ERNDIM DPT schemes 2014
common sample
HHH syndrome
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QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

ERNDIM DPT Centre Switzerland

Brian Fowler
University Children's Hospital
Basel /Zürich

email: brian.fowler@ukbb.ch


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www.ssiem2014.org
THE NORTH FACE

Patient information (child under care of Johannes Haeberle, left in photo with Matthis Gautschi)

Patient details provided:

Following uneventful pregnancy and birth this male child showed mild hypotonia at 6 months of age. A few months later, developmental delay and failure to thrive with elevated transaminases was observed. The urine was collected at the age of 8.75 years whilst receiving specific treatment.

Further information

The child showed the first symptoms at 6 months, then at 14 months liver dysfunction when increased ammonia (200 micromol/L) increased ornithine, orotic acid and mild increase of homocitrulline was found leading to diagnosis of HHH .

Follow up

Mutation analysis

Ornthine transporter gene *SLC25A15* gene

Homozygous mutation in exon 2 of SLC25A15 found - c.208_209delGCinsTT.

Treatment

Low protein diet

Citrulline

Review Article

Tessa et al. Human Mutation 2009 30:741-748

Table 1. Clinical and Molecular Features in New Hyperornithinemia-Hyperammonemia-Homoci

Patient/ Sex	AF	Family/ Origin	A.O.	Lethargy coma	Seizures/ myoclonus	Pyramidal signs	MR	Liver signs	Coagulopathy
1/M	6 y	A/Belgium	Infancy	No	No	No	Mild	Yes	Yes
2/F	2 m‡	B/Italy	Neonatal	Yes	No	na	na	No	No
3/M	5 y	C/Algeria	Infancy	No	No	Yes	Yes	No	No
4/M	3	C/Algeria	pr.d (2 m)	No	No	Yes	Yes	No	No
5/F	6 y	D/Senegal	Neonatal	Yes	Yes	Yes	Yes	No	Yes
6/F	6 y	D/Senegal	Neonatal	Yes	No	Yes	Mild	No	No
7/M	2 y	E/Spain	Infancy	na	na	na	Yes	na	na
8/F	2 y	F/Taiwan	Infancy	Yes	na	na	na	Yes (++)	Yes (++)
9/M	41 y	G/Italy	Childhood	Yes	No	Yes	No	No	Yes
10/M	54 y	H/Italy	Adulthood	Yes	No	Yes	No	Yes (++)	Yes
11/F	1 m	I/U.S.-Greece	Neonatal	Yes	na	na	na	no	Yes
12/M	1.5 y	J/Morocco	Infancy	Yes	No	Yes	Yes	Yes (++)	Yes (++)
13/F	3.8 y	K/Pakistan	Infancy	No	No	No	Yes	Mild	No
14/M	2 y	L/Morocco	Infancy	Yes	No	No	No	Yes (++)	Yes
15/M	4 y	L/Morocco	pr.d (6 y)	na	na	na	na	na	na
16/F	57 y	M/Pakistan	Adulthood	Yes	Yes	Yes	No	No	No

[a]GenBank reference sequence NM_014252.2.

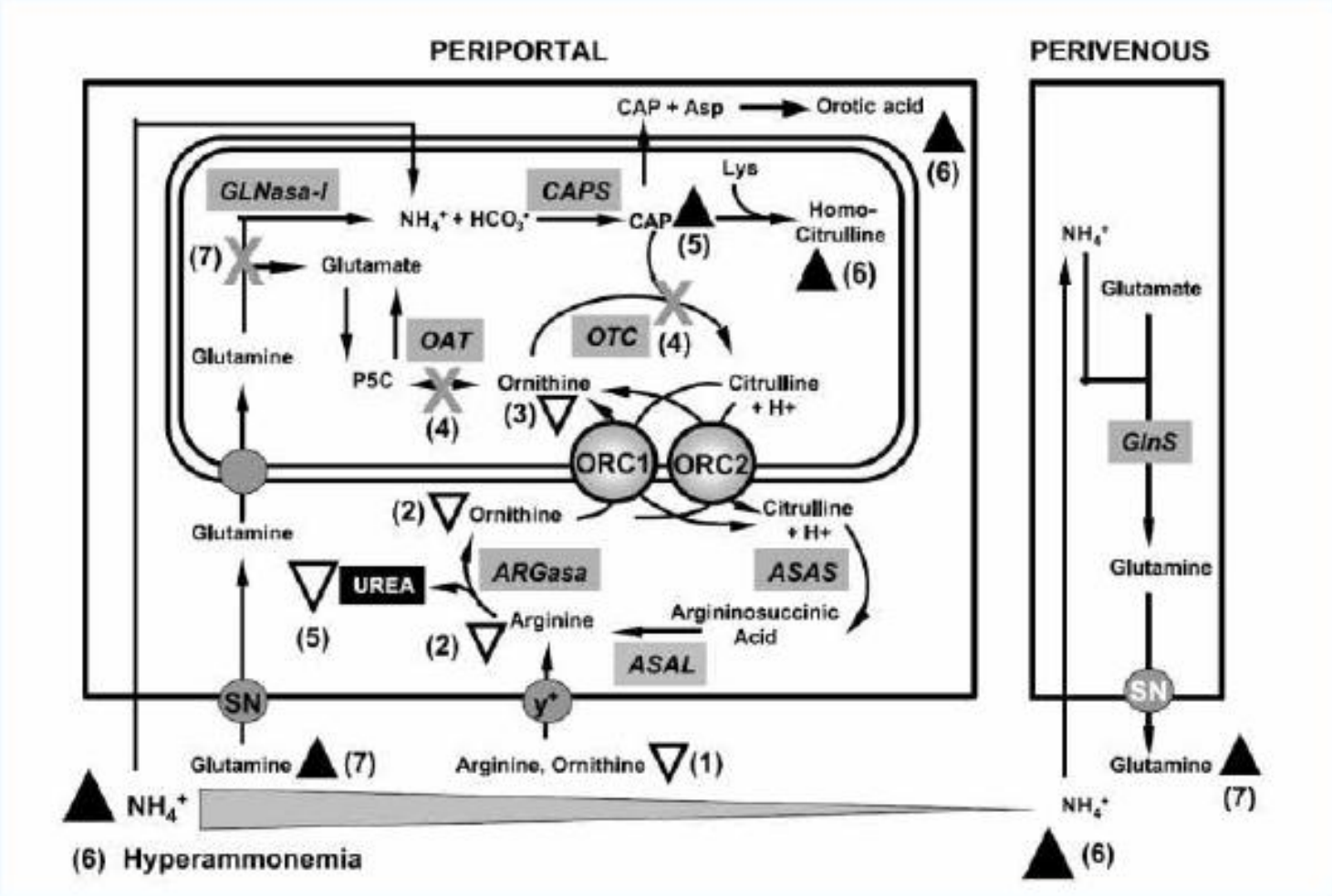
M, male; F, female; AF, age at follow-up; AO, age at onset; m, months; y, years; ‡, age at death; ++, severe; pr.d, prospective d

trullinuria (HHH) Patients

Ammonia	Ornithine	Homocitrulline	Orotate	AST/ALT	Mutation (cDNA level)[a]	Mutation (protein level)
137	951	1157	Increased	172/235	c.110T>G	p.M37R
na	na	na	na	na	p.212T>A	p.L71Q
370	822	10	4.03	25/45	p.733A>T	p.K245X
125	887	12	207	61/54	p.733A>T	p.K245X
700	509	Increased	109	66/40	c.535C>T	p.R179X
100	290	Increased	1.1	na	c.535C>T	p.R179X
75	419	1654	11.7	na	c.646G>A	p.G216S
321	450	Increased	4.7	2112/2331	c.815C>T	p.T272I
235	216	na	0.9	25/99	c.79G>A	p.G27R
222	537	Increased	12	1315/1930	c.56+1G>T/ intronic change	Frameshift
400	370	na	78	na	c.525insC/c.847C>T	p.S175fsX192/ p.L283F
200	700	139	600	na	c.208_209delCAinsTT	p.A70L
62	471	Increased	Increased	Increased	c.564C>G	p.F188L
96	493	Increased	75	na/880	c.535C>T	p.R179X
na	na	na	na	na	c.535C>T	p.R179X
306	na	na	na	na	c.208_208delCAinsTT	p.A70L

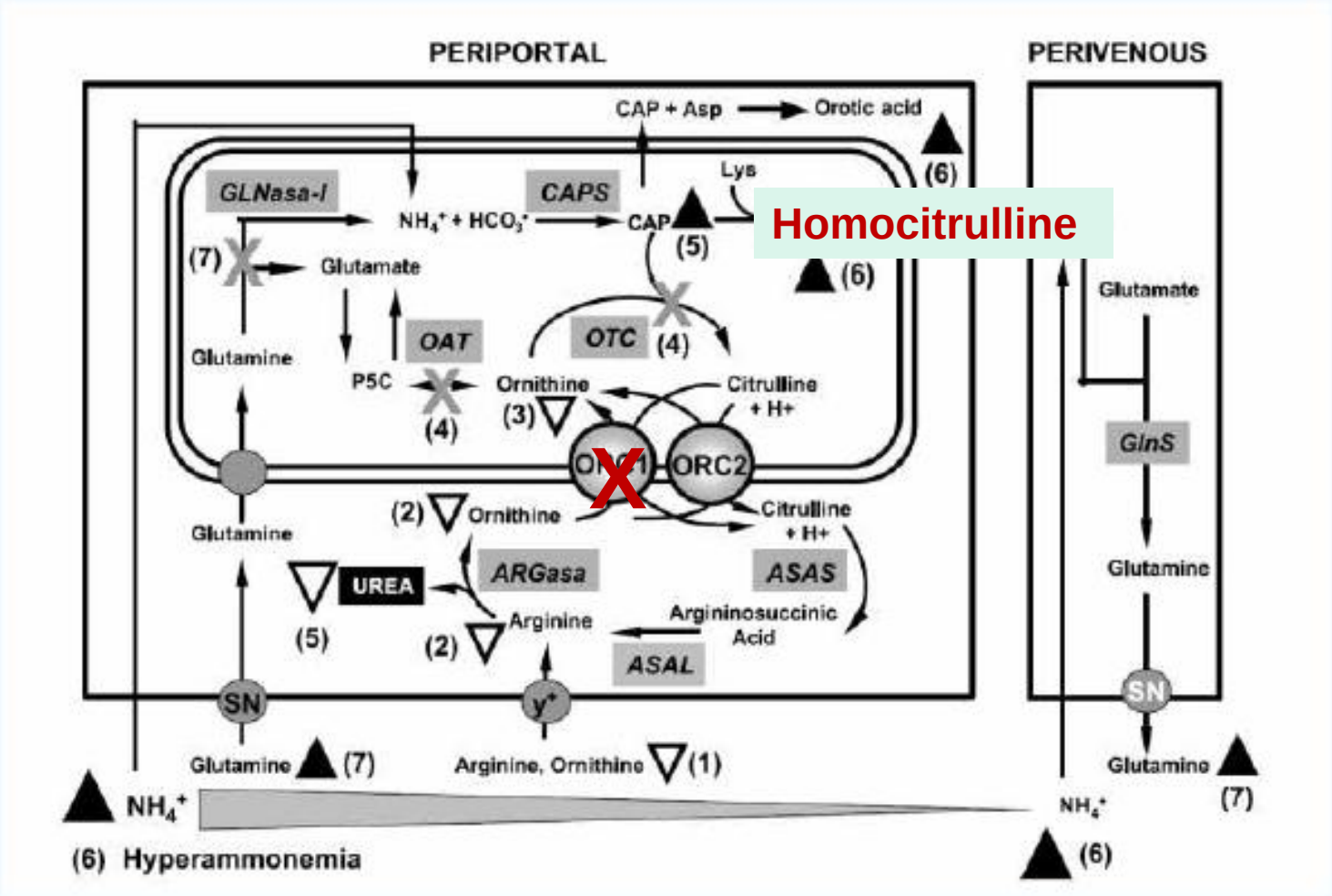
iagnosis; na, not available; MR, mental retardation. AST, aspartate transaminase; ALT, alanine transaminase.

Dysfunction of the urea cycle in HHH



Palacín M. Bertran J. Chillarón J. Estévez R. Zorzano A.: MGM 81. 2004

Dysfunction of the urea cycle in HHH



Palacín M. Bertran J. Chillarón J. Estévez R. Zorzano A.: MGM 81. 2004

DPT Centers

• Czech Republic	19	labs
• France	23	labs (2 no answer)
• Netherlands	20	labs (1 no answer)
• Switzerland	19	labs
• United Kingdom	21	labs (1 no answer)

Total Returns	98

Creatinine Values (mmol/L)

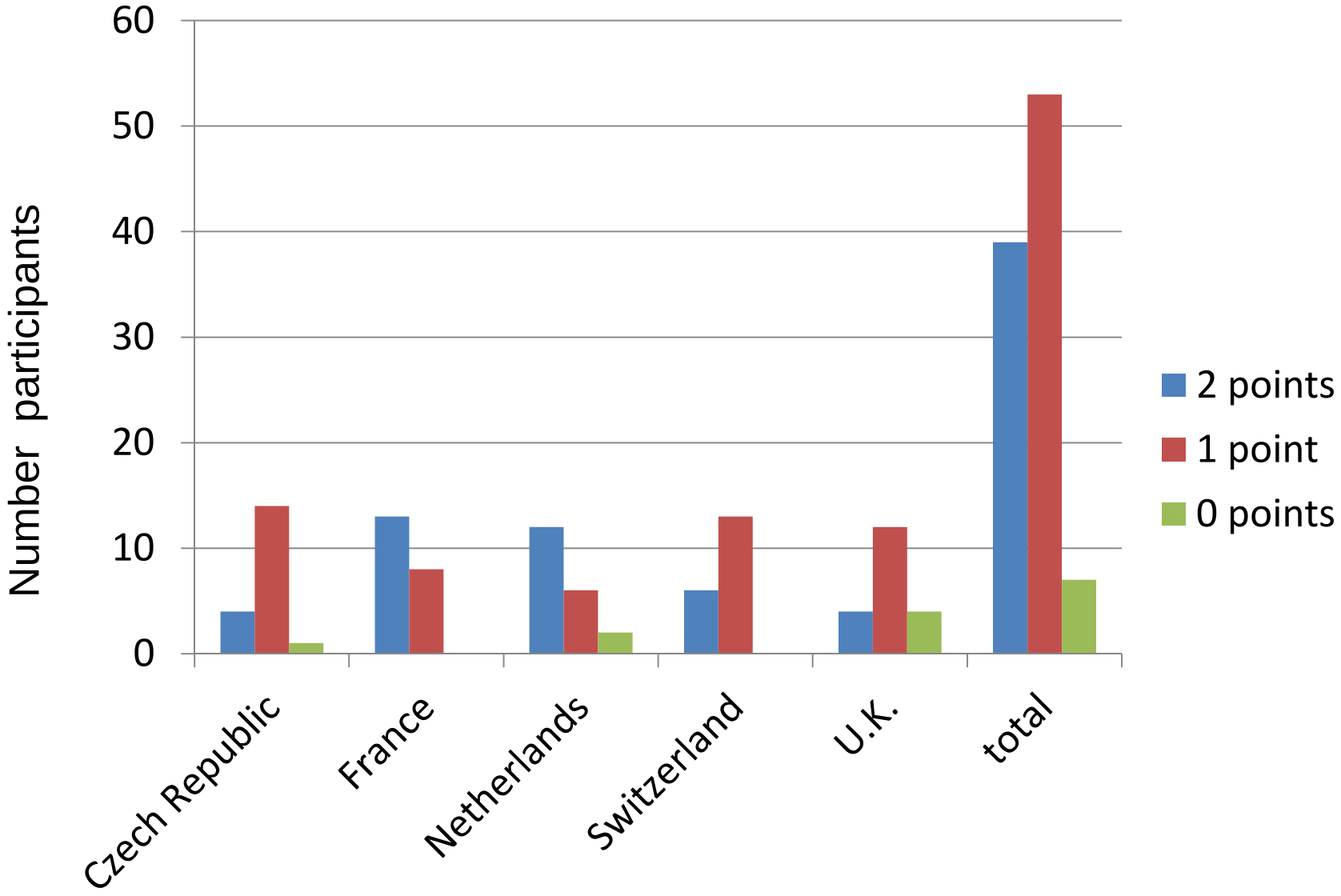
DPT Centre	n	Range	Median	Mean	Interlab CV%
Czech Republic	19	8.1 - 9.9	9.4	9.37	5%
France	21	7.9 -- 10.6	9.43	9.37	6%
Netherlands	18	8 - 11.6	9.26	9.33	8.90%
Switzerland	18	8.6 - 10.1	9.47	9.4	4%
U.K.	20	8.5 - 10.5	9.31	9.37	5%
total	96	7.9 - 11.6	9.35	9.38	5.90%

Scoring Scheme

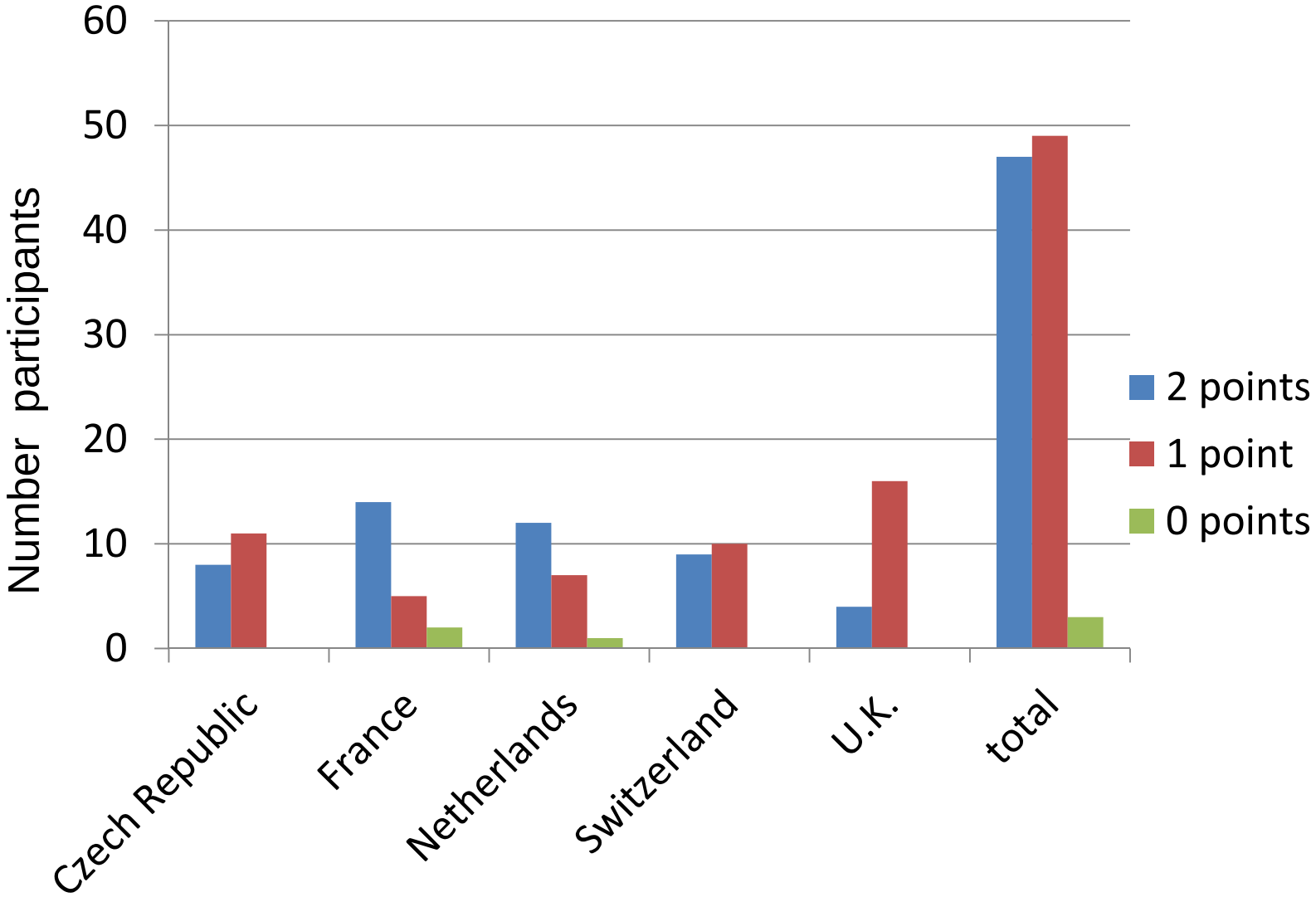
Analytical	
Homocitrulline	1 point
Orotic acid	1 point
Interpretation/ Diagnosis*	
HHH	2 points
Any urea cycle disorder	1 point

* Recommendations for confirmatory tests not scored but may be considered in scoring of interpretation

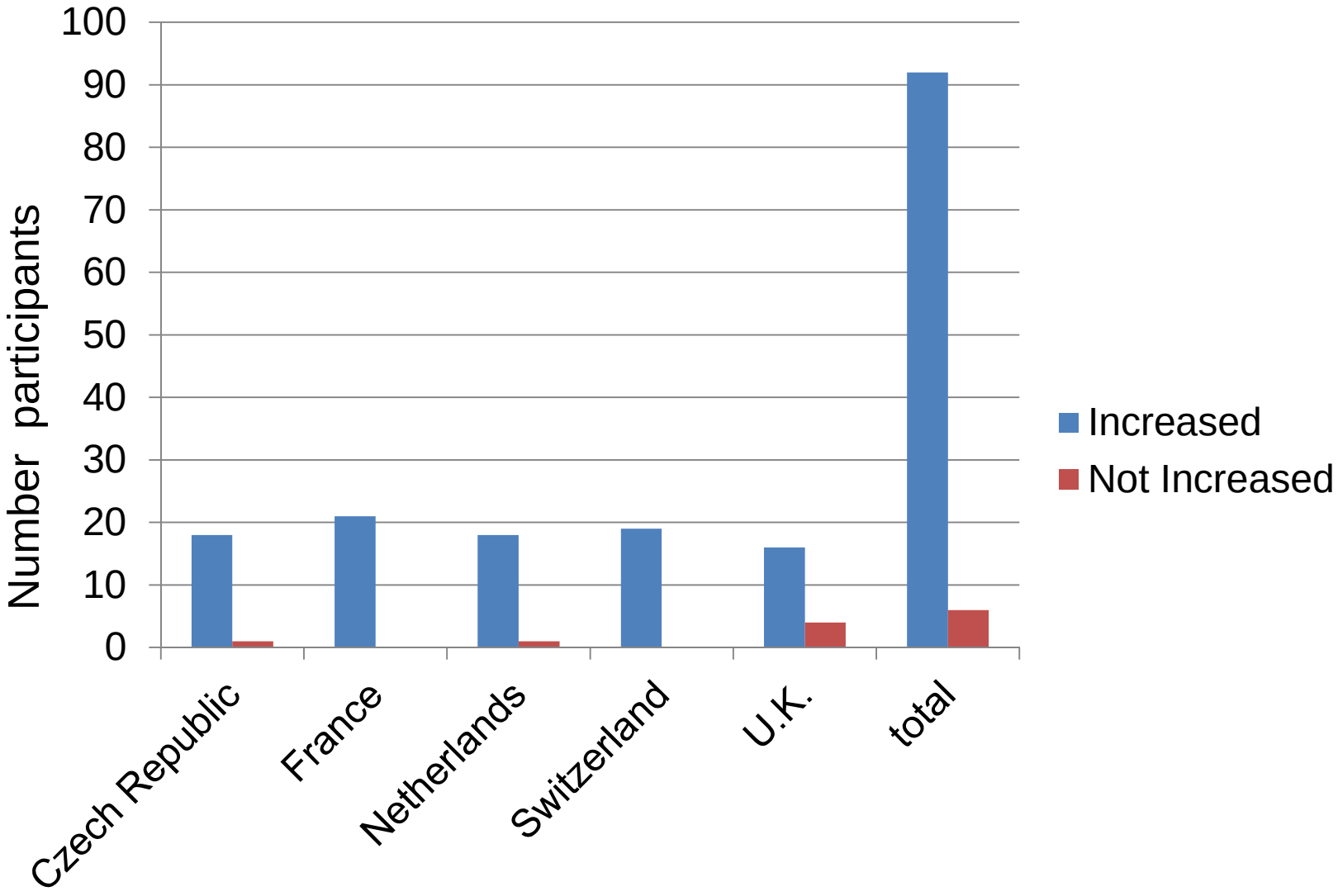
DPT 2014 Common Sample: Analytical Performance



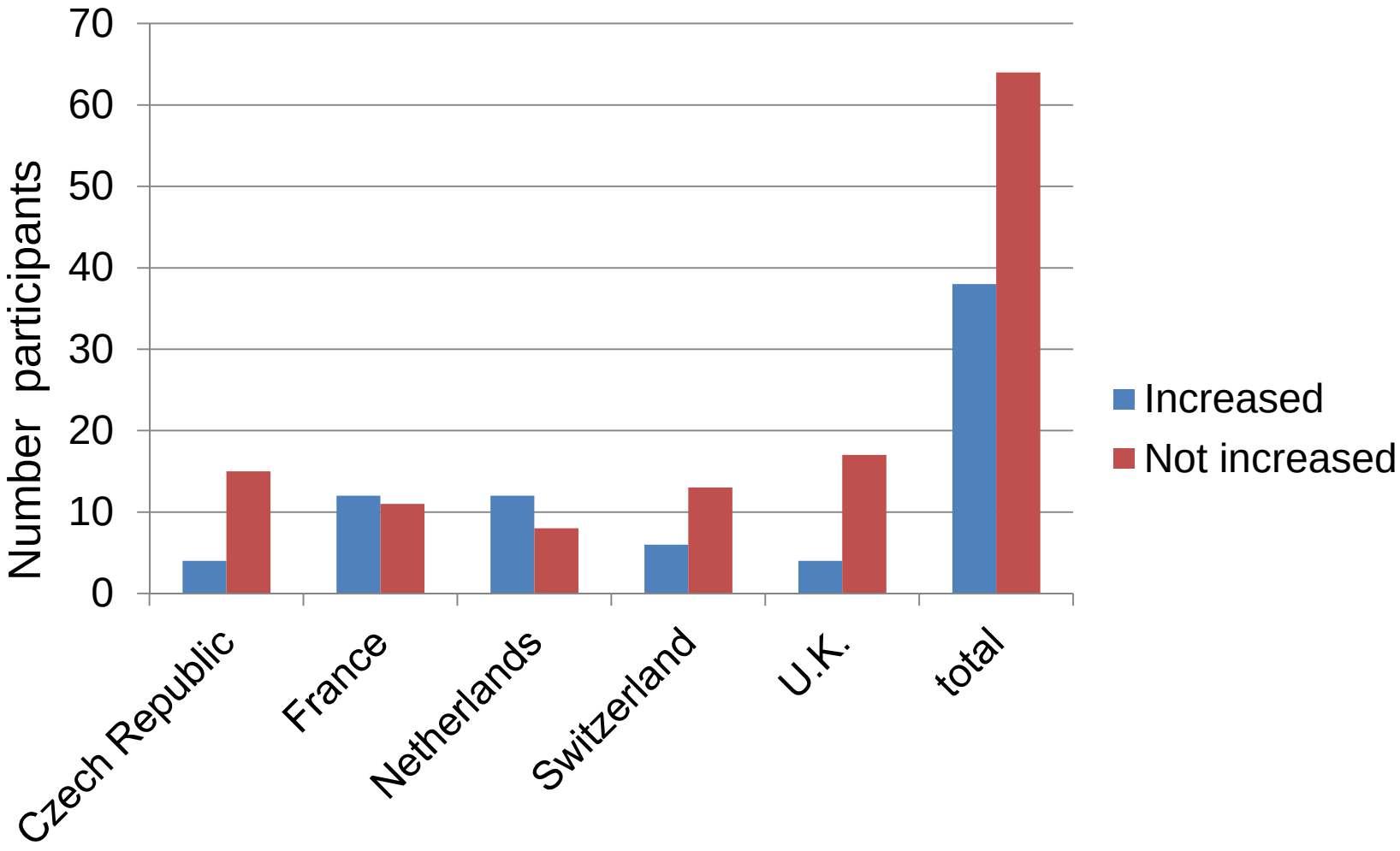
DPT 2014 Common Sample: Interpretative Performance



DPT 2014 Common Sample: **Orotic Acid** increased

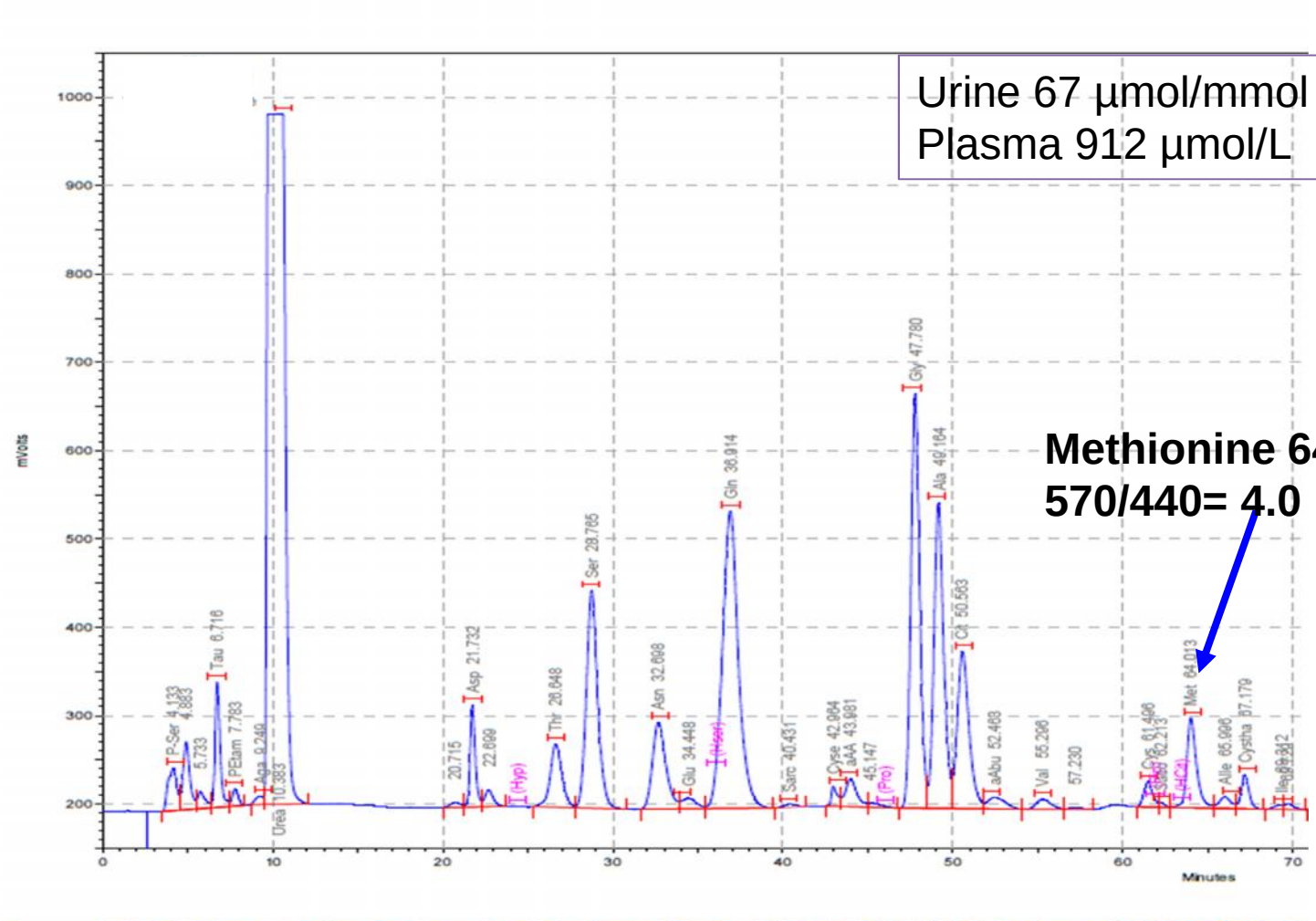


DPT 2014 Common Sample: **Homocitrulline** increased



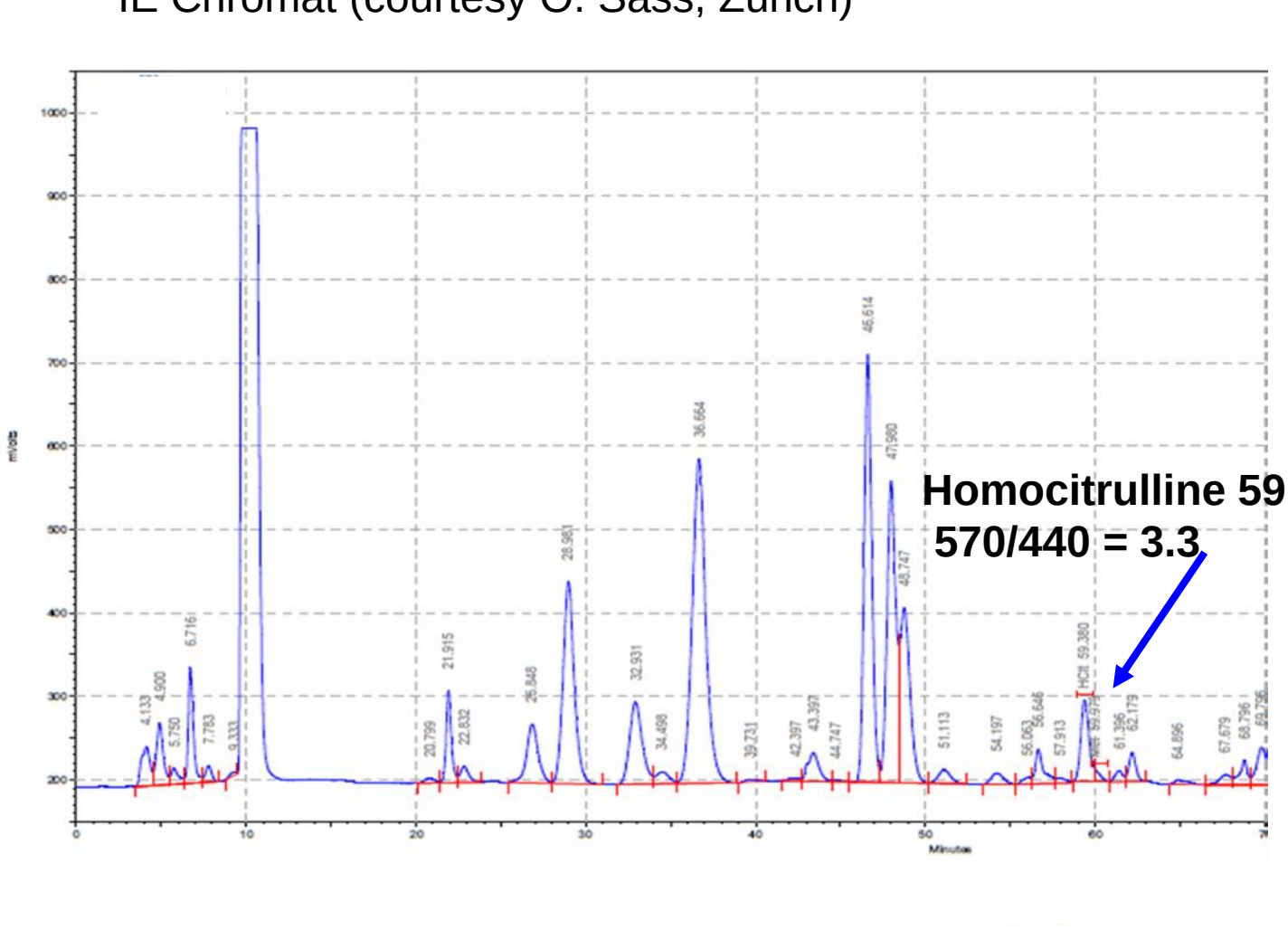
Sample E: Hypermethioninaemia due to methionine adenosyltransferase deficiency, OMIM 250850 (MAT1A gene).

IE Chromat (courtesy O. Sass, Zürich)



Sample F: Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome (treated with citrulline), mutation of the SLC25A15 gene, OMIM 238970

IE Chromat (courtesy O. Sass, Zürich)



Homocitrulline quantitation (mmol/mol Creat.)

	n	**range**	**median**	**S.D.**
France	9	18-30	24	3.35
NL	13	6.3 - 36	21	7.5
CH	6	28-199	53.5	64.9
All	28	6.3 - 199	25.5	40.4

Orotic acid quantitation (mmol/mol Creat.)

DPT Centre	n	Range	Median	SD
Czech Republic	14	13 - 60	22.7	12.9
France	19	10.3 - 70	20.4	12.8
Netherlands	16	5 -41.2	18.8	8.5
Switzerland	11	6 - 48.0	17.0	10.6
U.K.	-	-	-	-
total	60	5 - 70.0	19.4	11.4

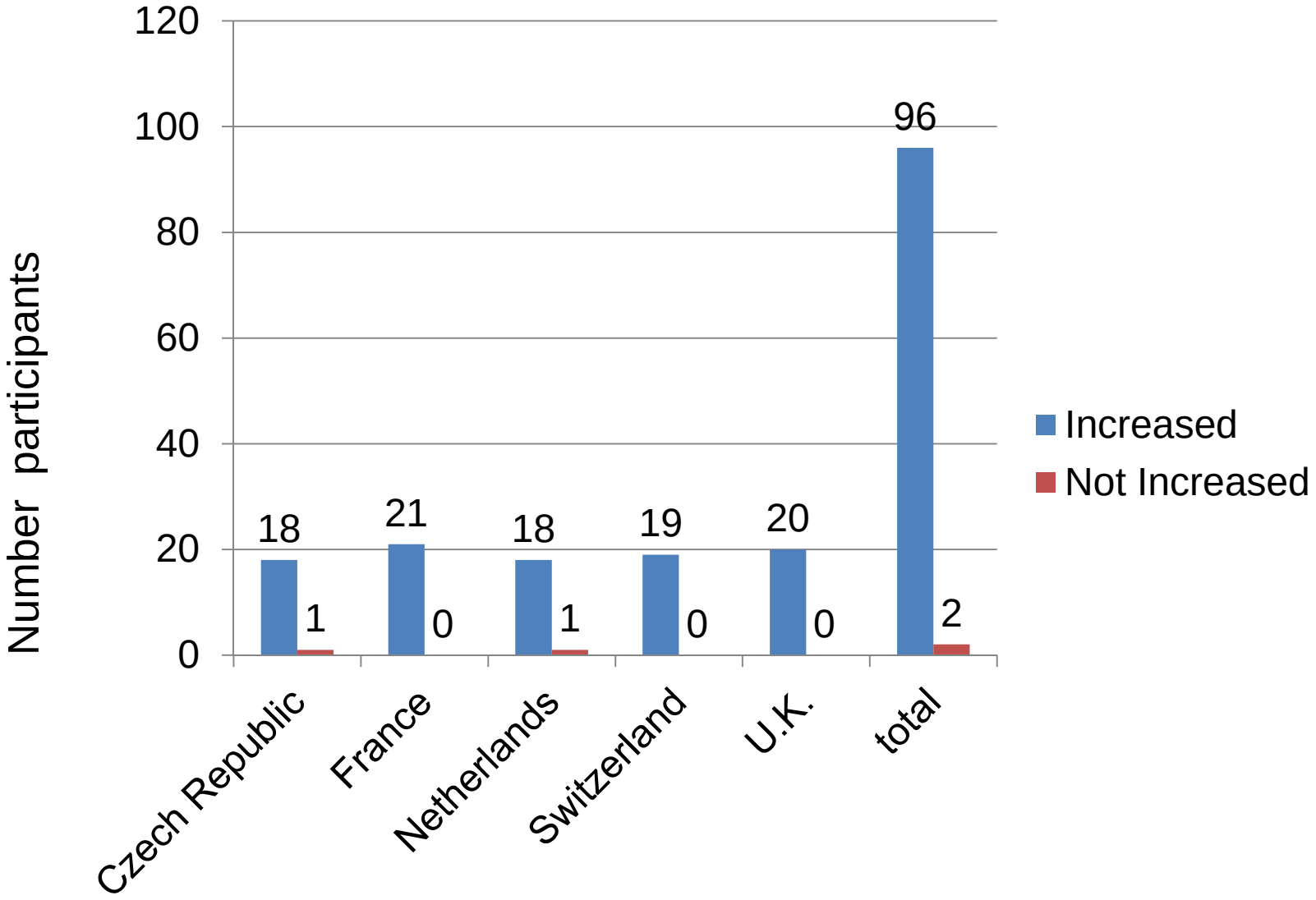
Citrulline quantitation (mmol/mol Creat.)

DPT Centre	n	Range	Median	SD
Czech Republic*	13	52-98	63.5	12.2
France	22	35.8-69	61.6	10.2
Netherlands	17	10 - 75	58.3	16.1
Switzerland**	17	52 - 88	60	8.0
U.K.	17	47 - 83	61	9.6
total	86	10 - 98	61.6	11.8

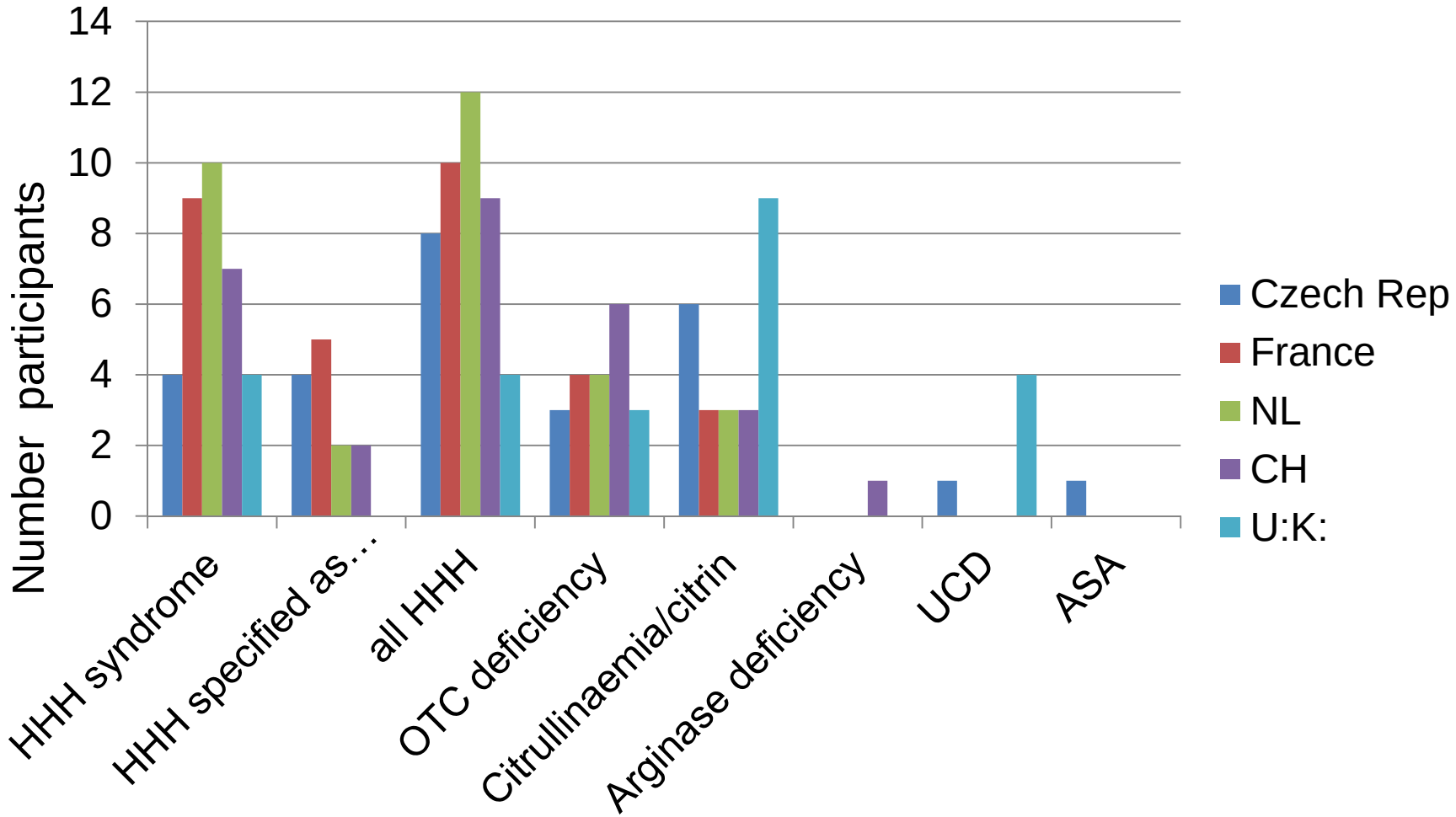
* One outlier (448) omitted

** Two outliers (390, 596) omitted

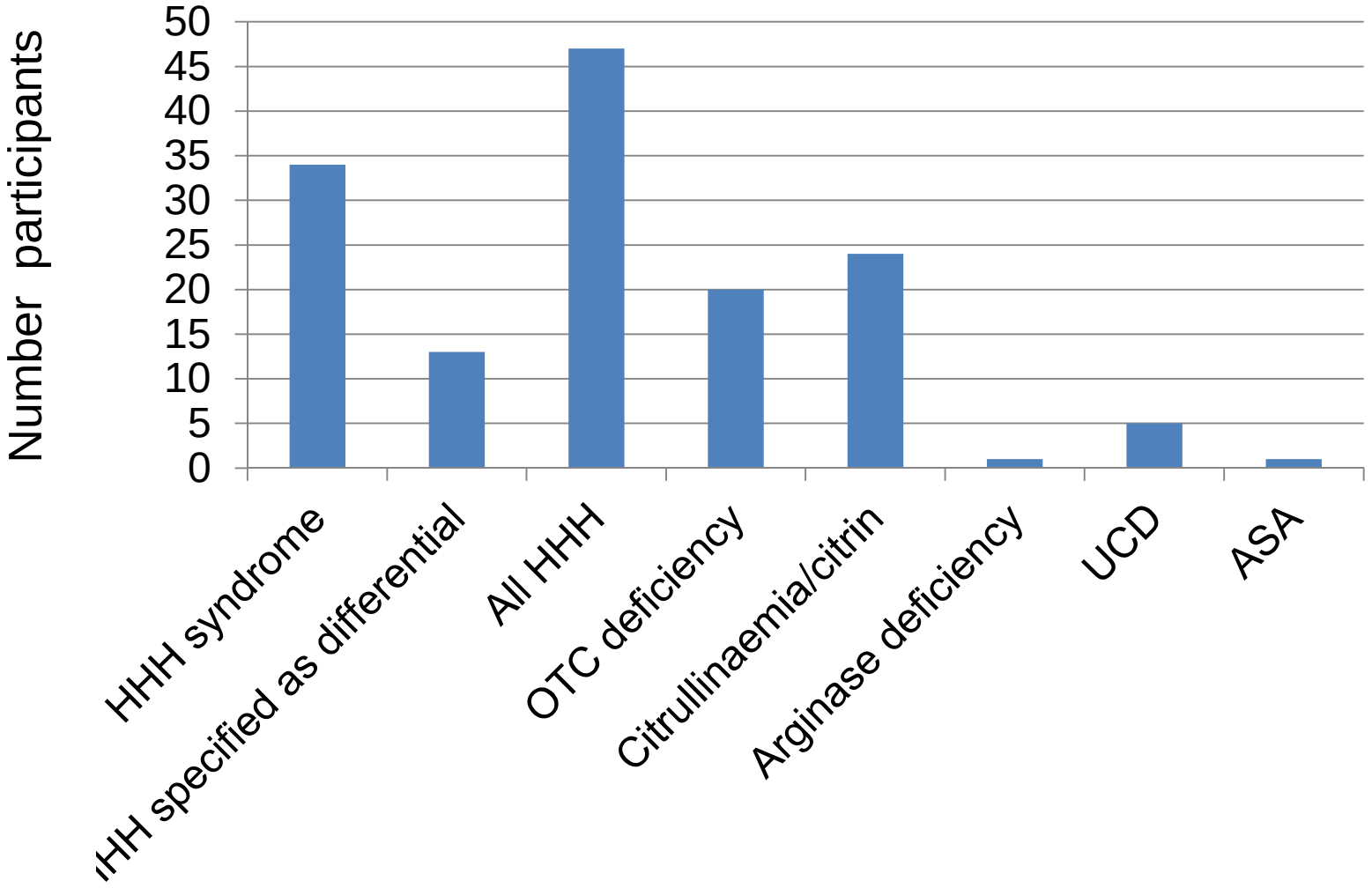
DPT 2014 Common Sample: **Citrulline** increased



Diagnoses made: individual centres



Diagnoses made: all labs



Message

- Identification of homocitrulline key to HHH diagnosis (accepting that the urine was collected whilst on treatment – Ongoing difficulty for DPT samples)
- Homocitrulline absence by current methods does not rule out HHH
- Ion exchange – difficult since retention time close to methionine but 440/570 ratio informative
- Tandem MS detected homocitrulline in a previous DPT sample (10.6 mmol/mol Creatinine)
- Correct measurement of orotic acid essential in differential diagnosis of hyperammonaemia
- Orotic acid found to be increased by most labs (all but six labs)
- Wide variation of performance between centres.