



**FOD/OAA Family Support Groups
National Metabolic Conference
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Newborn Screening and Diagnosis of Organic Acidurias

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Centers for Disease Control and Prevention**



SAFER • HEALTHIER • PEOPLE™



Newborn Screening

A Public Health Program

- Aimed at identification of conditions for which early intervention can prevent mortality, morbidity, and disabilities
- Performed by analysis of diagnostic markers in blood spots collected on filter paper at birth
- Largest population-based genetic screening effort in the US: ~ 4 Million babies screened annually

The Newborn Screening System

- **Screening**
 - Laboratory analysis of newborn bloodspot
- **Follow-up of an abnormal result**
 - Rapid location, follow-up and referral of the screen-positive infant
- **Diagnosis**
 - Clinical and biochemical evaluation to diagnose or rule out the disorder
- **Management**
 - Rapid planning and implementation of long-term therapy
- **Evaluation**
 - Assessment of the NBS program: efficiency of follow-up & intervention, benefit to society



**Alaska
Newborn Screening
Program**

Newborn Screening: Short Term Follow-up

Abnormal result obtained from the State Lab



CMS nurse from follow-up team notifies Genetics on-call Physician about the Abnormal Newborn Screen result



Genetics on-call Physician locates patient, makes clinical assessment of patient status – recommends clinic visit



**Infant sample collected (Plasma, Urine, Whole Blood)
Diagnostic evaluation performed**



Positive cases: long-term patient management & ongoing care

Follow-up Diagnostic Testing

- **Routine Chemistries**

- Electrolytes, blood gases, anion GAP
- Blood sugar, ketones, lactate, ammonia

- **Biochemical Genetics Testing**

- Plasma acylcarnitine (Green top tube – 0.5 ml min whole blood)
- Carnitine Status (Red top tube – 0.5 ml min whole blood)
- Urine organic acids (No preservatives – 3 ml min vol)
- Plasma amino acids (Green top tube – 0.5 ml min whole blood)

- **Other specialized testing**

- Enzyme analysis (eg Fatty acid oxidation Probe Assay)
- Mutation testing

Tandem Mass Spectrometry

Clinical Chemistry 49:11
1797–1817 (2003)

Review

Use of Tandem Mass Spectrometry for Multianalyte Screening of Dried Blood Specimens from Newborns

DONALD H. CHACE,* THEODORE A. KALAS, and EDWIN W. NAYLOR

***Tandem Mass
Spectrometer***



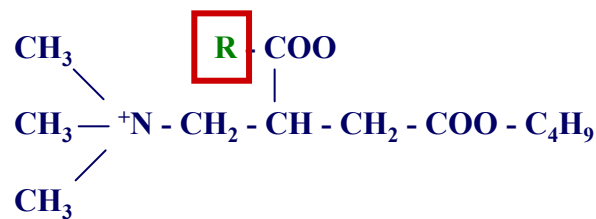
***A highly sensitive and
specific tool for diagnostic
sample analysis***

Tandem Mass Spectrometry

- Identification of unknown compounds
 - Quantitation of known analytes using stable isotopes
 - Provides structural and chemical information of molecules
 - High sensitivity and specificity
 - Fast analytical time
 - Potential for automation for high throughput tests
- (modified from ASMS, 1989)

Acylcarnitine Analysis by MS/MS

An Acylcarnitine molecule has the following structure:



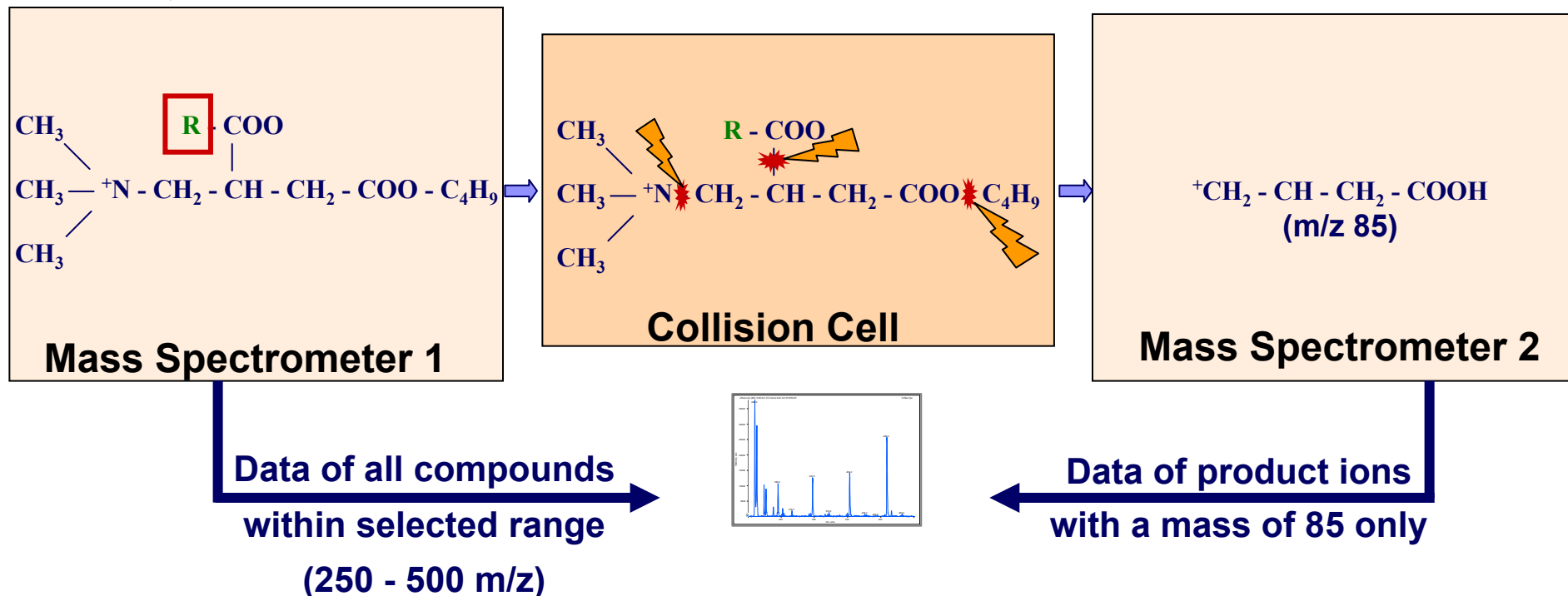
Members of this family of compounds have varying lengths of their R-Group

C3 acylcarnitine ... 3 carbon units
C8 acylcarnitine ... 8 carbon units
C16 acylcarnitine ... 16 carbon units

- Specific Acylcarnitines accumulate in fatty acid oxidation disorders (and certain organic acidemias) and form a distinct “pattern”
- Acylcarnitine analysis is very important in: prenatal diagnosis, newborn screening, evaluation of symptomatic patients, and postmortem screening

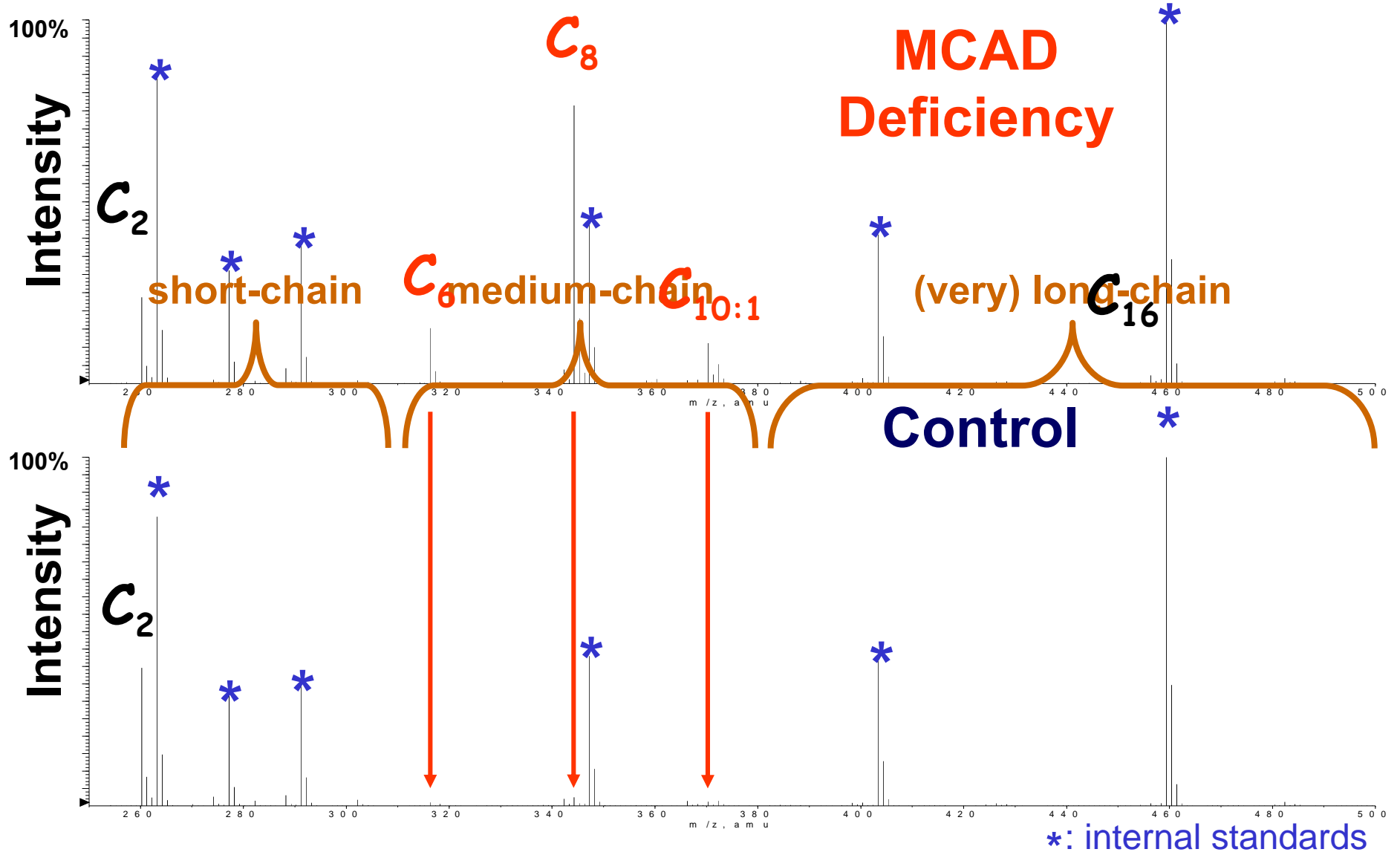
Acylcarnitine Analysis by MS/MS

3/16" blood spot
deproteinization
derivatization



Data system correlates m/z 85 to its precursor ion's mass and records the abundance of all precursors (parents of m/z 85)

Acylcarnitine Analysis



Newborn Screening by MS/MS

Disorders of fatty acid oxidation

2,4-Dienoyl-CoA reductase deficiency
Carnitine acylcarnitine translocase deficiency
Carnitine palmitoyltransferase I deficiency
Carnitine palmitoyltransferase II deficiency
Carnitine transport defect
Electron transfer flavoprotein deficiency
ETF ubiquinone oxidoreductase deficiency
Long-chain L-3-OH acyl-CoA dehydrogenase def.
Medium-chain acyl-CoA dehydrogenase deficiency
Medium-chain L-3-OH acyl-CoA dehydrogenase def.
Medium chain ketoacyl-CoA thiolase deficiency
Short-chain acyl-CoA dehydrogenase deficiency
Trifunctional protein deficiency
Very long-chain acyl-CoA dehydrogenase def.

Disorders of amino acid metabolism

Arginase deficiency
Argininosuccinate lyase deficiency
Argininosuccinate synthase deficiency
Maple syrup urine disease (MSUD)
Citrin deficiency
Cystathionine β -synthase deficiency
Methionine adenosyltransferase deficiency
Mitochondrial ornithine transport defect (HHH)
Phenylalanine hydroxylase deficiency (PKU)
Defects of bipterin metabolism
Fumarylacetoacetase deficiency
Tyrosine aminotransferase deficiency

Collective incidence

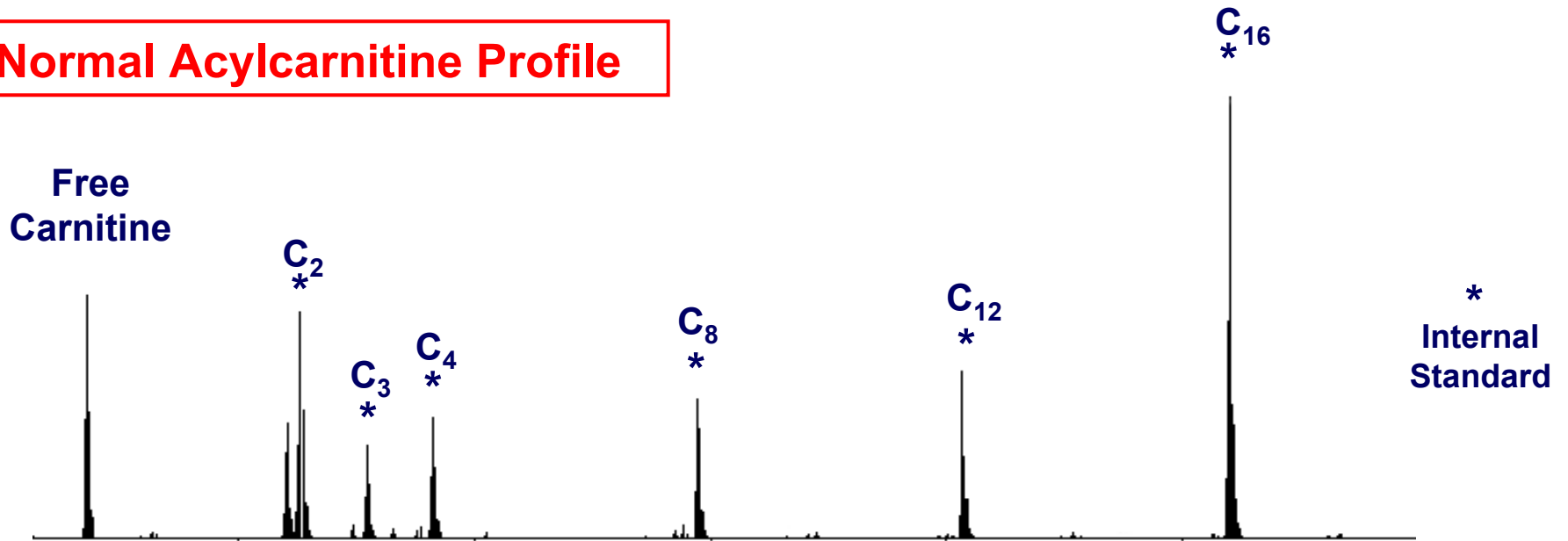
1:2-4,000 newborns

**Clinical impact (US):
Up to 2,000 cases/year**

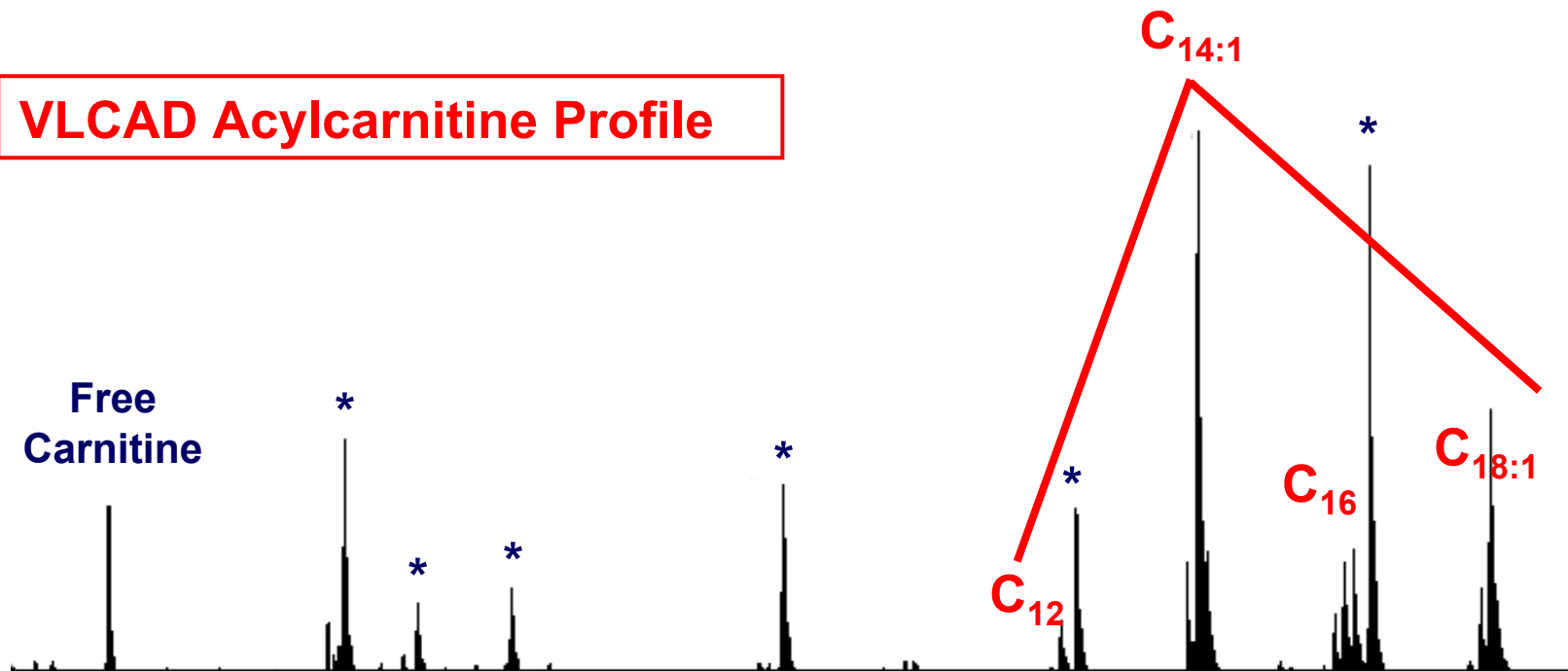
Disorders of organic acid metabolism

2-CH₃ butyryl-CoA dehydrogenase deficiency
2-CH₃ 3-OH butyryl-CoA dehydrogenase deficiency
3-OH 3-CH₃ glutaryl-CoA lyase deficiency
3-CH₃ crotonyl-CoA carboxylase deficiency
3-CH₃ glutaconyl-CoA hydratase deficiency
Isobutyryl-CoA dehydrogenase deficiency
Isovaleryl-CoA dehydrogenase deficiency
Glutaryl-CoA dehydrogenase deficiency
Malonyl-CoA carboxylase deficiency
Methylmalonyl-CoA mutase deficiency
Disorders of cobalamin metabolism
 β -ketothiolase deficiency
Multiple carboxylase deficiency
Propionyl-CoA carboxylase deficiency

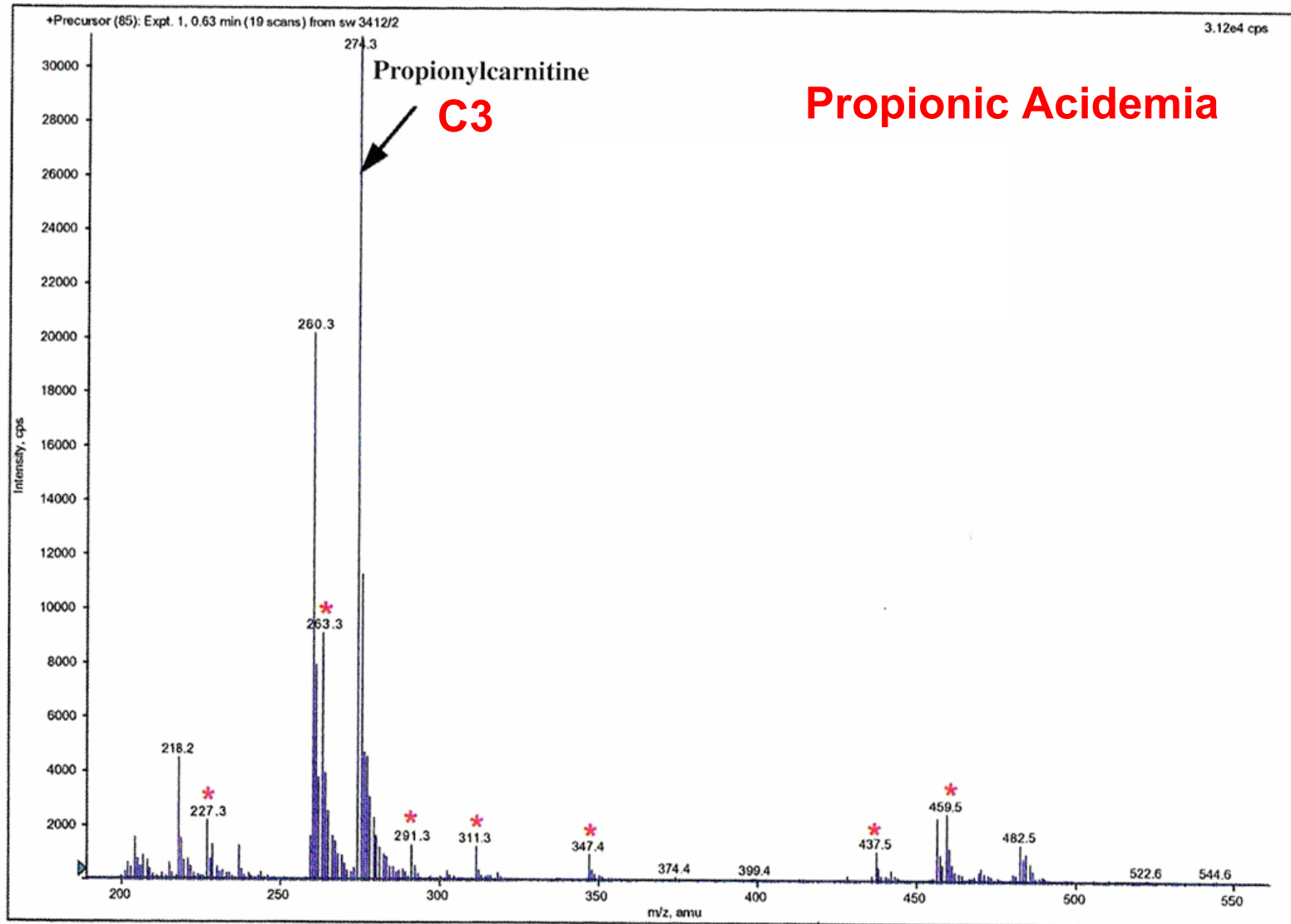
Normal Acylcarnitine Profile



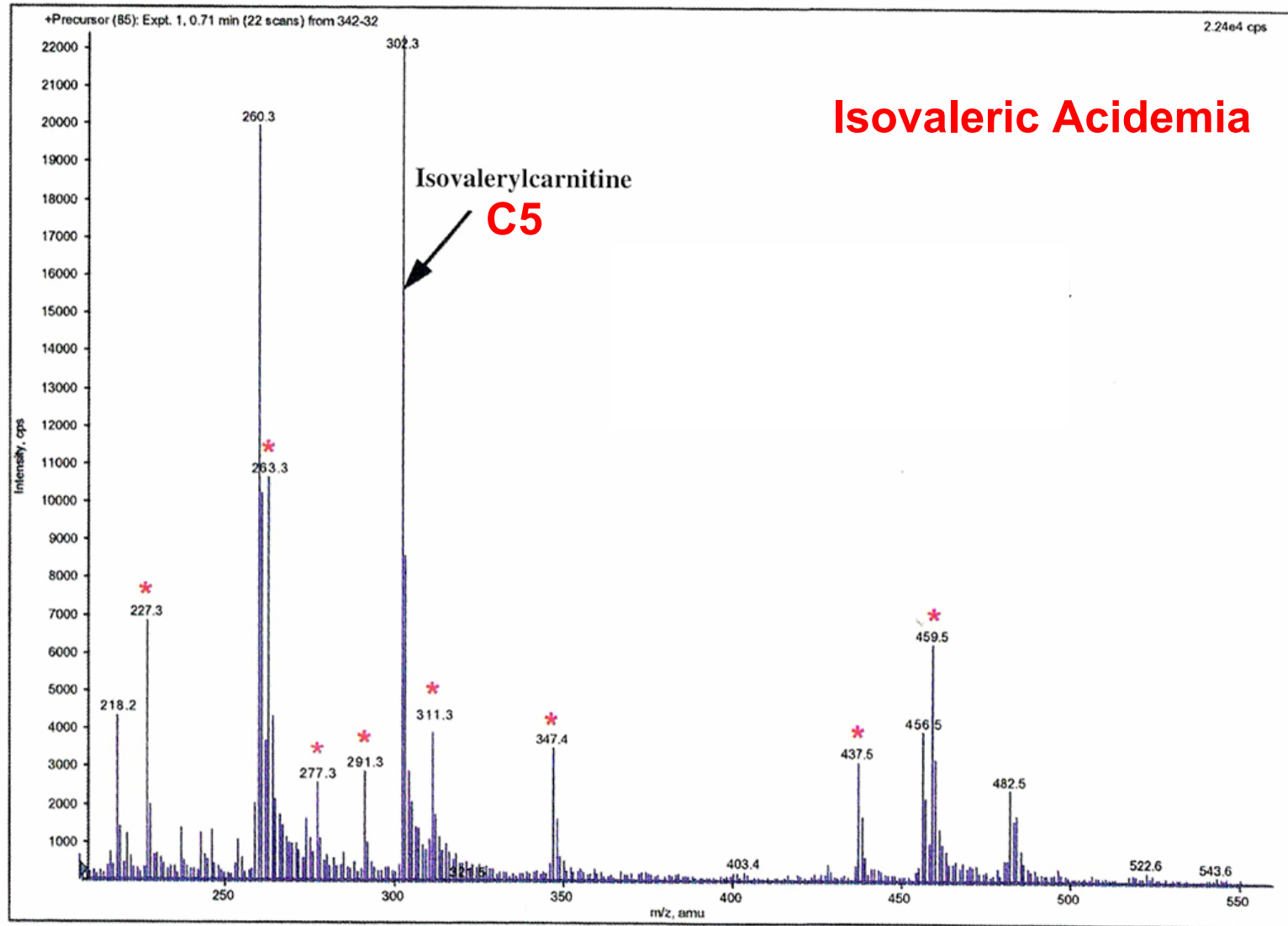
VLCAD Acylcarnitine Profile



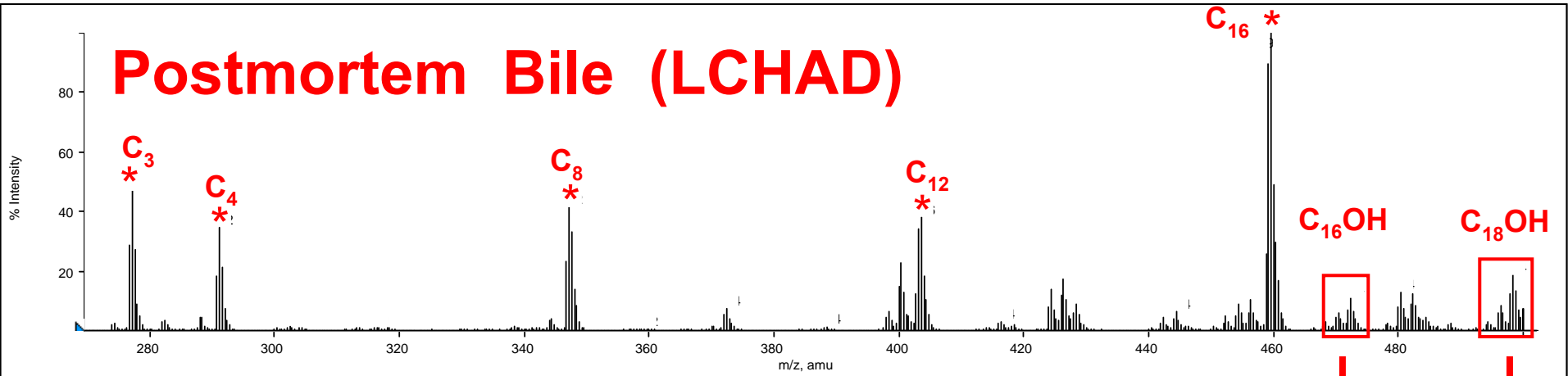
Acylcarnitine analysis



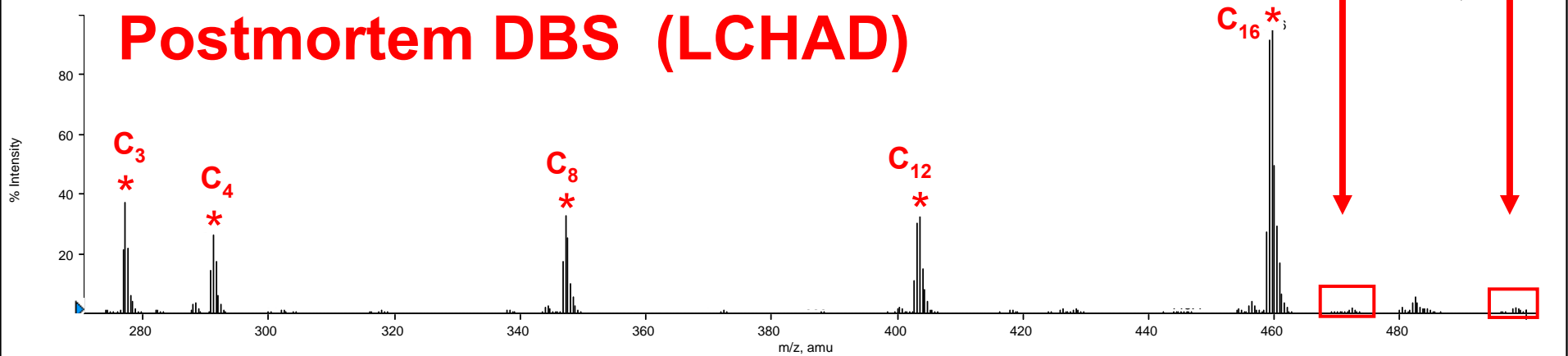
Acylcarnitine analysis



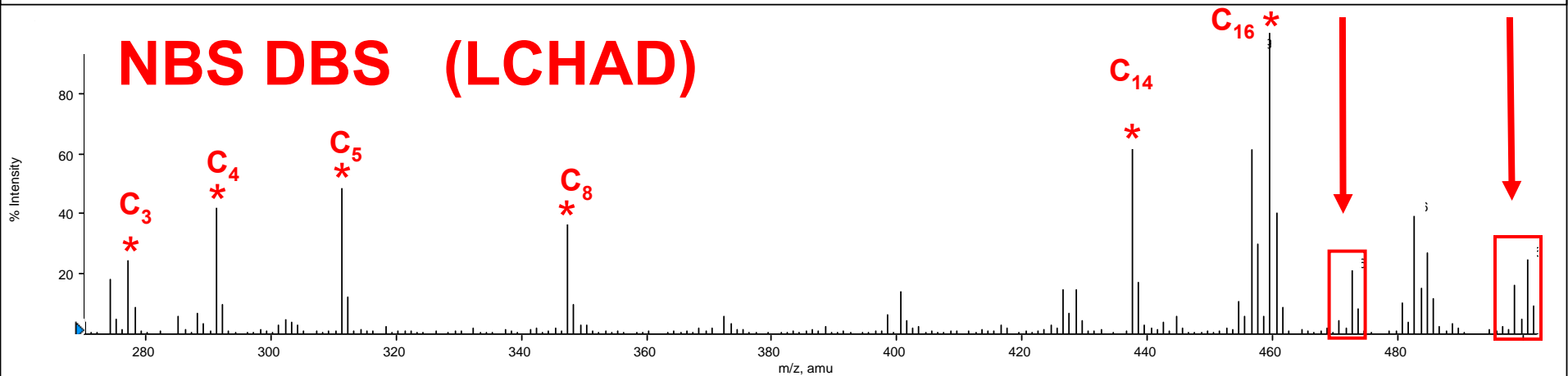
Postmortem Bile (LCHAD)



Postmortem DBS (LCHAD)

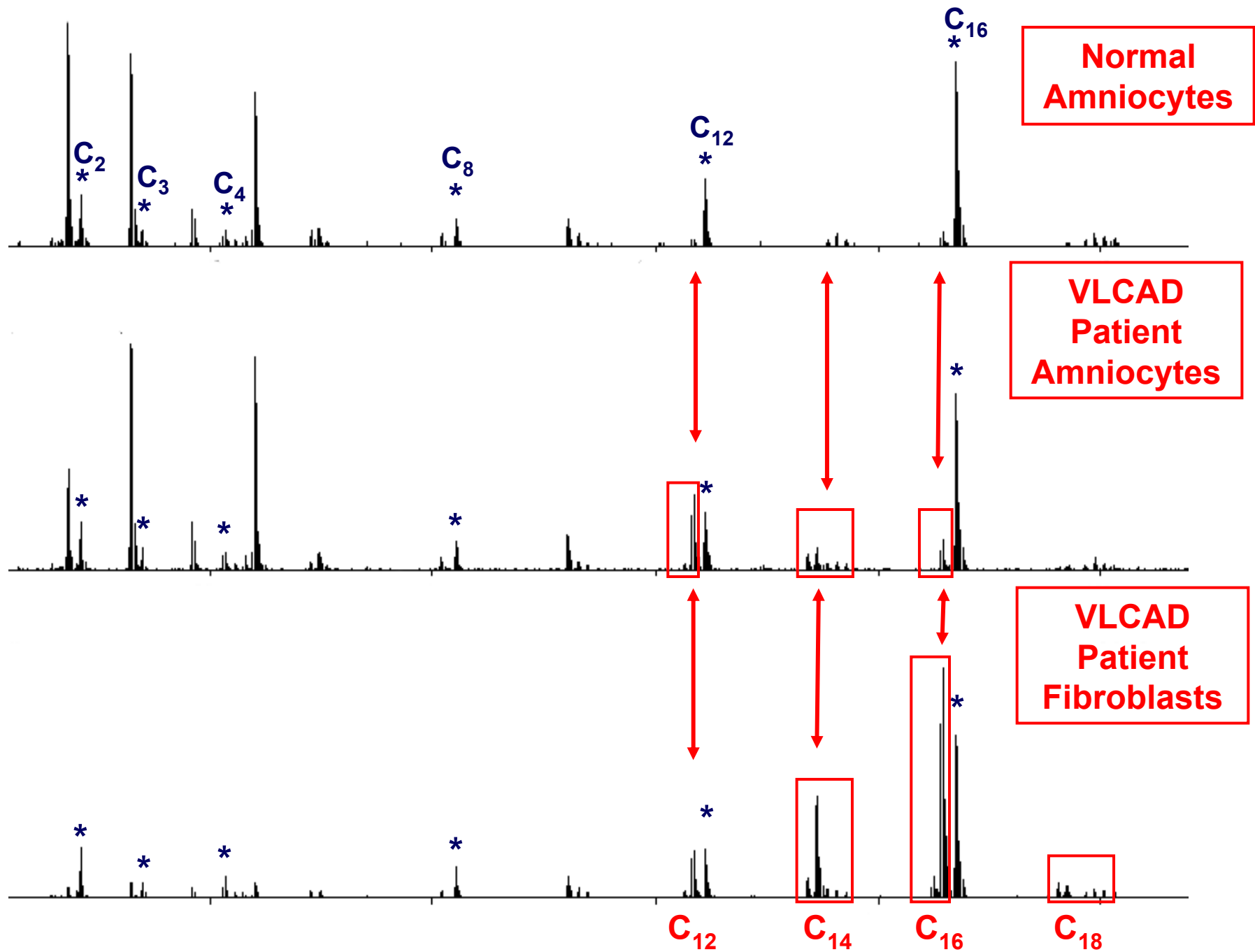


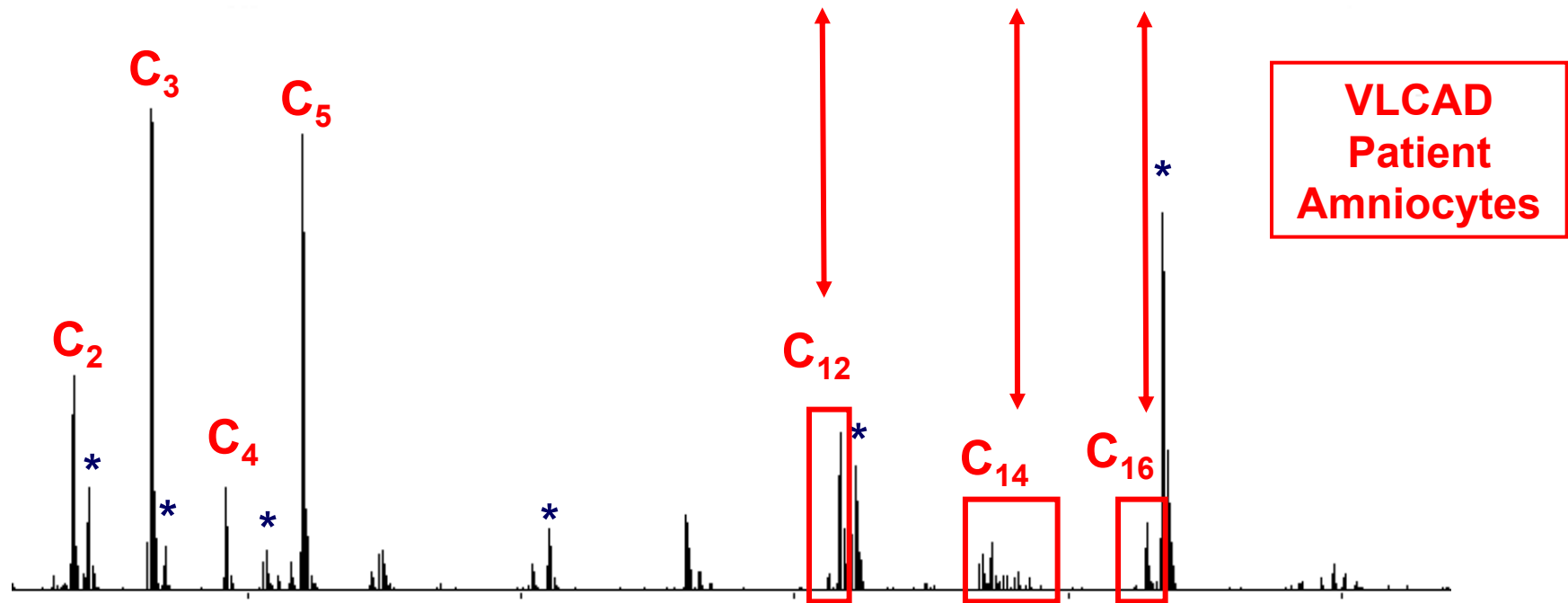
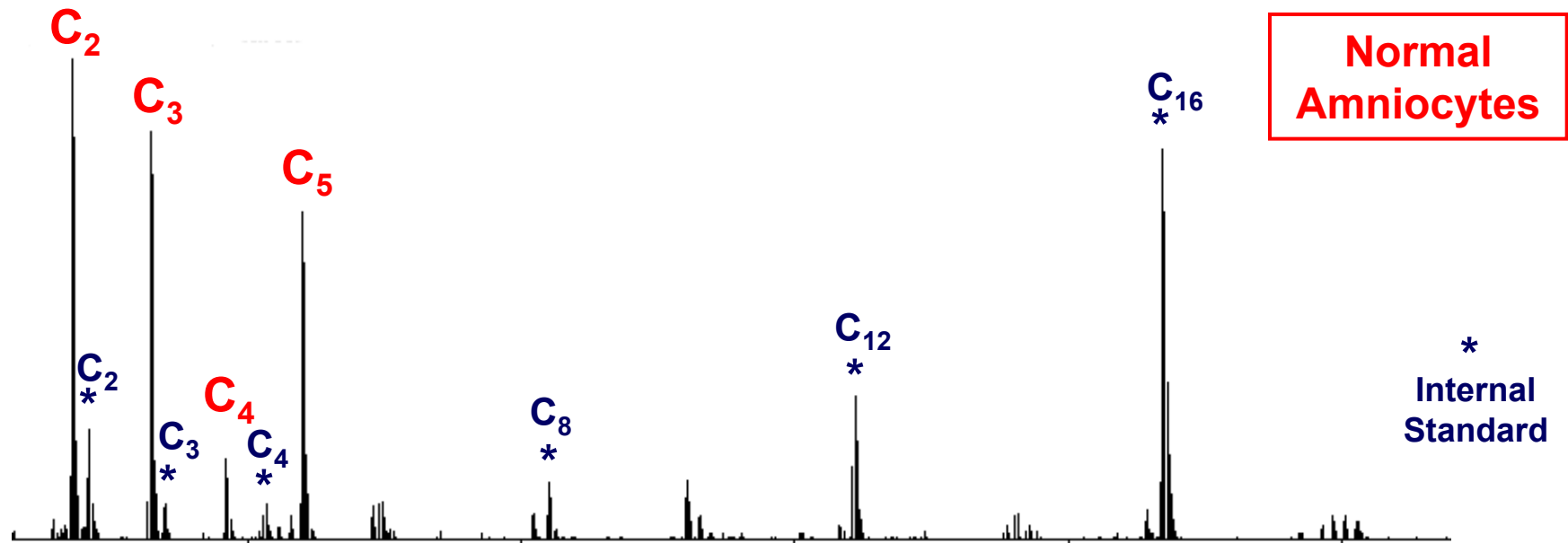
NBS DBS (LCHAD)



“In Vitro” Probe Assay

- **Monolayer of skin fibroblasts / amniocytes**
- **Growth media removed and replaced with “*in vitro* probe” media containing palmitic acid and L-carnitine**
- **Incubated for 72 hours, media is collected and subjected to acylcarnitine analysis**
- **Adherent cells are harvested - total protein analysis**





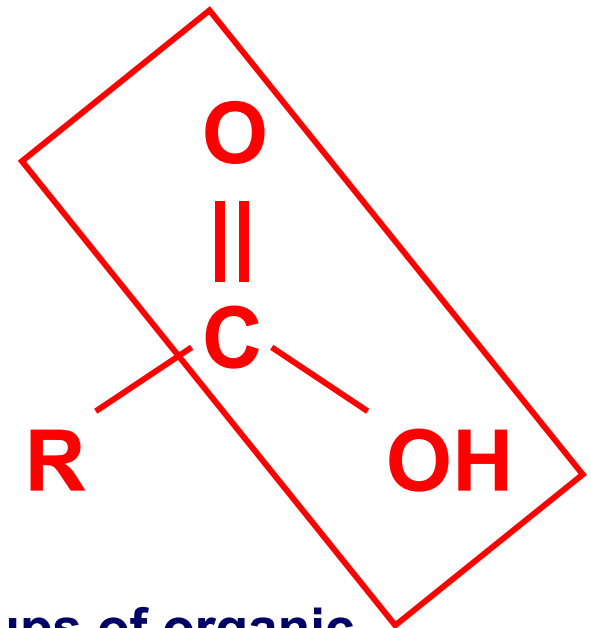
Enzyme Assay for VLCAD - Results

	Control Amniocytes	VLCAD Patient Amniocytes
MCAD Activity (mU/mg Protein)	1.01	1.21
VLCAD Activity (mU/mg Protein)	1.52	Not detected

	Control Fibroblasts	VLCAD Patient Fibroblasts
MCAD Activity (mU/mg Protein)	1.31	1.07
VLCAD Activity (mU/mg Protein)	3.61	Not detected

Urine Organic Acids

- **Organic Acids – What are they?**
 - Water-soluble compounds containing one or more carboxylic group as well as other functional groups (-keto, -hydroxy)
 - Intermediate metabolites of all major groups of organic cellular components: amino acids, lipids, carbohydrates, nucleic acids and steroids
- **Accumulation of metabolites which are not present under physiological conditions**

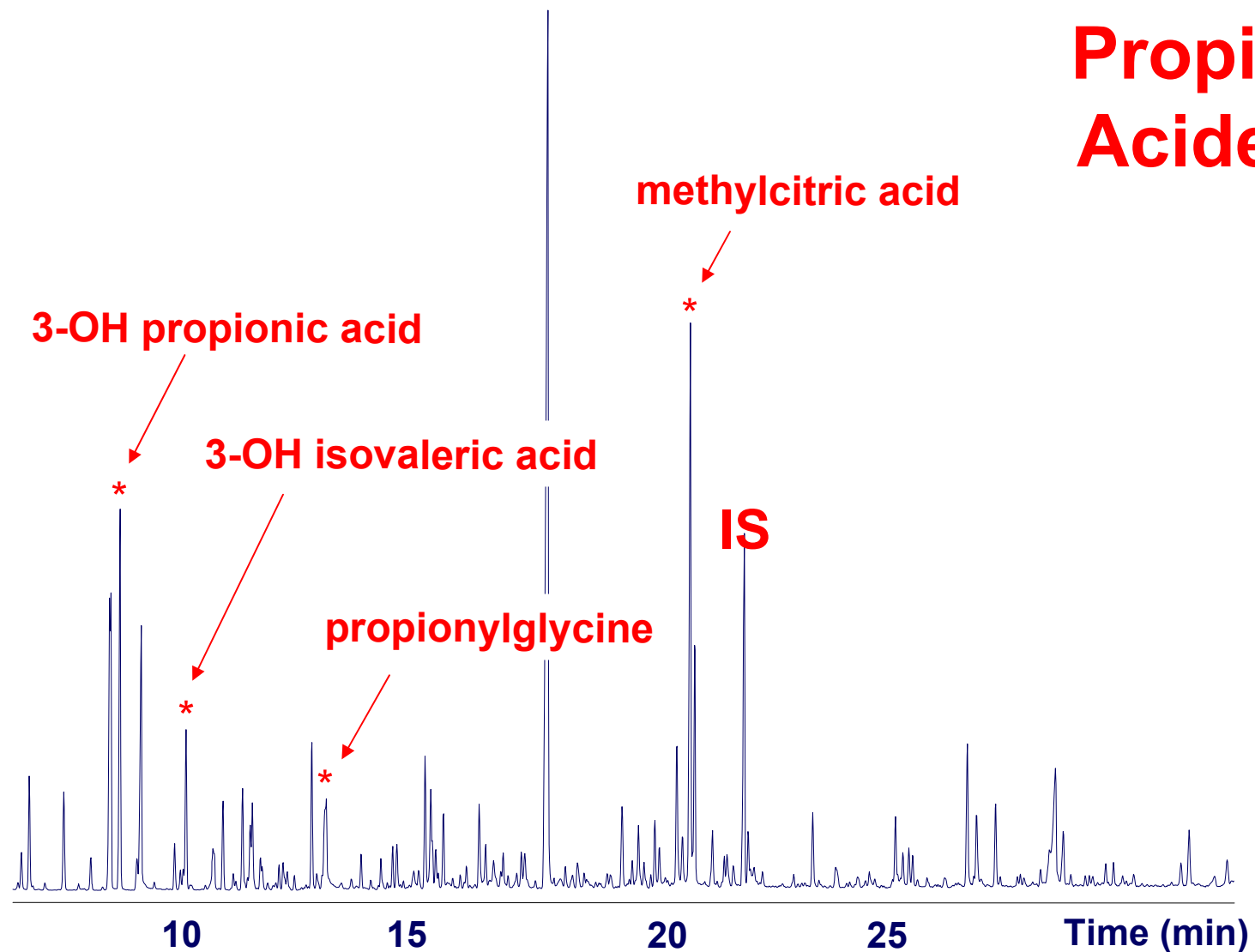


Urine Organic Acids

- **Urine Sample Collection**
 - Urine should be collected without preservatives
 - Collection should avoid fecal contamination
 - Frozen immediately
- **Sample Preparation and Analysis**
 - Liquid-liquid extraction of acidified urine into organic solvent
 - Evaporation of solvent and derivatization of residue
 - Gas chromatography – Mass spectrometry analysis
- **Specific diseases have characteristic organic acid elevations**

Organic Acid Analysis

Propionic Acidemia

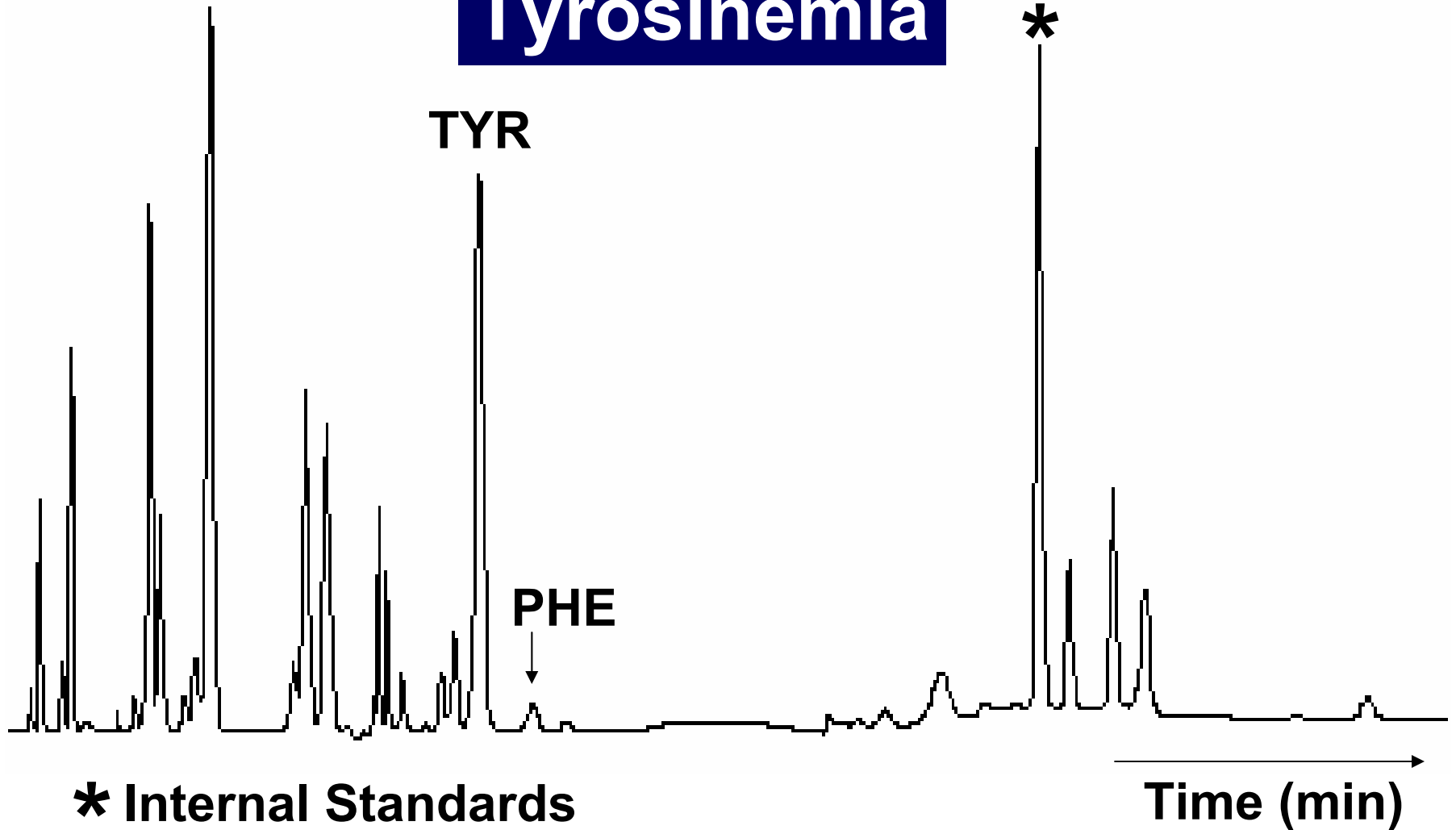


Plasma Amino Acids

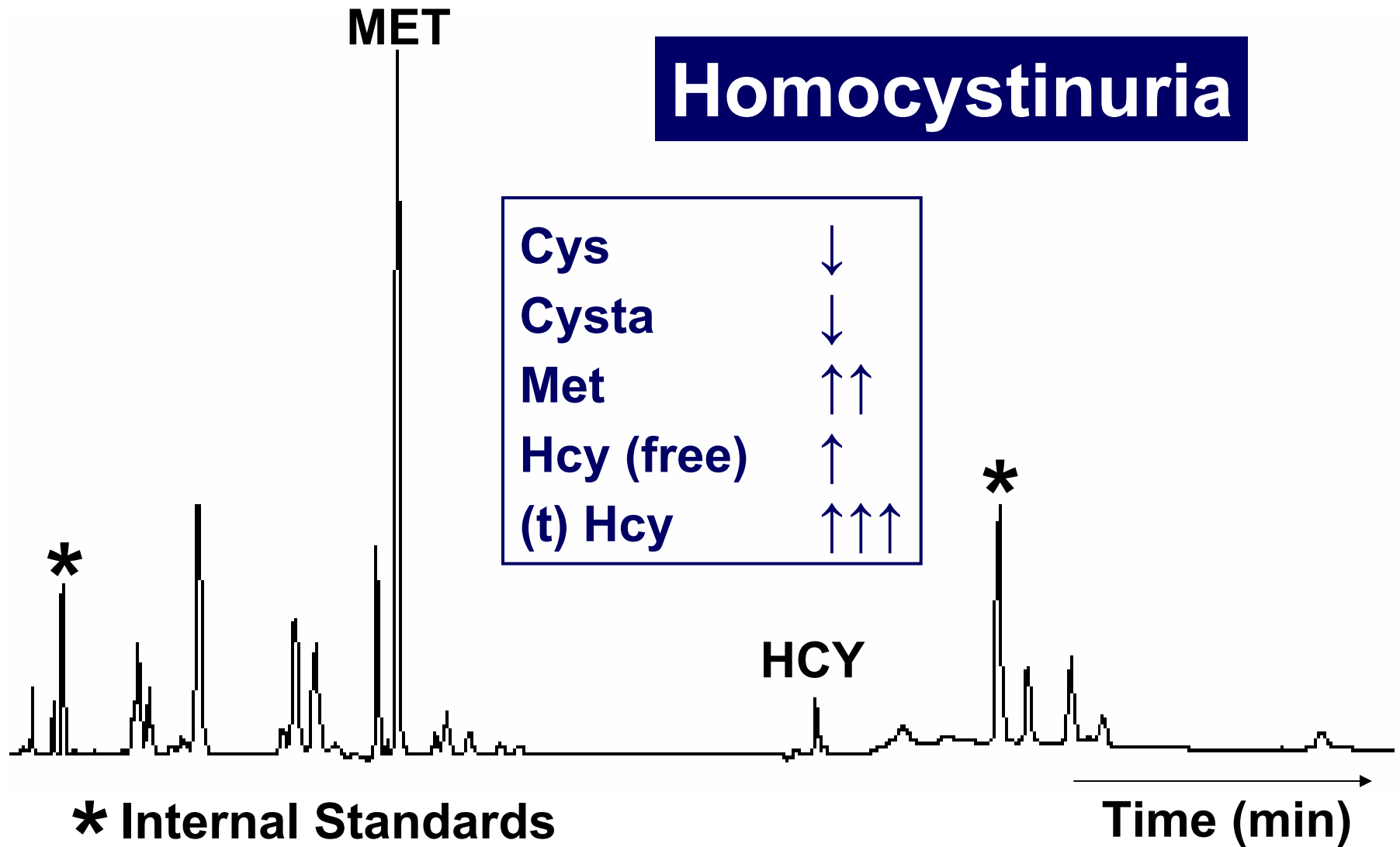
- **Plasma Sample Collection**
 - Timely centrifugation and separation of plasma specimens is critical to prevent artifacts
 - Must be refrigerated for the short term (< 4 hrs.) or frozen (-20°C) to arrest amino acid deterioration
 - Hemolyzed samples are not viable
- **Sample Preparation and Analysis**
 - Protein precipitation and centrifugation
 - Ion exchange chromatography to separate
 - Post-column derivatization & detection at 440 to 570 nm
- **Specific diseases have characteristic amino acid elevations**

Plasma Amino Acids Analysis

Tyrosinemia



Plasma Amino Acids Analysis



Follow-up Diagnostic Testing

- **Routine Chemistries**

- Electrolytes, blood gases, anion GAP
- Blood sugar, ketones, lactate, ammonia

- **Biochemical Genetics Testing**

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