Amino Acid Hydrogen Isotope ($\delta^2$H) Analysis
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 ($^1$H) has weaker bonds
(removing H should increase compound $\delta^2$H)

Less energy to make bonds with light isotope ($^1$H)
(adding H should decrease compound $\delta^2$H)
Major H Pools: NAD(P)H, FADH, H⁺  Body H₂O Intermediaries

dehydrogenases
hydrolases

Animal Metabolism: Hydrogen
Grew *E. coli* in water that varied in $\delta^{2}H$ 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 $\delta^2$H values (~300‰)
AA$_{ESS}$ are generally lower than AA$_{NESS}$

Evidence of direct routing of medium protein (tryptone) into nearly all AAs.

Alanine has high $P_{\text{water}}$ regardless of treatment

Fogel et al. 2016
Dietary protein (casein) varied from 5% to 35%; low $\delta^2H$: -108‰

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

Drinking water $\delta^2H$ varied from -95‰ to -50‰ to +5‰

Dietary fat was low and did not vary among diet treatments

Mauriel Rodriguez Curras
Bulk Tissue $\delta^2$H Results

$P_{\text{Water}}$: 15–26%
$P_{\text{Carbs}}$: 10–35%
$P_{\text{Protein}}$: 41–69%

Larger $P_{\text{carbs}}$ in low protein diet
Larger $P_{\text{protein}}$ 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

Gut Microbiome

Drinking H₂O (-95‰ -50‰ +5‰)

Metabolic H₂O (Variable)

NADPH (Variable)

De Novo Synthesis

Bulk Protein (-108‰)

Bulk Carbs (-16‰)
Essential Amino Acids

- Bulk Protein (-108‰)
- Bulk Carbs (-16‰)
- Corn Amino Acids
- Casein Amino Acids
- Drinking H$_2$O (-95‰ -50‰ +5‰)
- Metabolic H$_2$O (Variable)
- NADPH (Variable)
- Gut Microbiome

Routing
Non-Essential Amino Acids

- Corn Amino Acids
- Casein Amino Acids
- Drinking H₂O (-95‰ -50‰ +5‰)
- Metabolic H₂O (Variable)
- NADPH (Variable)
- Bulk Protein (-108‰)
- Bulk Carbs (-16‰)

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

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

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

Newsome et al. 2020
Essential Amino Acids (Low Protein Diet)

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

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

Significant effect of water $\delta^2H$ on nearly all $\text{AA}_{\text{NESS}}$

Newsome et al. 2020
How Does Amino Acid $\delta^{2}H$ Vary Among Trophic Levels?
Essential $\delta^2$H is Impacted by Metamorphosis: Gut Microbes?

Morra et al. 2021
Essential Amino Acid $\delta^2$H Fingerprints?

Linear Discriminant 1 (68.2%)

Linear Discriminant 2 (27.6%)

Linear Discriminant 3 (4.2%)

CAM Plants

$C_4$ Plants

$C_3$ Plants

Algae

Rio Grande, NM

Trees/Shrubs/Grasses

Algae

Besser et al. in review
**Tracing Region of Origin in Humans**

### Proline (Non-Essential)

\[ \delta^{2}H_{\text{Hair}} = 0.52(\delta^{2}H_{\text{Tap}}) + 214 \]

\[ R^2 = 0.55; \ p = 0.02 \]

### Leucine (Essential)

\[ \delta^{2}H_{\text{Hair}} = 0.19(\delta^{2}H_{\text{Tap}}) - 114 \]

\[ R^2 = 0.19; \ p = 0.008 \]

**Mancuso et al. in preparation**

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**Dr. Christy Mancuso**

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- Dillon MT
- Rifle CO
- Alamosa CO
- Roswell NM
- Pecos TX
- Big Spring TX

- Proline
- Leucine

- Mancuso et al. in preparation
Take Home Message(s): Amino Acid $\delta^2$H

For $\delta^2$H, 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 $\delta^2$H are faithful tracers of dietary protein $\delta^2$H values, while non-essential AA $\delta^2$H values are more influenced by environmental (drinking) water.

$\delta^2$H 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 $\delta^2$H analysis is a promising but relatively unexplored proxy. (empty niche waiting to be filled)