



# Newborn Screening of Premature Infants:

*Clinical Trial Data Suggests Gestational Age and Chronological Age are Key to Interpretation*

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# Objectives

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To describe the influence that gestational age and chronological age have on amino acid and acylcarnitine profiles in an at risk population of premature infants.

**Gestational Age and Age at Sampling Influence Metabolic Profiles in Premature Infants**

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# The Numbers

**Patient Samples**

3,579

995

**Premature Infants Studied**

**Collection Times (Days <1,7,24,42)**

4

0

**Metabolic Disorders Confirmed**

# The Analyses

## Tandem Mass Spectrometry

Amino Acid Profile (NL 102, butyl esters)

Acylcarnitine Profile (Pre 85, butyl esters)

179,500 data points (3,579 x 50 metabolites)

# Study Details

## ▶ Gestational Ages of Premature Infants

- ▶ 23 – 31 weeks

## ▶ 3 Groups

- ▶ Group 1: 23 to 26 weeks (n = 293)
- ▶ Group 2: 27 to 28 weeks (n = 277)
- ▶ Group 3: 29 to 31 weeks (n = 425)

## ▶ Median Birth Weight

- ▶ 1057 grams (644–1514 g)



## ▶ Study performed at 23 sites in 17 states

- ▶ 1 Central lab performed all tests
  - ▶ PE Genetics
- ▶ Interpretation of results
  - ▶ Pediatrix Analytical

## ▶ Samples collected using NBS consensus protocols

- ▶ State data shared from all abnormal results.
  - ▶ Received 15/17 state alert values

# Nutrition

## ▶ Parenteral (IV) Nutrition

- ▶ 2 Hours (median, initiated)

  - ▶ Dextrose, Amino Acids

- ▶ 24 Hours

  - ▶ Lipids

- ▶ 33% of patients

  - ▶ Carnitine

    - ▶ Increased use with Decreased gestational age

- ▶ Major Source of Nutrition < 7 days

## ▶ Enteral Nutrition

- ▶ > 24 Hours (median, initiated)

  - ▶ Human Breast Milk, Formulas

- ▶ Day 7

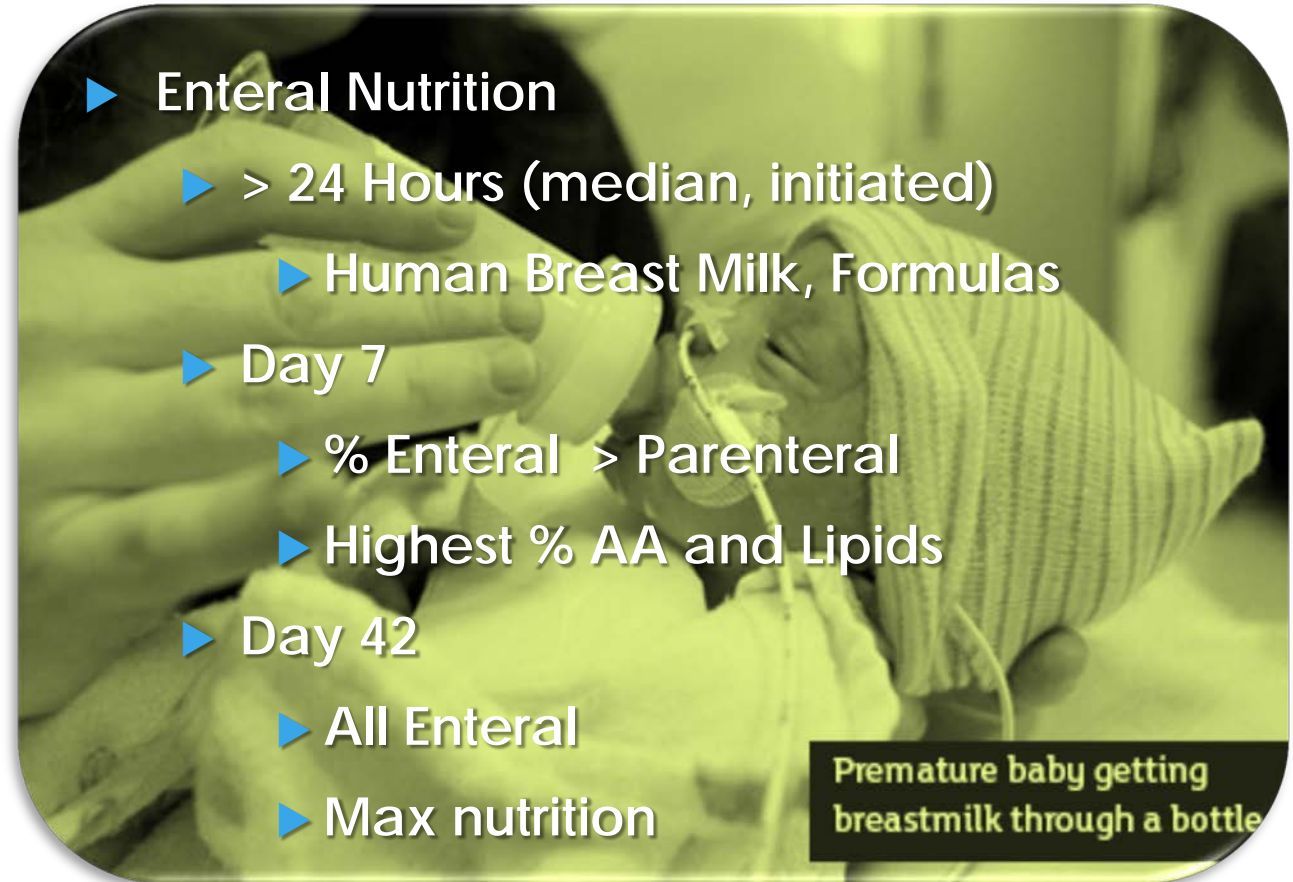
  - ▶ % Enteral > Parenteral

  - ▶ Highest % AA and Lipids

- ▶ Day 42

  - ▶ All Enteral

  - ▶ Max nutrition



Premature baby getting breastmilk through a bottle

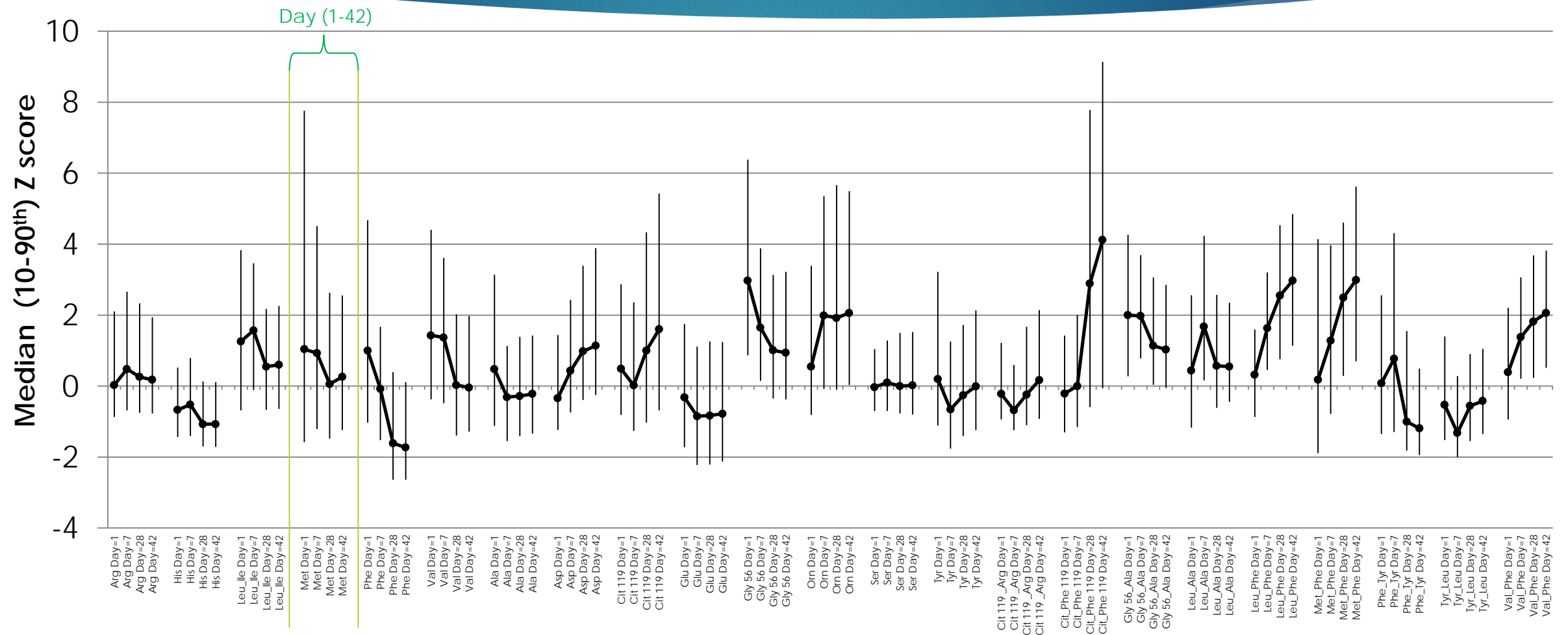
# Study Results



March of Dimes®

# Amino-Acids and Ratios

Quick Peak

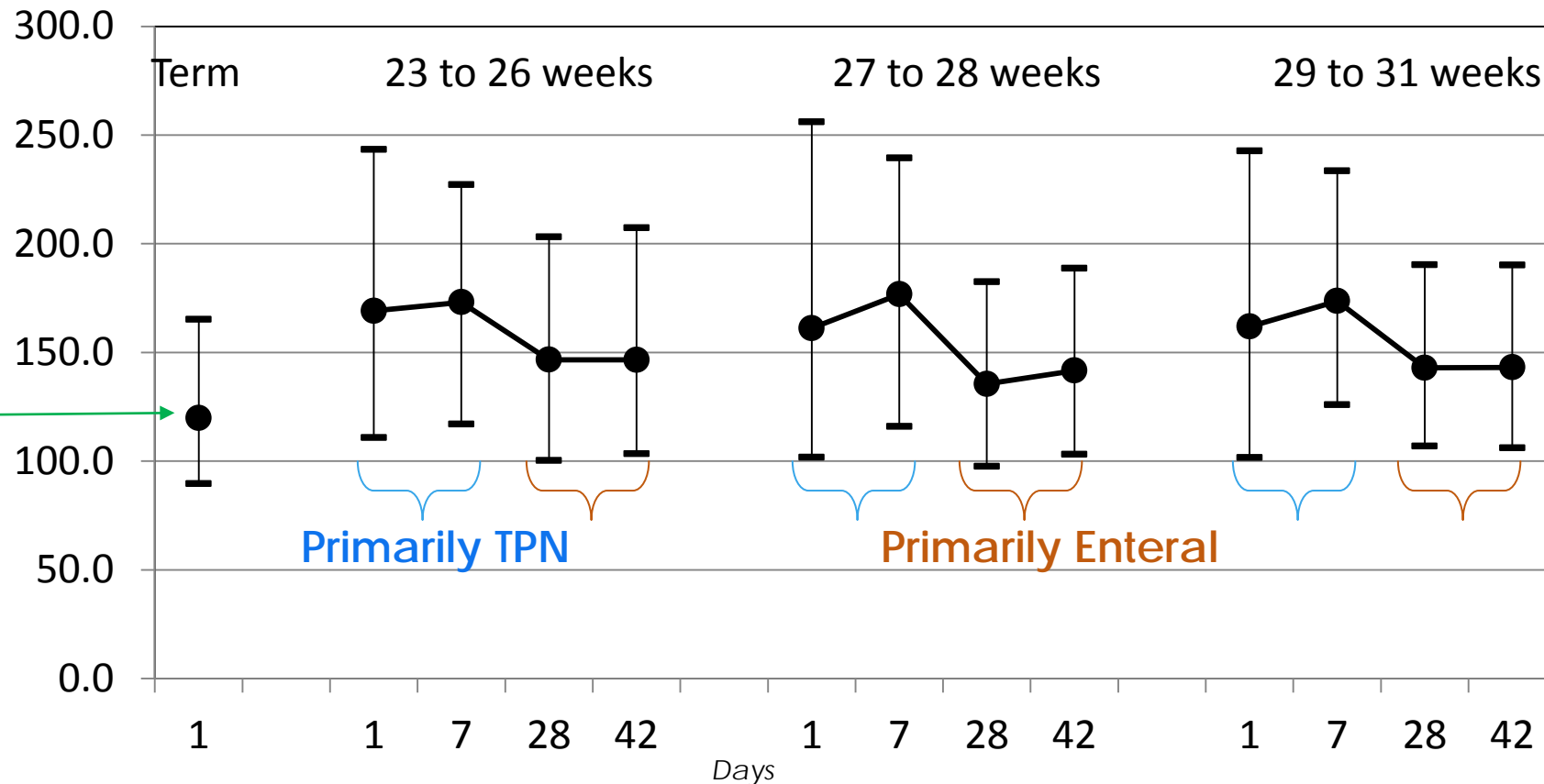




# Amino Acid Example: Leu+Ile

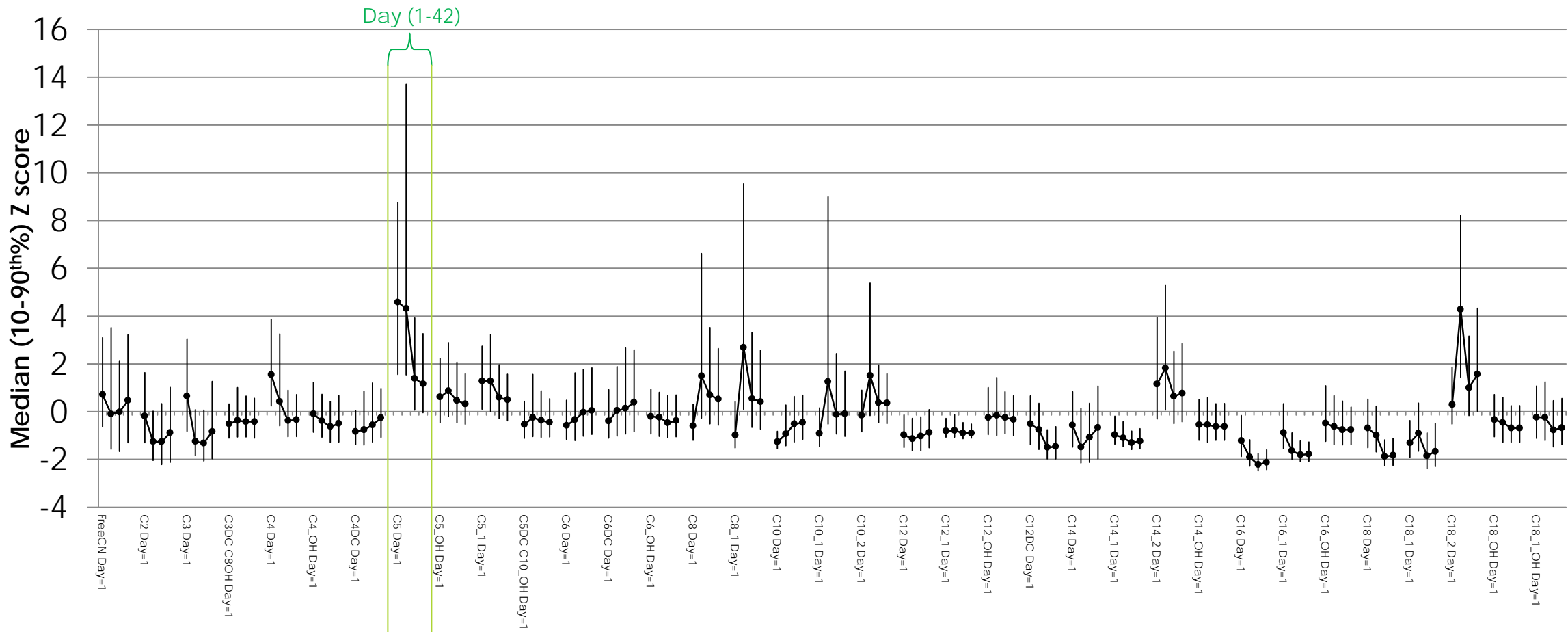
— Quantiles90 — Quantiles10 ● Median

From NBS  
Data

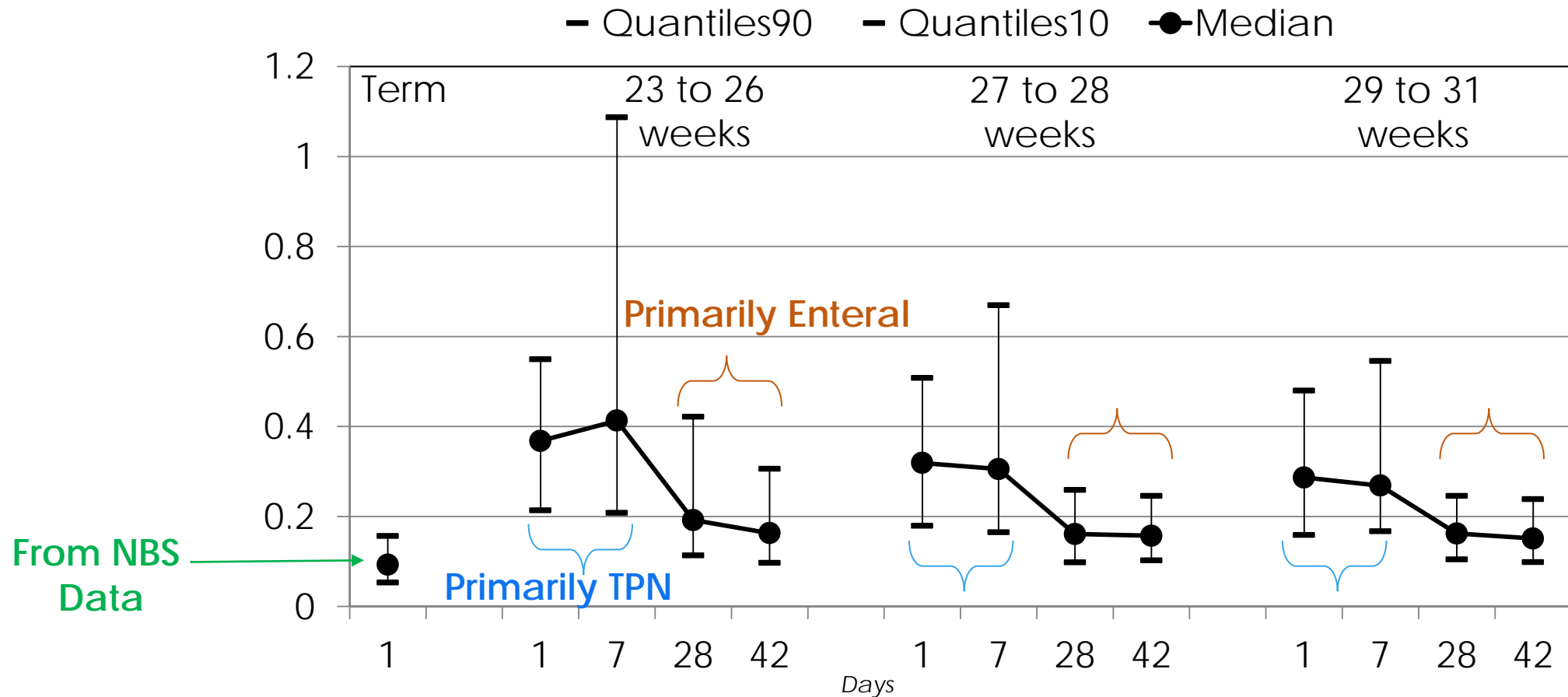


# All Acylcarnitines

Quick Peak

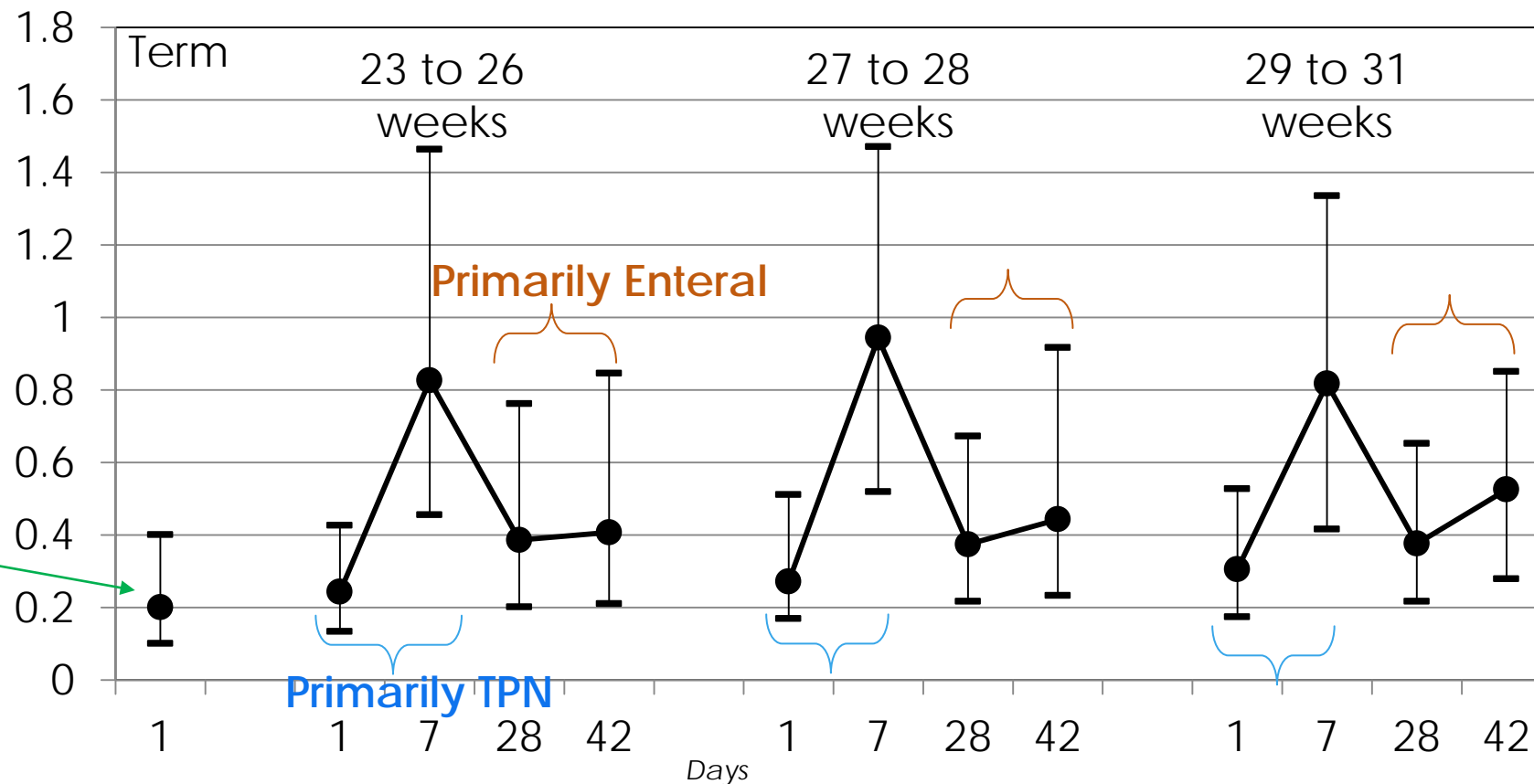


# Acylcarnitines (OA) Example: Isovaleryl Carnitine



# Acylcarnitines (FA) Example: Linoleyl Carnitine

— Quantiles90 — Quantiles10 ● Median



From NBS Data

# Generalized Findings (AA and AC)

## MS/MS Study (AC and AA)

- ▶ 214 alerts (21.5% based on standard NBS interpretation protocols)
  - ▶ 29% from Group 1 • 17% from Group 2 *(all days)*
  - ▶ 12% Day 1 • 2% Day 28 *(all groups)*
  - ▶ Highest rate of abnormal results were on day 7 from Group 1

## State NBS Screening (all tests)

- ▶ 461 alerts% (21.5% based on standard NBS interpretation protocols)
  - ▶ Premature Infants had most abnormal results: 64% versus 36% term
  - ▶ T4/TSH and CAH: highest percentage of abnormal results.
  - ▶ MS/MS: 8.9% and 2.6% for AC and AA, abnormal results respectively

**Study and State NBS results were similar in terms of abnormal % and metabolite elevations (harmonization)?**

# Key Amino Acid Findings

## ▶ Phe

- ▶ Higher in the 23-26 week group 1 than other groups.
- ▶ Above median at day 1 (62  $\mu\text{M}$ ). Median day 7 (55  $\mu\text{M}$ ).
  - ▶ **Well below median days 28-42 (38  $\mu\text{M}$ ).**
- ▶ Phe/Tyr ratio above median day 7 and below median days 28-42.
  - ▶ Tyrosine below median day 7, above median day 1

## ▶ Citrulline

- ▶ Increased with time for all groups (At days 28-42, 40% greater than day 1 and 7). Lowest on day 7.
- ▶ Interesting note (Citrulline not contained in protein or amino acid solution in TPN)
  - ▶ Made in small intestine, potential marker of gut health (low in unhealthy or abnormal GI tract )

# Key Acylcarnitine Findings

- ▶ Organic acid acylcarnitines higher in group 1 (C5, C5:1, C4DC, C5OH, C5DC) compared to group 2 and 3.
- ▶ Most ACs higher on day 0 than decrease through day 7. More variable pattern days 28-42.
- ▶ **Linoleic Acid**
  - ▶ **Significant increase by day 7.** Return to normal levels days 28-42
  - ▶ Due to Intralipid in TPN. Direct correlation between intralipid C18:2 content and C18:2 acylcarnitine)
- ▶ Free Carnitine
  - ▶ Supplemented patients with L-carnitine showed highest FC on day 7 (40  $\mu$ M versus 18 $\mu$ M)
  - ▶ Higher FC on day 1 compared to normal. Lower FC on day 7 for non supplemented patients).

# Summary

- ▶ 21 % of infants had an abnormal alert value
- ▶ 0% could be explained by TPN contamination with amino acids.
- ▶ Cutoff values inappropriate for premature infants (median values 1 SD above and below medians)
  - ▶ Normative values must correct for time and gestational age.
  - ▶ Must also correct for illness and nutritional support
- ▶ The most premature infants metabolically different that most mature premature infants.
- ▶ Organic acid acylcarnitines, branched chain amino acids, long chain acylcarnitines highest on day 7.
  - ▶ All feed into beta oxidation pathway
  - ▶ Elevating more toxic metabolites