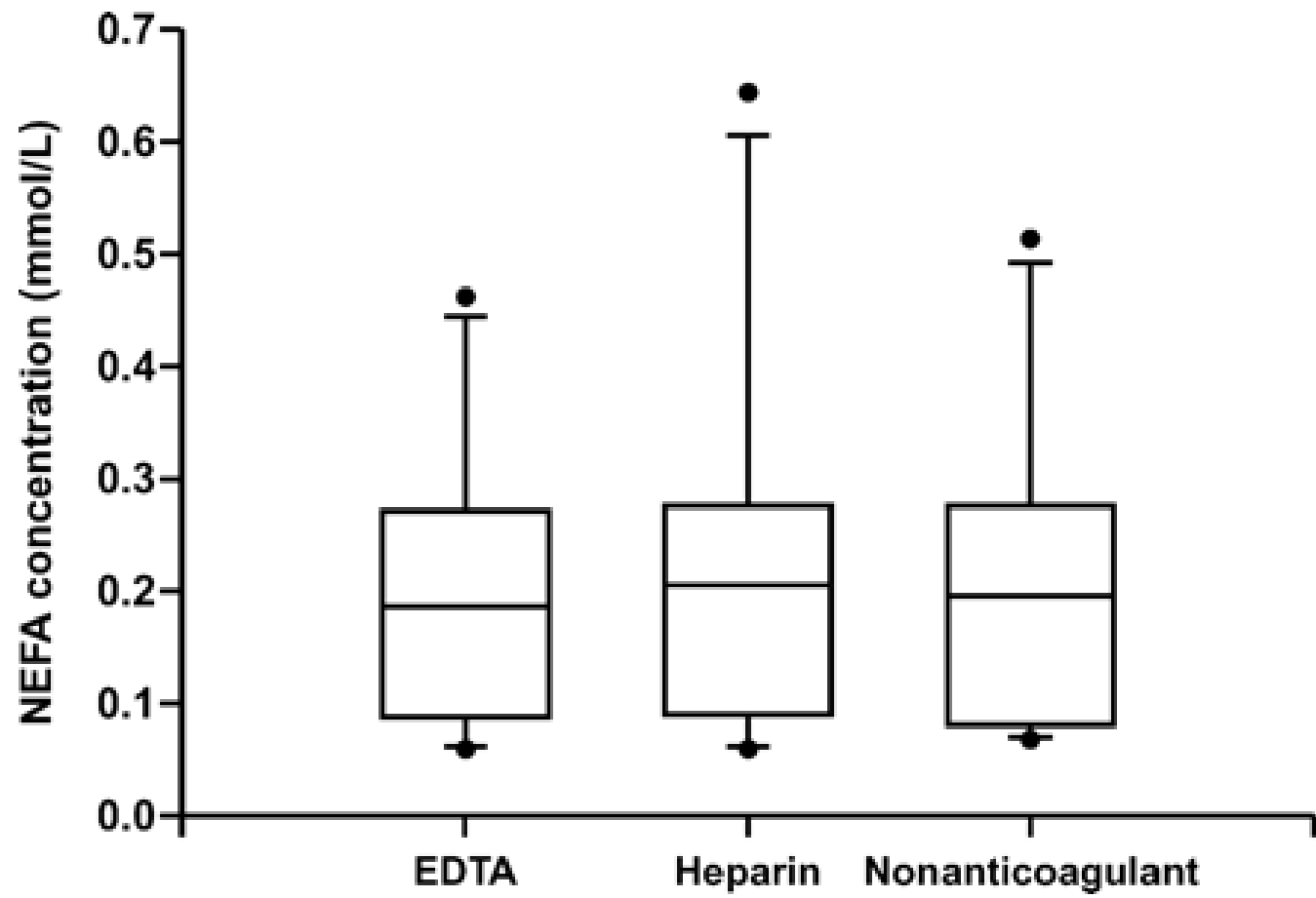
■ LiHep plasma

Glucose – Method Bias (WEQAS)



Glucose – Method Bias





Effect of storage and specimen type on plasma 3-OH Butyrate concentrations

