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Routine tests and IEM
A case-based approach

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Introduction

- Parts 1 and 2 things that can come up
  - Forearm exercise test
  - Organic acid disorders
  - Interpretation of sweat tests
  - Porphyria
  - Tyrosinaemia
- Part 2

~3/4 marks usually based around interpreting routine results and knowing how to collect appropriate samples for a possible inherited metabolic disease

Case 1

- Male infant normal term delivery with low birth weight
- Presented to Local hospital day 2 of life acutely unwell and hypothermic
- Initial thoughts what test would you run?
  - Possible sepsis, Blood gases, cultures, U and Es lactate and ammonia
- Blood gases day 2 of life

Test	Result
[H+]	75 nmol/L
pH	7.1
pCO2	4.2
Bicarbonate	12

Metabolic acidosis, with partial respirator compensation

Cultures negative
Ammonia 1200 mmol/L
Lactate 6 mmol/L

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Measuring ammonia

- Ideally measured on a free-flowing venous sample or arterial stab sent to the laboratory ASAP (ideally within 15 minutes and on ice).
  - Always send even if not under ideal conditions
- Elevated result should be confirmed by repeat analysis ASAP.
- An increasing trend in concentration makes a metabolic cause more likely
  - Levels >200 μmol/L more likely to be metabolic
- The commonest cause of a mildly raised level is contamination or sample deterioration.
  - If it is suspected result should be confirmed on a second sample prior to initiating treatment.

	Typical ammonia levels
Premature neonate	<150 μmol/L
Term neonate	<100 μmol/L
Infant/child	<50 μmol/L
Adult	<50 μmol/L

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First line investigations for hyperammonaemia

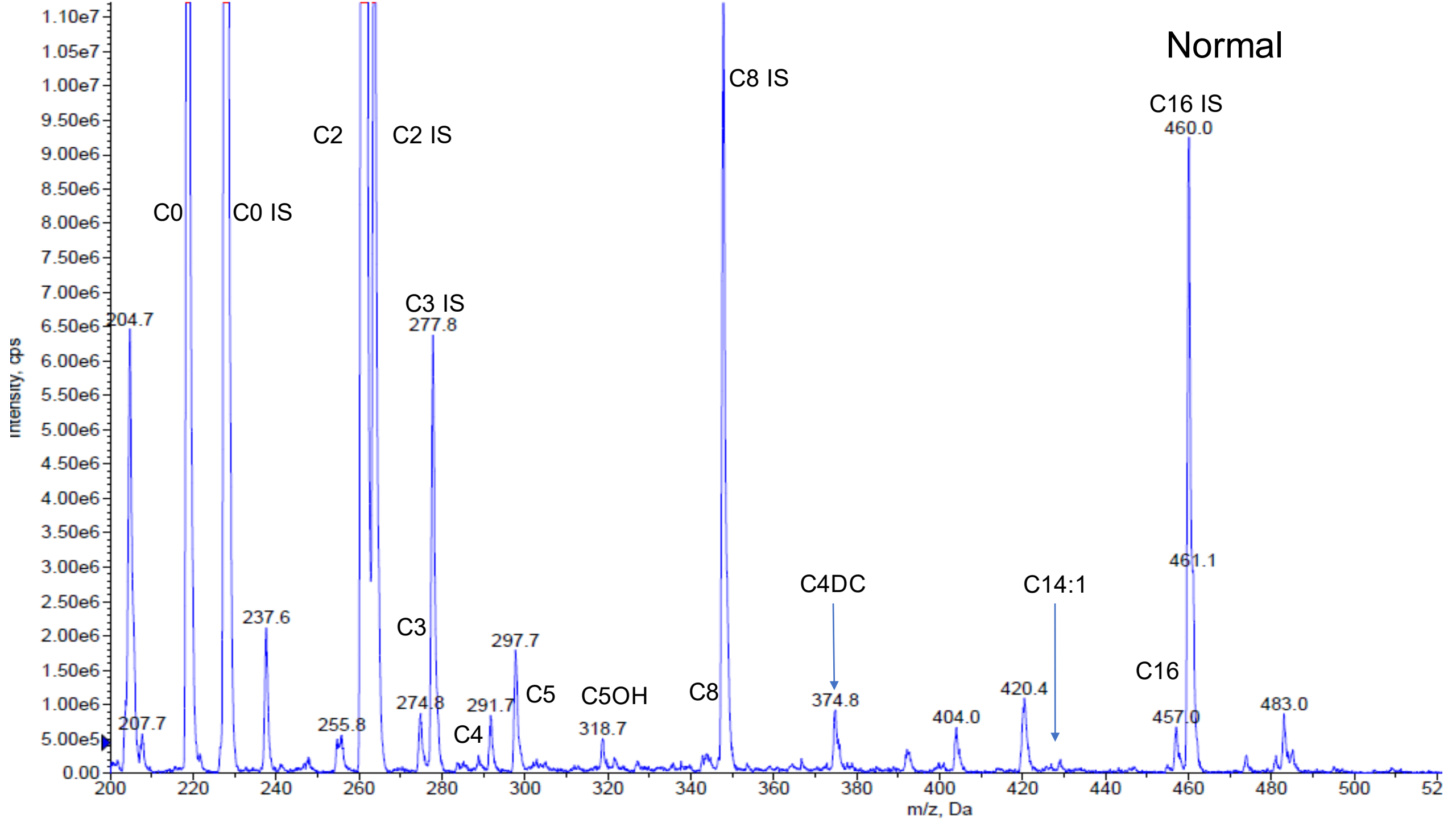
Investigations	Interpretation
Blood gases	Ammonia is a respiratory stimulant therefore hyperammonaemia causes a respiratory alkalosis. The presence of a metabolic acidosis may suggest an organic acid disorder or fatty acid oxidation defect.
Urea	May be inappropriately low compared to other markers of renal function / dehydration in urea cycle disorder.
Liver function test	Severely deranged in some acquired causes of hyperammonaemia. May be mild elevations in urea cycle defects and organic acidurias.
Glucose	Hypoglycaemia may occur in e.g. fatty acid oxidation disorders, hyperinsulinism and liver failure
Lactate	May be raised in a number of metabolic conditions and liver failure.
Calcium	Hypocalcaemia is a feature of some organic acid disorders.
Ketones	May differentiate organic acids disorders (increased) from fatty acid oxidation disorders and liver failure (not present/low).

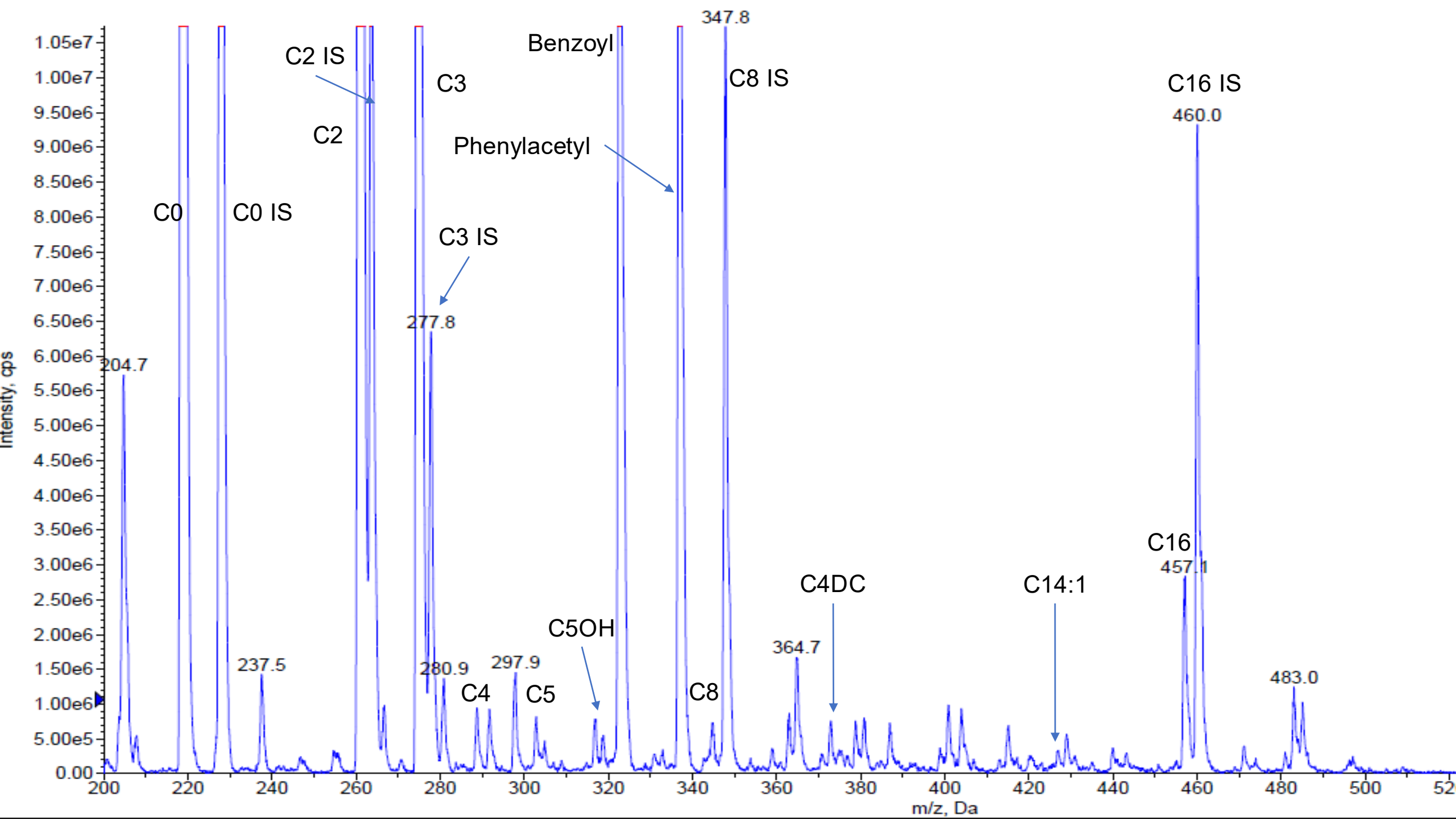
Test	Result	Test	Result
Sodium	135 (133-146)	ALP	120 (65-270)
Potassium	3.2 (3.2-6.0)	Total Bilirubin	24 (<100)
Urea	5.7 (0.7-5.0)	Corrected calcium	2.12 (2.17-2.44)
Creatinine	73 (47-82)	Phosphate	1.85 (1.50-2.60)
Bicarbonate	15 (20-30)	Magnesium	0.96 (0.66-1.00)
Chloride	94 (96-110)	Glucose	3.4
Albumin	27 (34-42)	Urine ketones	++
ALT	240 (9-52)	Ammonia	1200 (<50)

- Anion gap =29.2
- What does the anion gap indicate
  - Presence of an additional anion not measured
- Any other significant results?
  - Low bicarbonate and calcium, raised ALT elevated urine ketones

Case 1 review

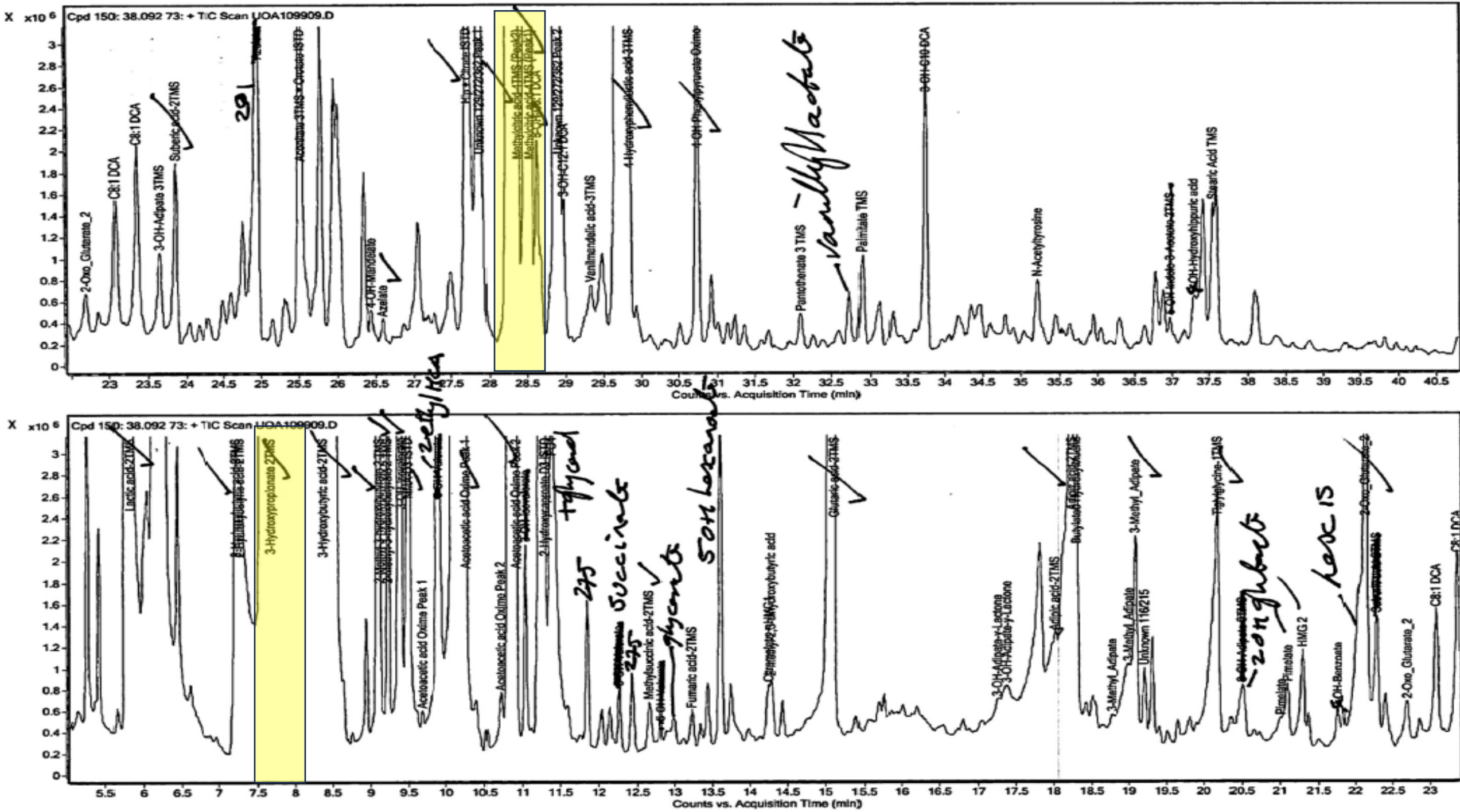
- Hyperammonemia
- Metabolic acidosis on blood gases with increased anion gap
- Mild abnormalities in LFTs
- Hypocalcemia
- Low normal glucose
- Raised urine ketones
- Possible organic acidaemia based upon routine biochemistry
- Follow up specialist test
  - Organic acids
  - Acylcarnitines


Normal
C0
C0 IS
C2
C2 IS
C3 IS
277.8
C8 IS
C16 IS
460.0
204.7
237.6
C3
297.7
C5
C5OH
318.7
C8
C4DC
374.8
C14:1
461.1
C16
457.0
420.4
483.0
404.0
255.8
274.8
291.7
C4
207.7
Intensity, cps
m/z, Da
1.10e7
1.05e7
1.00e7
9.50e6
9.00e6
8.50e6
8.00e6
7.50e6
7.00e6
6.50e6
6.00e6
5.50e6
5.00e6
4.50e6
4.00e6
3.50e6
3.00e6
2.50e6
2.00e6
1.50e6
1.00e6
5.00e5
0.00
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
52


C2 IS
C3
C2
Benzoyl
347.8
C8 IS
C16 IS
460.0
Phenylacetyl
C0
C0 IS
C3 IS
277.8
204.7
237.5
280.9
297.9
C4
C5
C5OH
C8
364.7
C4DC
C14:1
C16
457.1
483.0
Intensity, cps
m/z, Da

Acylcarnitine quantification day 8

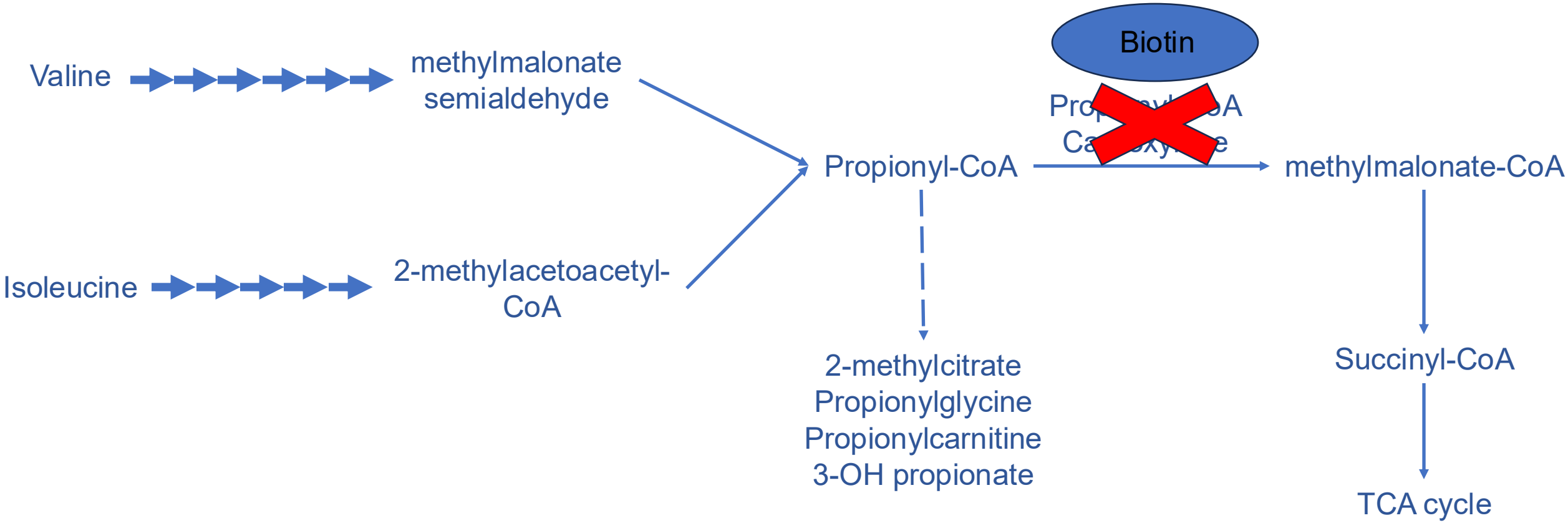
Carnitine species	Result umol/L (RR)
Free (C0)	23 (28.5-109.0)
Acetyl (C2)	25.1 (0-50.32)
Propionyl (C3)	7.05 (0.16-1.28)
Butyryl (C4)	0.26 (0-0.72)
Isovaleryl (C5)	0.11 (0-1.2)
Hexanoyl (c6)	0.07 (0-0.48)
Octanoyl (C8)	0.10 (0-0.72)
Tetradecenoyl (C14:1)	0.06 (0-0.55)
Palmitoyl (C16)	0.9 (0-8.43)
Succinyl/methylmalonyl (C4DC)	0.9 (<1.3)


Cpd 150: 38.092 73: + TIC Scan UOA109909.D
2-Oxo_Glutarate_2
C8:1 DCA
C8:1 DCA
3-OH-Adipate 3TMS
Suberic acid-2TMS
4-OH-Mandelate
Azelate
Vanillylmandelic acid-3TMS
4-Hydroxyphenylacetic acid-3TMS
4-OH-Phenylpyruvate-Oxime
Pantothenate 3 TMS
Palmitate TMS
3-OH-C10 DCA
N-Acetyltyrosine
4-OH-Hydroxyhippuric acid
Stearic Acid TMS
Counts vs. Acquisition Time (min)
Cpd 150: 38.092 73: + TIC Scan UOA109909.D
Lactic acid-2TMS
3-Hydroxypropionate 2TMS
3-Hydroxybutyric acid-2TMS
Acetoacetic acid Oxime Peak 1
Acetoacetic acid Oxime Peak 2
Fumaric acid-2TMS
Glutaric acid-2TMS
3-OH-Adipate-γ-Lactone
3-OH-Adipate-γ-Lactone
Adipic acid-2TMS
3-Methyl_Adipate
3-Methyl_Adipate
Unknown 116/215
Tiglylglycine-1TMS
Pimelate
Pimelate
HMG 2
4-OH-Benzoate
2-Oxo_Glutarate_2
C8:1 DCA
Counts vs. Acquisition Time (min)

Review of specialist investigations

- Acylcarnitines
  - Grossly elevated propionyl carnitine with normal methylmalonic carnitine
- Urine organic acids
  - Grossly elevated 3-hydoxypropinate with strongly raised methylcitrate
  - Strongly elevated lactate, 2hydroxybutyrate , 3-hydroxbutyrate, acetoacetate
- Final diagnosis
  - Propionic acidaemia

pathway


Valine
methylmalonate semialdehyde
Isoleucine
2-methylacetoacetyl-CoA
Biotin
Propionyl-CoA
methylmalonate-CoA
2-methylcitrate
Propionylglycine
Propionylcarnitine
3-OH propionate
Succinyl-CoA
TCA cycle

Case 2

- 21 month old male
- 15 month history of abdominal distention and loose stalls
- Eating and drinking well since birth
- But for last few days/week becoming more unwell
- No hypoglycaemia
- No abnormal movements
- No family history of note
- Older sibling age 3 years old no concerns

Case 2 blood gases

Test	Result	Test	Result
pH	7.32	Anion Gap	16.2
pCO2	5.6	Lactate	10.2
Bicarbonate	20.4	Glucose	5.9
BE	-4.2	Potassium	3.9

- Metabolic Acidosis with negative base excess and high anion gap, with raised lactate
- USS abdomen – No Ascites lots of free air in the intestine liver diffusely enlarged with right lobe of the liver noted to extended to the pelvis. No focal abnormality. Spleen not enlarged.

Case 2 routine bloods

Test	Result	Test	Result
Sodium	139 (133-146)	ALP	458 (145-320)
Potassium	4.1 (3.5-5.5)	Total Bilirubin	6 (<18)
Urea	4.8 (2.5-6.0)	AST	1218 (10-60)
Creatinine	15 (15-31)	gGT	180 (6-19)
Bicarbonate	15 (20-30)	Lactate	9.6 (0.7-2.1)
Chloride	99 (98-107)	Trigs	8.4 (0.27-1.55)
Albumin	48 (35-52)	Cholesterol	6.2 (1.2-4.7)
ALT	1010 (5-45)	Ammonia	73 mmol/L

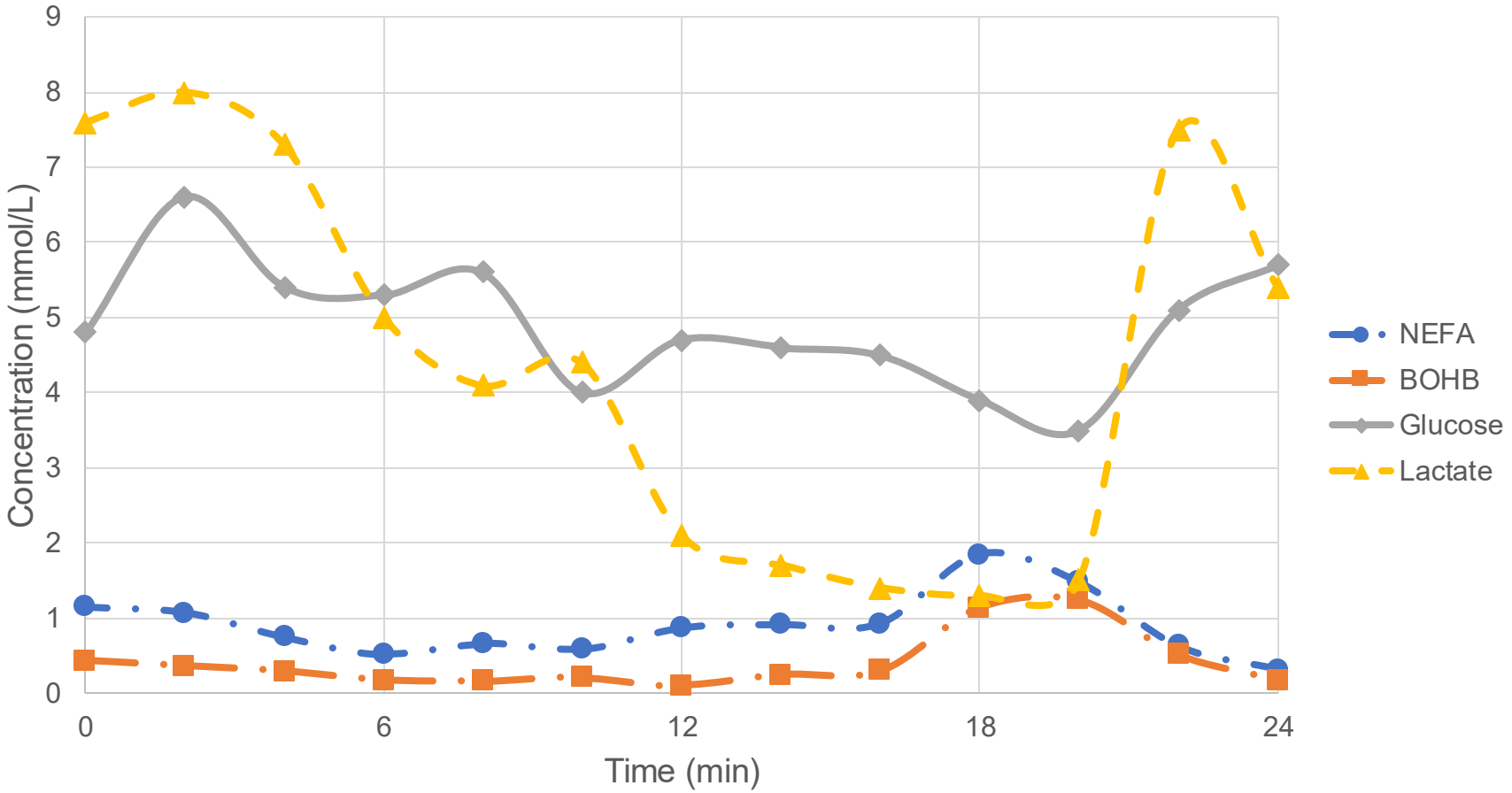
Haematology unremarkable

Case 2 review

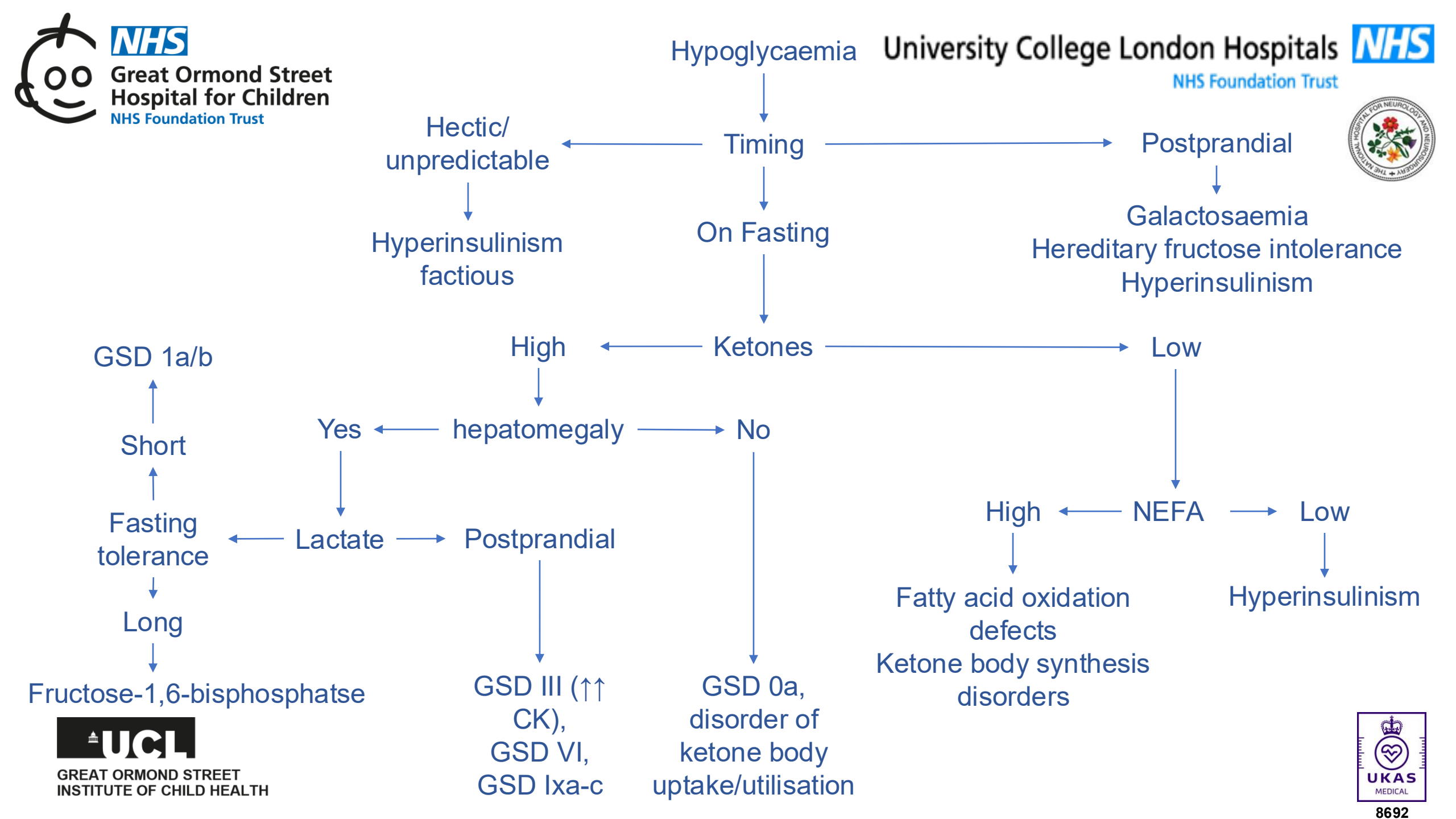
- Transaminitis and elevated Trigs, Chol and lactate
- Possible causes
  - Hepatises (viral/autoimmune)
  - Wilsons
  - A1AT deficiency
  - Tyrosinemia type 1
  - Galactosaemia
  - Lysosomal acid lipase
  - MPS disorders
  - Urea cycle defects
  - Congenital disorders of glycosylation
  - Fructose-1,6-bisphosphatase
  - Heredity fructose intolerance
  - Glycogen storage disease

- Coeliac screen negative
- A1AT Normal PIMM phenotype
- Copper Ceruloplasmin normal
- Gal-1-PUT and Gal-1-P normal
- Plasma amino acids : normal
- Organic acids
- Acylcarnitine profile normal
- Transferrin isoforms normal pattern
- ANA negative
- Normal total urine GAGs
- Anti mitochondrial AB negative
- Serology all negative
- Lysosomal white cell enzymes normal

Lactate is still persistently raised 3 days post admission 5.4 mmol/L.
Glucose at end of 12 hr fast 4.2 mmol/L



Lactate increases post prandially and falls upon fasting
Patient is able to mobile free fatty acid and generate BOHB in response to fasting
Glucose does fall at the end of the fast



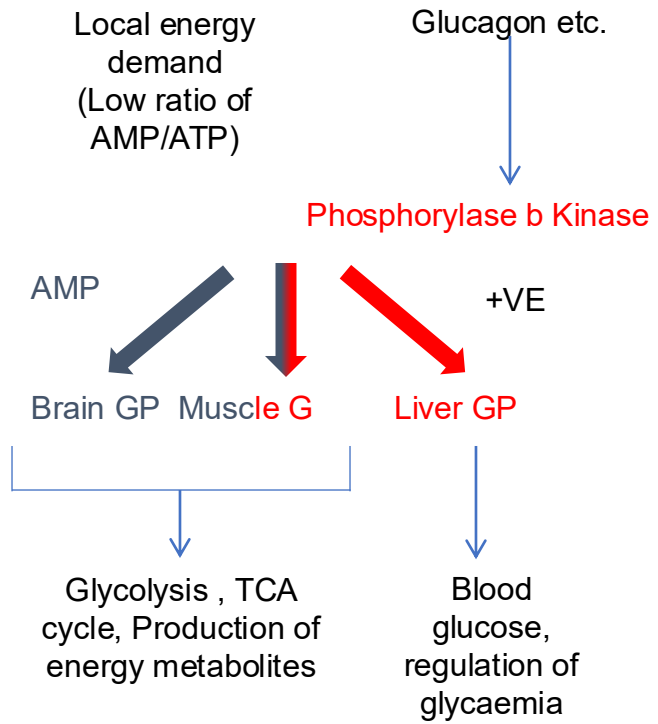
GSD screen

Test	Result
Erythrocyte Glycogen content	34 ug/g Hb (10-120)
Erythrocyte Phosphorylase B kinase	2 umol/min/ g Hb (10-90)
Leucocyte phosphorylase A	1.82 nmol/hr/mg ptn (2.70-8.32)
Leucocyte Total phosphorylase	0.32 nmol/hr/mg ptn (0.68-5.83)
Ratio Phosphorylase A/Total phosphorylase	0.18 (0.42-0.83)
Leucocyte debrancher activity	89 nmol/hr/mg ptn

Conclusion: Low Phosphorylase A activity, with low phosphorylase b kinase activity and low ratio of phosphorylase B kinase activity all in keeping with a diagnosis of GSD type IX a or b
Confirmed by genetic finding hemizygous mutation in PHKA2 (c.347A.G p.Y116C)

Phosphorylase b kinase (IX)

- Activator for Phosphorylase
- ~30% of GSDs
  - 75 % of cases x linked
- Muscle and Liver isoforms of some of the subunits
- Hepatic forms
  - Hypoglycemia, (worse than GSD VI)
  - Hepatomegaly
- Myopathic forms
  - Milder symptoms than GSD V



Phosphorylase b kinase

Type	Inheritance	Tissue	gene
IXa	X-linked	Liver	PHKA2
IXb	AR	all	PHKB
IXc	AR	Liver	PHKG2
IXd	X-linked	Muscle	PHKA1
IXe	AR	Muscle	PHKG1

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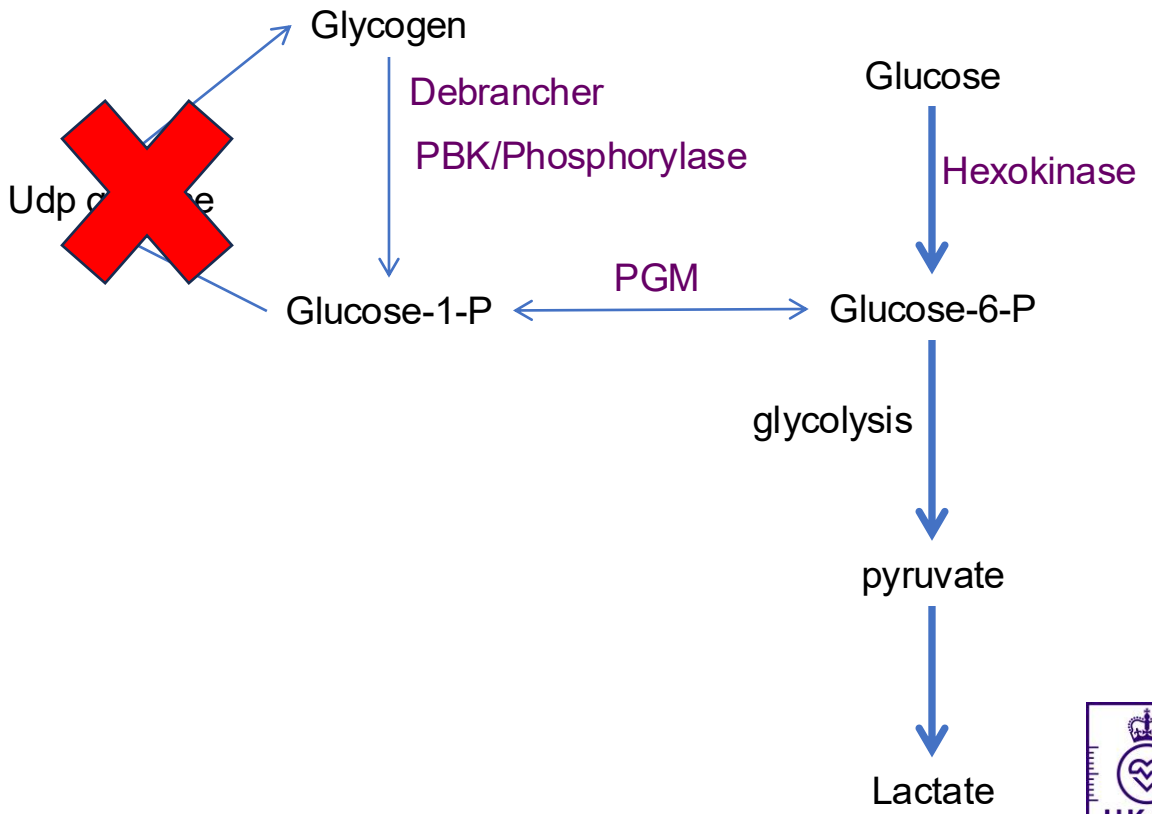
Routine biochemistry Investigation for Hepatic GSD's

Routine biochemistry in hepatic GSDs	
Liver function test	Derange in hepatic GSDs
Glucose	Frequent hypoglycaemia
Insulin NEBO etc	Rule out other causes of hypoglycaemia
Full blood count	GSD 1 b causes neutropoenia
Lactate	Will become elevated in hepatic GSD when patient given a glucose challenge
Urate	Elevated in GSD 1
triglycerides	Elevated in some hepatic GSDs



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Case 3

- 11 year old female
- Presents to local A + E with rapid deterioration in behaviour, and development of seizures
- Present 1 week prior with abdominal pain and vomiting given anti-emetics
- Similar episode in Pakistan 3 years earlier
- Parents second cousins
- On admission sodium 119
- Not on any medication associated with hyponatremia
- Given 3x NaCl boluses and transferred to specialist paediatric hospital

Test	Result	Test	Result
Sodium	120(133-146)	Plasma Osmo	260(282-300)
potassium	4.5 (3.5-5.5)	Urine osmo	612
Creatinine	75 (36-64)	Urine Sodium	190
Urea	7.4 (2.5-6.0)	TSH	0.22 (0.5-6.5)
Bilirubin	7 (<18)	FT4	34.1 ((10.3-24.2)
Albumin	41 (37-56)	Lipase	913(10-180)
ALP	180 (105-420)	Trigs	1.22 (0.42-1.47)
ALT	39 (10-55)	Cholesterol	5.5 (3.3-5.6)
Calcium	2.25 (2.19-2.66)	Urine toxicology	Unremarkable
Phosphate	1.45 (1.10-1.75)	Glucose	4.8

Trop I, BNP both normal

Case 3 review

- Hypotonic hyponatremia with raised lipase
  Raised lipase = probable Pancreatitis
- Causes of low sodium we can exclude
  - Hypothyroidism
  - Renal
- Clinical team felt that urine sodium appropriate given boluses of NaCl
  - Though maybe related to inappropriate antidiuretic hormone release
- Needed to exclude Adrenal

Adrenal

- Urine steroid profile
  - Undeletable allo-reduced cortisol metabolites and very low 5-alpha aldosterone metabolites
- Findings would be consistent with 5-alpha reductase deficiency
- Karyotype 46 XX, Tanners stage 2
- Pelvic US normal female structures
- Genetics negative for mutations in SRD5A2 gene
- Cortisol taken at 9 am 746 nmol/L
- Renin 1.1 nmol/L/hr
- Need to go back over clinical details
  - Hyponatremia, abdominal pain, and neurological disturbance
  - Upon further questioning patient noted to rash on her arms
  - ?possible porphyria

First line testing for Porphyria's

- ALL PROTECTED FROM LIGHT
- Acute
  - Urine porphobilinogen front line (ideally taken during acute attack)
    - Urine concentration assessed by measuring creatinine, request a repeat if urine creatinine < 2mmol/l
  - Confirmatory – Urine total porphyrins (usually done on same sample as PBG), faecal and plasma porphyrins

- Cutaneous
  - EDTA plasma scan (fluorescence emission spectroscopy)
  - Confirmatory – urine total porphyrins, whole blood porphyrins, faecal porphyrins

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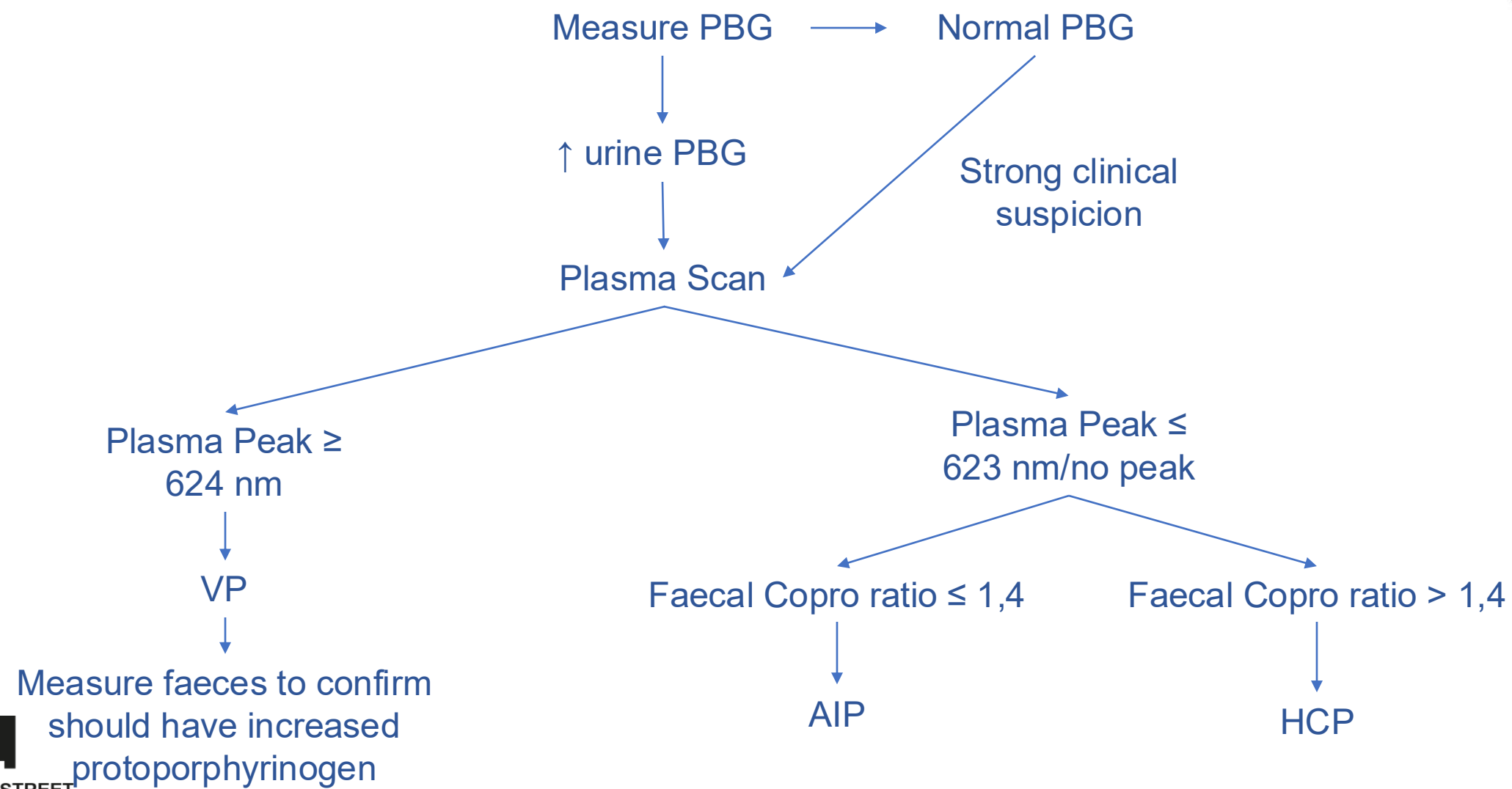
Acute porphyria



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Urine Porphyrin screen

Test	Result	Range
5-aminolaevulinic acid	6.6 μmol/mmol creatine	<3.8
Porphobilinogen	0.8 μmol/mmol creatine	<1.5
Total Porphyrin	43.3 μmol/mmol creatine	<35
Creatinine	5.5 mmol/L	

- Normal PBG, with slightly raised ALA and urine total porphyrin
- Results do not exclude inheritance of an acute porphyria, as could be in latent stage or in remission

Isolated ALA can be due to the following
Lead toxicity Lead levels normal
Alcohol or fasting Not picked up on Urine tox screen
Induction of ALA synthase by drugs e.g. barbiturates and sulphonamides Not on any medication that does
ALA dehydratase deficiency Very rare
Tyrosine type 1. No liver dysfunction

Final Porphyria results

Test	Result	
Erythrocyte free protoporphyrin	0.08 umol/l cells	<0.20
Erythrocyte total protoporphyrin	0.09 umol/l cells	<0.80
Erythrocyte HMB synthase	19.0 nmol/ml cells/hr	20-42
Plasma scan	Large peak at 627 nm	
Total Faecal porphyrins	710 nmol/g faeces	(<50)
Faecal HPLC	Increased protoporphyrinogen	

Likely diagnosis variegate porphyria
Plasma Peak at >624 nm
Increased protoporphyrinogen in faeces

Genetics PPOX heterozygote c.223-35_293del

Final thoughts on case 3

- Hyponatremia thought to be caused by excessive release of antidiuretic hormone
- Why the 5-alpha reductase deficiency metabolites
- Innala *et al* 2012
  - Demonstrated that females with AIP have lower 5a-reduced progesterone during luteal phase than healthy controls but no difference between patients with active AIP and latent AIP
- Pozo *et al* 2014
  - Case study of 24 patients with AIP and 24 healthy controls
  - Showed decreased levels of 41 steroids in AIP patients mainly hydroxylated steroids
  - Postulated maybe due to perturbed haem synthesis affecting cytochrome p450 involve in steroid biosynthesis or energy metabolism in the adrenals
- Most recent steroid profile: 5 alpha cortisol metabolites are now detectable

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Thank you

- Any Questions
  - Final 3 slides are some useful tables of information that may help with FRCPath exams.

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Useful OA disorders for RCPath exams

Disorder	Organics	Acylcarnitines	Other points
Propionic acidaemia	Elevated 3-hydroxypropionate, propionylglycine, methylcitrate	Elevated propionylcarnitine	Methylcitrate may be lost under Hippurate peak if patient on ammonia scavengers
Methylmalonic acidaemia	Elevated methylmalonic acid and methylcitrate	Elevated propionylcarnitine and methylmalonate	Methylcitrate may be lost under Hippurate peak if patient on ammonia scavengers
Isovaleric Acidaemia	Elevated 3-hydroxyisovalerate, 2-hydroxyisovalerate and isovalerylglycine	Elevated isovalerylcarnitine	Part of NBS false positives due pivoyl containing antibiotics isobaric for isovalerylcarnitine
Glutamic acidaemia	Glutarate and 3-hydroxyglutarte	Glutaryl-carnitine	Part of NBS, Normal C5DC carnitine does not exclude GA1 due to non-secretor phenotype, urine carnitine analysis can help with diagnosis

FAO disorders for RCPath exams

Disorder	Acyl-Carnitins	Other
Medium chain acyl-CoA Dehydrogenase deficiency	Elevated C8 with C8;C10 >2.0	Suberylglycine hexonylglycine and phenylacetylglycine can be present in urine organic acids
Very long chain acyl-CoA dehydrogenase deficiency	Diagnostic compound elevated C14:1 May also see elevated C12, C16, C16:1, C 18and C18:1	Elevated C6-C14 dicarboxylic and hydroxdicarboxylic acids on crisis urine
Long chain hydroxyacyl-CoA dehydrogenase deficiency	Elevated C16-OH, C16:1-OH, C18-OH and C18:1-OH	3-hydroxydicarboxylic aciduria with absent ketones on urine organic acids
Multiple acyl-CoA dehydrogenase deficiency	Can see elevated C4, C5, C6, C8, C10, C12, C14, C14:1, C5DC	Iosvalerylglycine, glutrate, 3-hydroxyglutarate, suberylglycine, hexanoylglycine can be present in urine organic acids
Carnitine palmitoyl transferase 1 CPT1	Elevated free carnitine with low C16 C0/(C16+C18 ratio)	Blood spot preferred sample type
Carnitine palmitoyl transferase CPT2	Elevated C16 carnitine with low free carnitine C2/(c16+C18:1) ratio	Plasma preferred sample type

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Porphyria

Disease	Enzyme	inheritance	presentation	tissue	Urine	Faecal	Plasma	erythrocyte
ALA dehydrogenase deficiency porphyria (ADP)	ALA dehydrogenase	AR	Neurological	Erythropoietic	↑ALA	Normal	Normal	Protoporphyrin (zinc)
Acute intermittent porphyria	HMB synthase	AD	Neurological	Hepatic	↑PBG	Normal	Peak at 615-620 nm	Normal
Hereditary coproporphyria (HCP)	Coproporphyria oxygenase	AD	Neurological/ Photosensitivity	Hepatic	Coproporphyrin III	Coproporphyrin III	Peak at 615-620 nm	Normal
Variegate porphyria (VP)	Protoporphyrinogen oxidase	AD	Neurological/ Photosensitivity	Hepatic	Coproporphyrin III	Protoporphyrin >Coproporphyrin III	Peak at 624-627 nm	Normal
Porphyria cutanea tarda PCT	Uroporphyrinogen decarboxylase	AD/ acquired	Photosensitivity	Hepatic	Uroporphyrin I and III	Isocoproporphyrin	Peak at 615-620 nm	Normal
Congenital erythropoietic porphyria CEP	Uroporphyrinogen cosynthase	AR	Photosensitivity	Erythropoietic	Uroporphyrin I Coproporphyrin	Coproporphyrin I	Peak at 615-620 nm	Protoporphyrin (zinc) Uroporphyrin I free
Erythropoietic porphyria	ferrochelatase	AD	Photosensitivity	Erythropoietic	Normal	↑protoporphyrin (40% of cases)	Peak at 626-634 nm	Protoporphyrin