

Case of A Puzzling Acylcarnitine Profile

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Male patient

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04/08/2022 Bloodspot acylcarnitine sample received from Royal Cornwall Hospital

Clinical details: jaundice, reduced eating, hereditary spherocytosis

Bloodspot Acylcarnitine Profile by MS/MS

Bloodspot Free Carnitine	*	122.54	umol/L	8.8 - 41.8	* high
C2	*	39.98	umol/L	5.2 - 28.1	* high
C3	*	7.74	umol/L	0.47 - 2.50	* high
C4	*	0.68	umol/L	< 0.44	* high
C5:1		0.02	umol/L	< 0.04	
C5	*	0.23	umol/L	< 0.21	* high
C4-OH	*	0.38	umol/L	< 0.38	* high
C6		0.05	umol/L	< 0.09	
C5-OH		0.32	umol/L	< 0.36	
C8		0.05	umol/L	< 0.15	
C3DC		0.06	umol/L	< 0.09	
C10:1		0.03	umol/L	< 0.12	
C10		0.03	umol/L	< 0.24	
C4DC		0.38	umol/L	< 1.0	
C5DC		0.03	umol/L	< 0.09	
C12:1		0.03	umol/L	< 0.12	
C12		0.05	umol/L	< 0.15	
C6DC		0.02	umol/L	< 0.07	
C6DC-OH		0.01	umol/L	< 0.03	
C14:2		0.04	umol/L	< 0.06	
C14:1		0.09	umol/L	< 0.15	
C14		0.10	umol/L	< 0.33	
C8DC		0.01	umol/L	< 0.05	
C14:1-OH		0.04	umol/L	< 0.06	
C14-OH		0.01	umol/L	< 0.04	
C16:1	*	0.15	umol/L	< 0.15	* high
C16		1.63	umol/L	0.28 - 1.63	
C10DC		0.09	umol/L	< 0.17	
C16:1-OH		0.07	umol/L	< 0.1	
C16-OH		0.02	umol/L	< 0.04	
C18:2	*	0.64	umol/L	0.09 - 0.48	* high
C18:1	*	2.87	umol/L	0.44 - 1.81	* high
C18		1.03	umol/L	0.19 - 1.22	
C18:1-OH	*	0.03	umol/L	< 0.03	* high
C18:OH		0.01	umol/L	< 0.02	
FC/ (C16+C18)	*	46.07		< 39.3	* high
(C16+C18:1)/C2		0.11		< 0.28	

Bloodspot Acylcarnitine Profile by M S/M S

Report Comment:

Free carnitine (C0) = 122.5 umol/L

(ref range 8.8 - 41.8)

Acetylcarnitine (C2) = 39.9 umol/L

(ref range 5.2 - 28.1)

Marked increase in free carnitine, together with increases in acetylcarnitine, propionylcarnitine and a number of other short and long chain acylcarnitine species. Is patient on carnitine supplementation? If not, advise repeat bloodspot and plasma acylcarnitines, with urine organic acids, to investigate further.

23/11/2022

Repeat bloodspot sample received

Bloodspot Acylcarnitine Profile by MS/MS

Bloodspot Free Carnitine	*	119.01	umol/L	8.8 - 41.8	* high
C2	*	61.23	umol/L	5.2 - 28.1	* high
C3	*	10.08	umol/L	0.47 - 2.50	* high
C4		0.39	umol/L	< 0.44	
C5:1		0.02	umol/L	< 0.04	
C5	*	0.21	umol/L	< 0.21	* high
C4-OH		0.21	umol/L	< 0.38	
C6		0.07	umol/L	< 0.09	
C5-OH		0.16	umol/L	< 0.36	
C8		0.05	umol/L	< 0.15	
C3DC		0.07	umol/L	< 0.09	
C4DC		0.46	umol/L	< 1.0	
C5DC		0.04	umol/L	< 0.09	
C12:1		0.09	umol/L	< 0.12	
C12		0.07	umol/L	< 0.15	
C6DC		0.01	umol/L	< 0.07	
C6DC-OH		0.02	umol/L	< 0.03	
C14:2		0.04	umol/L	< 0.06	
C14:1		0.11	umol/L	< 0.15	
C14		0.24	umol/L	< 0.33	
C8DC		0.02	umol/L	< 0.05	
C14:1-OH	*	0.07	umol/L	< 0.06	* high
C14-OH		0.01	umol/L	< 0.04	
C16:1	*	0.22	umol/L	< 0.15	* high
C16	*	2.72	umol/L	0.28 - 1.63	* high
C10DC		0.12	umol/L	< 0.17	
C16:1-OH	*	0.10	umol/L	< 0.1	* high
C16-OH		0.03	umol/L	< 0.04	
C18:2	*	0.60	umol/L	0.09 - 0.48	* high
C18:1	*	2.73	umol/L	0.44 - 1.81	* high
C18		1.16	umol/L	0.19 - 1.22	
C18:1-OH	*	0.05	umol/L	< 0.03	* high
C18:OH		0.01	umol/L	< 0.02	
FC/ (C16+C18)		30.67		< 39.3	
(C16+C18:1)/C2		0.09		< 0.28	

Further clinical information:

Seen in eating disorders clinic – had been acutely unwell with acute food restriction and associated complications of hepatitis, anaemia.

Prescribed vitamin supplements (folic acid, thiamine).

Interested in eating high protein diet to “bulk up”, ?taking carnitine (also, for weight loss effects).

Mum confirmed not taking any other supplements.

Bloodspot Acylcarnitine Profile by M S/M S
Report Comment:

Free carnitine (C0) = 119 $\mu\text{mol/L}$ (ref 8.8-41.8)

Acetylcarnitine (C2) = 61.2 $\mu\text{mol/L}$ (ref 5.2-28.1)

Propionylcarnitine (C3) = 10.1 $\mu\text{mol/L}$ (ref 0.47-2.5)

Similar profile to previous results with a notable persistent increase in total free carnitine, acetylcarnitine and propionylcarnitine and mild increase in long chain acylcarnitine species. In the absence of a history of carnitine supplementation, along with the significant increase in C3 carnitine, further investigation is warranted. Advise urine organic acids and quantitative methylmalonic acid, total plasma homocysteine, plasma amino acids, ammonia, a check of B12 status, plasma carnitine and repeat blood spot acylcarnitines. Please provide results of liver function at the time of sampling.

Further samples received 23/05/2023

Bloodspot Acylcarnitine Profile by MS/MS

Bloodspot Free Carnitine	*	174.50	umol/L	8.8 - 41.8	* high
C2	*	63.52	umol/L	5.2 - 28.1	* high
C3	*	9.92	umol/L	0.47 - 2.50	* high
C4		0.33	umol/L	< 0.44	
C5:1		0.02	umol/L	< 0.04	
C5	*	0.23	umol/L	< 0.21	* high
C4-OH		0.17	umol/L	< 0.38	
C6		0.04	umol/L	< 0.09	
C5-OH		0.19	umol/L	< 0.36	
C8		0.03	umol/L	< 0.15	
C3DC		0.02	umol/L	< 0.09	
C10:1		0.03	umol/L	< 0.12	
C10		0.03	umol/L	< 0.24	
C4DC		0.30	umol/L	< 1.0	
C5DC		0.06	umol/L	< 0.09	
C12:1		0.03	umol/L	< 0.12	
C12		0.06	umol/L	< 0.15	
C6DC		0.00	umol/L	< 0.07	
C14:2		0.03	umol/L	< 0.06	
C14:1		0.07	umol/L	< 0.15	
C14	*	0.36	umol/L	< 0.33	* high
C14-OH		0.01	umol/L	< 0.04	
C16:1	*	0.17	umol/L	< 0.15	* high
C16	*	2.73	umol/L	0.28 - 1.63	* high
C16:1-OH		0.09	umol/L	< 0.1	
C16-OH		0.02	umol/L	< 0.04	
C18:2		0.42	umol/L	0.09 - 0.48	
C18:1		1.77	umol/L	0.44 - 1.81	
C18		1.22	umol/L	0.19 - 1.22	
C18:1-OH		0.02	umol/L	< 0.03	
C18:OH		0.01	umol/L	< 0.02	
FC/ (C16+C18)	*	44.18		< 39.3	* high
(C16+C18:1)/C2		0.07		< 0.28	

Paired plasma sample received:

Plasma Acylcarnitine Profile by MS/MS

Free Carnitine	38.32	umol/L	15.0 - 53.0
Acetylcarnitine C2	8.85		5.5 - 27.2
Propionylcarnitine C3	0.68		< 1.3
Butyrylcarnitine C4	0.19		< 0.4
Tiglylcarnitine C5:1	0.02		< 0.04
Isovalerylcarnitine C5	0.05		< 0.5
3-OH-Butyrylcarnitine C4-OH	0.02		< 0.1
Hexanoylcarnitine C6	0.01		< 0.12
3-OH-isovalerylcarnitine C5-OH	0.02		< 0.06
Octanoylcarnitine C8	0.01		< 0.22
Malonylcarnitine C3DC	0.01		< 0.1
C10:1	0.02		< 0.22
Decanoylcarnitine C10	0.02		< 0.3
Methylmalonylcarnitine C4DC	0.03		
Glutaryl carnitine C5DC	0.05		< 0.06
C12:1	0.02		< 0.1
C12	0.02		< 0.1
C6DC	0.02		
C14:2	0.01		
cis-5-Tetradecenoylcarnitine C14:1	0.02		< 0.18
Tetradecanoylcarnitine C14	0.04		< 0.2
C14-OH	0.00		
C16:1	0.03		< 0.08
Palmitoylcarnitine C16	0.17		< 0.24
C16:1OH	0.01		
3-OH-Palmitoylcarnitine C16-OH	0.00		< 0.05
C18:2	0.08		
Oleoylcarnitine C18:1	0.20		< 0.28
Stearoylcarnitine C18	0.06		< 0.1
C18:1-OH	0.00		< 0.05
3-OH-Stearoylcarnitine C18-OH	0.00		
FC/ (C16+C18)	166.61		
(C16+C18:1)/C2	0.04		

Other investigations:

Urine organic acids

Profile shows a mild increase in excretion of orotic acid with a trace of uracil also detected. Patient is noted to have hereditary spherocytosis. Orotic acid may be mildly increased secondary to the increased cell turnover seen in haemolytic anaemia. Advise sending a repeat fresh urine sample for organic acids to check for persistence. In view of previous bloodspot acylcarnitine findings, please note no increases in methylmalonic acid or associated metabolites were detected.

Total plasma homocysteine

5.1 umol/L (2.0 – 14.3)

Plasma amino acids

Profile is not suggestive of an amino acid or urea cycle disorder. Low levels of some amino acids noted, may be reflecting nutritional status - ?low protein intake. The variation in glutamine and cystine from normal is likely due to changes caused by delay in sample handling.

How to explain discrepant acylcarnitine profiles?

? analytical problem/interference

? secondary to haematological condition



When stuck....email/phone a (metabolic) friend!

Contacted Sheffield metabolic laboratory to discuss

Forwarded DBS and plasma sample for analysis

Confirmed discrepant DBS vs plasma results

Metabolic Fingerprint in Hereditary Spherocytosis Correlates With Red Blood Cell Characteristics and Clinical Severity

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Hereditary spherocytosis (HS)

Common cause of hereditary chronic haemolytic anaemia

Characterised by impaired red blood cell membrane integrity

RBCs from HS patients show enhanced membrane loss

Study compared DBS of HS patients with healthy controls to investigate metabolic impact of the membrane defect

Demonstrated increases in DBS C0, C2, C3, and some long chain species

Final acylcarnitine reports:

Plasma Acylcarnitine Profile by MS/MS

Free Carnitine

Report comment

38.32 umol/L

15.0 - 53.0

Normal free carnitine and acylcarnitine profile. The abnormal acylcarnitine species detected in patient's bloodspot samples noted to be normal in this plasma sample. Results confirmed by analysis at an alternative laboratory. No evidence of a fatty acid oxidation disorder detected. (Note: the diagnostic sensitivity of acylcarnitine analysis is not fully defined. In particular, patients with VLCAD deficiency, some cases of glutaryl-CoA dehydrogenase deficiency, and carnitine depleted patients with some other disorders are known to give false negative results).

Bloodspot Acylcarnitine Profile by M S/M S

Report Comment:

Persistent marked increases in bloodspot free carnitine, acetylcarnitine and propionylcarnitine. Free carnitine (C0) = 174.5 umol/L (ref 8.8-41.8) Acetylcarnitine (C2) = 63.5 umol/L (ref 5.2-28.1) Propionylcarnitine (C3) = 9.92 umol/L (0.47-2.50) Results confirmed by analysis at an alternative laboratory. These findings have been described in the literature in patients with hereditary spherocytosis, and likely to be secondary to the decreased red blood cell integrity. Plasma acylcarnitine profile noted to be normal (see report for specimen number 23B00097589).

Learning points

- Requesting both DBS and plasma
- Discuss with other labs/colleagues
- Full clinical history, awareness of other conditions