

# Metabolomic Analysis for Newborn Screening and Diagnosis of Metabolic Disorders

## *Mass Spectrometry and Separation Sciences for Laboratory Medicine*

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## Learning goals

- The audience will be made aware of the public health significance of screening newborns for metabolic diseases
- The role that medium-chain acyl-CoA dehydrogenase deficiency played in defining the expansion of newborn screening will be outlined
- The role of tandem mass spectrometry as a screening tool for metabolic disorders will be described
- Future trends in newborn screening using mass spectrometry will be discussed
- The use of untargeted metabolomics for the diagnosis of metabolic diseases will be described.

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## Conflicts of Interest

no conflicts to declare

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## Phenylketonuria and blood spot collection

Nc1ccc(cc1)C(=O)O  $\xrightleftharpoons{\text{Phenylalanase}}$  O=C(O)c1ccc(cc1)C(=O)O  
 Phenylalanine  $\rightleftharpoons$  Phenylpyruvic acid  
 $\downarrow$  Phenylalanine Hydroxylase  
Nc1ccc(O)cc1C(=O)O  
 Tyrosine

March of Dimes

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## Robert Guthrie and the bacterial inhibition assay

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## Improved outcomes for patients with PKU

- In the unscreened population:
  - patients are inevitably institutionalized with severe developmental delay
  - \$188K US per year in 2009\*
- Screened population:
  - essentially normal development on a restricted dietary regimen, no institutionalization
  - Concerns about maintaining good metabolic control, particularly in girls of conceptional age

\* National Council for Disability

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## Other diseases added

- congenital adrenal hyperplasia
  - congenital hypothyroidism
  - sickle cell disease (other hemoglobinopathies)
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- All demonstrate much improved clinical outcomes following early diagnosis

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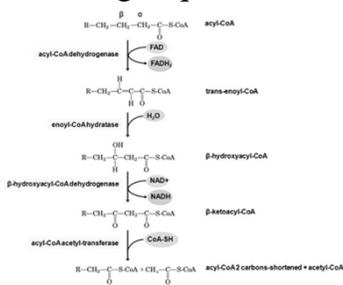
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## MCAD deficiency fulfills most screening requirements




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Science, 1983 Jul 1;221(4605):73-5.

**Dicarboxylic aciduria: deficient [1-14C]octanoate oxidation and medium-chain acyl-CoA dehydrogenase in fibroblasts.**

Rhead WJ, Amendt BA, Fritchman KS, Felts SJ.

Pediatr Res, 1983 Nov;17(11):877-84.

**Medium-chain acyl-CoA dehydrogenase deficiency in children with non-ketotic hypoglycemia and low carnitine levels.**

Stanley CA, Hale DE, Coates PM, Hall CL, Corkey BE, Yang W, Kelley RI, Gonzales EL, Williamson JR, Baker L.

Br Med J (Clin Res Ed). 1984 Mar 31; 288(6422): 976.

PMCID: PMC1442514

**Deficiency of medium chain fatty acylcoenzyme A dehydrogenase presenting as the sudden infant death syndrome.**

A J Howat, M J Bennett, S Variend, and L Shaw

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## The word about MCAD deficiency gets out

- 1980's-90's many more cases of MCAD described often associated with sudden death, from neonates to adults(46y).
- Estimated that 25% of cases die at first presentation. High morbidity in survivors
- Very high incidence of previous sibling deaths
- Population frequency estimated to be same as Phenylketonuria in Northern European populations (1 in 8,000-16,000)
- Regarded as treatable if diagnosed early (sibling studies)

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## The advent of tandem mass spectrometry as a tool for NBS

- David Millington's group at Duke University pioneered tandem mass spectrometry methodologies for measurement of amino acids for amino acid disorders and acylcarnitines for MCAD deficiency and other fatty acid oxidation defects and some organic acidemias
- The use of flow injection and Multiple Reaction Monitoring allows for simultaneous measurement of many acylcarnitine and amino acid species without chromatographic separation in a 2 minute analytic time
- Stable isotope-labeled internal standards allows for accurate quantitation

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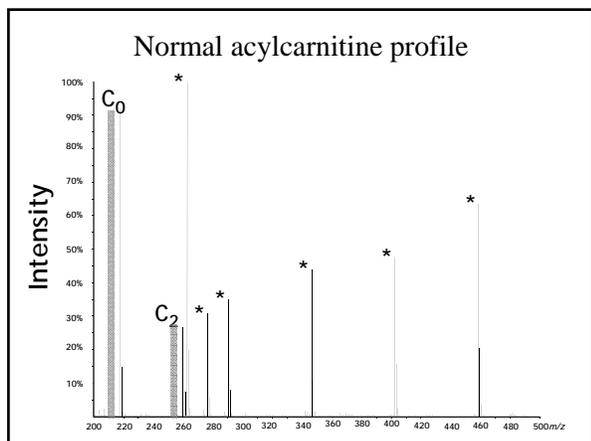
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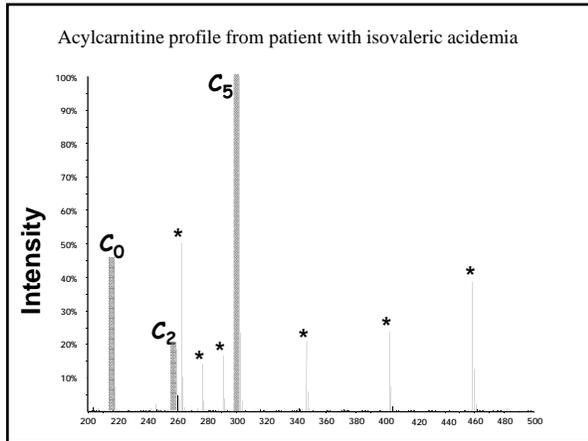
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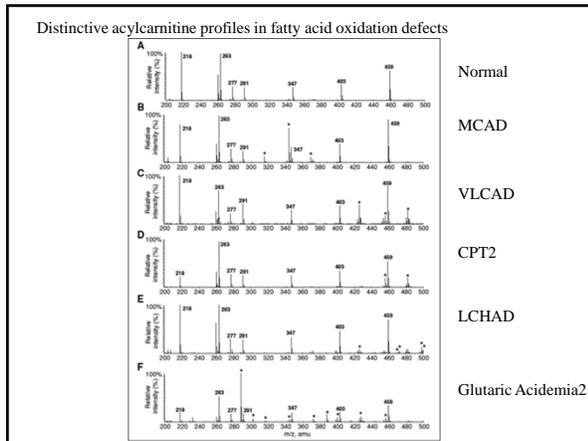
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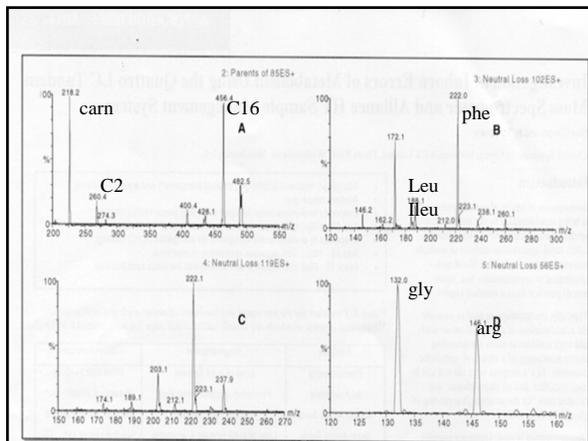
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### Current status of newborn screening

- USA: Most states follow ACMG guidelines
- Australia: Includes most of ACMG recommended disorders
- UK: MCAD plus 4 more
- Germany: 14 conditions mandated
- Netherlands: 14 conditions
- Programs starting in many countries

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### 10 Great public health achievements: Centers for Disease Control

- Vaccine preventable diseases
- Prevention and control of infectious diseases
- Tobacco control
- **\*Maternal and child health\* folate supplementation, Newborn screening**
- ~~Motor vehicle safety~~
- Cardiovascular disease prevention
- Occupational safety
- Cancer prevention
- Childhood lead poisoning prevention
- Public health preparedness and response

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### Metabolomic analysis for metabolic diseases

- Screening designed for maximum sensitivity (least false negatives)
- Generates some false positives
- Requirement for confirmatory testing usually requires mass spectrometric analysis
- GC-MS for organic acids, untargeted metabolomics assay
- TMS or LC-MS/MS for plasma acylcarnitines, amino acids, lysosomal enzymes

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## Follow-up guidelines

- National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines: Follow-Up Testing for Metabolic Disease Identified by Expanded Newborn Screening Using Tandem Mass Spectrometry; Executive Summary

Clin Chem 55: 1615-1626, 2009

[www.aacc.org/nacb/guidelines](http://www.aacc.org/nacb/guidelines)

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## Urine organic acid analysis

- Untargeted approach to identification of metabolic intermediates indicating a specific defect of metabolism.
- amino acids, fatty acids, neurotransmitters, vitamin metabolism, cholesterol biosynthesis, other mitochondrial pathways, oxalate/peroxisomal metabolism, glutathione pathway, ketone body metabolism.

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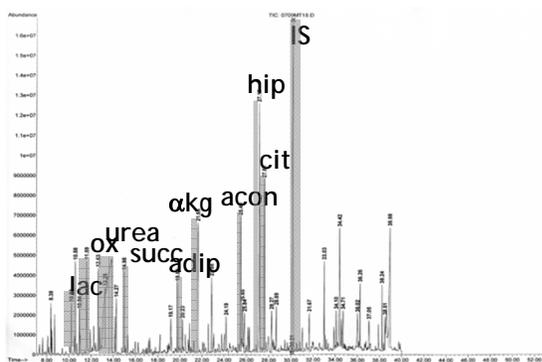
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## Normal organic acid chromatogram



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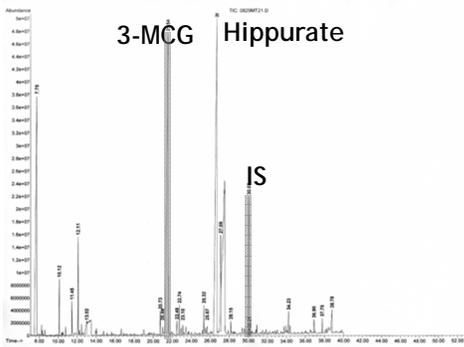
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### 3-methylcrotyl-CoA carboxylase deficiency



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### How low can we go?

- GC-MS with full scan 10 $\mu$ mol/mmol creatinine
- GC-MS with heavy isotope-labelled internal standard, selected ion monitoring 100nmol – 10  $\mu$ mol/mmol creatinine

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### Pre-analytical sources of urine metabolomic artifacts

- Endogenous, Metabolic
  - Symbiotic
  - Clinical
- Exogenous, Pharmacologic, prescribed
  - Pharmacologic, not prescribed
  - Nutritional/Xenobiotic

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### Metabolic artifacts

- Artifacts related to fasting:
- ketones up to 20mmol/mmol creatinine (DKA)
- dicarboxylic and 3-hydroxydicarboxylic acids ( ? mitochondrial leakage)
- Intermediates of ketogenic amino acid catabolic pathways
- Artifacts related to stress
- phenolic acid metabolites of catecholamines (HVA, VMA,)

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### Clinical artifacts

- Other disease states
- *Liver disease of any etiology*  
tyrosine metabolites, N-acetylated amino acids
- *Catecholamine-secreting tumors*  
catechol metabolites VMA, HVA, VLA
- *Maternal disease (artifacts in newborns)*  
maternal metabolic disease

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### Products of the microbiome

- Products of GI flora metabolism
- *Volatile fatty acids*  
propionate, butyrate (often further metabolized endogenously). Direct oxidation in gut epithelium
- *TCA cycle intermediates*  
succinate, citrate, isocitrate
- *Products of bacterial metabolism of plant membranes*  
*p, m and o* cresol, 4-hydroxyphenylacetate, 3 and 4-hydroxyhippurate, but not 2-hydroxyhippurate

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## Pharmacologic artifacts

- Prescribed and non-prescribed
- *Anticonvulsants and their metabolites*  
Phenytoin. Phenobarbital, valproate, carbamazepine
- *NSAIDS*  
Salicylates, acetaminophen, ibuprofen
- *Treatment for hyperammonemia*  
benzoate → hippurate,  
phenylacetate → phenylacetylglutamate
- *Any drug that is metabolized and excreted as a glucuronide*

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## Nutritional/xenobiotic artifacts

- Nutritional support for failure to thrive
- *Medium-chain triglycerides*  
Medium chain dicarboxylic acids
- *Additives to formula*  
octenylsuccinic acid added to Nutramigen
- *Products that make foods more palatable*  
adipic acid added to yoghurt to make smooth, benzoic acid in soda to protect taste, metabolized to hippuric acid

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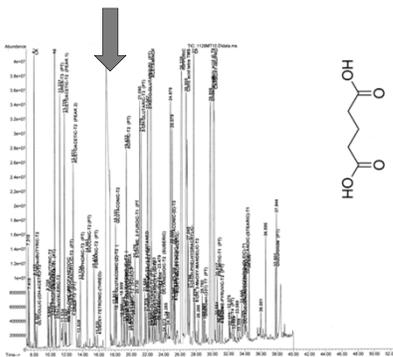
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## Metabolomic Profile 1




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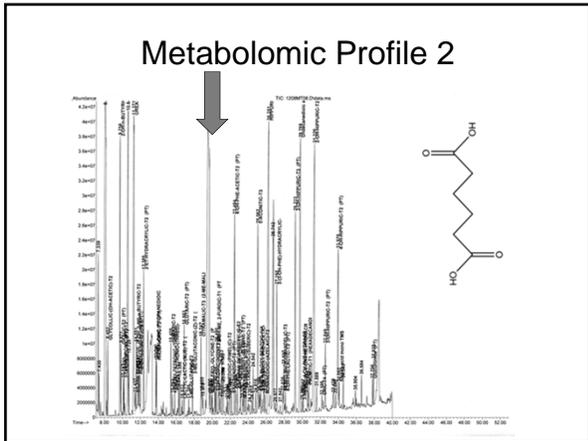
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**Measurement of individual metabolites:**  
targeted disease-related products of discovery investigations

Requires a stable isotope labeled internal standard,  
[<sup>13</sup>C], [<sup>15</sup>N], [<sup>3</sup>H]

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**Ornithine trans carbamylase (OTC) deficiency**

X-linked urea cycle defect  
Males relatively easy to diagnose  
Females have variable X-chromosomal inactivation

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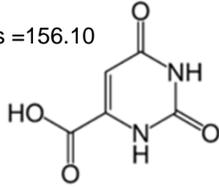
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### Orotic Acid

Molar mass = 156.10



Internal standard, [1,3-15N] orotic acid, Molar mass = 158.10

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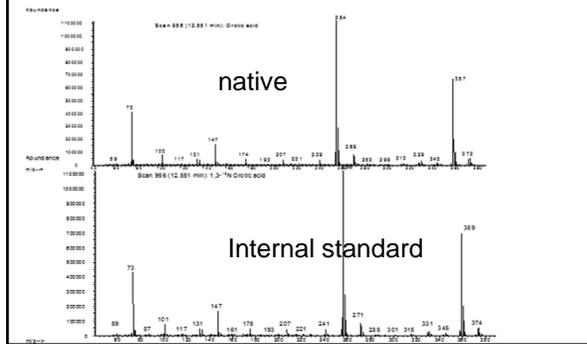
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### Native and labeled spectra



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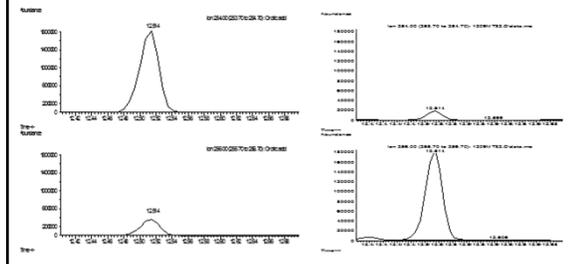
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### Isotope-dilution-selected ion monitoring for gold standard quantification



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## Mass spectrometry for additional metabolic conditions

- Lysosomal enzymes- Gaucher, Pompe, Fabry disease- TMS
- Glycosylated proteins- carbohydrate deficient glycoprotein diseases- Time of Flight MS
- Acyl-CoA's, mitochondrial diseases-TMS

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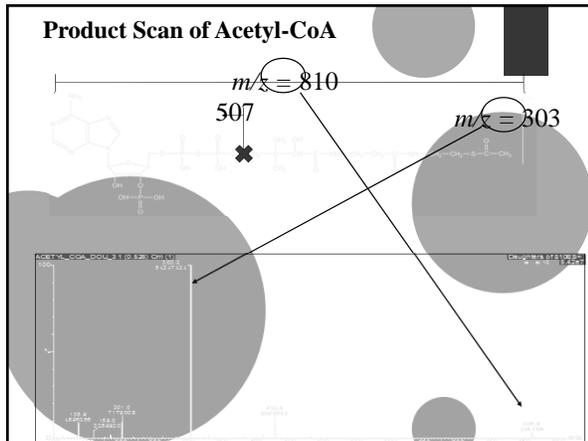
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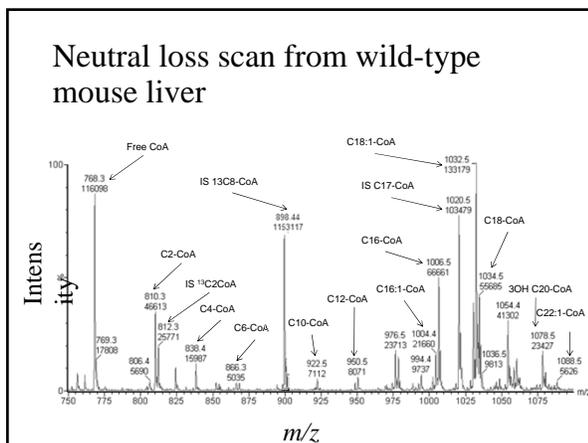
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