FOD™ All in This Together
Fatty Oxidation Disorders Family Support Group
www.fodsupport.org

FOD/OAA Family Support Groups
National Metabolic Conference
Atlanta, Georgia July 30 & 31, 2010

Organic Acidemia Association
www.oaanews.org

Newborn Screening and Diagnosis of Fatty Acid Oxidation Disorders

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National Center for Environmental Health
Centers for Disease Control and Prevention

Newborn Screening

A Public Health Program

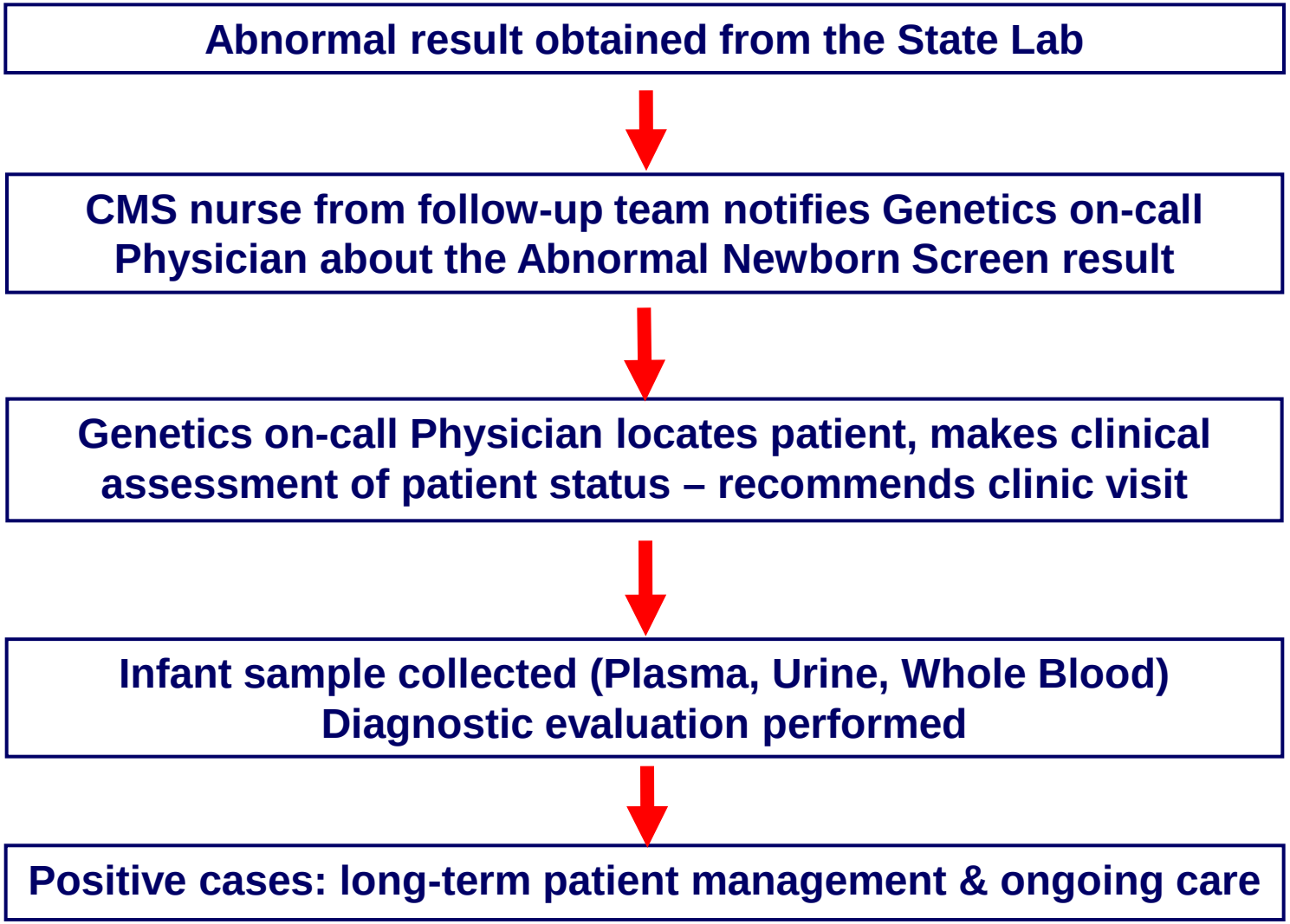
- **Aimed at identification of conditions for which early intervention can prevent mortality, morbidity, and disabilities**
- **Performed by analysis of diagnostic markers in blood spots collected on filter paper at birth**
- **Largest population-based genetic screening effort in the US:** ***~ 4 Million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



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

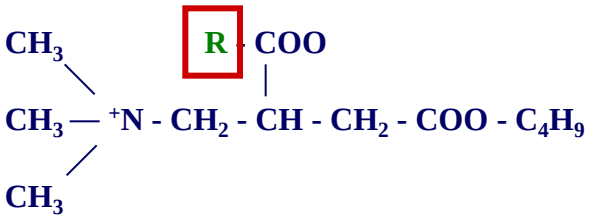
Tandem Mass Spectrometry

- 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**

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 trasport defect (HHH)
Phenylalanine hydroxylase deficiency (PKU)
Defects of biopterin metabolism
Fumarylacetoacetase deficiency
Tyrosine aminotransferase deficiency

Collective incidence

1:2-4,000 newborns

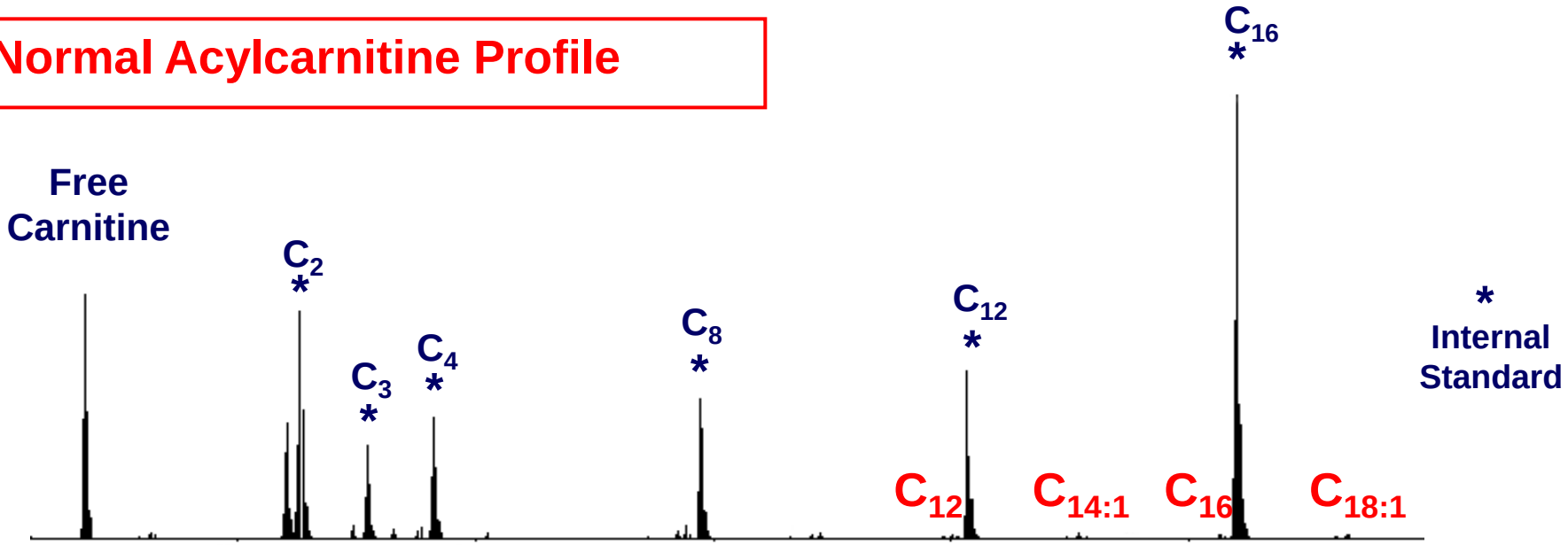
Clinical impact (US):

Up to 2,000 cases/year

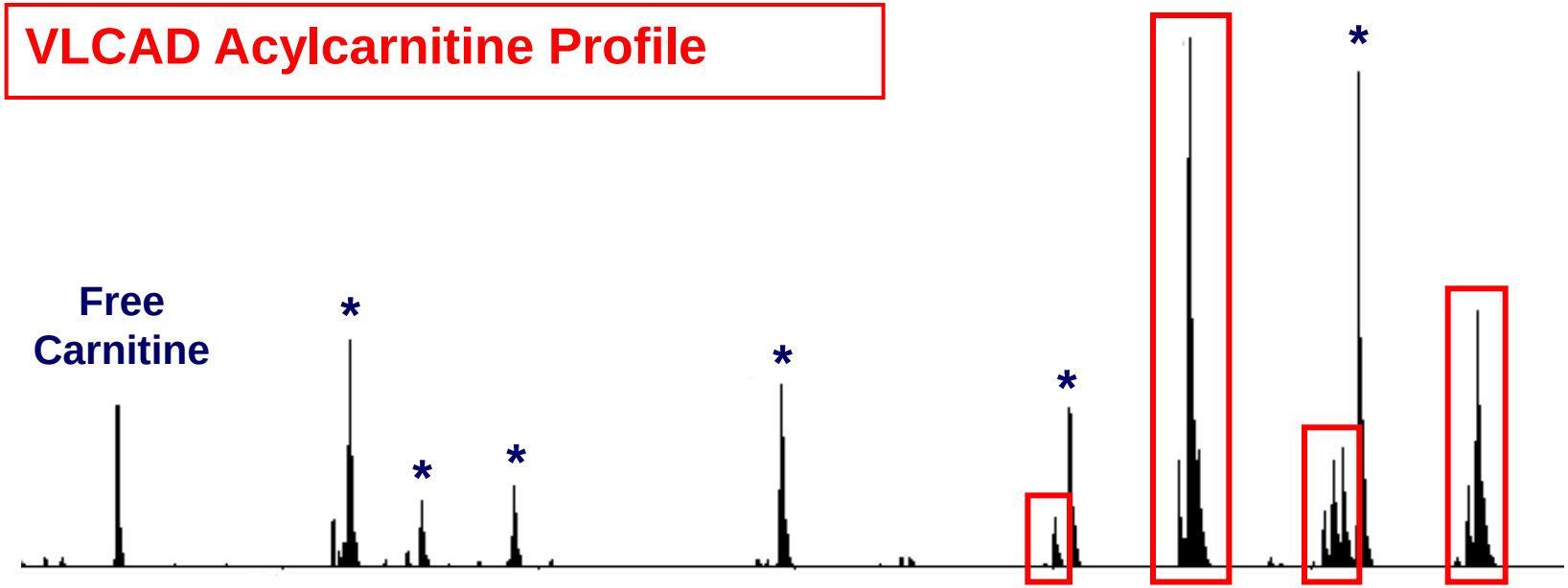
Disorders of organic acid metabolism

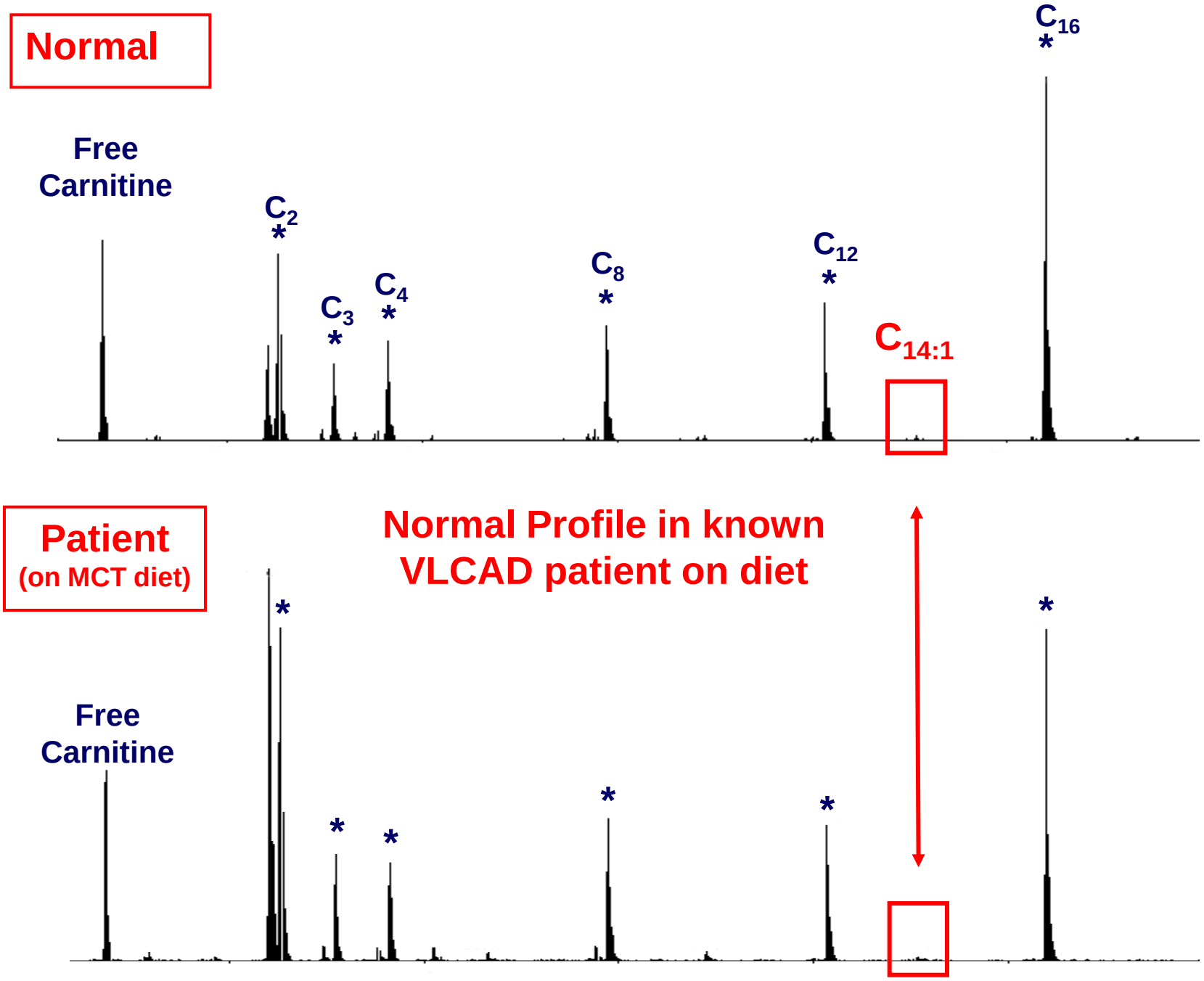
2-CH3 butyryl-CoA dehydrogenase deficiency
2-CH3 3-OH butyryl-CoA dehydrogenase deficiency
3-OH 3-CH_3 glutaryl-CoA lyase deficiency
3-CH_3 crotonyl-CoA carboxylase deficiency
3-CH_3 glutaconyl-CoA hydratase deficiency
Isobutyryl-CoA dehydrogenase deficiency
Isovaleryl-CoA dehydrogenase deficiency
Glutaryl-CoA dehydrogenase deficiency
Malonyl-CoA carboxylase deficiency
Methylmalonyl-CoA mutase deficiency
Disorders of cobalamin metabolism
β-ketothiolase deficiency
Multiple carboxylase deficiency
Propionyl-CoA carboxylase deficiency

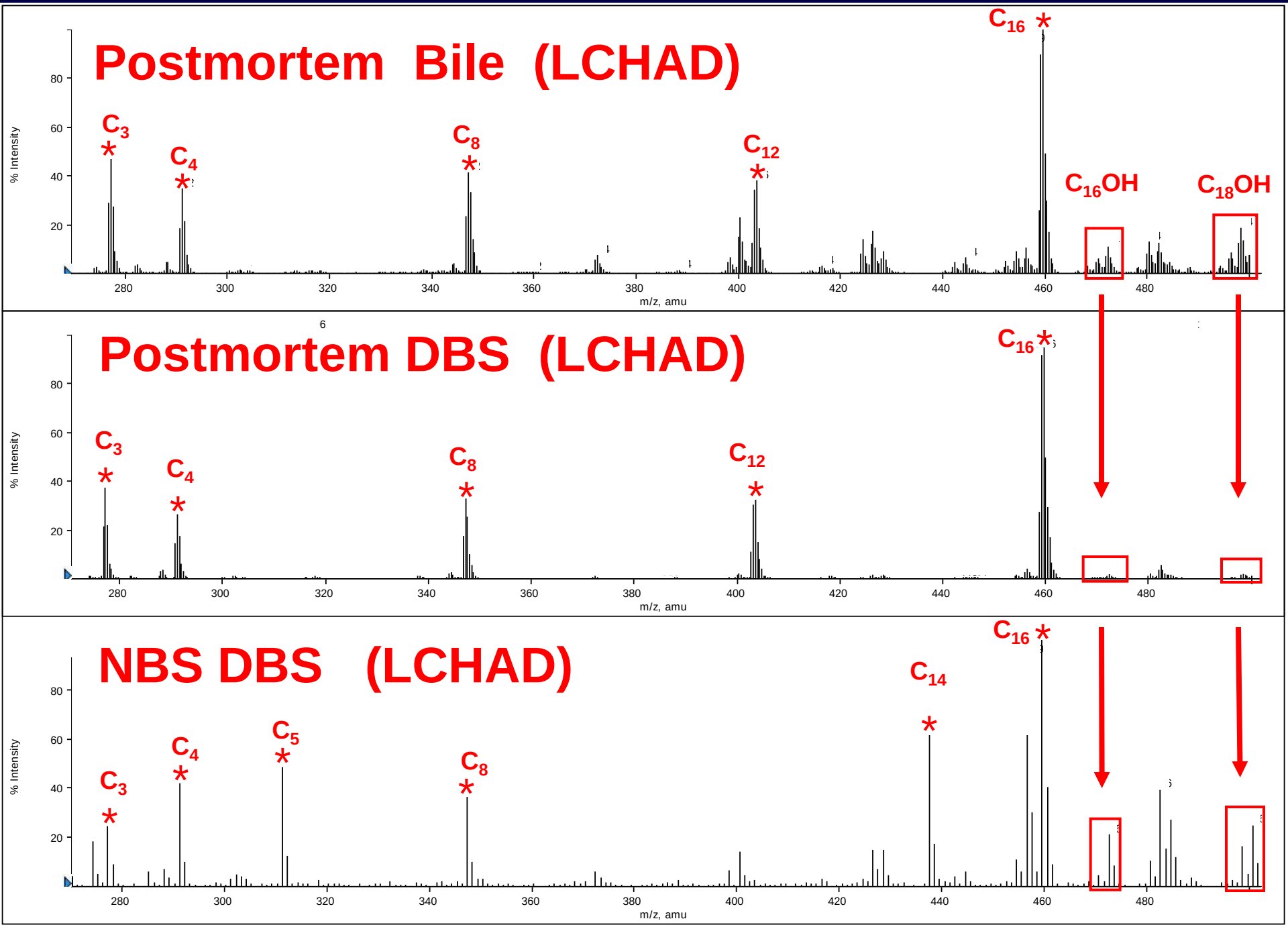
Normal Acylcarnitine Profile



VLCAD Acylcarnitine Profile

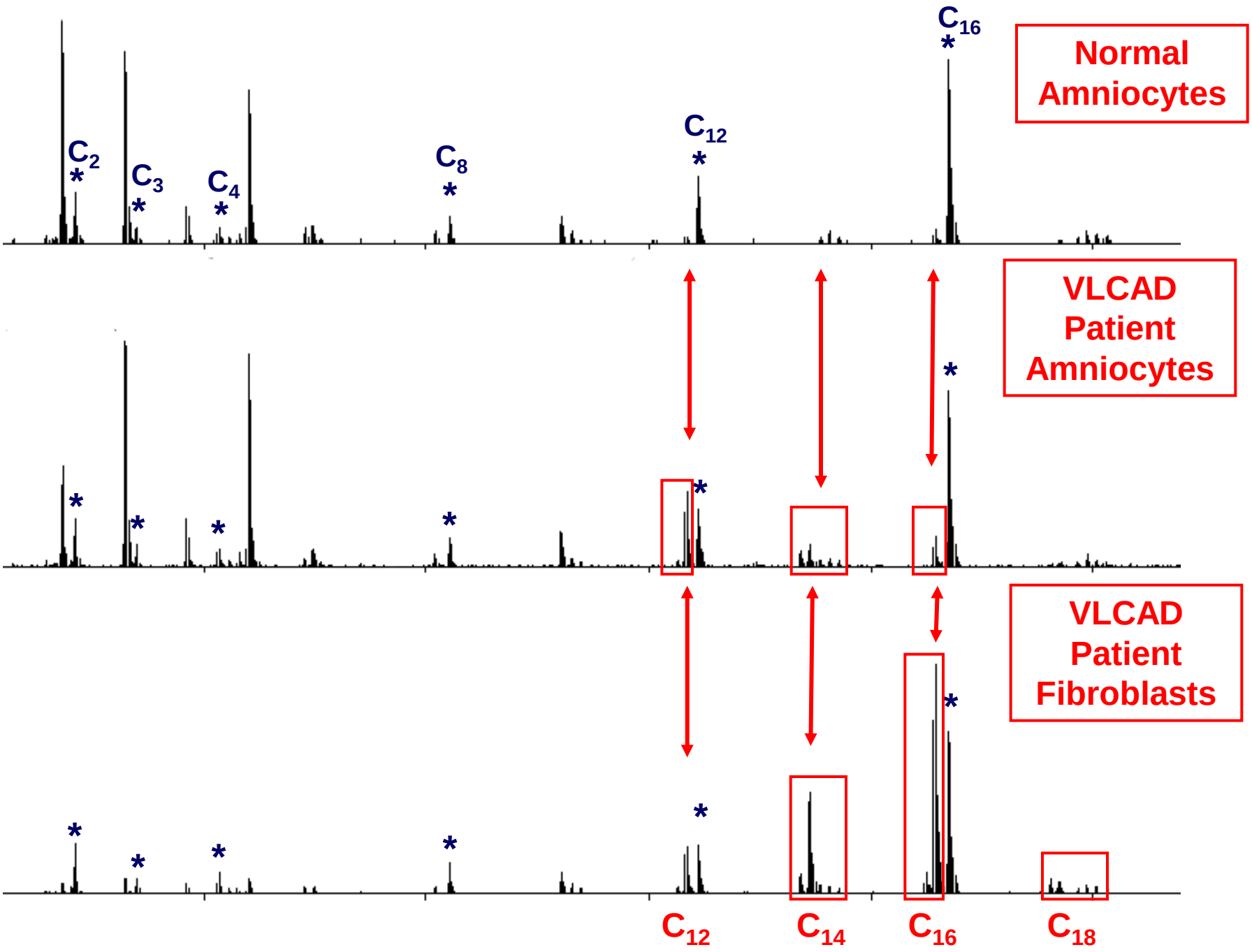



Normal
Free Carnitine
C2
C3
C4
C8
C12
C14:1
C16
Patient
(on MCT diet)
Normal Profile in known
VLCAD patient on diet
Free Carnitine


Postmortem Bile (LCHAD)
C3 *
C4 *
C8 *
C12 *
C16 *
C16OH
C18OH
% Intensity
m/z, amu
Postmortem DBS (LCHAD)
C3 *
C4 *
C8 *
C12 *
C16 *
% Intensity
m/z, amu
NBS DBS (LCHAD)
C3 *
C4 *
C5 *
C8 *
C14 *
C16 *
% Intensity
m/z, amu

Fatty Acid Oxidation 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**


Normal Amniocytes
VLCAD Patient Amniocytes
VLCAD Patient Fibroblasts
C2
C3
C4
C8
C12
C16
C12
C14
C16
C18

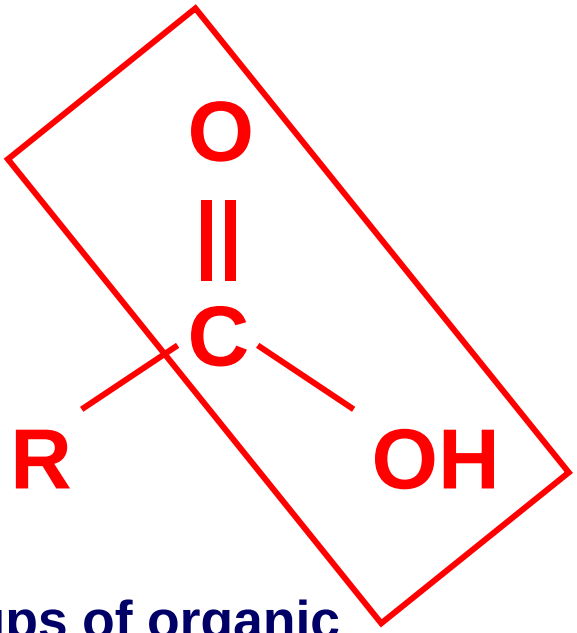
Enzyme Assay for VLCAD - Results

	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)**

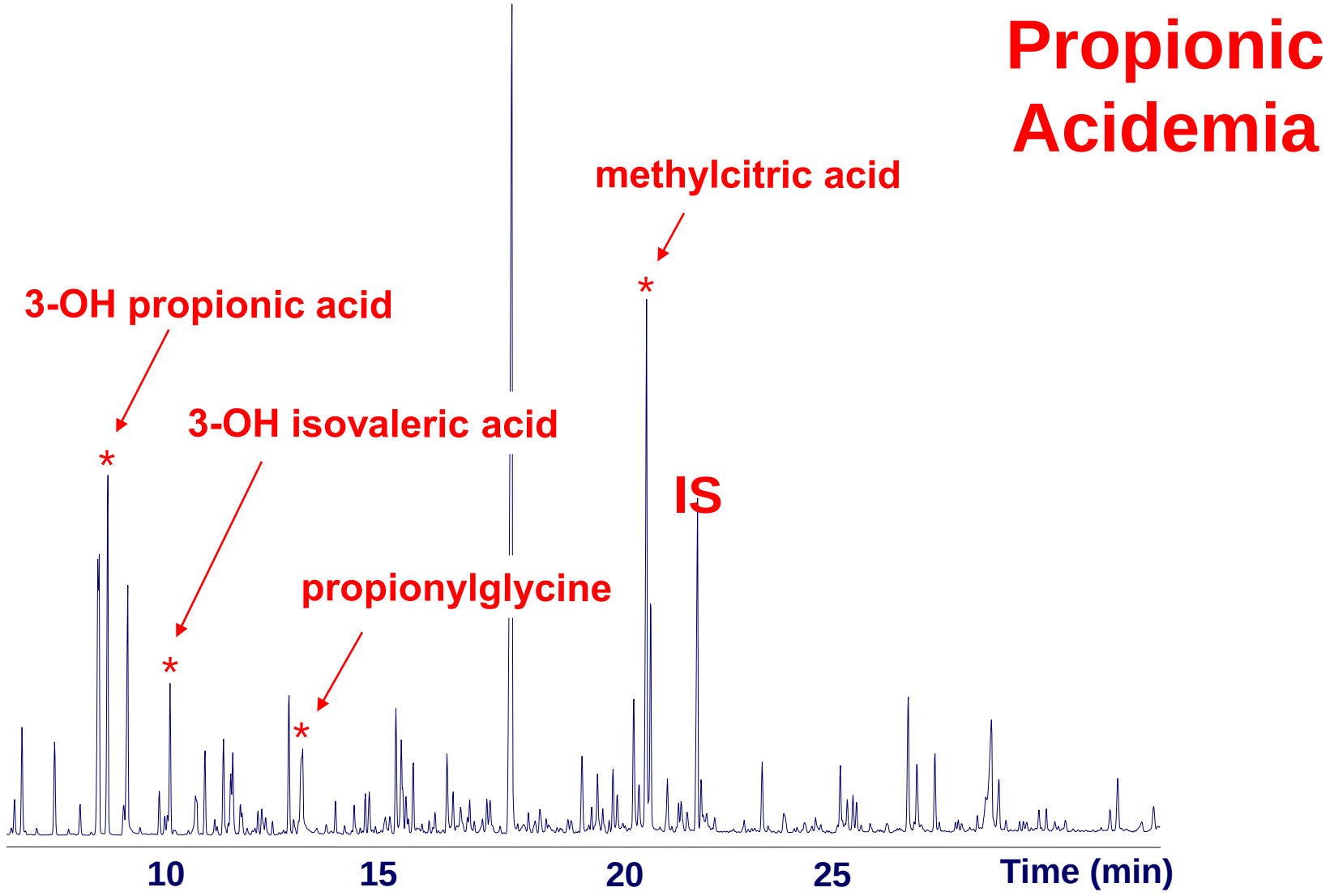


  - **Intermediate metabolites of all major groups of organic cellular components: amino acids, lipids, carbohydrates, nucleic acids and steroids**
- **Accumulation of metabolites which are not present under physiological conditions**

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



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^{o}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