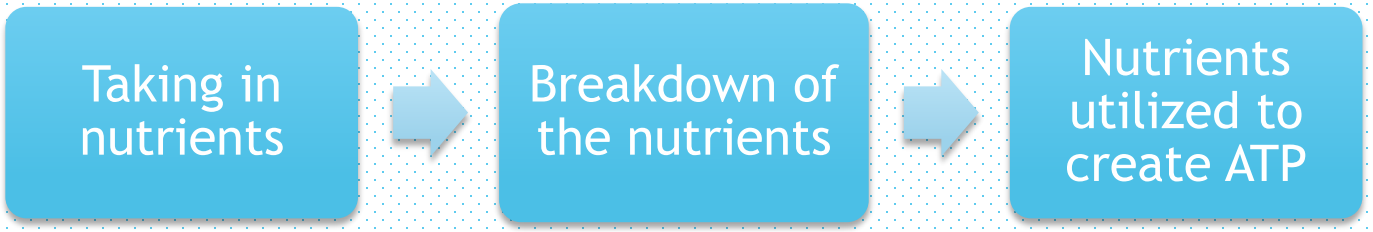
The Feasibility of Improving Positive Predictive Values of MCAD Deficiency Screening by Using Additional Acylcarnitine Markers

Jacob Voudren

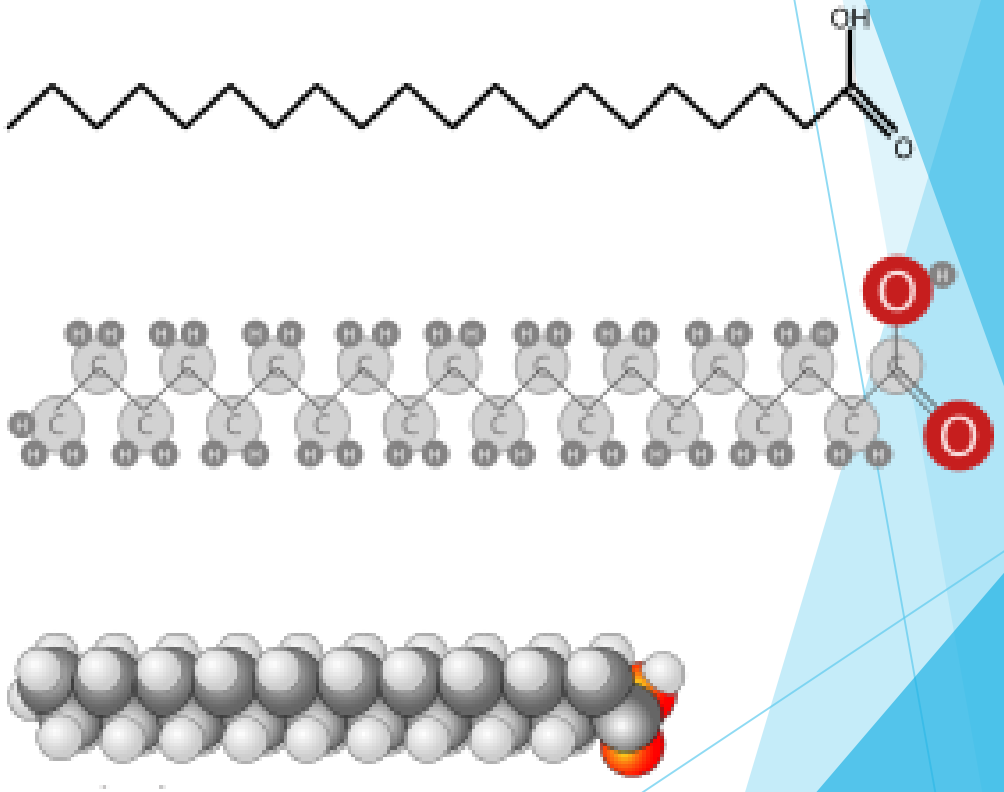
Introduction

- Metabolism
  - Series of chemical changes that result in creation of ATP (1)
    - ATP essential for (1)
      - Signal Transduction
      - Metabolism
      - Basic biochemical functions within cells



Introduction

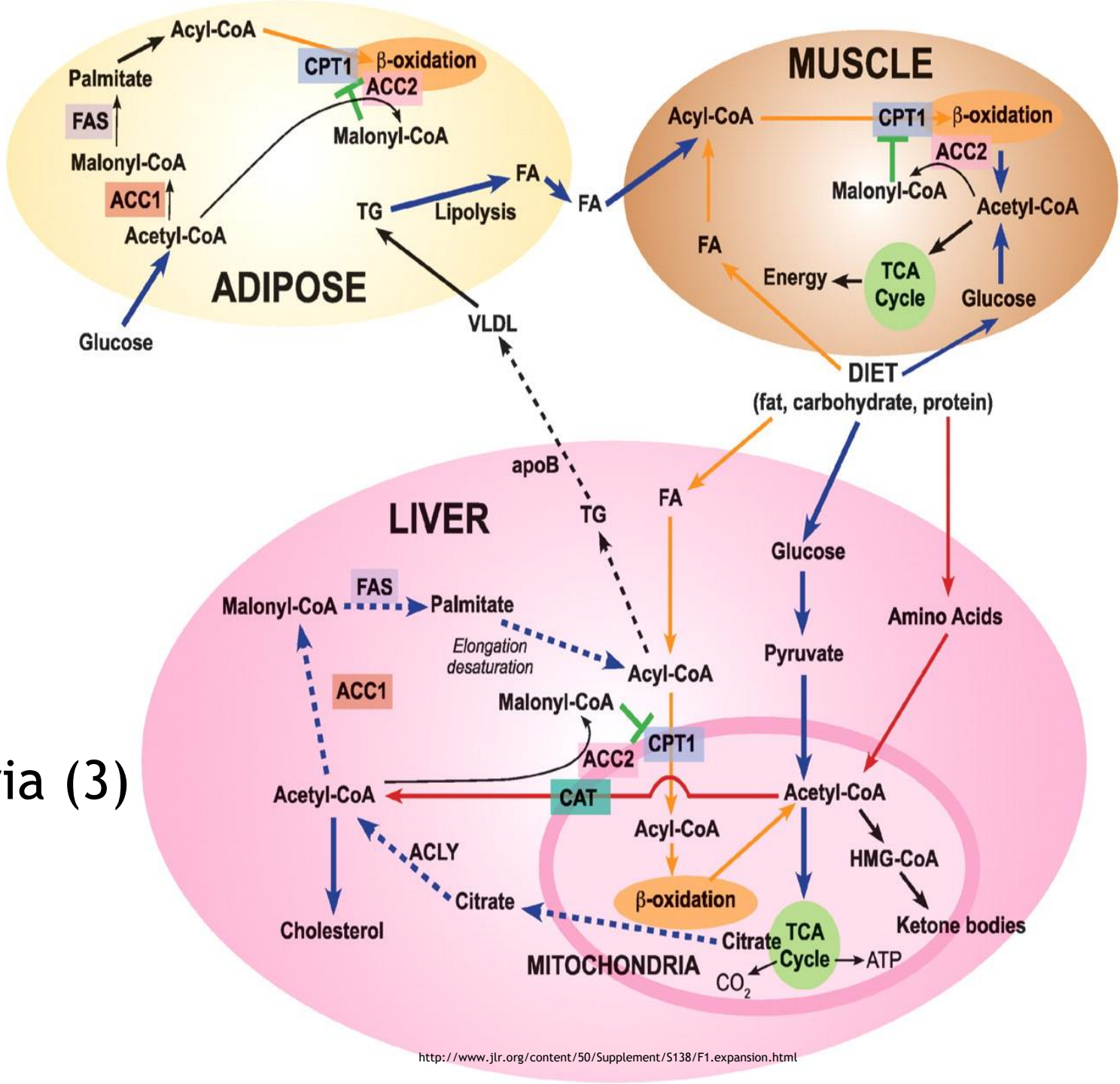
- Fatty Acids: most basic fat in the body(1)
  - Supports:
    - Brain Function & Growth
  - Controls:
    - Inflammation by providing the building blocks to create prostaglandins(1)



https://en.wikipedia.org/wiki/Fatty_acid

Introduction

- Metabolism of Fatty Acids (3)
  - Energy
  - Synthesizes new lipids
- Broken down in mitochondria (3)

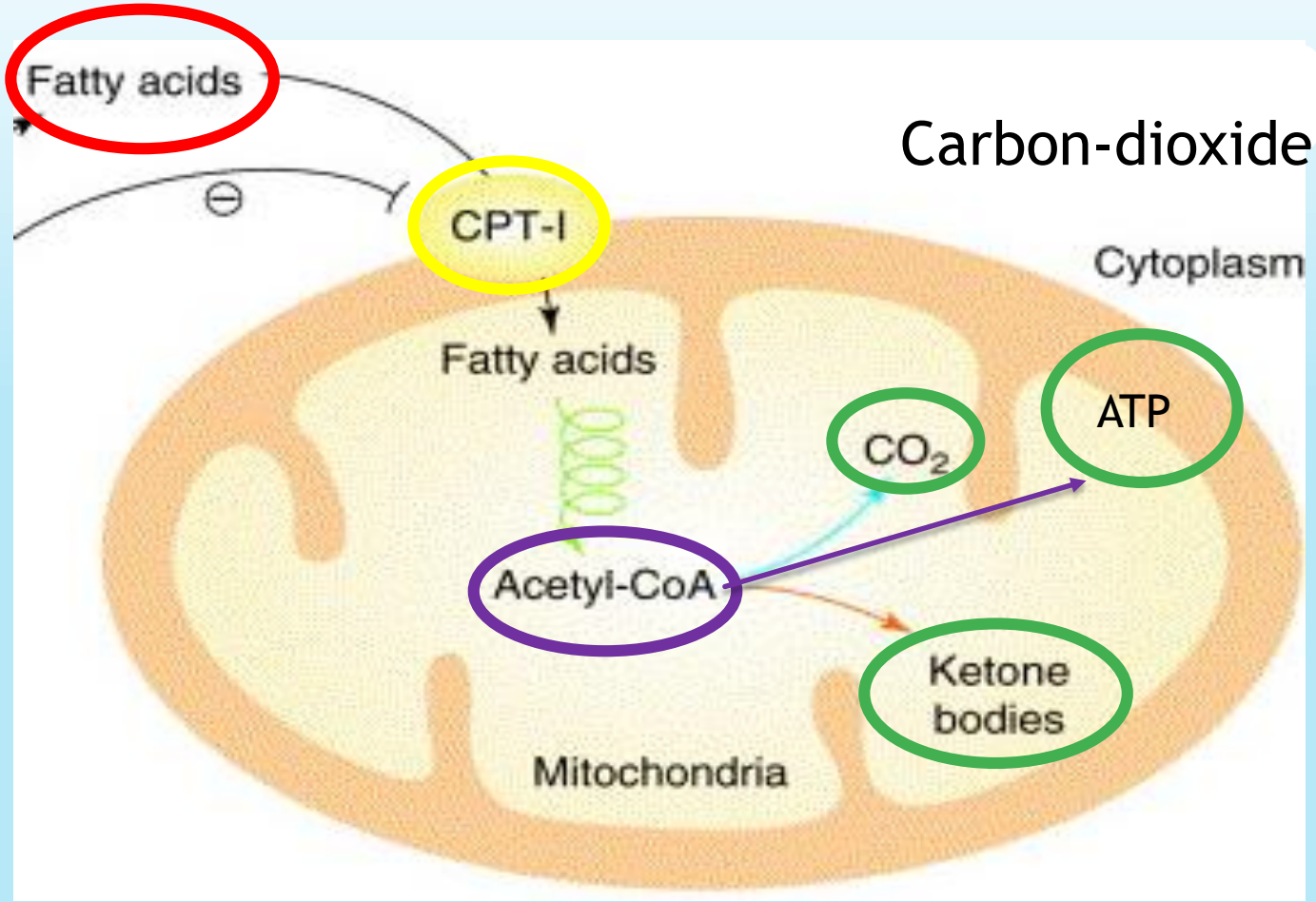


http://www.jlr.org/content/50/Supplement/S138/F1.expansion.html

Fatty Acid Oxidation

CPT-1 bring fatty-acids into mitochondria (3)

Enzyme Acetyl-CoA metabolizes fatty-acids (3)



Carbon-dioxide: expelled

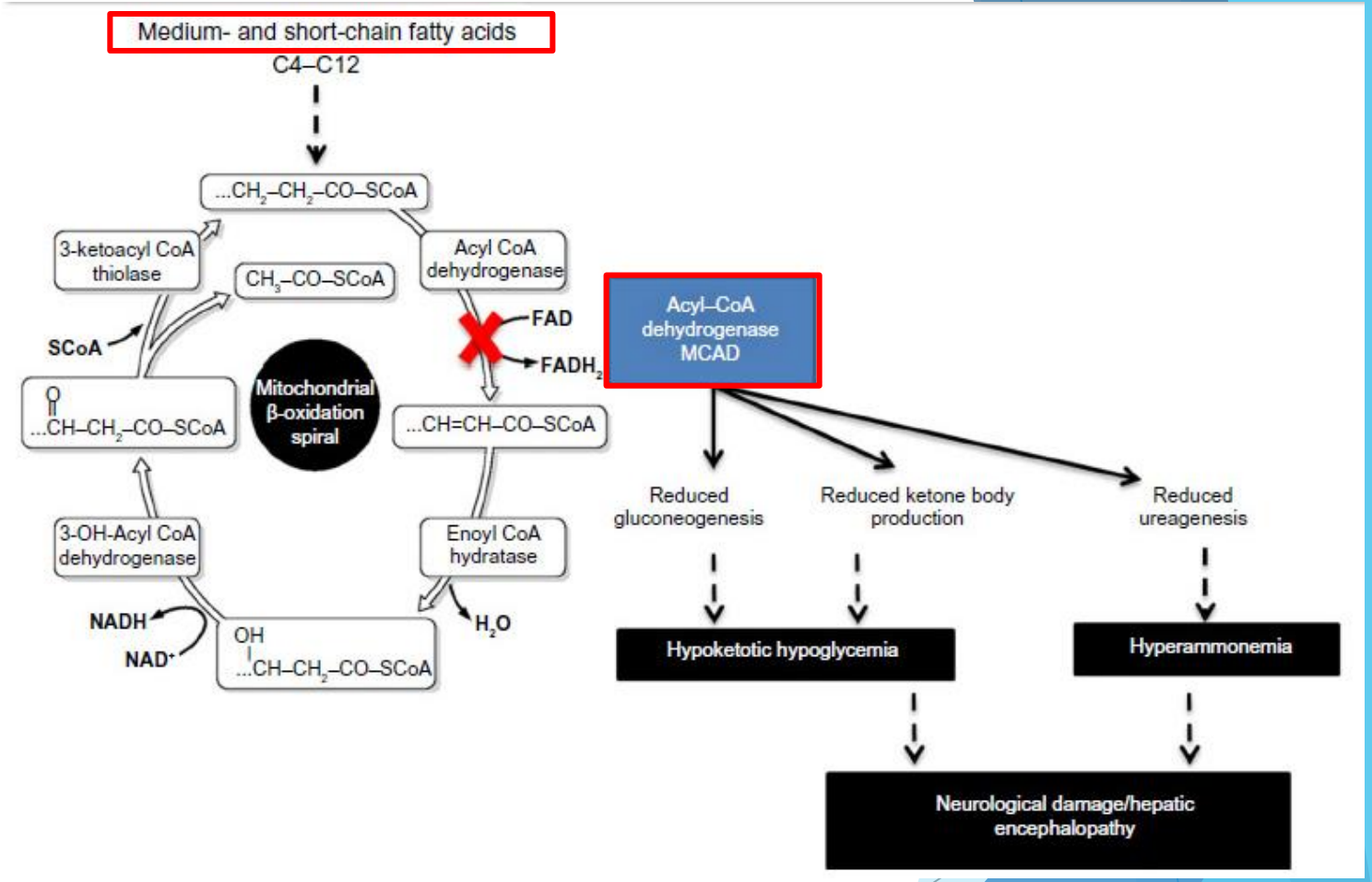
ATP: provides energy to heart and muscles

Ketones: used as energy sources when glucose is unavailable

Introduction

- **Fatty Acid Oxidation Disorders (FOD)**
  - Inherited metabolic disorders
    - **S**hort **C**hain **A**cetyl-CoA **D**ehydrogenase **D**eficiency (**SCADD**)
    - **M**edium **C**hain **A**cetyl-CoA **D**ehydrogenase **D**eficiency (**MCADD**)
    - **L**ong **C**hain 3 **H**ydroxyl **A**cyl-CoA **D**ehydrogenase **D**eficiency (**LCHADD**)
    - **V**ery **L**ong **C**hain **A**cetyl-CoA **D**ehydrogenase **D**eficiency (**VLCADD**)
    - **M**ultiple **A**cetyl-CoA **D**ehydrogenase **D**eficiencies (**MADD**)

Introduction



https://www.dovepress.com/cr_data/article_fulltext/s60000/60617/img/fig1small.jpg

Multiple **A**cetyl-CoA **D**ehydrogenase **D**eficiency

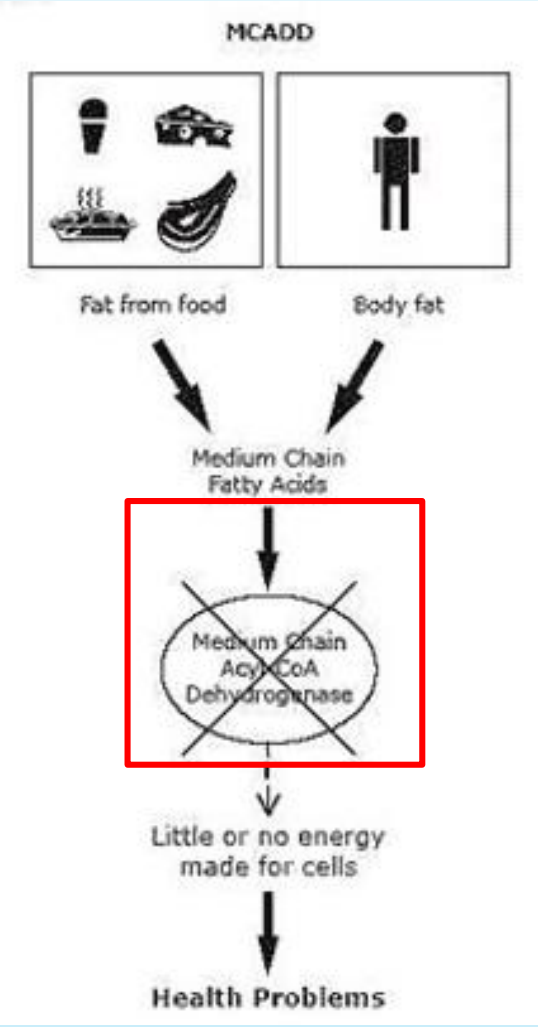
Lack of catalysts required to convert stored fat into energy (38)

Screened for by the newborn screening system (NBS)

Introduction

Medium Chain Acetyl-CoA Dehydrogenase Deficiency (MCADD)

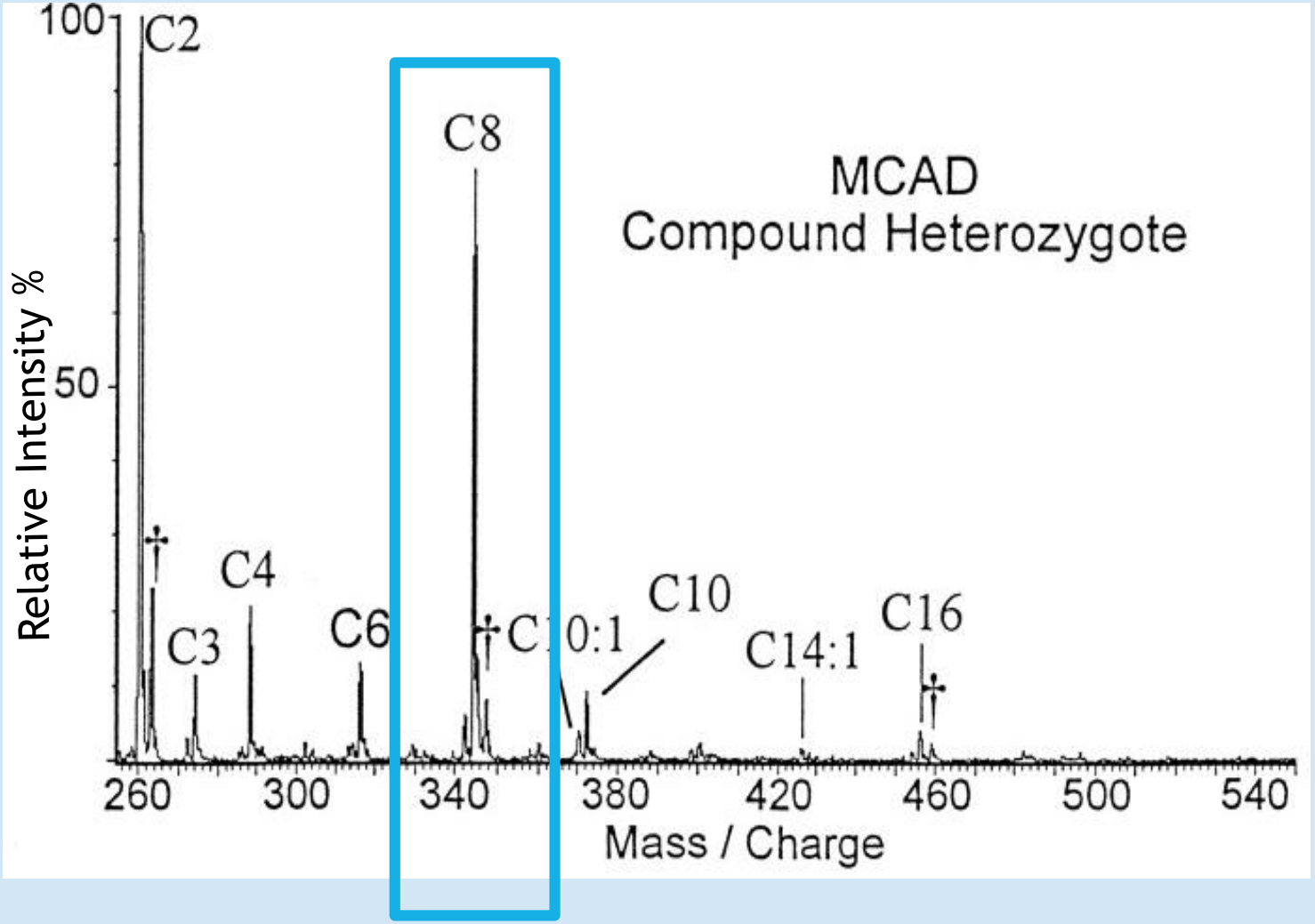
- Lack of catalyst required to break down medium chain fatty-acids
  - Effecting approximately 1 in 50,000 children (37)
- Screened for by the newborn screening system (NBS)



https://www.newbornscreening.info/Parents/fattyaciddisorders/MCADD.html

Introduction

- **Newborn Screening System (NBS)**
  - Series of diagnostic tests to **analyze inherited genetic disorders** of infants (35)
  - **MCADD** determined by **elevated C8** levels in the bloodstream



http://neoreviews.aappublications.org/content/6/12/e539.figures-only

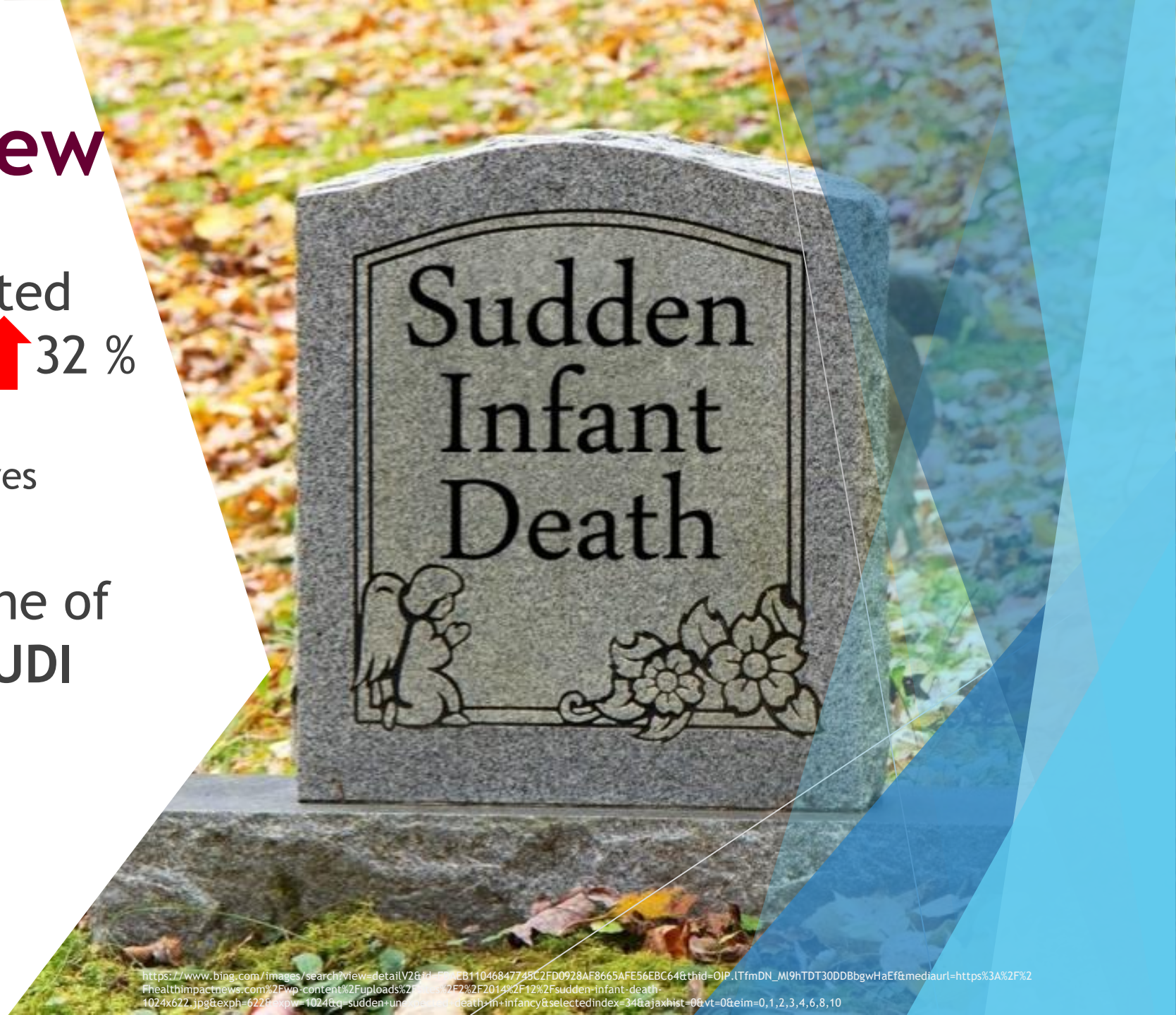
Literature Review

Rate of sudden unexpected death in infancy (SUDI) ↑ 32 % from 1990-2000

- Red Nose: Saving Little Lives

Confirmed **FODs** were one of the **genetic causes of SUDI**

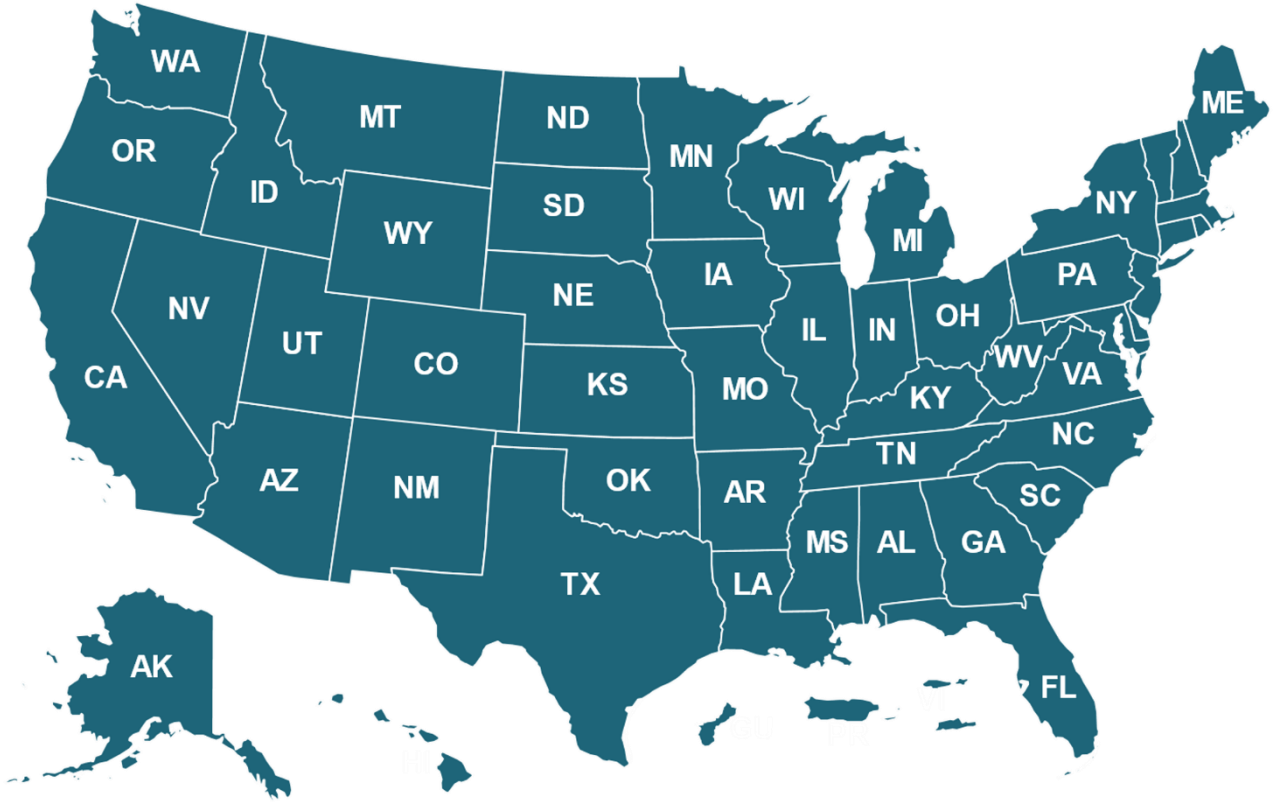
- Lovera et al. 2003



https://www.bing.com/images/search?view=detailV2&id=[illegible]EB1104684774[illegible]5C2FD0928AF8665AFE56EBC64&thid=OIP.lTfmDN_Ml9hTDT30DDBbgwHaEf&mediaurl=https%3A%2F%2Fhealthimpactnews.com%2Fwp-content%2Fuploads%2[illegible]%2F2%2F2014%2F12%2Fsudden-infant-death-1024x622.jpg&exph=622&expw=1024&q=sudden+unexpected+death+in+infancy&selectedindex=34&ajaxhist=0&vt=0&eim=0,1,2,3,4,6,8,10

Literature Review

- 2007 Congress enacted the Newborn Screening Saves Lives Act
  - Promote NBS Education
  - Funded various state's NBS
  - Mandated all 50 states

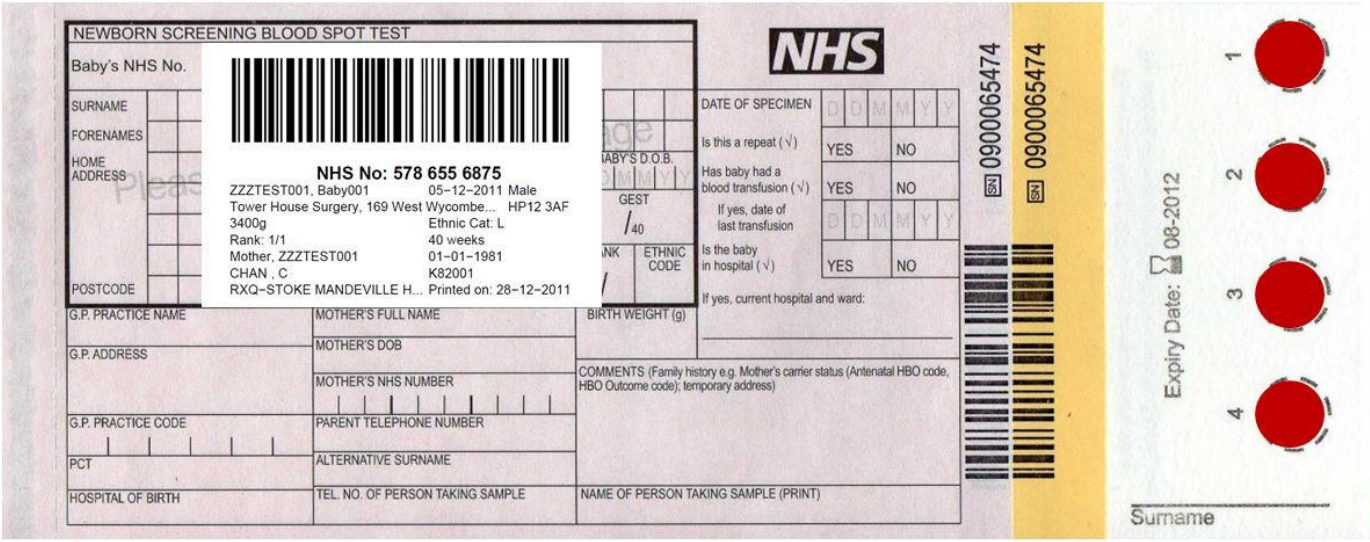


https://www.babysfirsttest.org/newborn-screening/rusp-conditions#

Literature Review

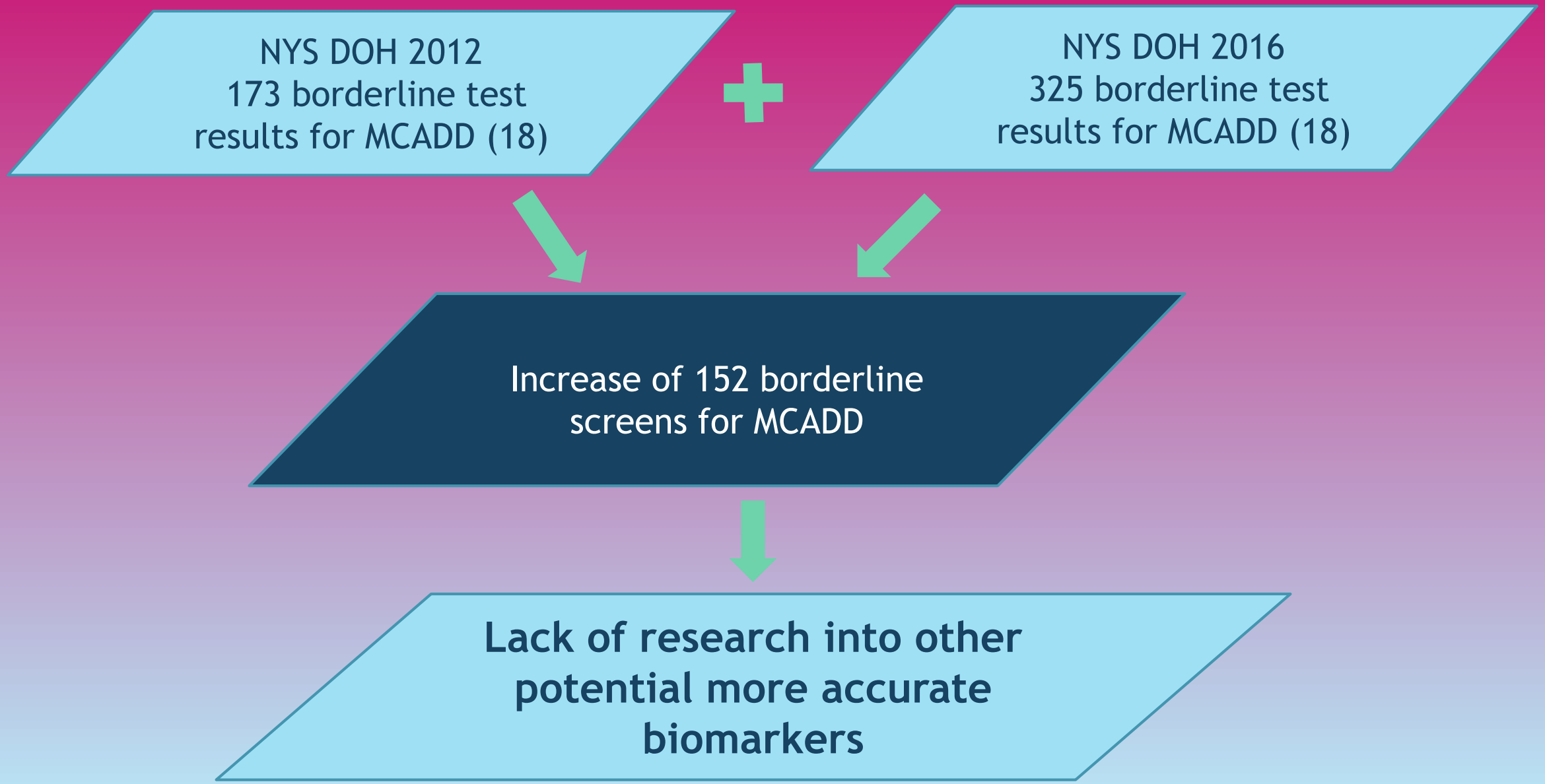
Tarini et al. 2011

- Gestation age ***did impact*** newborn screening results (24)
- Neonatal screening reduces incidences of metabolic crisis and death (17,18)



https://www.newswire.com/files/x/02/dd/57a11689afaa254fc31700876290.jpg

Gap in the Research

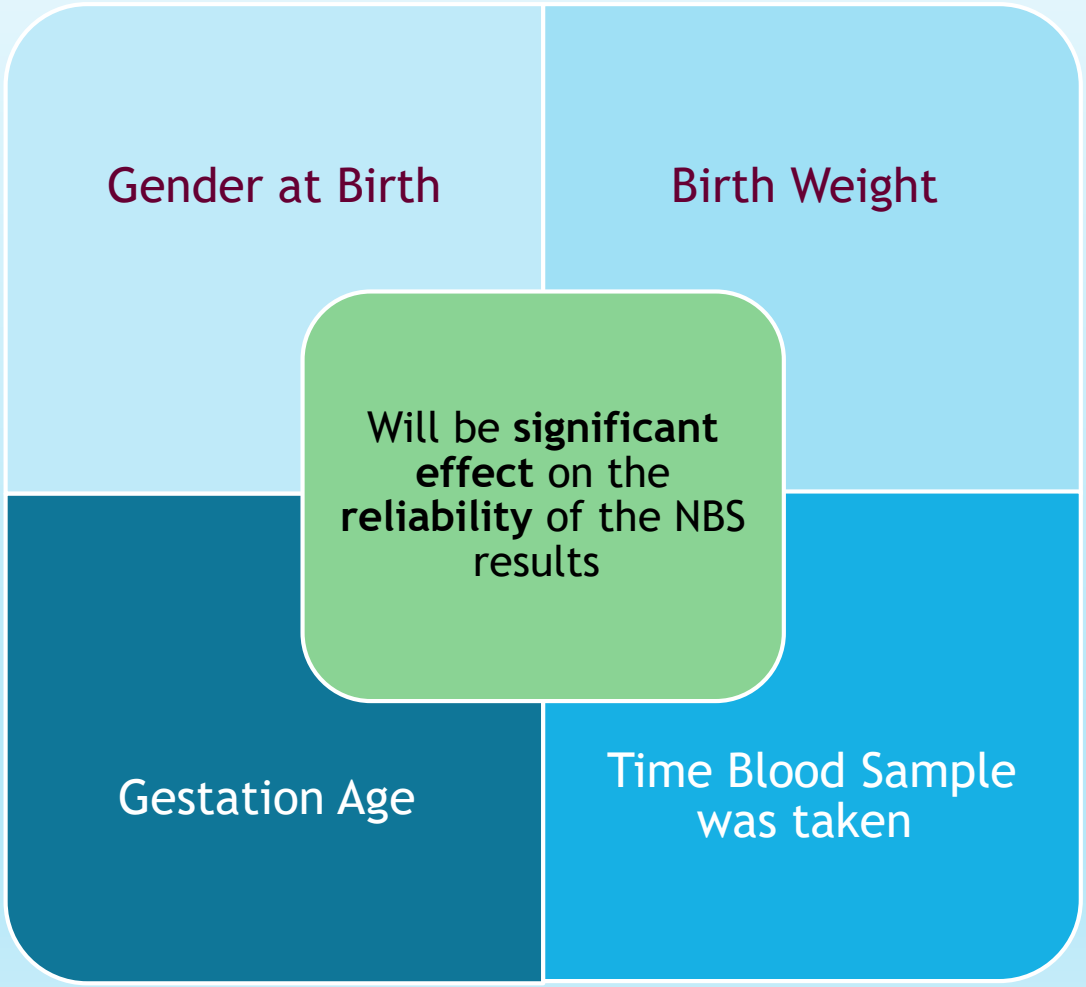


Goals of Research

Identify potential **secondary acylcarnitine markers** and ratios to **improve** the **NBS** for **MCAD deficiency**

Improve the **reliability** of the **NBS biomarkers** for **MCAD deficiency**

Hypotheses



Significant **correlation** ***between*** screening results for biomarkers ***C14*** and ***C16*** in infants with ***MCADD and MADD***

Methodology

Heel prick test sent to lab within 48 hrs after birth

https://www.medgadget.com/2018/11/global-newborn-screening-lc-ms-reagent-kit-revenue-is-nearly-212-m-usd.html

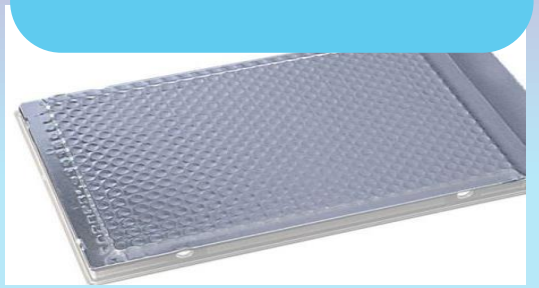
Desired samples were hole punched to fit into well-plates

https://guide.alibaba.com/shopping-guides/hand-held-metal-hole-punch.html

https://www.perkinelmergenomics.com/india/healthcare-providers/newborn-health/basic-screening/index.html

200 µL of Internal Standard were added

Foil was placed over the samples



Sample were incubated at 60°C for 45 minutes

Methodology

40 µL is extracted and placed into a new well plate

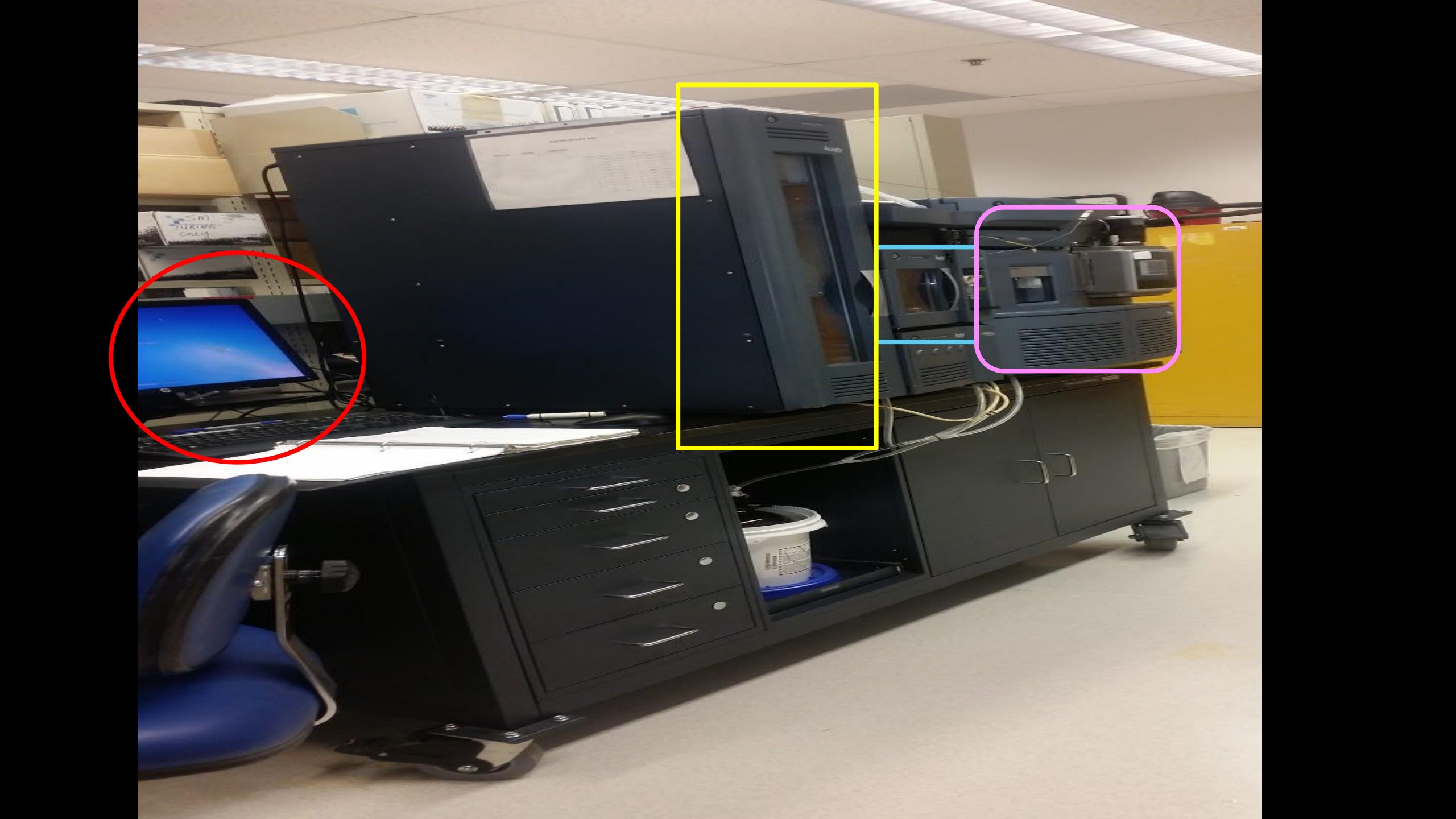
75 µL of butanolic HCl were added to the sample

200 µL of Internal Standard

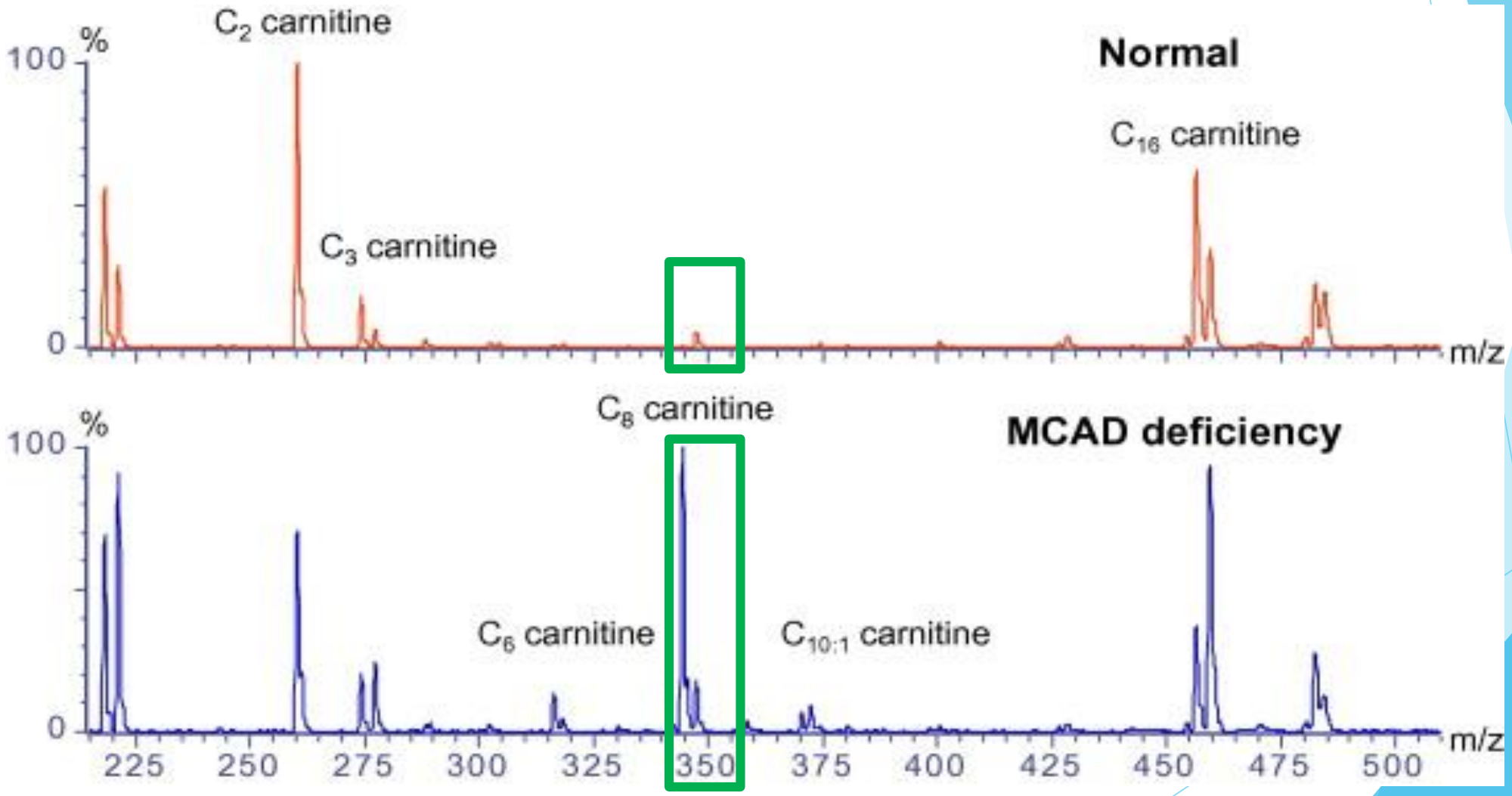
Incubated for 20 minutes at 60°C

200 µL of reconstitution solution

Foil was then placed on the well-plate



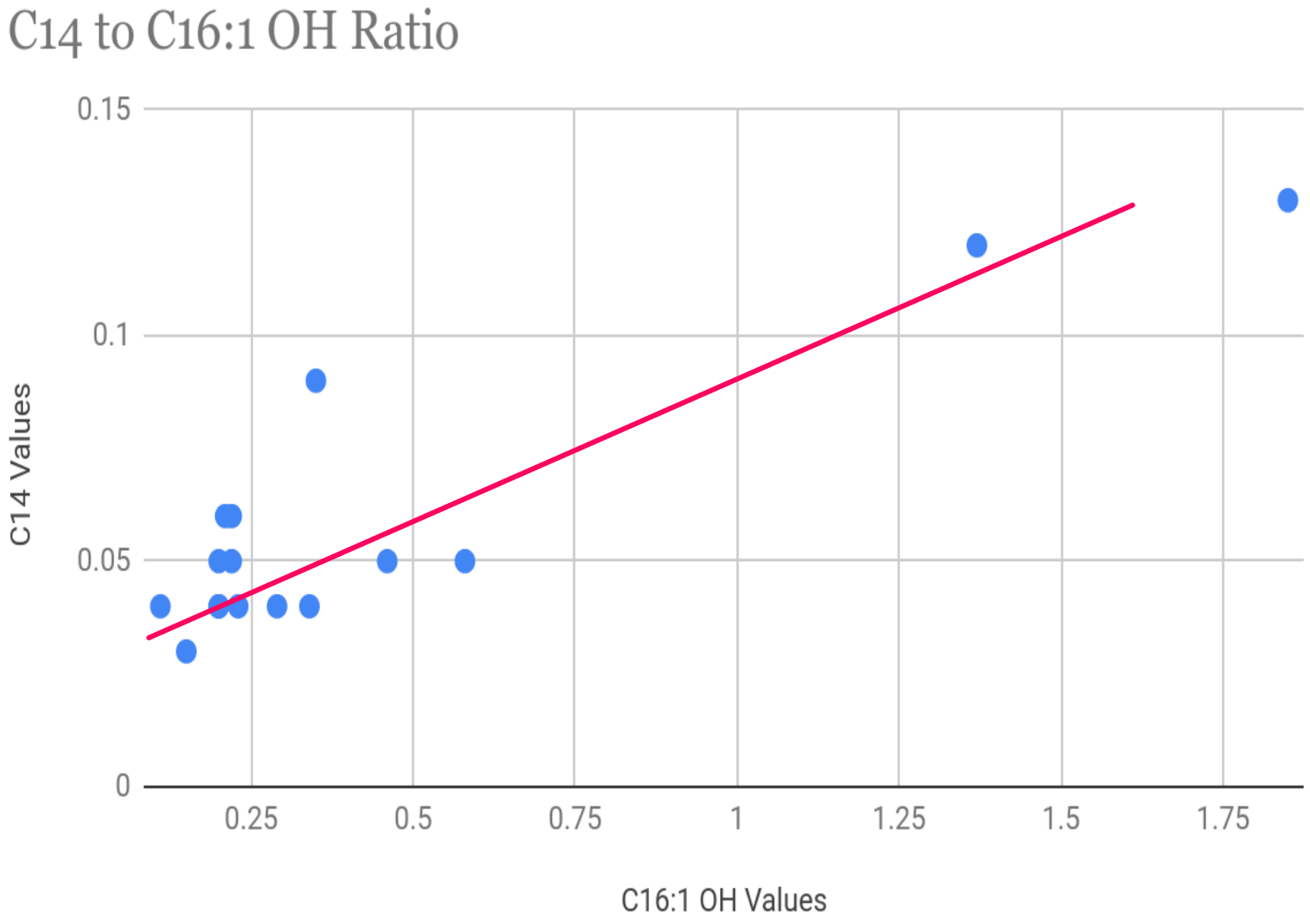
Methodology



MAYO CLINIC

Collaborative Laboratory Integrated Reports

Results



C14 and C16:1 OH levels

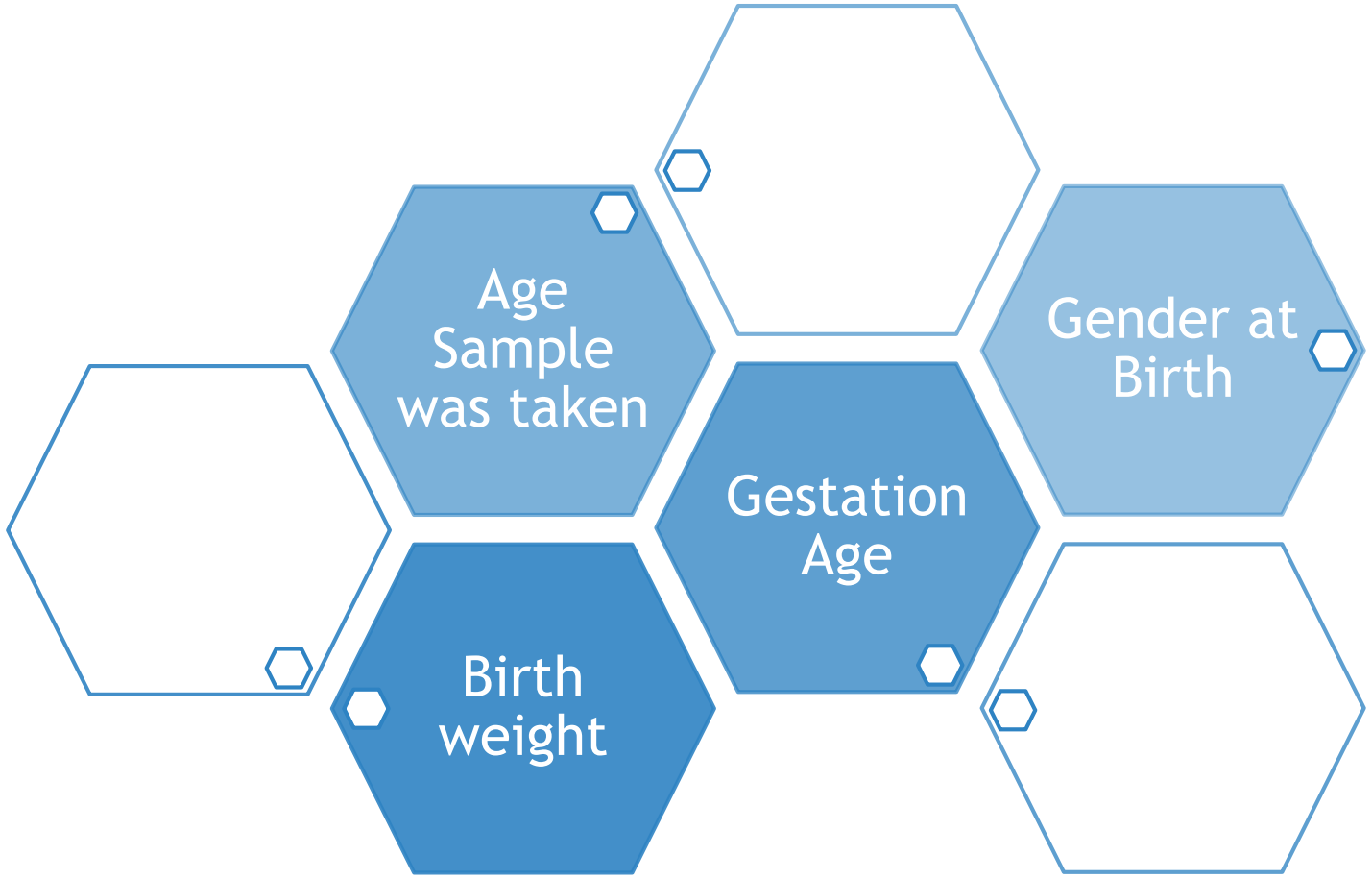
- Positive correlation 0.887

C14/C16:1 OH ratio & C8

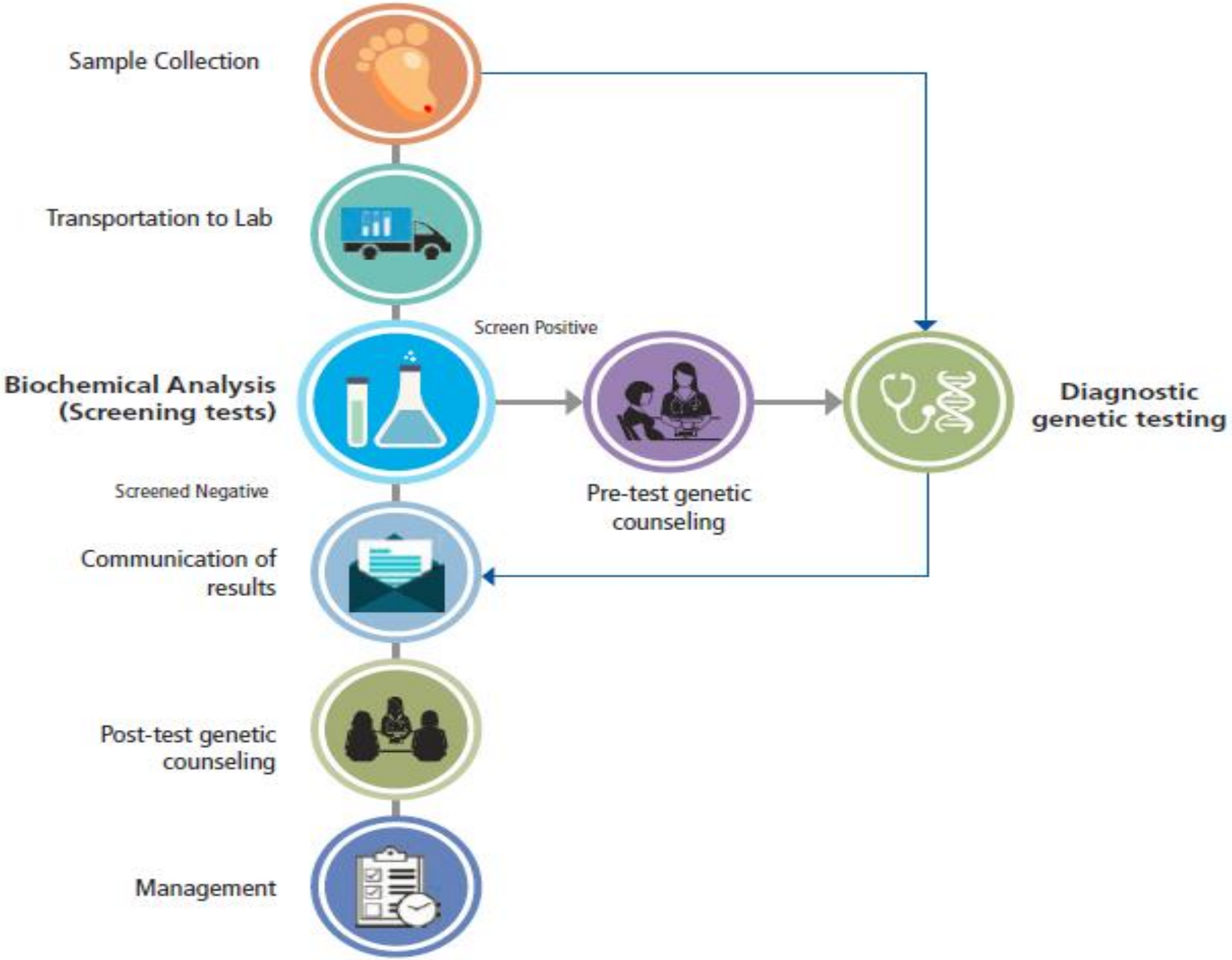
- Statistically significant negative correlation

Results

No significant correlation involving levels of acylcarnitine blood profile

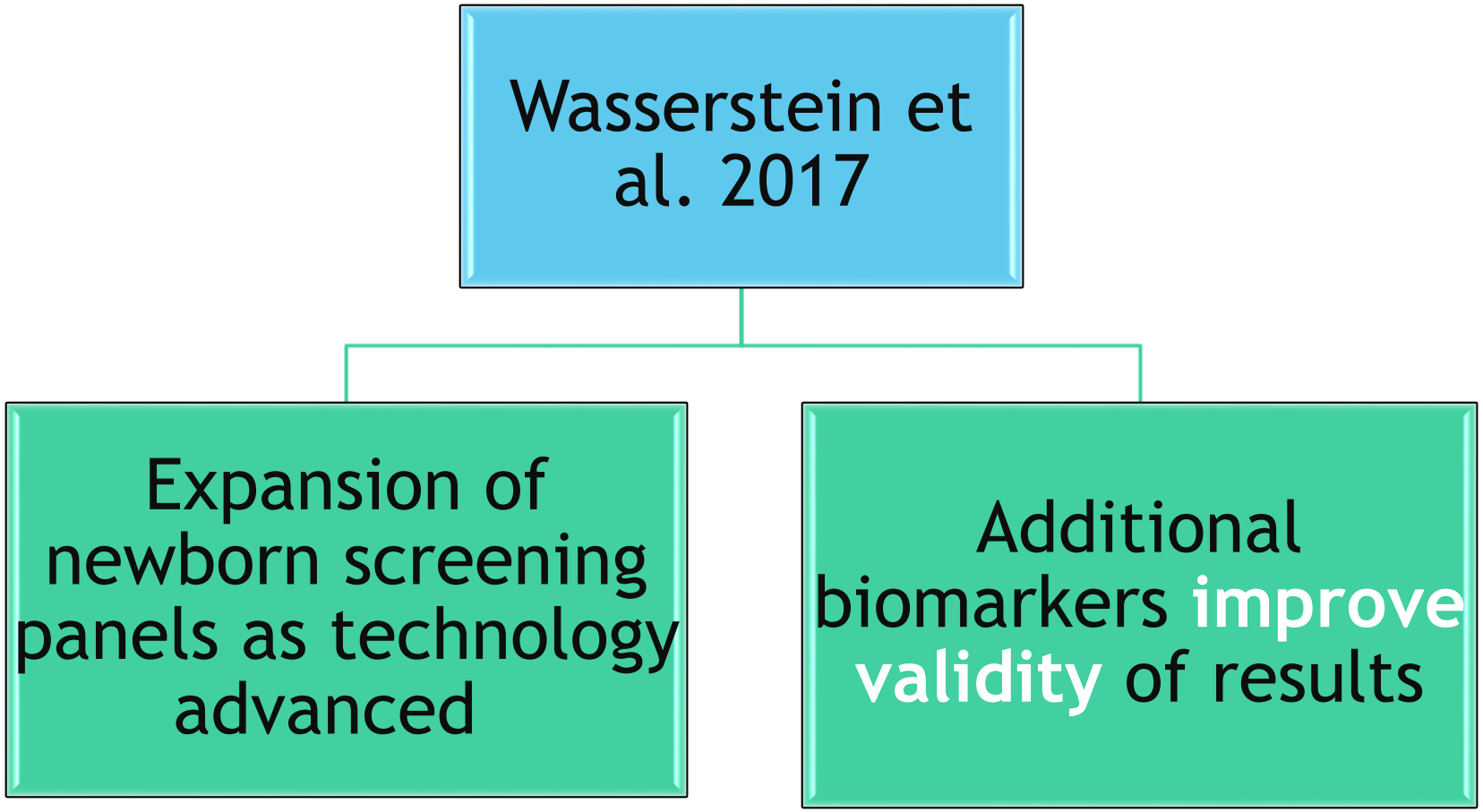


Discussion



https://www.perkinelmergenomics.com/india/healthcare-providers/newborn-health/basic-screening/index.html

Discussion


Wasserstein et al. 2017
Expansion of newborn screening panels as technology advanced
Additional biomarkers improve validity of results

Discussion

Lack of correlation between

- Gestation age
- Time of sample taken
- Gender
- Birth weight

Results of NBS are independent of these Factors

INCREASES **VALIDITY**

https://jaxmecfs.com/2018/05/30/ny-state-department-of-health-new-webpage-about-me-cfs/

Discussion

Correlation of C14 & C16:1 OH

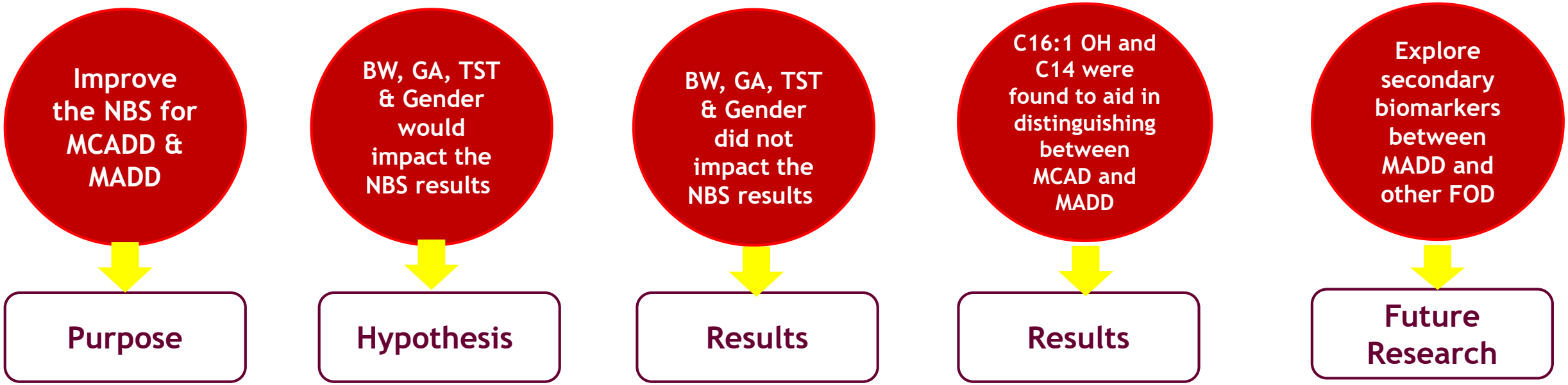
- Improves screening for MCADD & MADD
- Decreases the SUDI population

Limitations

- Sample degradation
- Sample size

https://jaxmecfs.com/2018/05/30/ny-state-department-of-health-new-webpage-about-me-cfs/

Conclusion


Improve the NBS for MCADD & MADD
Purpose
BW, GA, TST & Gender would impact the NBS results
Hypothesis
BW, GA, TST & Gender did not impact the NBS results
Results
C16:1 OH and C14 were found to aid in distinguishing between MCAD and MADD
Results
Explore secondary biomarkers between MADD and other FOD
Future Research

Acknowledgements

- My Little Brother Noah
- Dr. Mark Morrissey
- Ms. Gillian Rinaldo
- My Parents & Grandma
- My Science Research Peers

References

1. Matsubara, Y., Narisawa, K., Tada, K., Ikeda, H., Yao, Y. Q., Danks, D. M., . . . McCabe, E. R. (1991, August 31). Prevalence of K329E mutation in medium-chain acyl-CoA dehydrogenase gene determined from Guthrie cards. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1678810
2. Figure 2f from: Irimia R, Gottschling M (2016) Taxonomic revision of Rochefortia Sw. (Ehretiaceae, Boraginales). Biodiversity Data Journal 4: E7720. https://doi.org/10.3897/BDJ.4.e7720. (n.d.). doi: 10.3897/bdj.4.e7720.figure2f
3. Houten, S.M.; Violante, S.; Ventura, F.V.; Wanders, R.J. The Biochemistry and Physiology of Mitochondrial Fatty Acid Beta-Oxidation and Its Genetic Disorders. Annu. Rev. Physiol. 2016, 78, 23–44. [CrossRef] [PubMed]
4. Banta-Wright SA and Steiner RD. Tandem Mass Spectrometry in Newborn Screening A Primer for Neonatal and Perinatal Nurses. J Perinat Neonat Nurs 2004; 18(1): 41-5
5. Chace Dh, Kalas TA, Naylor EW. The application of tandem mass spectrometry to neonatal screening for inherited disorders of intermediary metabolism. Annual Rev Genomics Hum Genet 2002; 3:17-45
6. McHugh DMS, Cameron CA, Abdenu JE, et. al. Clinical validation of cutoff target ranges in newborn screening of metabolic disorders by tandem mass spectrometry: A worldwide collaborative project. Genetics in Medicine 2011; 13(3): 230-254.
7. Marquardt G, Robert Currier R, McHugh DMS, et. al. Enhanced interpretation of newborn screening results without analyte cutoff values. Genetics in Medicine 2012; 14(7): 648-645
8. Newborn Screening Program. (2015, November 27). Retrieved from https://www.wadsworth.org/programs/newborn/screening
9. Van Hove, D. (1993). Figure 2f from: Irimia R, Gottschling M (2016) Taxonomic revision of Rochefortia Sw. (Ehretiaceae, Boraginales). Biodiversity Data Journal 4: E7720. https://doi.org/10.3897/BDJ.4.e7720. *Medium-chain Acyl-CoA Dehydrogenase (MCAD) Deficiency: Diagnosis by Acylcarnitine Analysis in Blood.* doi: 10.3897/bdj.4.e7720.figure2f
10. W. (2008, September 27). Retrieved February 13, 2018, from https://www.youtube.com/watch?v=J-wao0O0_qM
11. Vishwanath, V. A. (2016, March). Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4934411/
12. Yokota, I., Coates, P. M., Hale, D. E., Rinaldo, P., & Tanaka, K. (1991, December). Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1686456/
13. Nsiah-Sefaa, A.; McKenzie, M. Combined defects in oxidative phosphorylation and fatty acid beta-oxidation in mitochondrial disease. Biosci. Rep. 2016, 36, e00313. [CrossRef] [PubMed]
14. Smeitink, J.; van den Heuvel, L.; DiMauro, S. The genetics and pathology of oxidative phosphorylation. Nat. Rev. Genet. 2001, 2, 342–352. [CrossRef] [PubMed]
15. Wajner, M.; Amaral, A.U. Mitochondrial dysfunction in fatty acid oxidation disorders: Insights from human and animal studies. Biosci. Rep. 2016, 36, e00281. [CrossRef] [PubMed]

References

16. Bartlett, K.; Eaton, S. Mitochondrial beta-oxidation. Eur. J. Biochem. 2004, 271, 462-469. [CrossRef] [PubMed]
17. DiMauro, S.; DiMauro, P.M. Muscle carnitine palmitoyltransferase deficiency and myoglobinuria. Science 1973,182, 929-931. [CrossRef] [PubMed]
18. Karpati, G.; Carpenter, S.; Engel, A.G.; Watters, G.; Allen, J.; Rothman, S.; Klassen, G.; Mamer, O.A. The syndrome of systemic carnitine deficiency. Clinical, morphologic, biochemical, and pathophysiologic features. Neurology 1975,25, 16-24. [CrossRef] [PubMed]
19. Gregersen, N.; Lauritzen, R.; Rasmussen, K. Suberylglycine excretion in the urine from a patient with dicarboxylic aciduria. Clin. Chim. Acta 1976,70, 417-425. [CrossRef]
20. Kelly, D.P.; Whelan, A.J.; Ogden, M.L.; Alpers, R.; Zhang, Z.F.; Bellus, G.; Gregersen, N.; Dorland, L.; Strauss, A.W. Molecular characterization of inherited medium-chain acyl-CoA dehydrogenase deficiency. Proc. Natl. Acad. Sci. USA 1990,87, 9236-9240. [CrossRef] [PubMed]
21. Yokota, I.; Indo, Y.; Coates, P.M.; Tanaka, K. Molecular basis of medium chain acyl-coenzyme A dehydrogenase deficiency. An A to G transition at position 985 that causes a lysine-304 to glutamate substitution in the mature protein is the single prevalent mutation. J. Clin. Investig. 1990,86, 1000-1003. [CrossRef] [PubMed]
22. Matsubara, Y.; Narisawa, K.; Miyabayashi, S.; Tada, K.; Coates, P.M. Molecular lesion in patients with medium-chain acyl-CoA dehydrogenase deficiency. Lancet 1990,335, 1589. [CrossRef]
23. The citric acid cycles. Retrieved from https://www.khanacademy.org/test-prep/mcat/biomolecules/krebs-citric-acid-cycle-and-oxidative-phosphorylation/a/the-citric-acid-cycle-2
24. Kompare, M.; Rizzo, W.B. Mitochondrial fatty-acid oxidation disorders. Semin. Pediatr. Neurol. 2008,15, 140-149. [CrossRef] [PubMed]
25. Tiny, T.; Pihko,H.Long-chain-hydroxyacyl-CoA dehydrogenase deficiency.Acta Paediatr.1999,88,237-245.[CrossRef] [PubMed]
26. Houten, S.M.; Wanders, R.J. A general introduction to the biochemistry of mitochondrial fatty acid beta-oxidation. Inherit. Metab. Dis.2010,33, 469-477. [CrossRef] [PubMed]
27. DeLonlay, P.; Giurgea,I.;Touati,G.;Saudubray,J.M.Neonatal Hypoglycaemia:Etiologies.Semin.Neonatol.2004,9,49-58. [CrossRef] [PubMed] Cells 2018,7, 46
28. Kotter's, M.; Jaksch, M.; Ketelsen, U.P.; Weiner, S.; Glocker, F.X.; Lucking, C.H. Valproic acid triggers acute rhabdomyolysis in a patient with carnitine palmitoyltransferase type II deficiency. Neuromuscul. Disord. 2001,11, 757-759. [CrossRef]
29. Moczulski, D.; Majak, I.; Mamczur, D. An overview of beta-oxidation disorders. Postepy Hig. Med. Dosw. 2009,63, 266-277.
30. Emery, J.L.;Howat,A.J.;Variend,S.;Vawter,G.F.Investigationofinbornerrorsofmetabolisminunexpected infant deaths.Lancet1988,2, 29-31. [CrossRef]
31. Wanders, R.J.; Duran, M.; Ijlst, L.; de Jager, J.P.; van Gennip, A.H.; Jakobs, C.; Dorland, L.; van Sprang, F.J. Sudden infant death and long-chain 3-hydroxyacyl-CoA dehydrogenase. Lancet 1989,2, 52-53. [CrossRef]
32. Sim, K.G.;Hammond,J.;Wilcken,B.Strategiesforthediagnosisofmitochondrialfattyacidbeta-oxidation disorders.Clin. Chim. Acta 2002,323, 37-58. [CrossRef]
33. Mansouri, A.;Fromenty,B.;Durand,F.;Degott,C.;Bernuau,J.;Pessayre,D.Assessment The Prevalence Of genetic metabolic defects in acute fatty liver of pregnancy J. Hepatol.1996,25, 781. [CrossRef]
34. (n.d.). Retrieved from https://www.stat.washington.edu/thompson/Genetics/1.3_genotypes.html
35. Waisbren, J. (2003). Figure 2f from: Irimia R, Gottschling M (2016) Taxonomic revision of Rochefortia Sw. (Ehretiaceae, Boraginales). Biodiversity Data Journal 4: E7720. https://doi.org/10.3897/BDJ.4.e7720. doi: 10.3897/bdj.4.e7720.figure2f

The Feasibility of Improving Positive Predictive Values of MCAD Deficiency Screening by Using Additional Acylcarnitine Markers

Jacob Voudren