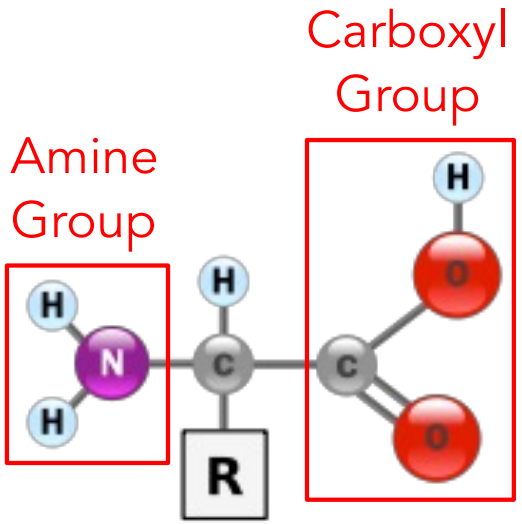
Consumer Amino Acid Hydrogen Isotopes

Wet Chemistry: Hydrolysis and Derivatization

(1) Lipid-Extraction & Dowex (purify protein)

2:1 Chloroform:Methanol (3 X 24hr soaks, DI rinse, freeze-dry)
Dowex resin (acid:base) to remove carbohydrates

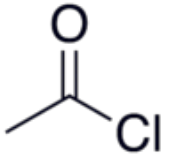


(2) Acid Hydrolysis

~5mg of tissue hydrolyzed in 6N HCl at 110˚C for 20 hours
Glutamine/Glutamate converted to glutamic acid during hydrolysis

(3) Esterification of Carboxyl Terminus

Add 1ml of 1:4 acetyl chloride:isopropanol
Heat at 110˚C for 60 minutes

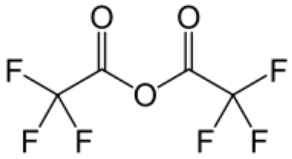


CH_3COCl

Adding C and H, but we know how much (where) and δ value

(4) Trifluoroacetylation of Amine Group

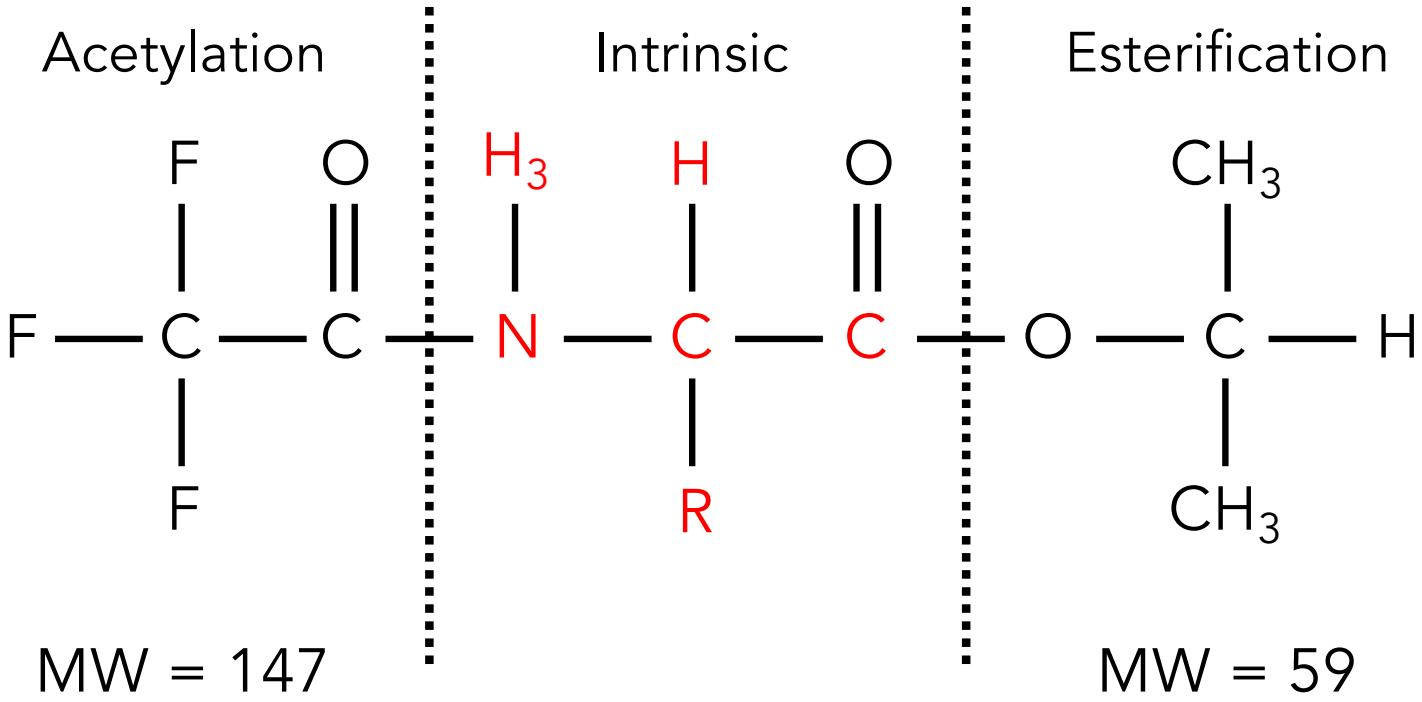
Add 1ml of 1:1 dichloromethane:trifluoroacetic anhydride (TFAA)
Heat at 110˚C for 10 minutes



$C_4F_6O_3$

Amino Acid Derivatization

Black is what you add, red is what you want to measure…



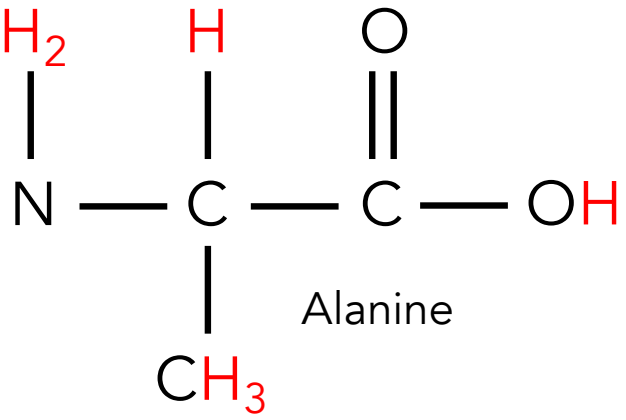
Basic molecular weight (MW) of compound +206!

Unless there are multiple active groups:
Asp and Glu (two propyl esters)
Ser, Thr (two per fluoro groups, OH^-)

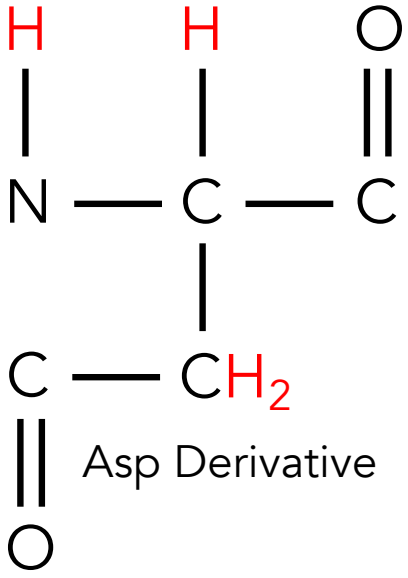
What Happens to Hydrogen During Derivatization?

H bound to C (alpha and methyl) less likely to be replaced

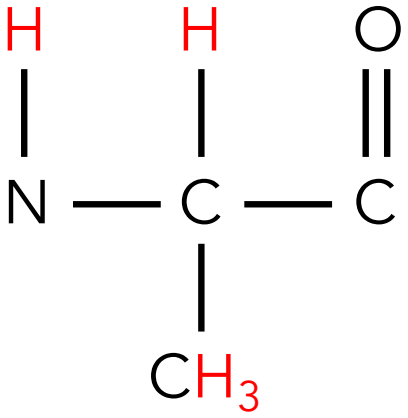
H bound to N or O: not as strongly bonded



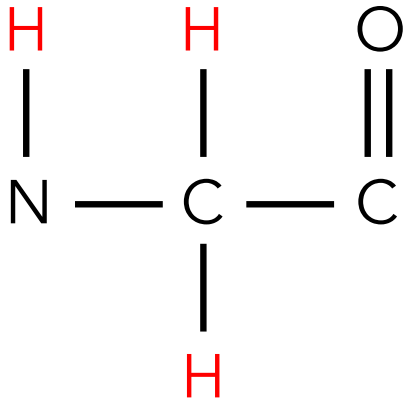
Alanine



Asp Derivative



Alanine Derivative



Gly Derivative

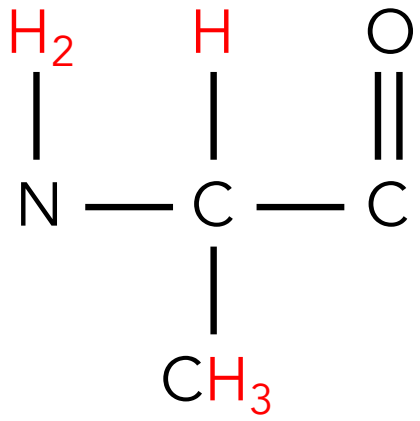
Fogel et al. 2016

How is Hydrogen Swapped On and Off of Molecules? Enzymes

via dehydrogenases: transfers H to electron acceptor (e.g., NAD or NADP)

via hydrolases: use (body) water to break chemical bonds

via transaminases: replace keto group with NH_2

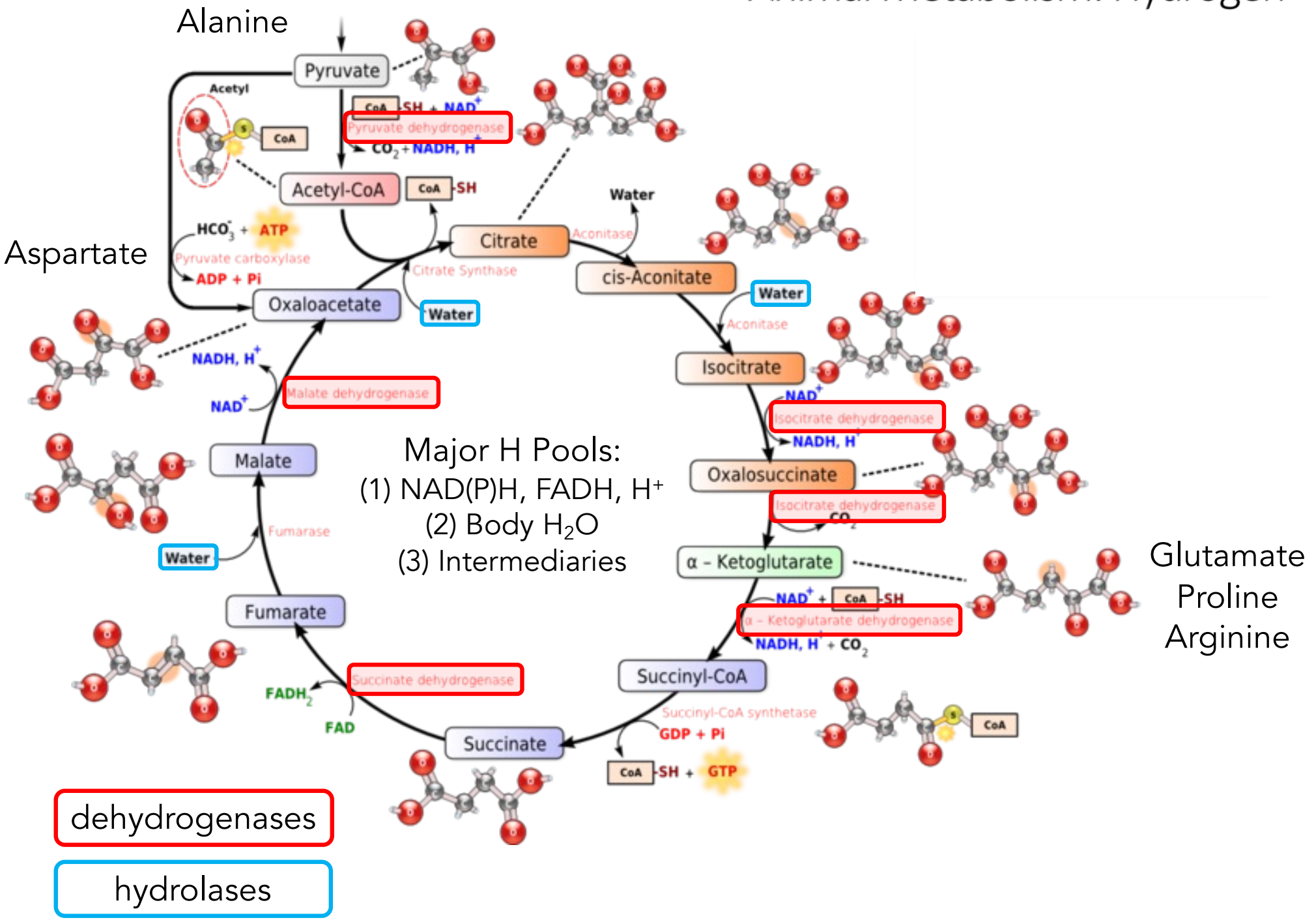


Isotope Rules:

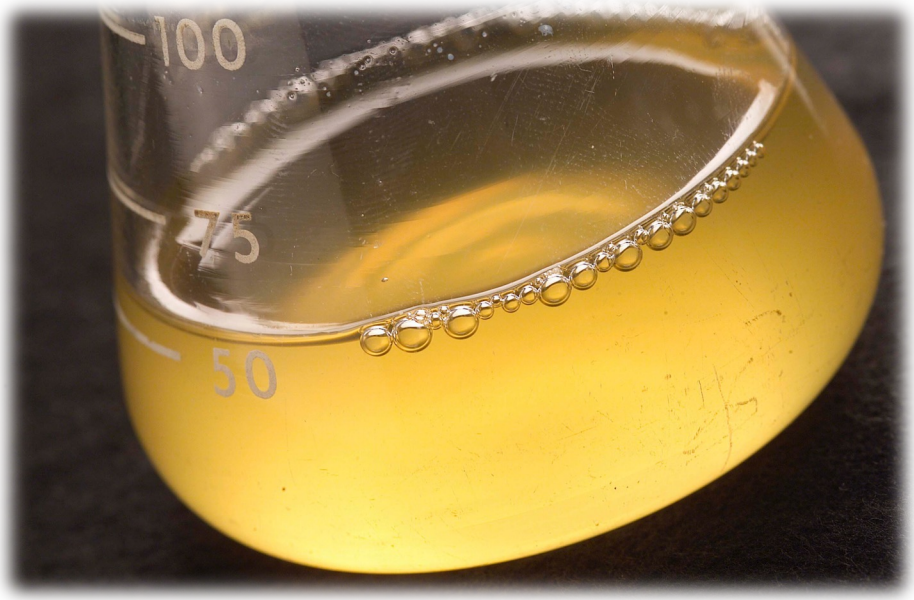
Light isotope (^1H) has weaker bonds
(removing H should increase compound δ^2H)

Less energy to make bonds with light isotope (^1H)
(adding H should decrease compound δ^2H)

Animal Metabolism: Hydrogen



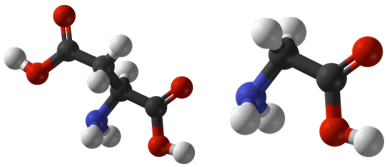
Our First Experiment with Amino Acid δ^2H: Prokaryotes



Grew *E. coli* in water that varied in δ^2H from -55 ‰ to +1070‰ (4 Treatments)
2 "Diet" Treatments: Glucose+NH_3 OR Tryptone (protein-rich)

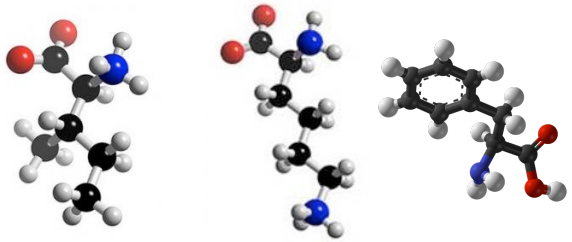
Non-Essential AA

Aspartate Glycine



Essential AA

Isoleucine Lysine Phenylalanine



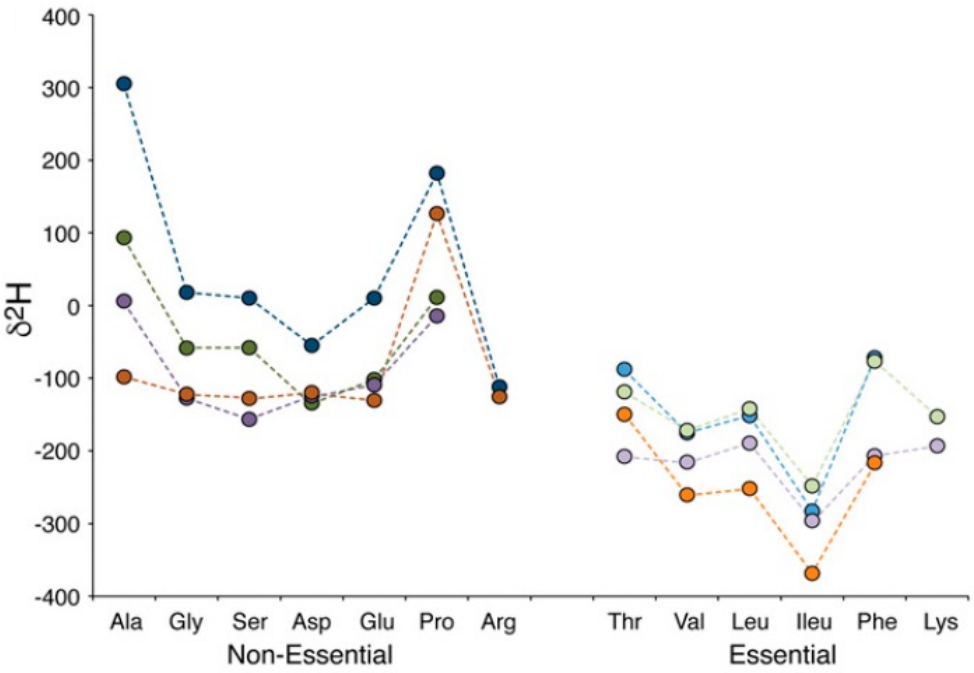
Fogel et al. 2016

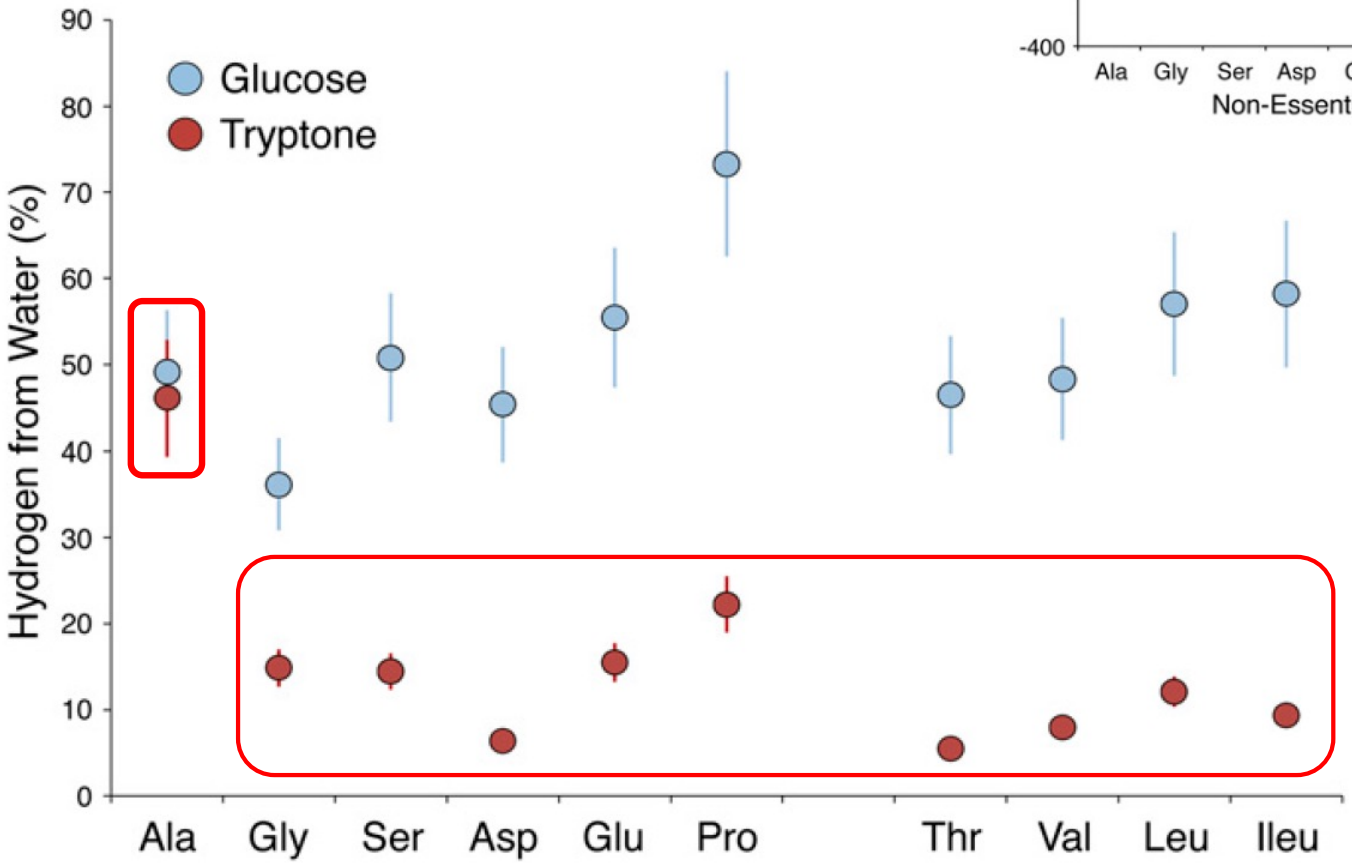
Highlights

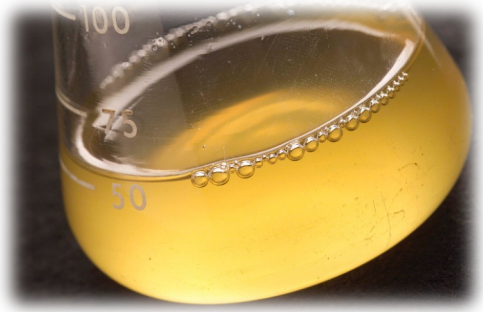
Huge range in AA δ^2H values (>300‰)
AA_{ESS} are generally lower than AA_{NESS}

Evidence of direct routing of media protein into nearly all AAs (exchange w/cellular water?)

Alanine has high P_{water} regardless of treatment







Fogel et al. 2016

How About a Eukaryote?

Ingredient	Macromolecule	Diet 1	Diet 2	Diet 3	δ^2H (SD)	[H] (SD)	δ^{13}C (SD)	[C] (SD)	δ^{15}N (SD)	[N] (SD)
Casein	Protein	0.05	0.20	0.35	−107±4.2	6.0±0.02	−24.8±0.2	48.4±0.32	5.5±0.3	14.1±0.35
Sucrose	Carbohydrates	0.45	0.30	0.15	−14±4.1	6.3±0.04	−11.7±0.8	41.9±0.15	–	–
Corn Meal	Carbohydrates	0.15	0.15	0.15	−18±4.4	5.8±0.05	−10.8±0.2	47.2±0.05	1.5±0.8	0.8±0.05
Corn Oil	Lipids	0.02	0.02	0.02	−154±4.6	9.6±0.21	−15.5±0.2	73.9±2.65	–	–
Cellulose	Binder	0.25	0.25	0.25	−25±4.0	5.8±0.02	−25.6±0.2	41.9±0.41	–	–
Fortified Salt	Salt	0.04	0.04	0.04	–	–	–	–	–	–
Brewer's Yeast	Yeast	0.02	0.02	0.02	−66±4.1	5.8±0.05	−21.4±0.3	44.0±0.30	3.2±0.2	7.3±0.15
Vitamin Mix	Vitamins	0.01	0.01	0.01	1±4.4	6.3±0.05	−12.9±0.3	39.9±0.38	–	–
	Protein:Carb Ratio	5:60	20:45	35:30						
	Bulk δ^2H	−26.0±3.0	−41.0±3.0	−56.0±3.0						
	Bulk δ^{13}C	−16.2±0.2	−18.3±0.2	−20.3±0.2						
	Bulk δ^{15}N	2.6±0.7	3.8±0.6	4.3±0.5						

Mauriel Rodriguez Curras

Dietary protein (casein) varied from 5% to 35%; low δ^2H: -108‰

Dietary carbohydrates varied from 30% to 60%; high δ^2H: -16‰

Drinking water δ^2H varied from -95‰ to -50‰ to +5‰

Dietary fat was low and did not vary among diet treatments

Rodriguez Curras et al. 2018

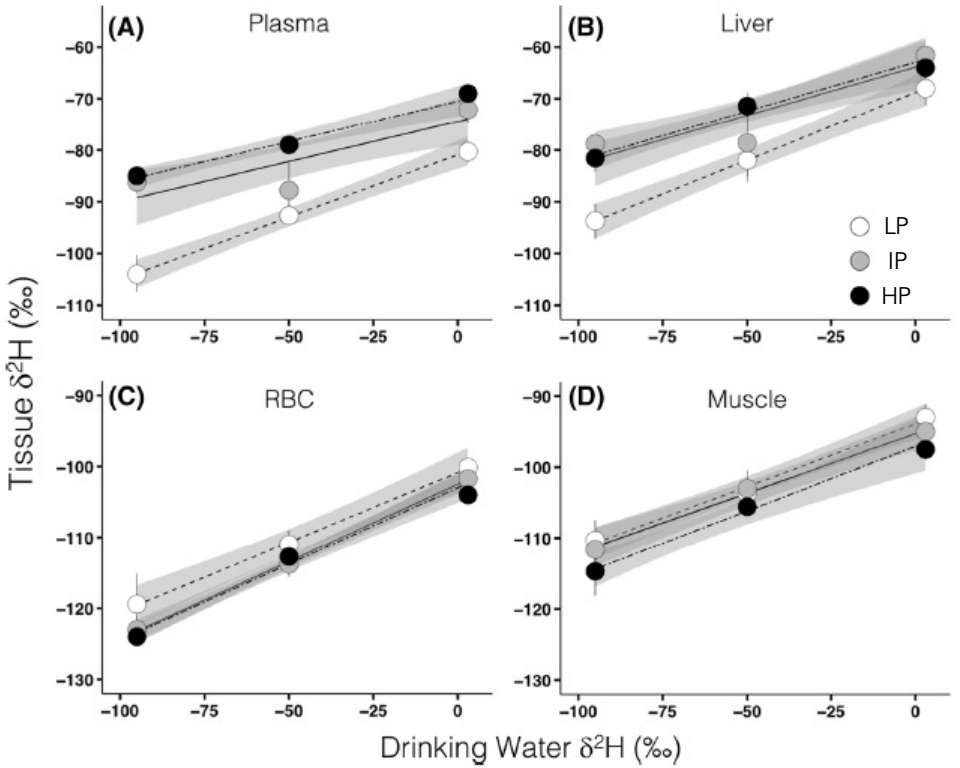
Bulk Tissue δ^2H Results

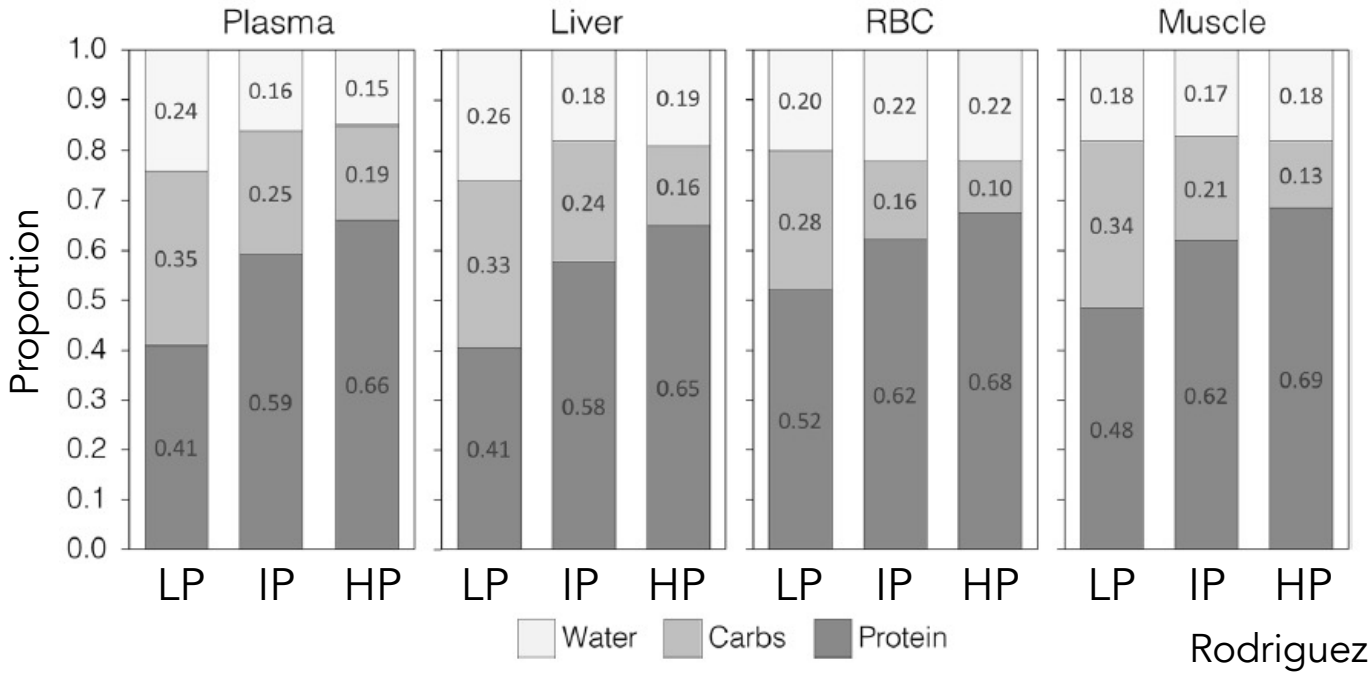
P_{Water}: 15–26%

P_{Carbs}: 10–35%
$P_{Protein}$: 41–69%

Larger P_{carbs} in low protein diet
Larger $P_{protein}$ in high protein diet

More AA *de novo* synthesis in low protein diet
More AA routing in high protein diet





Rodriguez Curras et al. 2018

Our (Working) Model

Cornmeal Amino Acids

Routing

Casein Amino Acids

Body H_2O

Drinking H_2O
(-95‰ -50‰ +5‰)

Metabolic H_2O
(Variable)

NADPH
(Variable)

De Novo Synthesis

Bulk Protein
(-108‰)

Bulk Carbs
(-16‰)

Newsome et al. 2020

Essential Amino Acids

Corn Amino Acids

Routing

Casein Amino Acids

Body H_2O

Drinking H_2O
(-95‰ -50‰ +5‰)

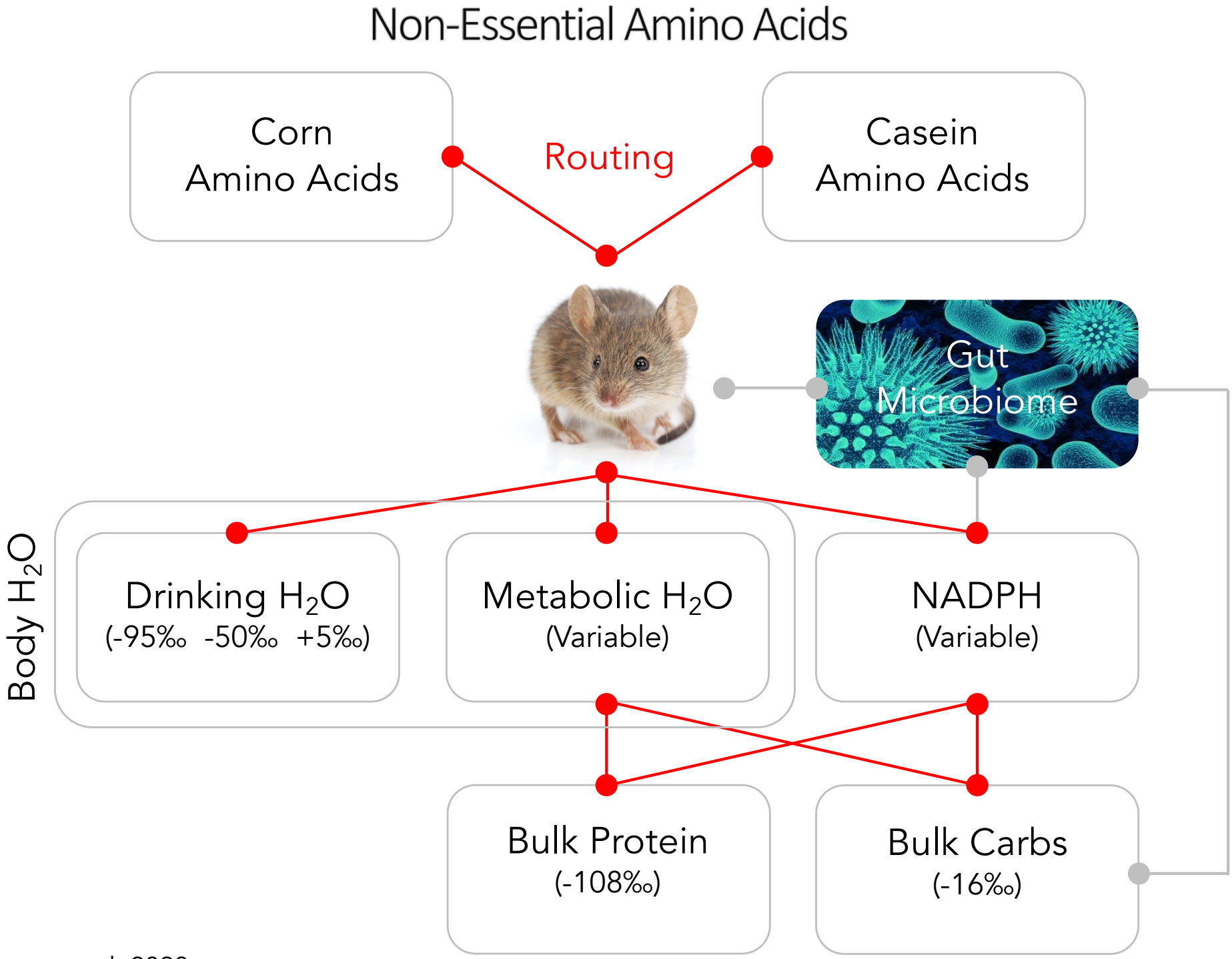
Metabolic H_2O
(Variable)

NADPH
(Variable)

Bulk Protein
(-108‰)

Bulk Carbs
(-16‰)

Newsome et al. 2020



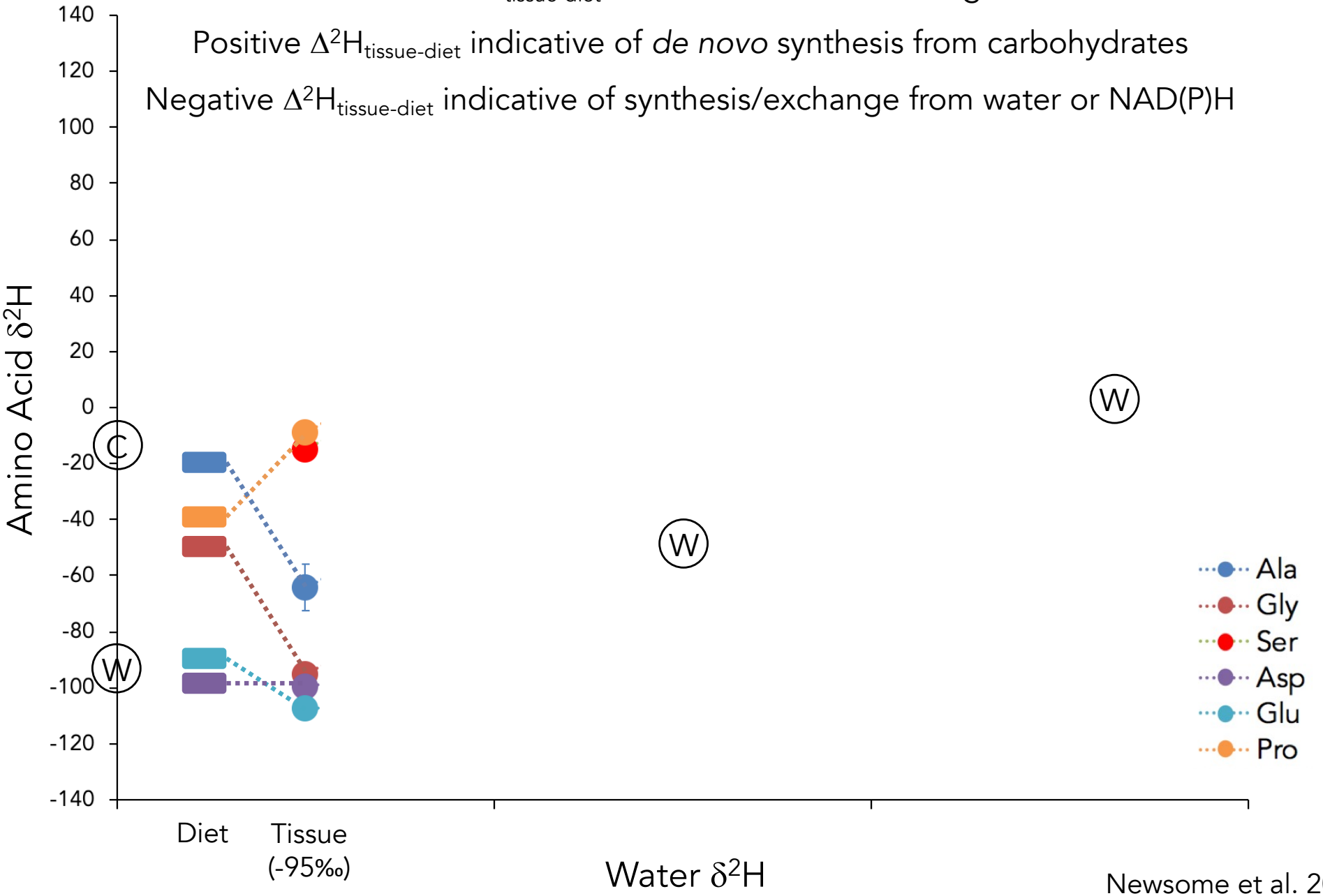
Newsome et al. 2020

Example

No $\Delta^2H_{tissue\text{-}diet}$ indicative of direct routing

Positive $\Delta^2H_{tissue\text{-}diet}$ indicative of *de novo* synthesis from carbohydrates

Negative $\Delta^2H_{tissue\text{-}diet}$ indicative of synthesis/exchange from water or NAD(P)H

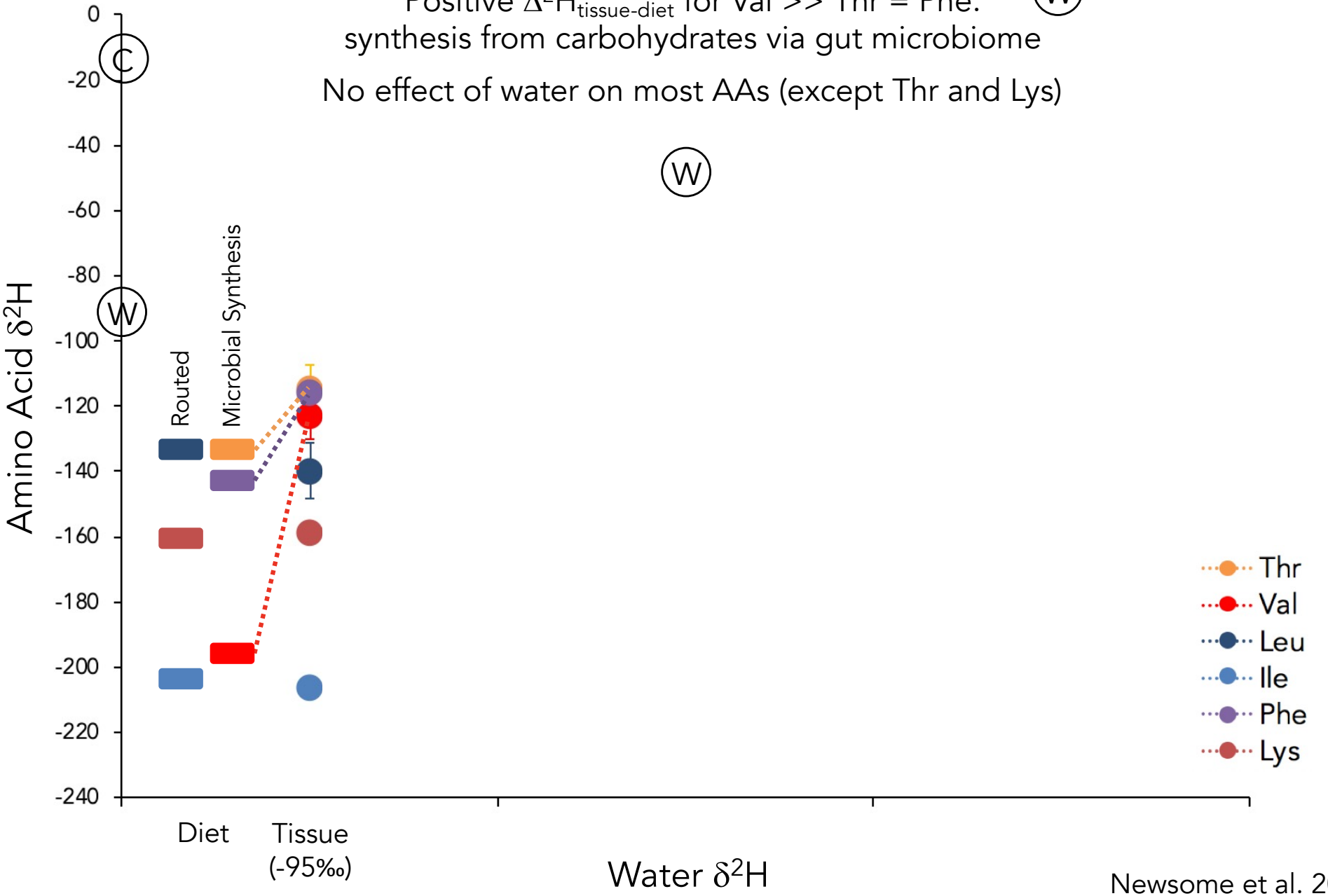


Newsome et al. 2020

Essential Amino Acids (Low Protein Diet)

Positive $\Delta^2H_{tissue\text{-}diet}$ for Val >> Thr = Phe:
synthesis from carbohydrates via gut microbiome

No effect of water on most AAs (except Thr and Lys)



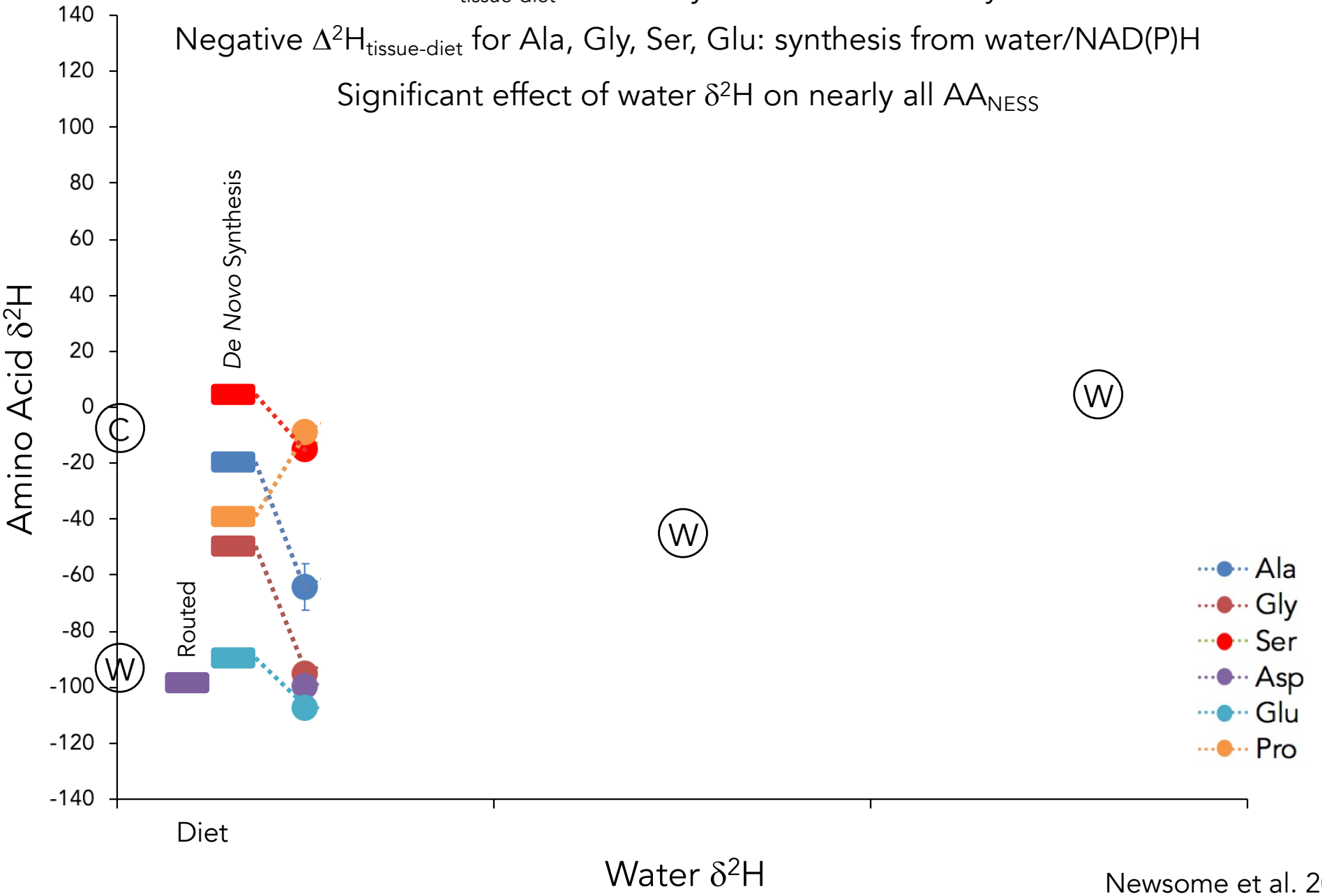
Newsome et al. 2020

Non-Essential Amino Acids (Low Protein Diet)

Positive $\Delta^2H_{tissue\text{-}diet}$ for Pro: synthesis from carbohydrates

Negative $\Delta^2H_{tissue\text{-}diet}$ for Ala, Gly, Ser, Glu: synthesis from water/NAD(P)H

Significant effect of water δ^2H on nearly all AA_{NESS}

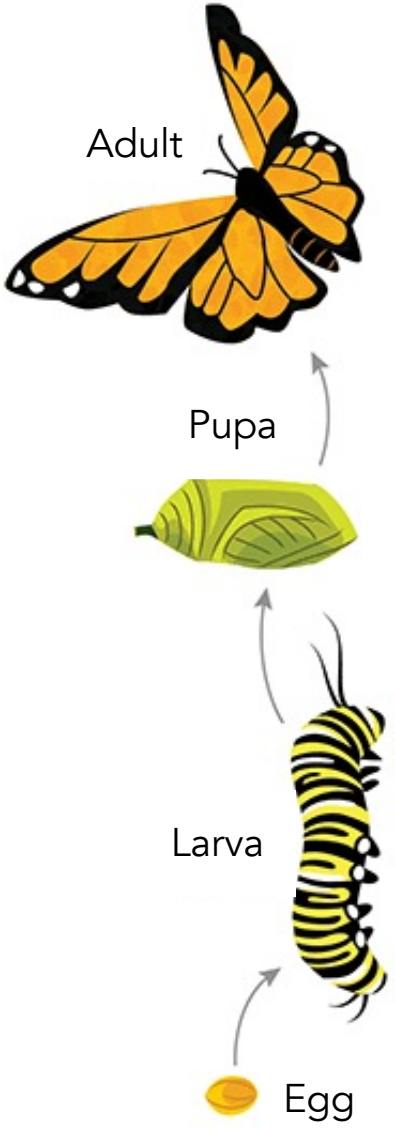


Newsome et al. 2020

How Does Amino Acid δ^2H Vary Among Trophic Levels?

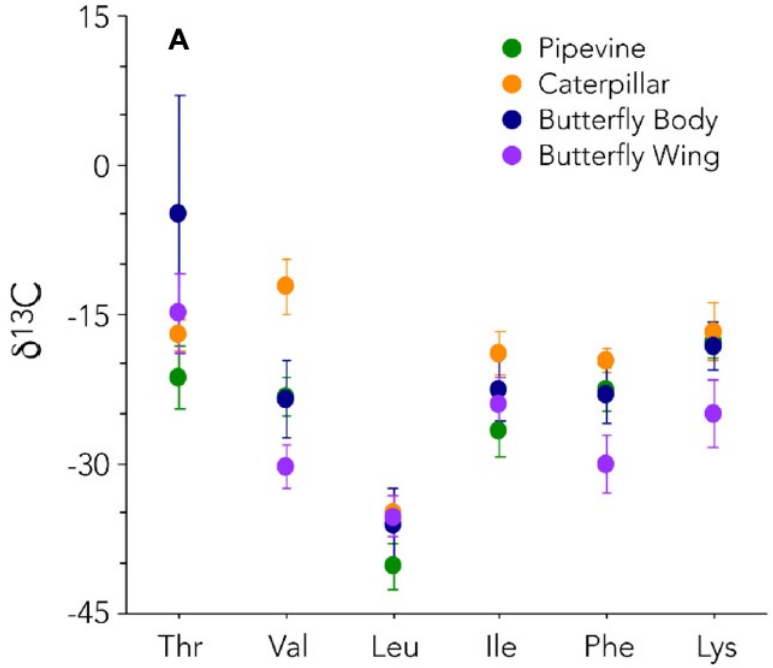
Pipevine Swallowtails (*Battus philenor*)

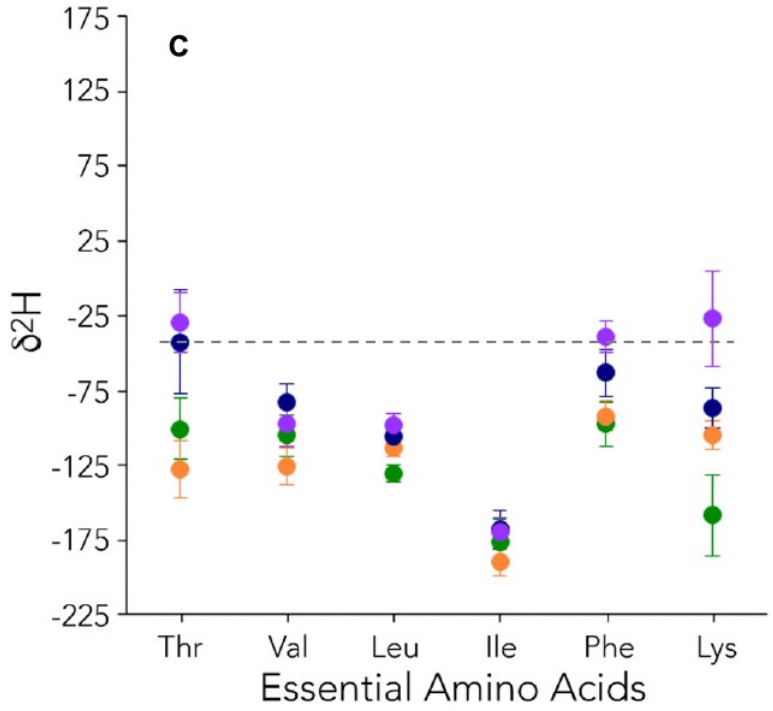
Pipevine
(*Aristolochia*)

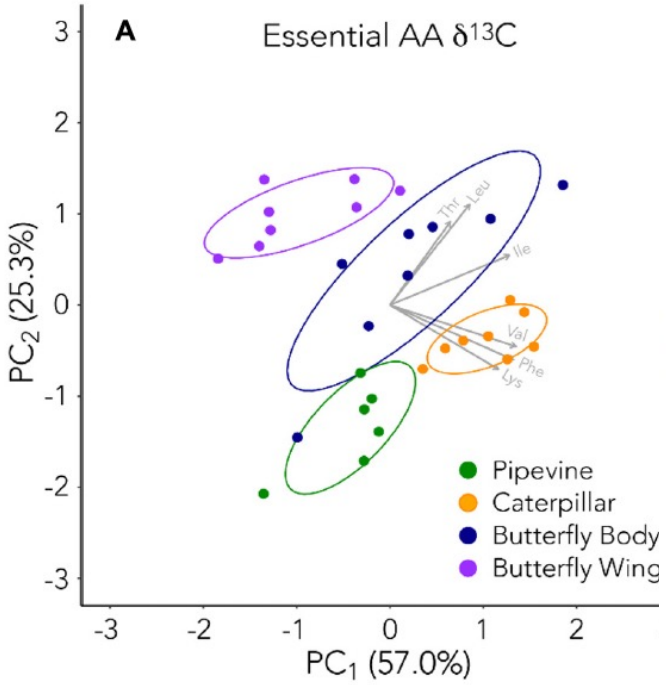


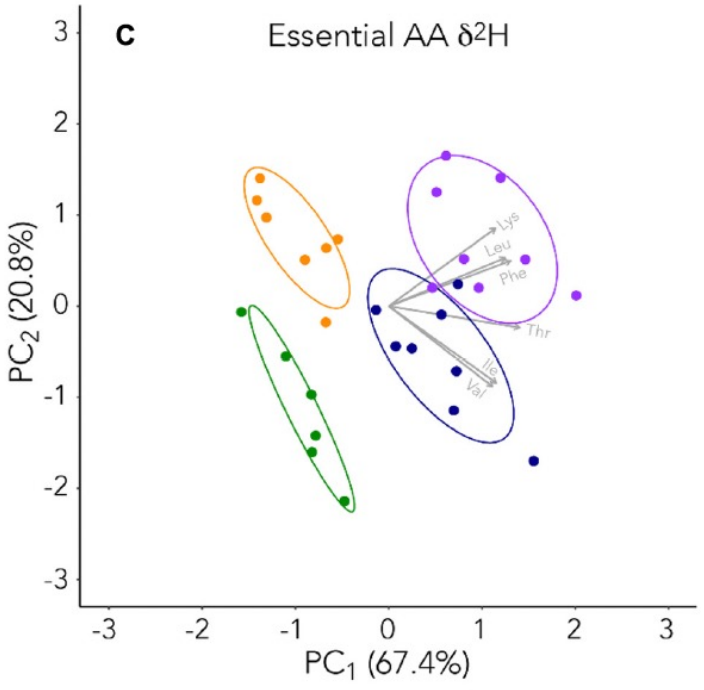
Morra et al. 2021

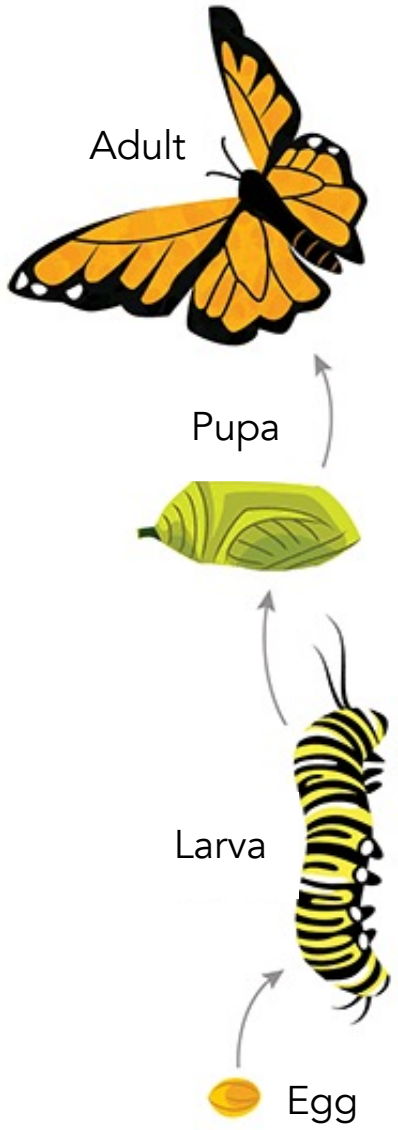
Essential δ^2H is Impacted by Metamorphosis: Gut Microbes?





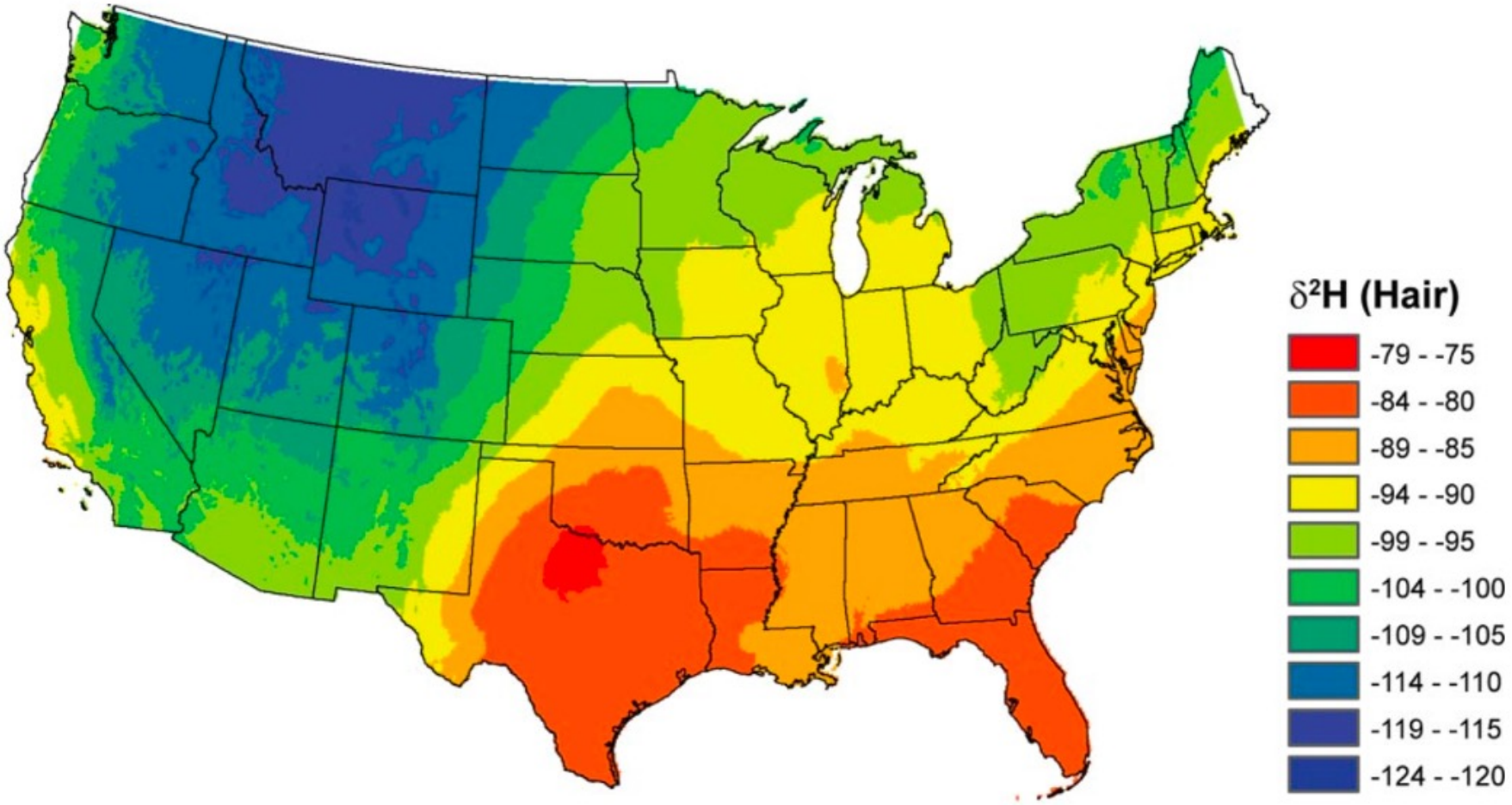






Morra et al. 2021

δ^2H: Geographic Assignment of Humans



Ehleringer et al. 2008

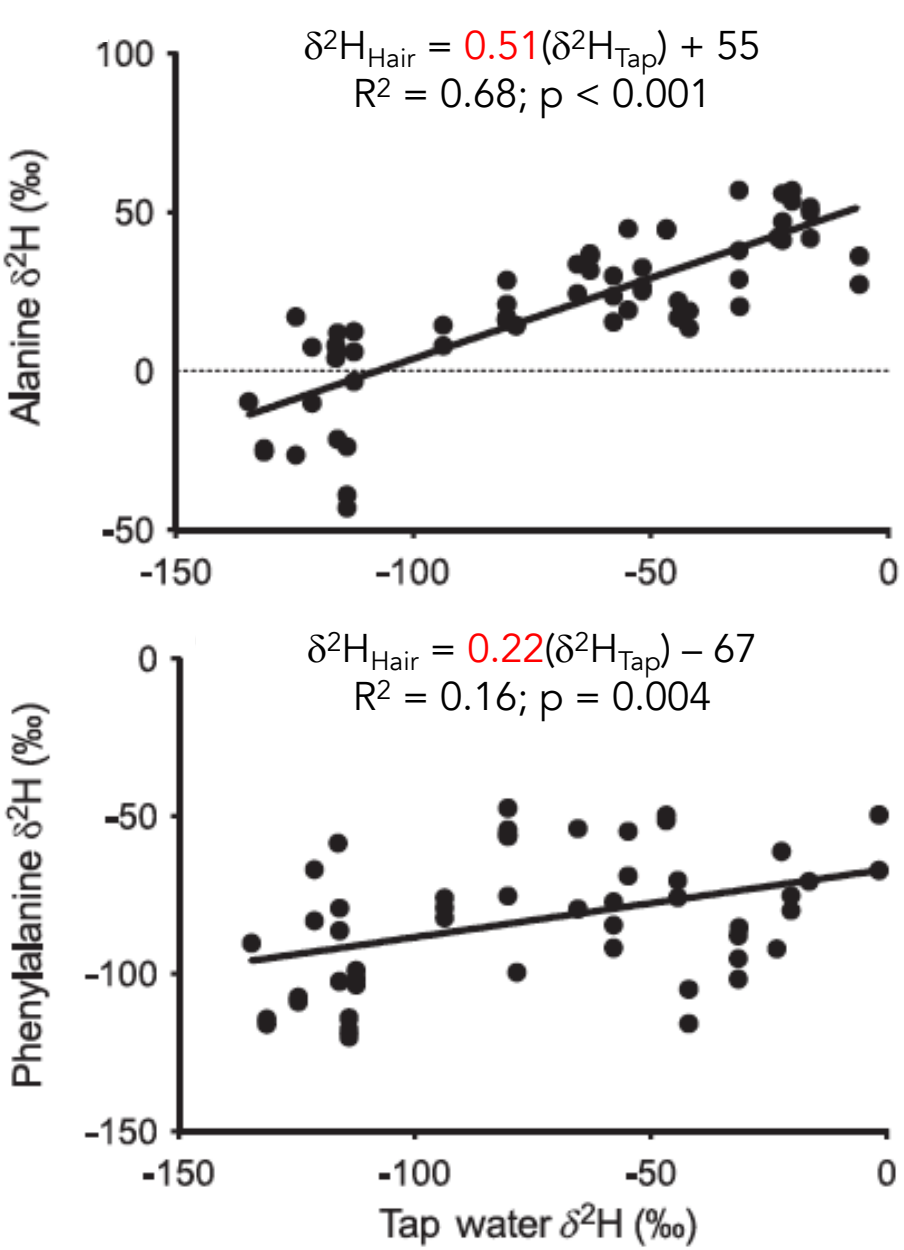
Tracing Region of Origin in Humans

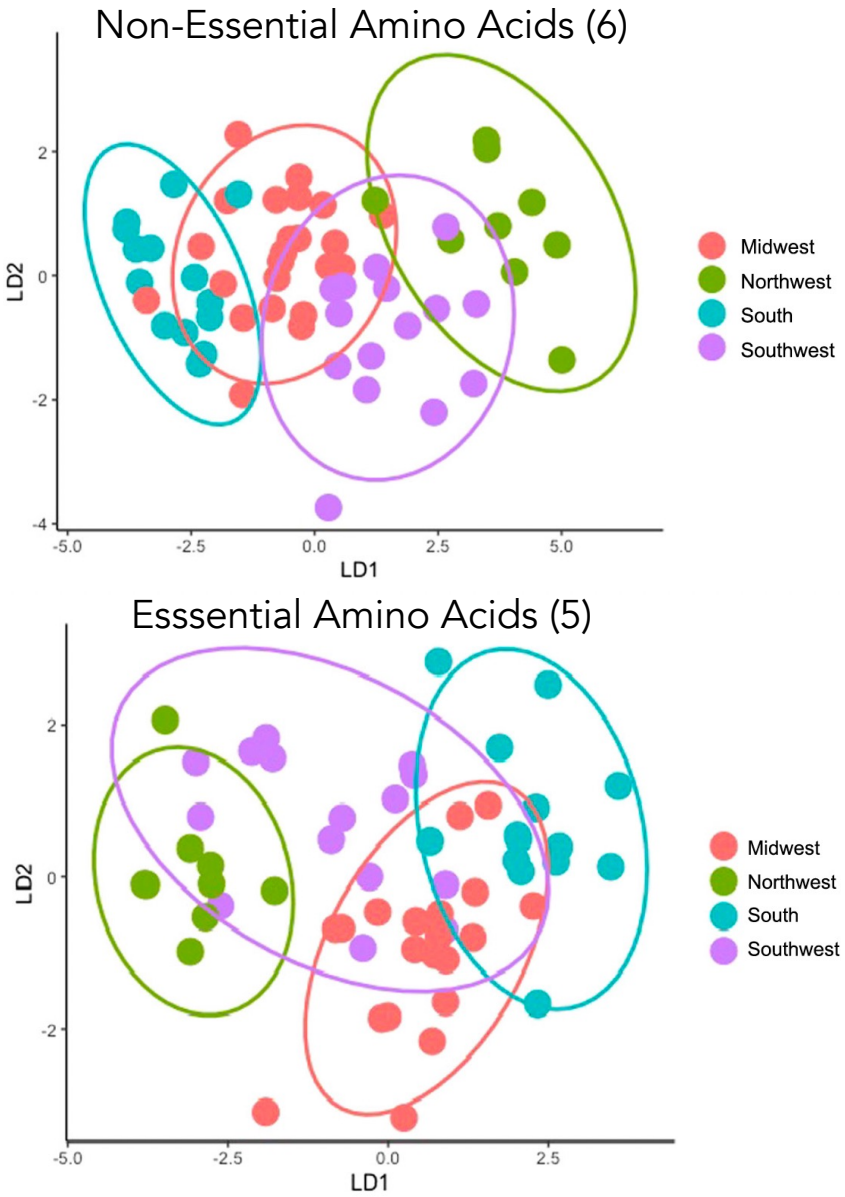
Location	Region	Scalp hair mean (± SD) δ^2H, ‰	N	Tap water mean (± SD) δ^2H, ‰	n
Alamosa, CO	Southwest	−108 ± 3.2	3	−111 ± 0.8	3
Alexandria, LA	Southern	−80 ± 2.2	3	−22 ± 3.0	4
Big Spring, TX	Southern	−79 ± 1.6	2	−6 ± 0.6	3
Bryon, IL	Midwest	−90 ± 3.7	3	−52 ± 2.2	3
Casper, WY	Northwest	−112 ± 3.7	3	−114 ± 0.8	5
Chicago, IL	Midwest	−100 ± 5.2	2	−44 ± 0.2	3
Conway, AR	Southern	−84 ± 0.8	2	−20 ± 0.7	3
Cut Bank, MT	Northwest	−119 ± 1.8	2	−132 ± 1.2	3
Daluth, MN	Midwest	−91 ± 7.1	2	−66 ± 3.2	4
Dillon, MT	Northwest	−125	1	−133 ± 0.8	3
Evanston, WY	Northwest	−111 ± 7.1	2	−125 ± 3.3	6
Fort Smith, Ar	Southern	−85	1	−31 ± 1.3	3
Lincoln, NE	Midwest	−95 ± 5.7	3	−58 ± 0.6	3
Lusk, WY	Northwest	−114 ± 0.2	2	−131 ± 1.4	3
Mahomet, IL	Midwest	−95 ± 0.2	2	−42 ± 3.0	3
Monroe, LA	Southern	−85 ± 5.9	3	−17 ± 0.7	3
Monticello, UT	Southwest	−103 ± 2.2	3	−94 ± 1.0	3
Muskogee, OK	Southern	−81	1	−23 ± 1.9	3
Paduach, KY	Midwest	−88 ± 1.6	3	−32 ± 1.9	3
Pecos, TX	Southern	−88 ± 0.9	2	−54 ± 2.0	3
Price, UT	Southwest	−115 ± 3.8	3	−116 ± 2.6	3
Rifle, CO	Southwest	−108 ± 1.7	4	−120 ± .8	3
Roosevelt, UT	Southwest	−119.0	1	−113 ± 3.9	3
Roswell, NM	Southwest	−94 ± 0.3	2	−58 ± 1.2	3
Valentine, NE	Midwest	−103 ± 7.3	5	−80 ± 1.0	3
Vaughn, NM	Southwest	−99.0	1	−78 ± 1.9	3
Vernal, UT	Southwest	−93 ± 0.6	3	−113 ± 2.5	4
Wykoff, MN	Midwest	−103 ± 7.5	3	−63 ± 2.7	5

Dr. Christy Mancuso

Mancuso et al. 2022

Tracing Region of Origin in Humans





Mancuso et al. 2022

Take Home Message(s): Amino Acid δ^2H

For δ^2H, amino acids (AA) are classified as non-essential and essential and generally mirror patterns seen in $\delta^{13}C$.

Experiments on bacteria and mice show that essential AA δ^2H are faithful tracers of dietary protein δ^2H values, while non-essential AA δ^2H values are more influenced by carbohydrates and (drinking) water.

δ^2H analysis of non-essential AA (Ala) may provide a method for assessing region of origin (and movement/migration) at higher resolution than bulk tissue analysis for humans where diet is more controlled.

This approach may allow for tracing sources of water and food in a single tissue sample.

Amino acid δ^2H analysis is a promising but relatively unexplored proxy. (empty niche waiting to be filled)