Urinary Acylglycine LC-MSMS method Analytical concerns & clinical use

R.G.Edwards

I23-P

MEASUREMENT OF URINARY ACYLGLYCINES BY TANDEM MASS SPECTROMETRY

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J Inherit Metab Dis (2007) 30 (Suppl 1)

Clinical Chemistry 48:11
1970–1980 (2002)

Automation and
Analytical Techniques

Comprehensive Screening of Urine Samples for Inborn Errors of Metabolism by Electrospray Tandem Mass Spectrometry

James J. Pitt,* Mary Eggington, and Stephen G. Kahler

Quantitative analysis of urinary acylglycines for the diagnosis of β-oxidation defects using GC-NCI-MS

C.G. Costa [a,b,c], W.S. Guérand [b], E.A. Struys [b], U. Holwerda [b], H.J. ten Brink [b], I. Tavares de Almeida [a], M. Duran [c], C. Jakobs [b,*]

Journal of Pharmaceutical and Biomedical Analysis
21 (2000) 1215–1224

Measurement of Urinary Acylglycines by TMS to Facilitate the Investigation of Metabolic Disorders

P.M. Marchetti, A. Patterson, T. Williams, M. Henderson
Department of Clinical Chemistry, St James's University Hospital, Leeds, UK

Evaluation of Urinary Acylglycines by Electrospray Tandem Mass Spectrometry in Mitochondrial Energy Metabolism Defects and Organic Acidurias

Luisa Bonafé,*[,1] Heinz Troxler,*[,1,2] Thomas Kuster,* Claus W. Heizmann,* Néstor A. Chamoles,† Alberto B. Burlina,‡ and Nenad Blau*

Molecular Genetics and Metabolism 69, 302–311 (2000)

A Method for Comprehensive Analysis of Urinary Acylglycines by Using Ultra-Performance Liquid Chromatography Quadrupole Linear Ion Trap Mass Spectrometry

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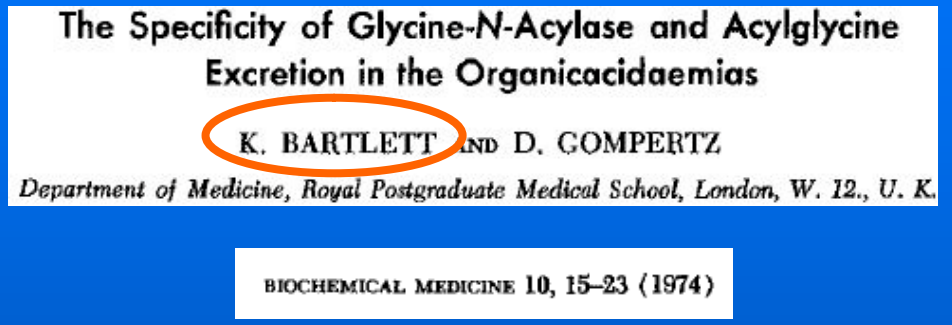
J Am Soc Mass Spectrom 2010, 21, 2105–2116

Acylglycine formation

- Glycine-N-acylase
- Acyl-CoA esters conjugated glycine (Crn, GluN)
- Polar compounds urine excreted in urine
- Acylglycines reflect mitochondrial acyl-CoA esters
- Acyl-CoA dehydrogenases
- Leu/ Ile/ Val/ Lys catabolism



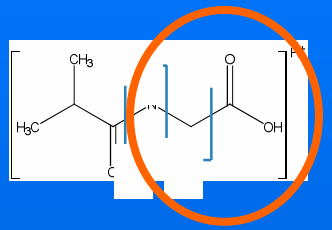
The Specificity of Glycine-*N*-Acylase and Acylglycine Excretion in the Organicacidaemias

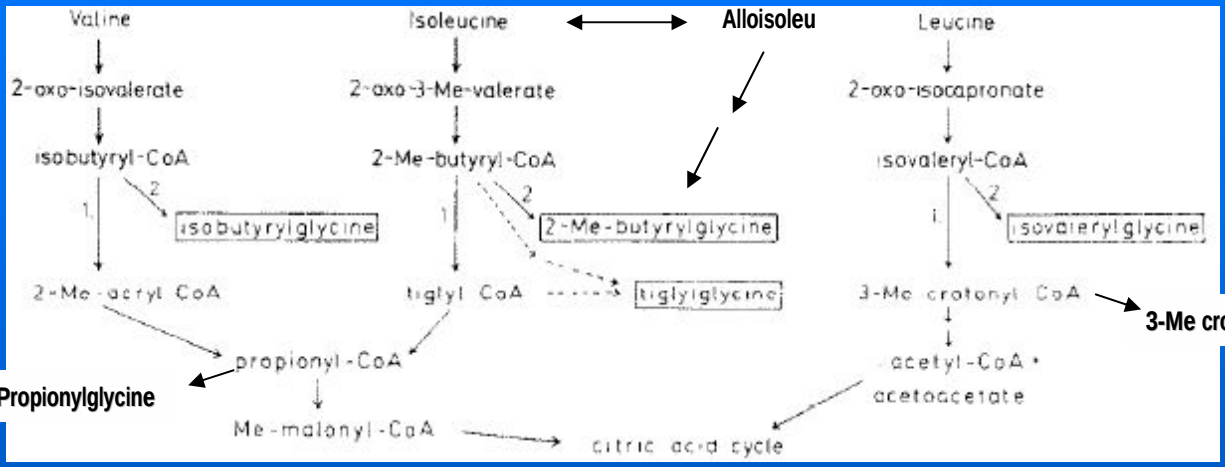
K. BARTLETT AND D. COMPERTZ

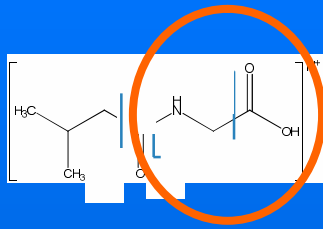
Department of Medicine, Royal Postgraduate Medical School, London, W. 12., U. K.

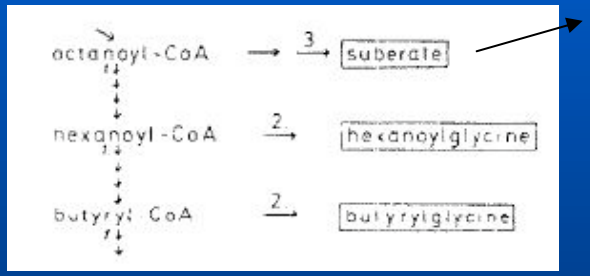
BIOCHEMICAL MEDICINE 10, 15–23 (1974)

Biosynthesis of Acylglycines









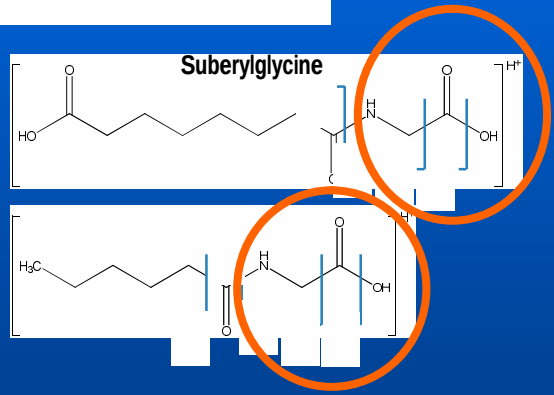


Fig. 1. Scheme for the proposed biosynthesis of the compounds found elevated in urine from the patient (marked with squares). a, The degradation of branched-chain amino acids; b, the fatty acid β-oxidation. 1, Acyl-CoA dehydrogenases; 2, glycine-*N*-acylase; 3, ω-oxidating system.

Clinica Chimica Acta, 106 (1980) 215—221

Method Characteristics

- Micromass Quattro Micro, Waters 2795 HPLC
- Negative ion
- Urine ‘Dilute & shoot’, 20 µL
- 2.5 min, isocratic 80% AcN
- Hexanoylglycine $^{13}C_2$ (IS)
- 9 acylglycines; propionyl, tigly, 3-methylcrotonyl, isovaleryl, 2-methylbutyryl, hexanoyl, octanoyl, phenylpropionyl, suberyl

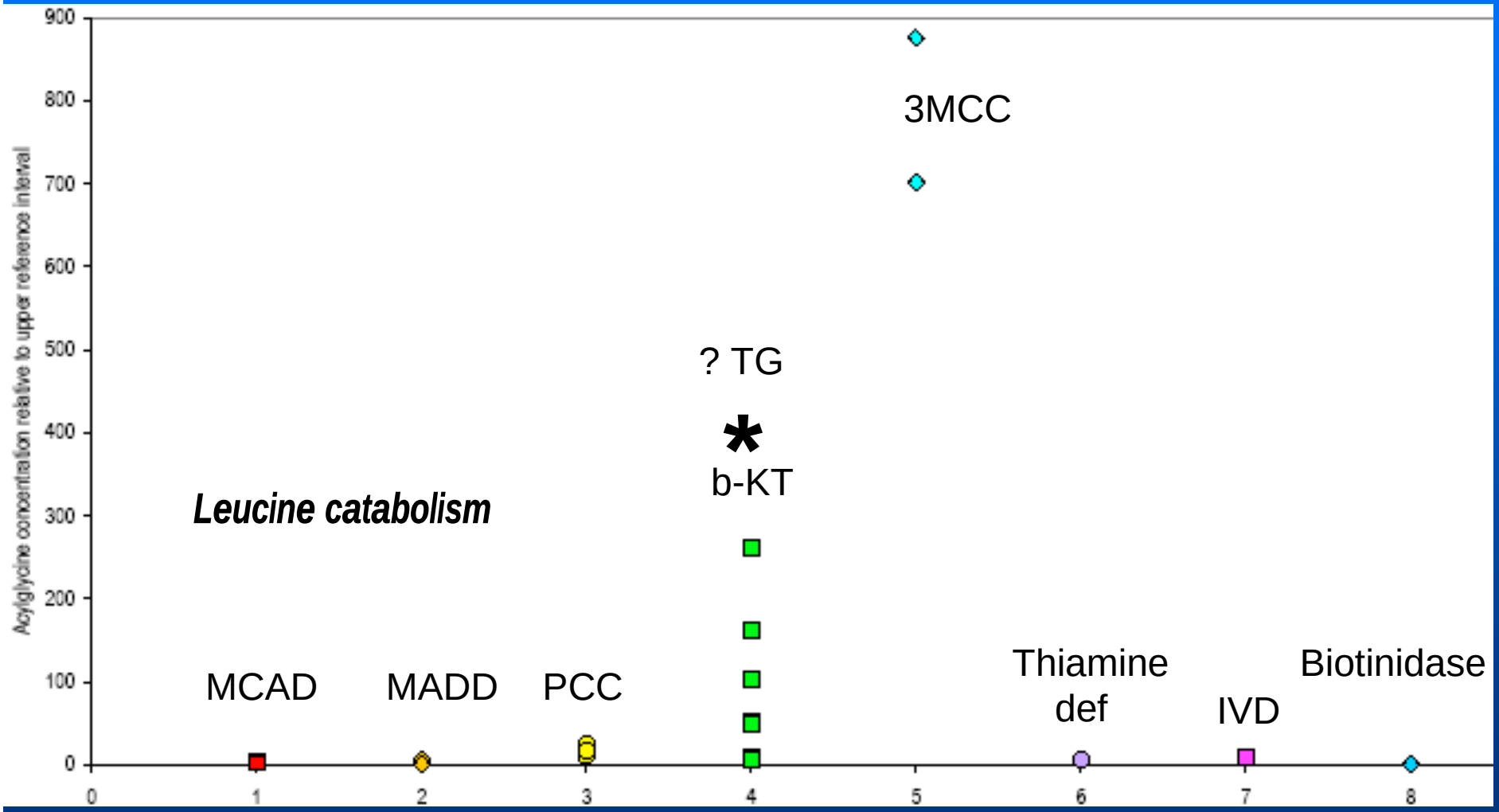
- Current use; NNS MCAD urinary hexanoylglycine

Acylglycine excretion

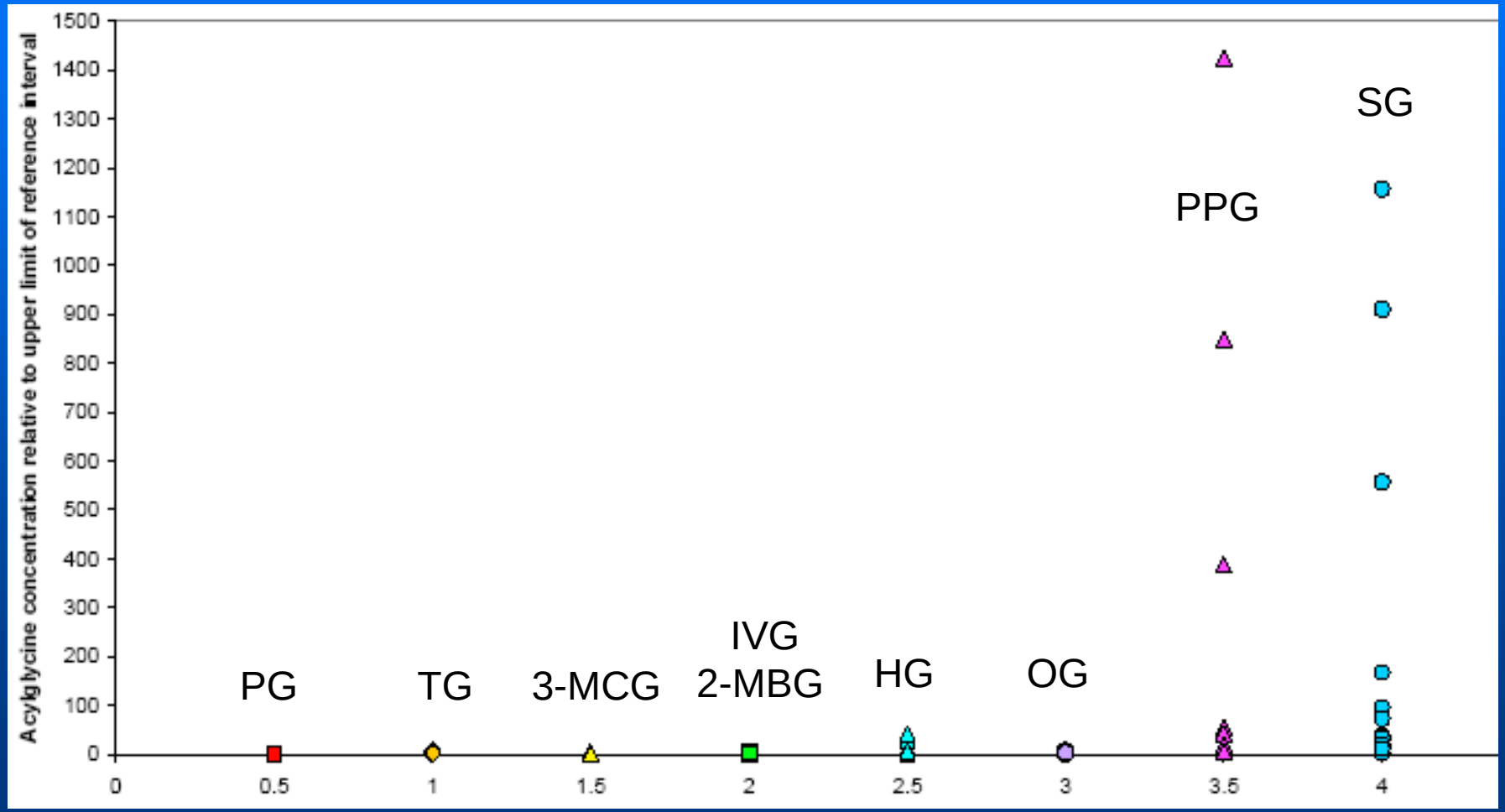
Urine Glycine	Parent ion m/z	Daughter ion m/z	Enzyme deficiency
Propionyl	130.1	74	PCC, MUT, HLCS
Tigly (2-mecrotonyl)	156.1	112	PCC, HLCS, BKT
3-methylcrotonyl	156.2	74	HMGCL, 3MCC, HLCS, {BKT}, {thiamine def}
Isovaleryl	158.0	74	IVD, MADD, {EMA-A}, {Valp}
2-methylbutyryl	158.0	74	MADD, SBCAD
Hexanoyl	172.2	74	MCAD, MADD, PCC, BKT, {Valp}
Octanoyl	200.3	74	MCAD, MADD
3-phenylpropionyl	206.2	74	MCAD
Suberyl	230.2	74	MCAD, {MCT feeds}
C13 Hexanoyl	174.2	75	

Butyryl	144	~	SCAD, MCAD, MADD
Isobutyryl	144	~	MADD
Glutarylglycine	202	~	GCDH (decompensated)

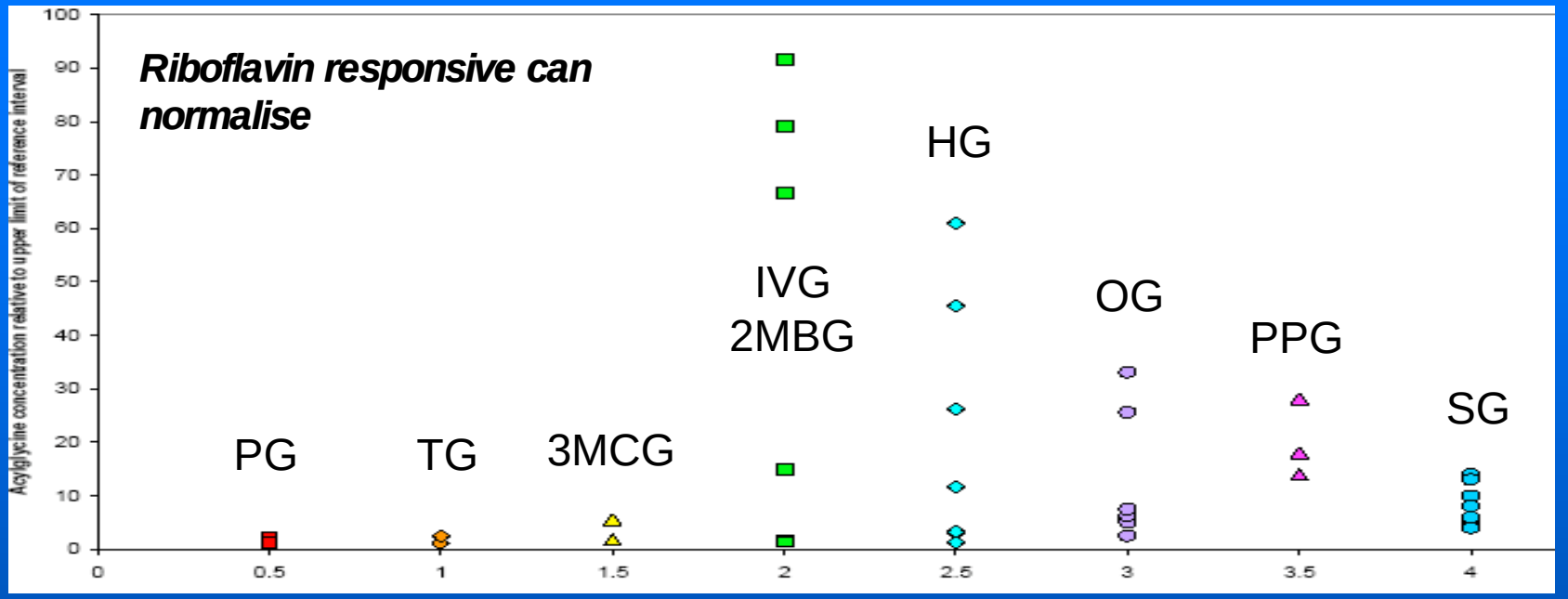
3-Methylcrotonylglycine excretion

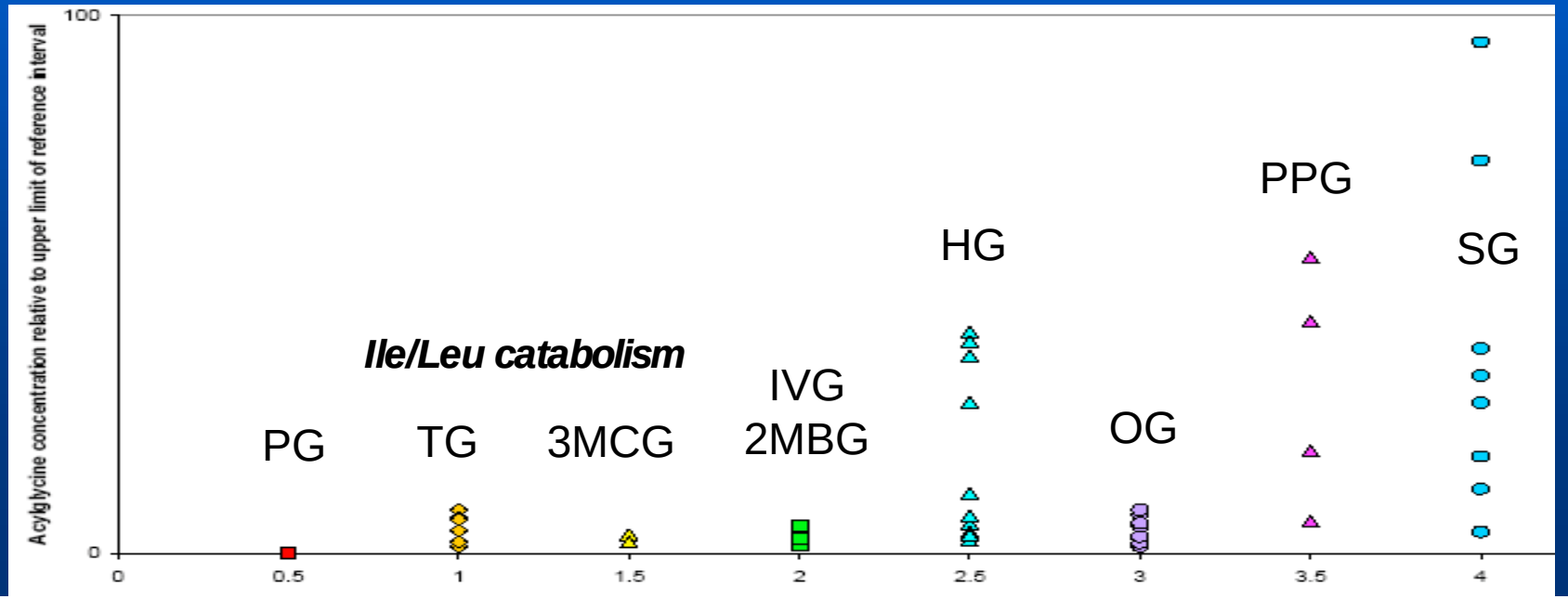


Medium Acyl-CoA Dehydrogenase deficiency



MADD & MCAD


Riboflavin responsive can normalise
Acylglycine concentration relative to upper limit of reference interval
100
90
80
70
60
50
40
30
20
10
0
PG
TG
3MCG
IVG
2MBG
HG
OG
PPG
SG
0
0.5
1
1.5
2
2.5
3
3.5
4


Ile/Leu catabolism
Acylglycine concentration relative to upper limit of reference interval
100
0
PG
TG
3MCG
IVG
2MBG
HG
OG
PPG
SG
0
0.5
1
1.5
2
2.5
3
3.5
4

Analytical issues

- Separation of IVG/ 2MBG (TG/3MBG)
- Deterioration of suberylglycine
- Alternative source of pure standards (synth SBC)
- Appropriate control material - ? Spiked, patient
- Mixed standards for all or some glycines

- Addition of butyryl/ isobutyryl glycine

Clinical & Diagnostic Use

Elevated C4 acylcarnitine

- SCAD – {butyrylglycine} (EMA +/- MeSuccinate)
- Isobutyryl CoA dehydrogenase – isobutyrylglycine
- EMA encephalopathy – isovalerylglycine (EMA)

Elevated C5 acylcarnitine >

- IVD - isovalerylglycine
- SBCAD – 2-methylbutyrylglycine (2-EHA)

- Equivocal Organic acids & acyls (?acylgly detected)

- 'Quick screen method' Pitt et al. Negative ion method; additional MRM orotic acid, sulphocysteine etc.