The Axis of Contamination

Dextrose and Amino Acids

False Positives due to TPN for VLBW Infants

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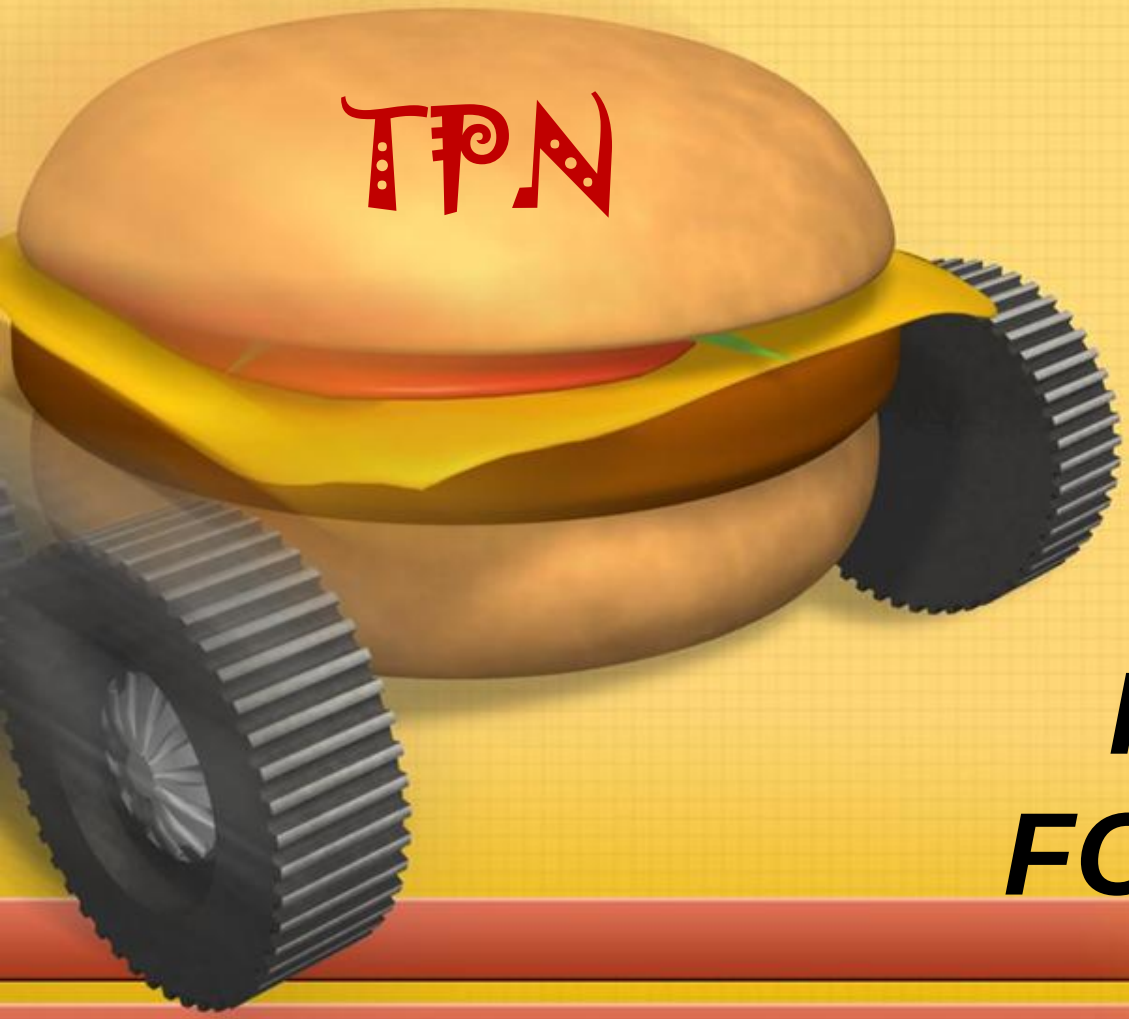
The title I would like to use…



FAST FOOD FOR PREEMIES

VLBW – Very Low Birth Weight

* Hypothesis:
  * Extremely high concentrations of amino acids in DBS are **not** due to a biochemical defect.
  * False Positive Results are ***in part*** due to mixing (contamination) of IV feeds (total parenteral nutrition solutions) that contain very high concentrations of amino acids.
  * A marker for contamination by TPN would help identify infants whose DBS sample is invalid / unacceptable and reduce presumptive positives.
    * Would not reduce repeat sampling in the short term.
    * Reduce repeat sampling in the long term by identifying laboratories that do not collect samples properly

Brief History

* High FP rate for premature infants historically.
  * even with better analytical instruments and methods.

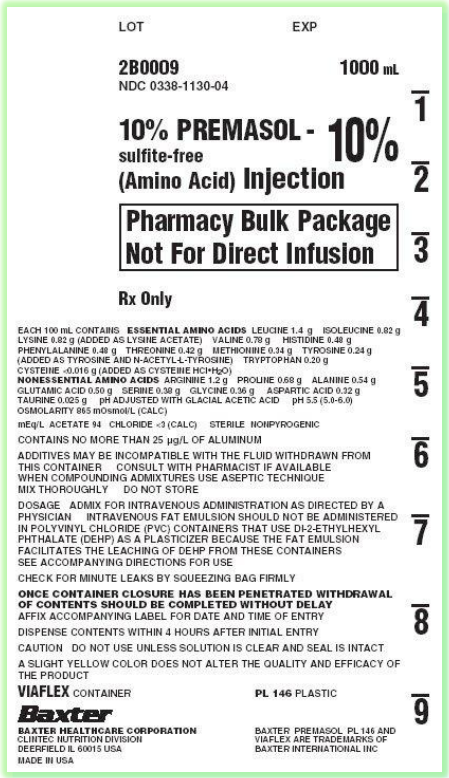
* False Positives characterized by very high Leu/Ile, Phe, Met, Ala but not Tyr.
  * Interpretation guidelines in some labs act on milder elevations of Tyr and/or Met but not on much higher concentrations of Met when Leu/Phe/Ala are also high.

* Clinical trial of 100 premature infants with multiple collections points on TPN did not have these very abnormal profiles.

* Examination of all very high AA profiles revealed markers in the acylcarnitine profiles that were not acylcarnitines.
  * Process of elimination indicated dextrose
  * Mass spectra indicated a carbohydrate
  * Mayo suggested dextrose in an abstract at an SIMD meeting.

What is TPN?

* Source of Energy
  * DEXTROSE (D-glucose)
    * 5, 10 and 12.5% (% = g per 100mL, dL)
* Source of Protein
  * Free L-Amino Acids
    * 2 – 4 g per kg per day
    * Proprietary mix of AA
* Isotonic Saline
  * Salts, minerals etc…



LOT EXP

2B0009 1000 mL

NDC 0338-1130-04

10% PREMASOL - 10%
sulfite-free
(Amino Acid) Injection

Pharmacy Bulk Package
Not For Direct Infusion

Rx Only

EACH 100 mL CONTAINS **ESSENTIAL AMINO ACIDS** LEUCINE 1.4 g ISOLEUCINE 0.82 g LYSINE 0.82 g (ADDED AS LYSINE ACETATE) VALINE 0.78 g HISTIDINE 0.48 g PHENYLALANINE 0.48 g THREONINE 0.42 g METHIONINE 0.34 g TYROSINE 0.24 g (ADDED AS TYROSINE AND N-ACETYL-L-TYROSINE) TRYPTOPHAN 0.20 g CYSTEINE <0.016 g (ADDED AS CYSTEINE HCl•H2O) **NONESSENTIAL AMINO ACIDS** ARGININE 1.2 g PROLINE 0.68 g ALANINE 0.54 g GLUTAMIC ACID 0.50 g SERINE 0.38 g GLYCINE 0.36 g ASPARTIC ACID 0.32 g TAURINE 0.025 g pH ADJUSTED WITH GLACIAL ACETIC ACID pH 5.5 (5.0-6.0) OSMOLARITY 865 mOsmol/L (CALC)

mEq/L ACETATE 94 CHLORIDE <3 (CALC) STERILE NONPYROGENIC

CONTAINS NO MORE THAN 25 µg/L OF ALUMINUM

ADDITIVES MAY BE INCOMPATIBLE WITH THE FLUID WITHDRAWN FROM THIS CONTAINER CONSULT WITH PHARMACIST IF AVAILABLE WHEN COMPOUNDING ADMIXTURES USE ASEPTIC TECHNIQUE MIX THOROUGHLY DO NOT STORE

DOSAGE ADMIX FOR INTRAVENOUS ADMINISTRATION AS DIRECTED BY A PHYSICIAN INTRAVENOUS FAT EMULSION SHOULD NOT BE ADMINISTERED IN POLYVINYL CHLORIDE (PVC) CONTAINERS THAT USE DI-2-ETHYLHEXYL PHTHALATE (DEHP) AS A PLASTICIZER BECAUSE THE FAT EMULSION FACILITATES THE LEACHING OF DEHP FROM THESE CONTAINERS SEE ACCOMPANYING DIRECTIONS FOR USE

CHECK FOR MINUTE LEAKS BY SQUEEZING BAG FIRMLY

ONCE CONTAINER CLOSURE HAS BEEN PENETRATED WITHDRAWAL OF CONTENTS SHOULD BE COMPLETED WITHOUT DELAY
AFFIX ACCOMPANYING LABEL FOR DATE AND TIME OF ENTRY

DISPENSE CONTENTS WITHIN 4 HOURS AFTER INITIAL ENTRY

CAUTION DO NOT USE UNLESS SOLUTION IS CLEAR AND SEAL IS INTACT

A SLIGHT YELLOW COLOR DOES NOT ALTER THE QUALITY AND EFFICACY OF THE PRODUCT

VIAFLEX CONTAINER **PL 146** PLASTIC

Baxter

BAXTER HEALTHCARE CORPORATION
CLINTEC NUTRITION DIVISION
DEERFIELD IL 60015 USA
MADE IN USA

BAXTER PREMASOL PL 146 AND VIAFLEX ARE TRADEMARKS OF BAXTER INTERNATIONAL INC

Unusual m/z values in the AC-BE Profiles (Pre 85)

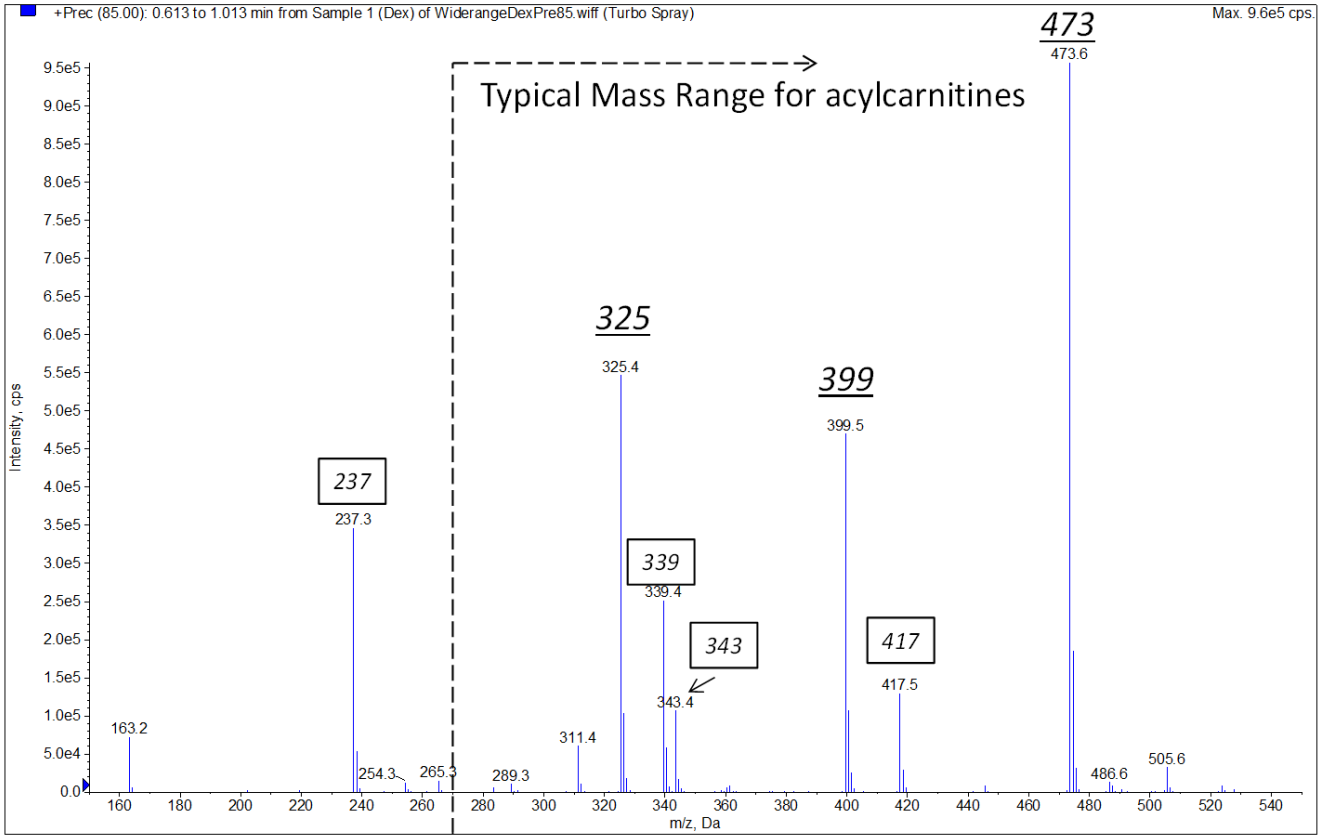
* Nearly all false positive results with high amino acid concentrations had the following markers in the acylcarnitine profile:

m/z: **325 399 473**

* Dextrose suspected based on
  * Product ion profile had fragmentation profiles similar to carbohydrates.
  * Eliminated other components of TPN

* Conclusive evidence for dextrose not easily obtained because:
  * Analysis of pure dextrose did not show markers
    * (subsequently found that preparation in same manner of a DBS did show markers)
  * m/z values did not add up to dextrose (MW = 260)

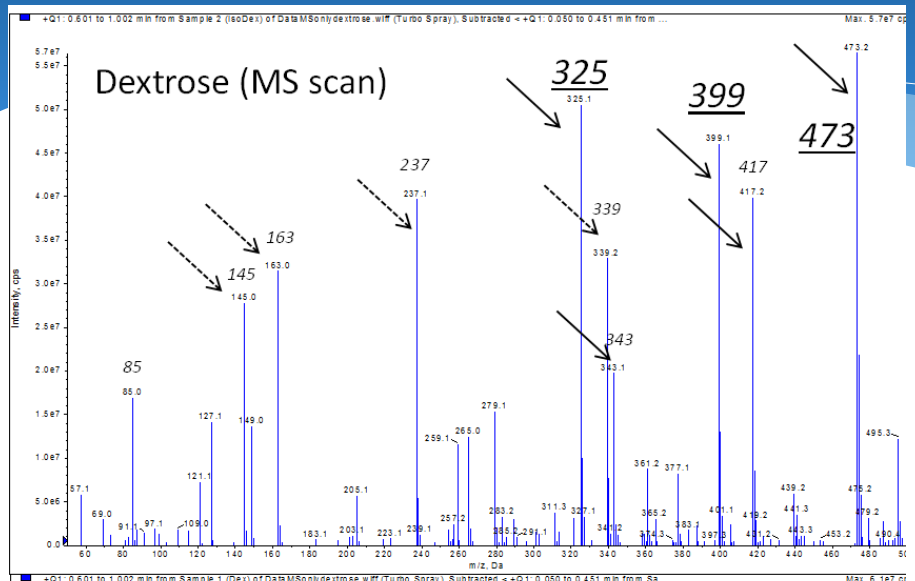
Precursor Ion m/z 85, extended range (dextrose only – BE treatment)

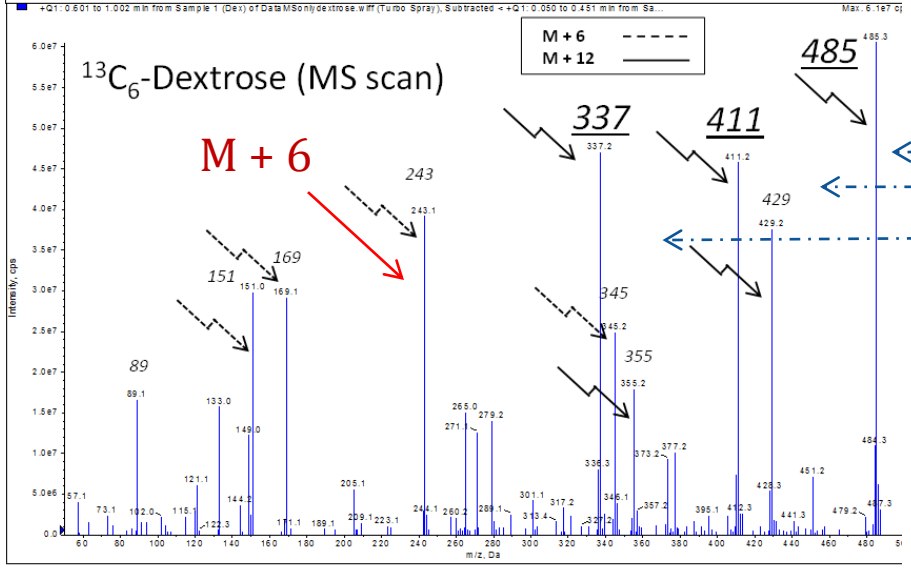


Pre – 85 (AC profile)

Dextrose – *(prepared in same manner as DBS – butyl esterification)*

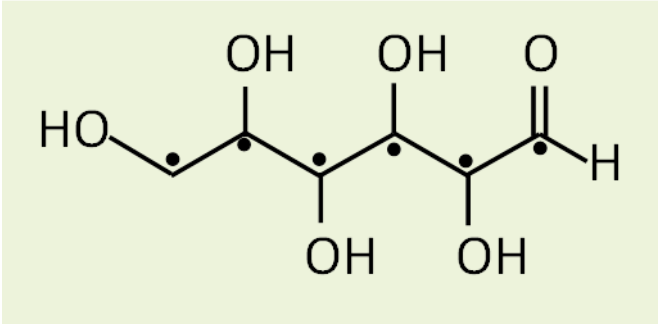
Solution: Isotope Labeled D-glucose (m/z 180)





M + 12

$^{13}C_6H_{12}O_6$ 186 Da

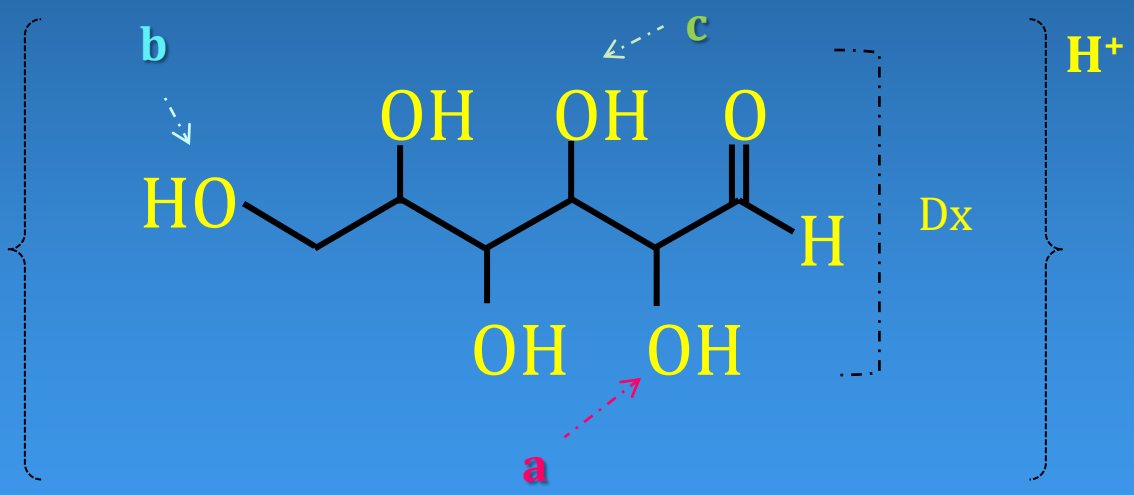


Shift of fragments by <u>12 Daltons</u>

Can only occur if "2" dextrose molecules

Found a dextrose monomer at m/z 237

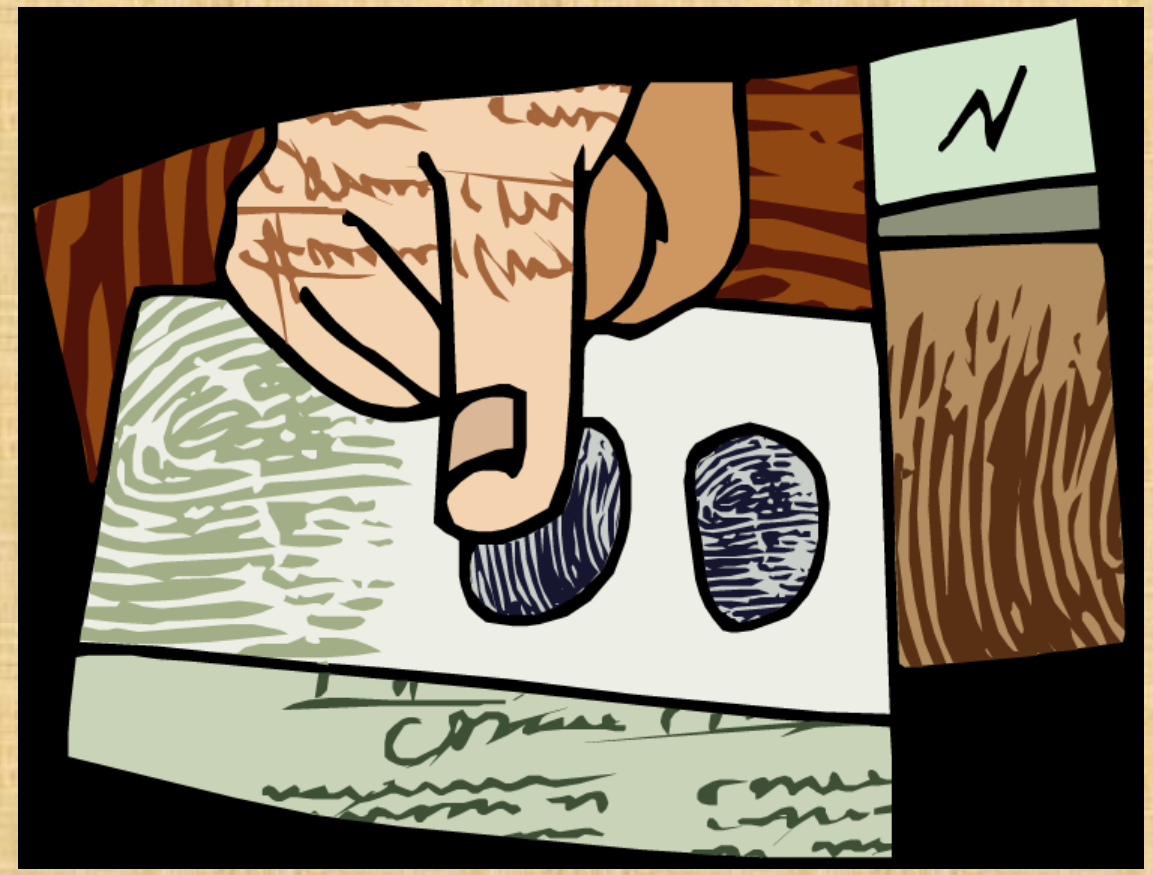
m/z	Dx	a	b	c	Formula	Comments
237	Dex	Butyl	-	-	$[C_{10}H_{21}O_6]^+$	butyl ether
325	Dex	Dex-H_20	-	-	$[C_{12}H_{21}O_{10}]^+$	Dimer – water
399	Dex	Dex	Butyl	-	$[C_{16}H_{31}O_{11}]^+$	Dimer + butyl ether
417	Dex	Dex	Butyl	-	$[C_{16}H_{33}O_{12}]^+$	237 + 180
473	Dex	Dex	Butyl	Butyl	$[C_{20}H_{41}O_{12}]^+$	2 x 237 – H^+



Butyl <u>ethers</u> with dextrose!

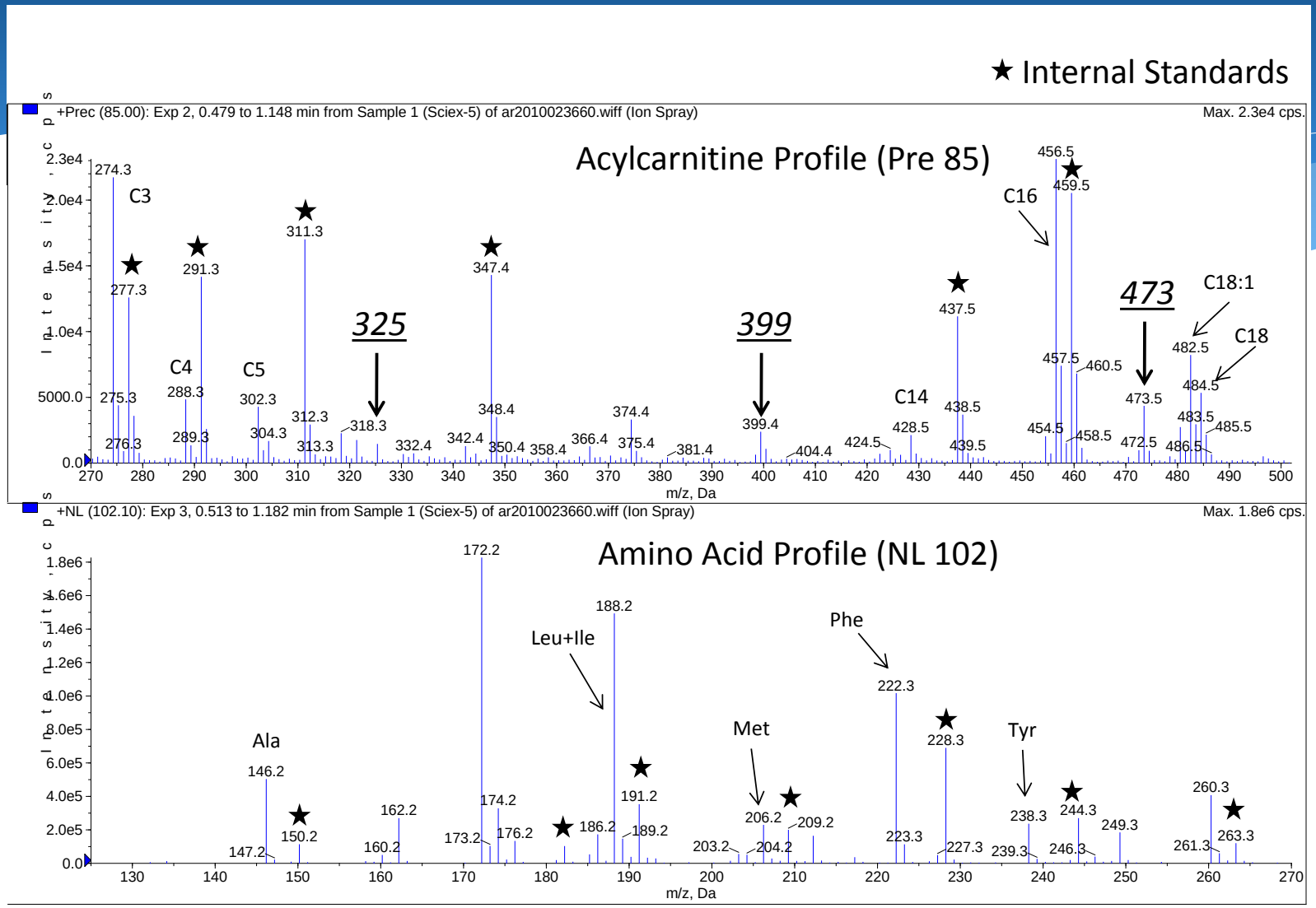
Monomers, dimers and trimers – Oh My!

DEXTROSE IDENTITY CONFIRMED

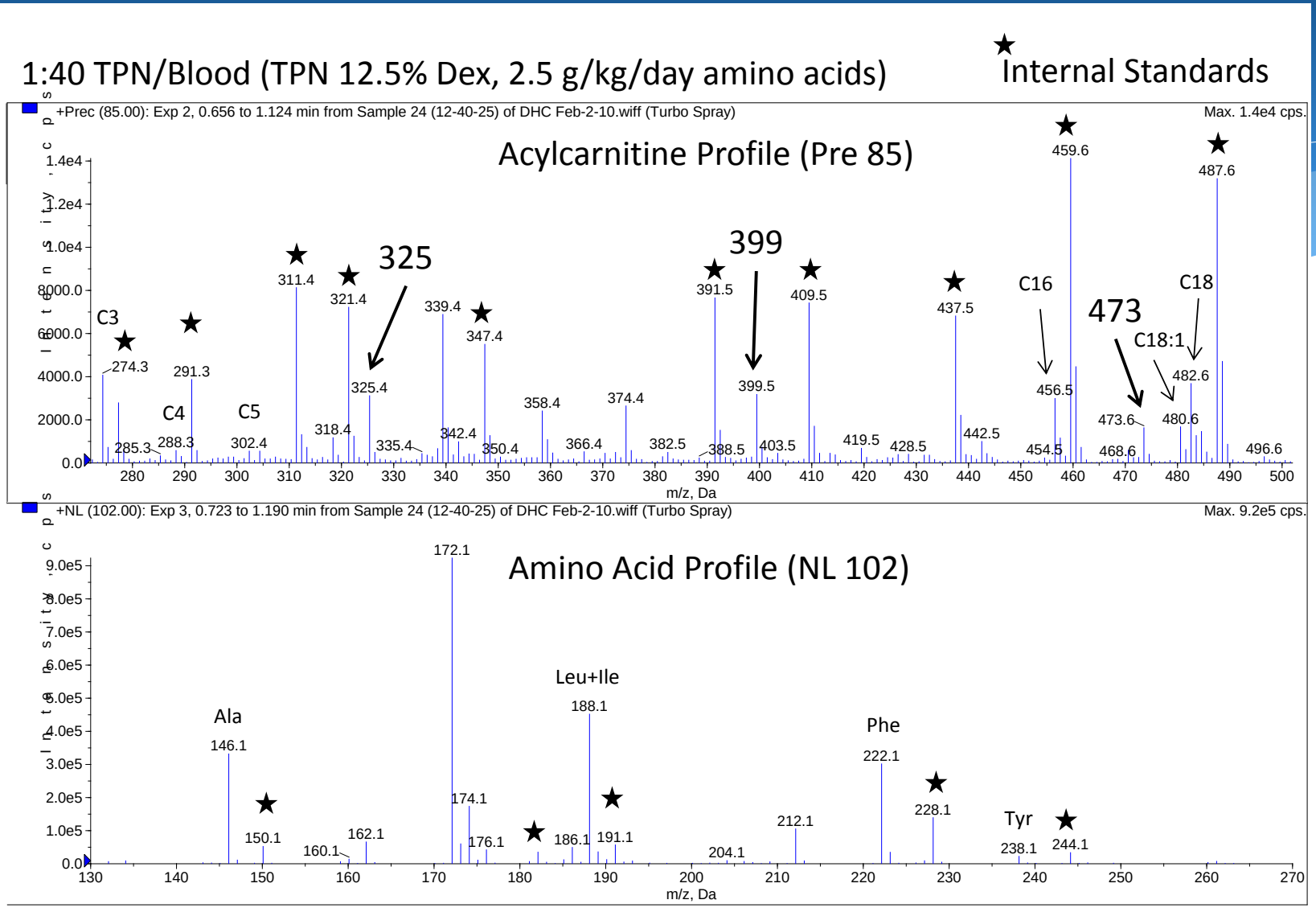


BUT WAS THE CRIME SCENE CONTAMINATED?

Classic High FP from a Preemie DBS



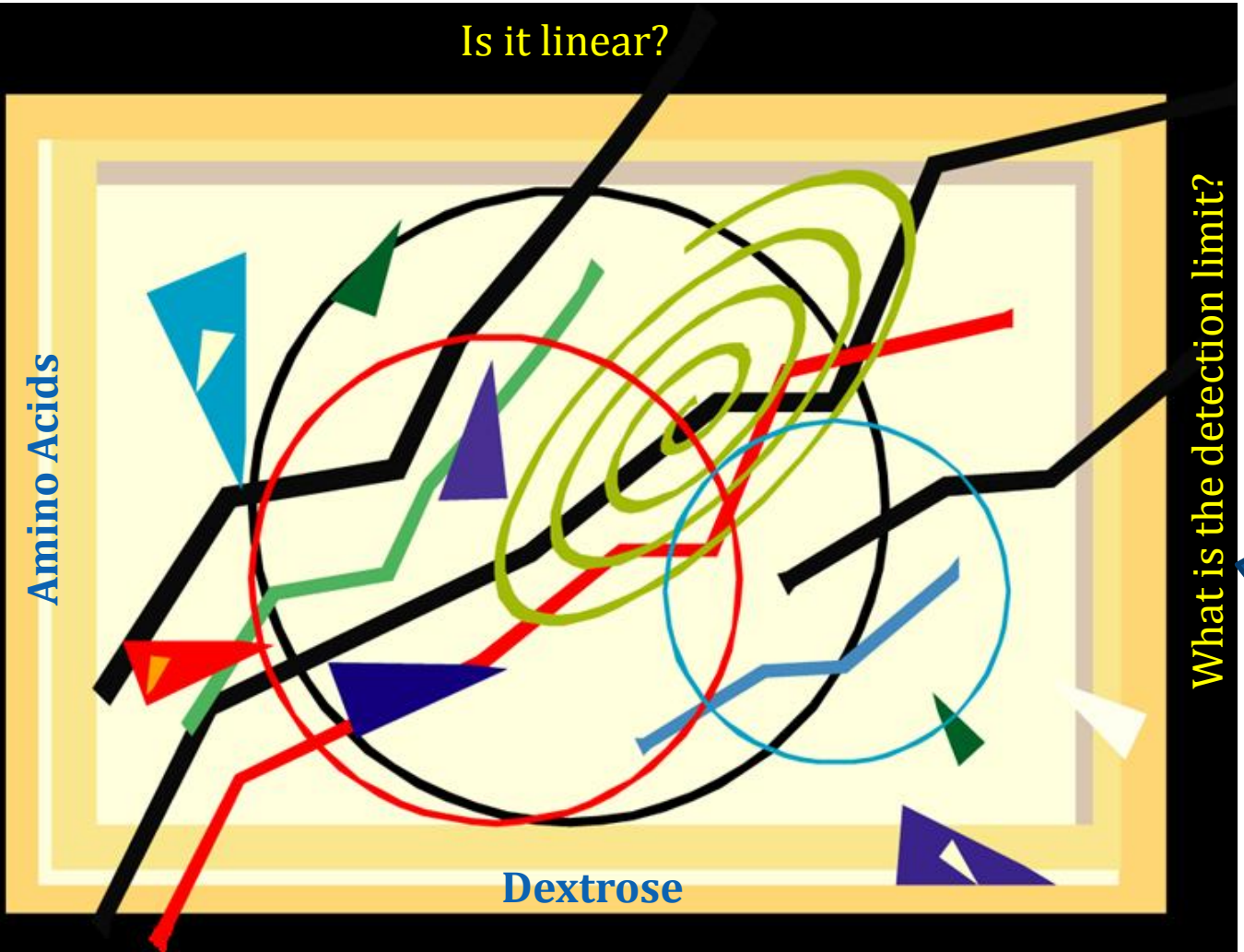
DBS spiked with TPN for Comparison



1:40 dilution of TPN solution in Blood before making DBS

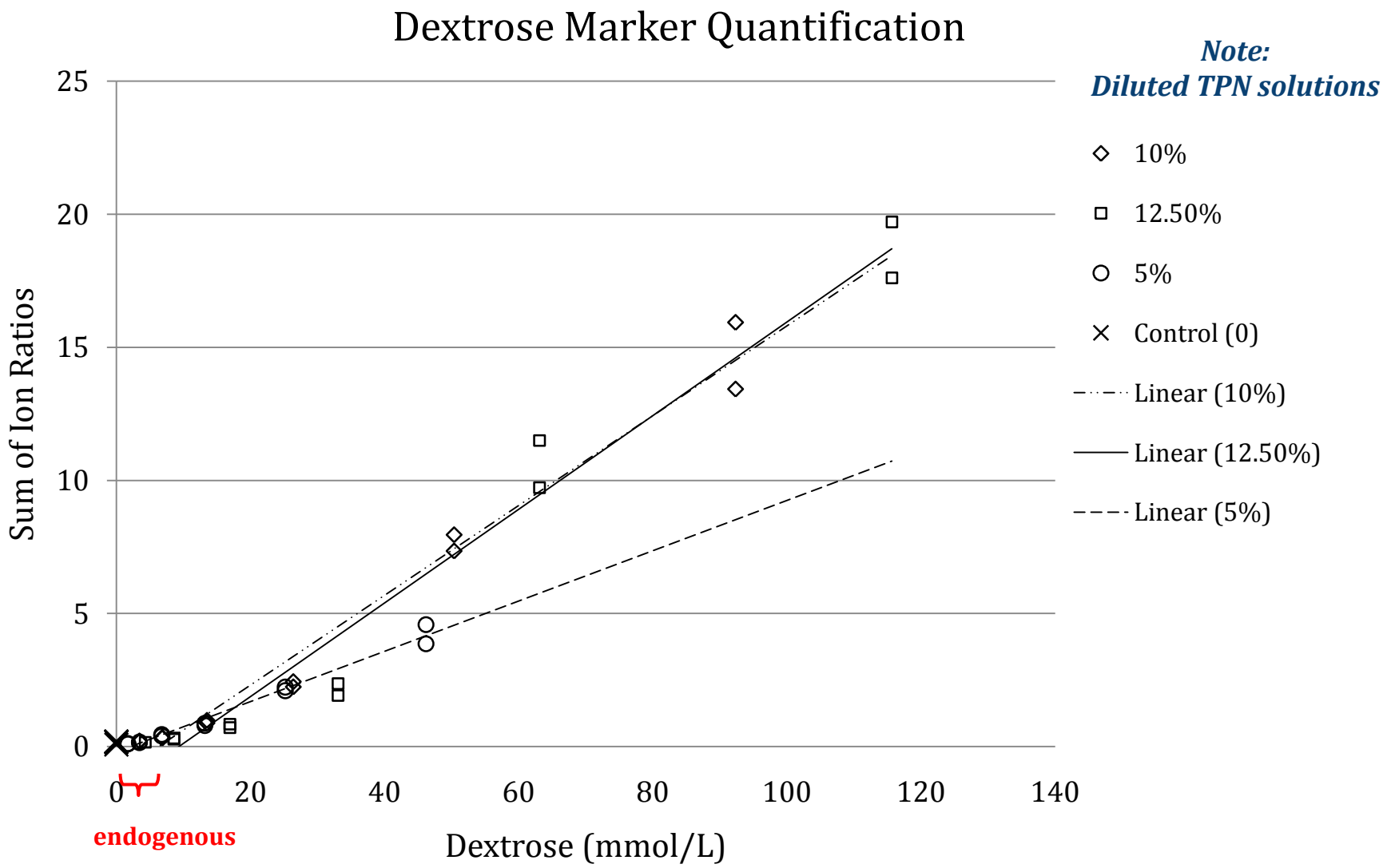
Dextrose Marker Quantification – Amino Acid Relationship

Can I use data in my interpretation?

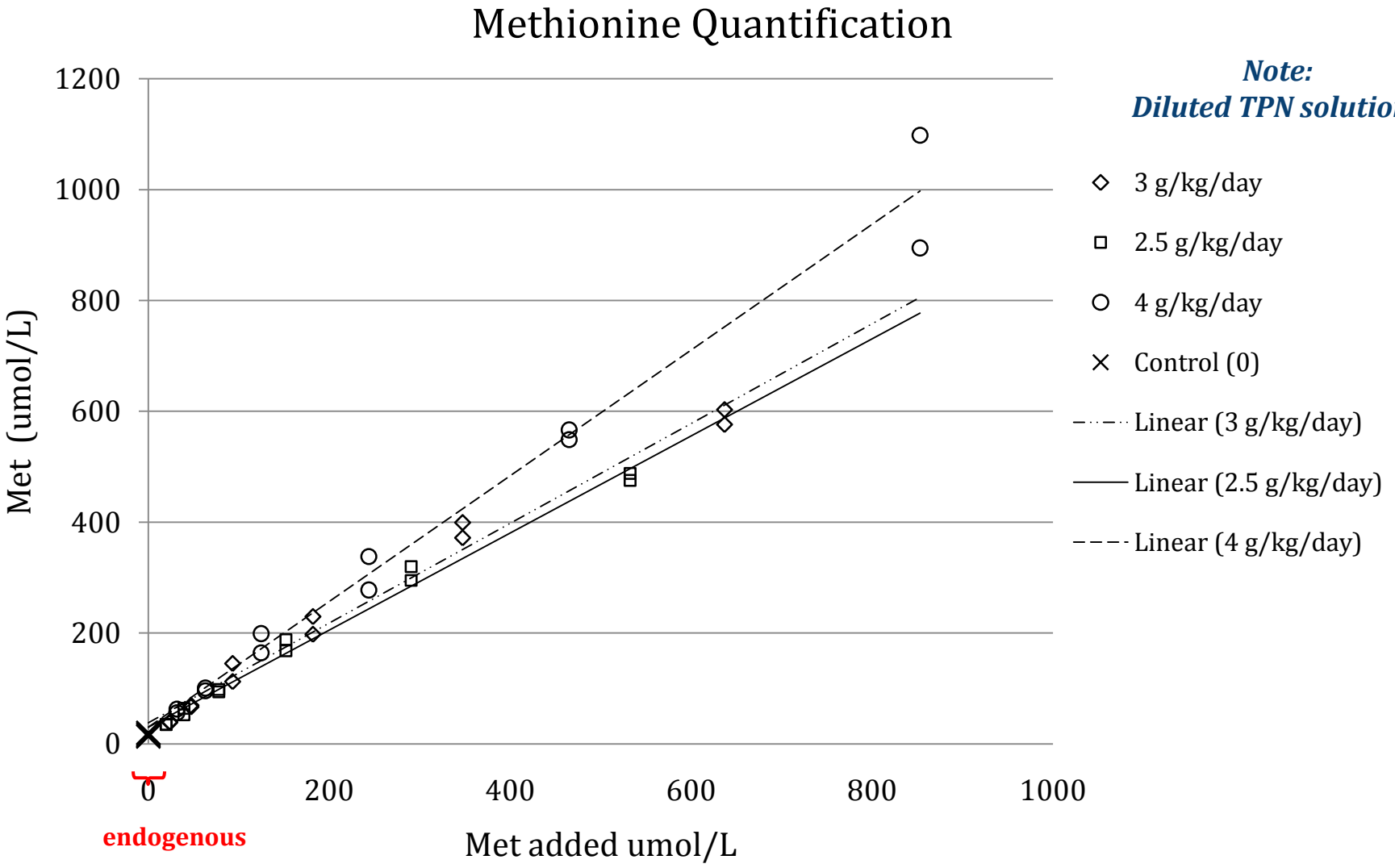


Is there a direct relationship between Dextrose and Amino Acids?

Dextrose Blood Concentration vs Sum of Dextrose Marker Pseudo-concentrations



Methionine blood Concentrations (a representative marker of amino acids)



A bit more data….

Table 4
Selected actual and estimated amino acid concentrations (μmol/l).

3.0 g/kg/day	Ala	Leu + Ile	Met	Phe	Tyr
Endogenous	330.59	136.44	18.78	47.6435	42.39
1:5 saline/endog	262.82	106.64	13.97	37.02	34.15
1:80	504.21	576.66	68.18	128.15	51.69
1:5	2119.49	4338.00	589.44	865.53	119.16
Estimated 1:5	1706.80	4829.77	636.95	829.09	367.35

Table 2
Regression analysis for dextrose marker ion ratios and dextrose enrichments.

Dex marker	Correlation coefficient	Slope	Intercept
325 (solutions A, B and C)	0.98	0.05	−0.20
399 (solutions A, B and C)	0.97	0.07	−0.39
473 (solutions A, B and C)	0.96	0.04	−0.39
DxSum (solutions A, B and C)	0.98	0.16	−0.98
DxSum (solution A)	0.98	0.18	−1.6
DxSum (solution B)	0.99	0.16	−0.57
DxSum (solution C)	0.99	0.09	−0.2

Note that a 1:80 contamination increases Leu+Ile above most cutoffs and Met and Phe are borderline. Tyr remains normal.

1:80 is a detectable dex marker concentration

Dextrose / Amino Acids correlate with calculated concentrations in TPN

Table 3
Regression analysis of added TPN and selected amino acids.

Amino acid	Correlation coefficient	Slope	Intercept
Ala	0.99	0.99	407
Leu + Ile	0.99	0.89	301
Met	0.98	1.0	21
Phe	0.98	1.1	54
Tyr	0.94	0.22	44

TPN's Signature Profile

* Molar Ratios
  * As the TPN contamination increases, the molar ratios of amino acids approach a signature TPN profile rather than a normal endogenous profile
  * Reducing FP for PKU via an elevated Phe/Tyr ratio does not work.
    * As published – a secondary ratio (Phe/Leu) is necessary to rule out PKU in preemies. These results confirm why.

Table 5
Amino acid molar ratios of selected blood pools.

3.0 g/kg	Phe/Tyr	Phe/Leu	Leu/Phe	Met/Phe	Leu/Ala
Endogenous	1.12	0.35	2.86	0.39	0.41
1:5 saline/endog	1.08	0.35	2.88	0.38	0.41
1:80	2.48	0.22	4.50	0.53	1.15
1:5	7.26	0.20	5.00	0.68	2.05

Approach for use in the NBS lab…

* Verify markers on your instrument…
  * Ionization efficiency of instruments vary thus the three markers may also vary in relative intensity
    * It is why the sum of the markers was used in the study
* An exact determination of the increase concentration of amino acids cannot be made based on the dextrose marker sum.
  * It can be approximated based on information of what was given to infant.
* Further study in ongoing in a 1000 premature infant clinical trial where the marker is measured at 5 different time points.

Conclusions

* Detection of dextrose markers together with elevated amino acids indicate that a DBS was contaminated by TPN solution.
  * Profile does not reflect infants metabolism
* Higher concentrations of TPN contamination more closely reflect the TPN solution.
* It is likely that dextrose markers may be more frequent in certain collection facilities/nurseries.
  * An opportunity to revisit collection procedures
* False positive rates reduced. Short term follow-up / repeat sample increased. Long term follow up /repeat sample reduced with improved collection.

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Tandem mass spectrometric identification of dextrose markers in dried-blood spots from infants receiving total parenteral nutrition

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Detection of TPN contamination of dried blood spots used in newborn and metabolic screening and its impact on quantitative measurement of amino acids

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Evidence Suggest
Markers are Present in

UNDERIVATIZED METHOD USING HYDRAZINE

Hydrazine derivatizes succinylacetone and…

New Markers at m/z 177,195,339,357

This crime (not doing butylesters) is under investigation!

PS – if you do butyl esterification and hydrazine you get even more markers! Just what we need in our profiles!

AMERICA's MOST Wanted …. *(Scientists at the CDC)*

Partners in ~~Crime~~ Research–

The ~~Line-Up~~ spot check