Discovering Diet-Dependent Changes in Amino Acid Metabolism

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Introduction

The Price Lab ran a mouse diet experiment to see if we could detect changes in amino acid metabolism according to diet. **The diets used were low protein ad libitum (LP AL), low protein CR (LP CR), high protein ad libitum (HP AL), and high protein CR (HP CR)**. Multiple individuals were randomly assigned to each diet. Extracting D_2O labelled samples across several time points allows us to use mass spectrometry to measure both protein concentration and turnover (**figure 1**) (1). These are measured by analyzing the shift in isotopic peaks (2). **Using these changes in isotopic peaks we can also calculate n-values (see figure 2 below), which are metabolic indicators for the associated molecule.**

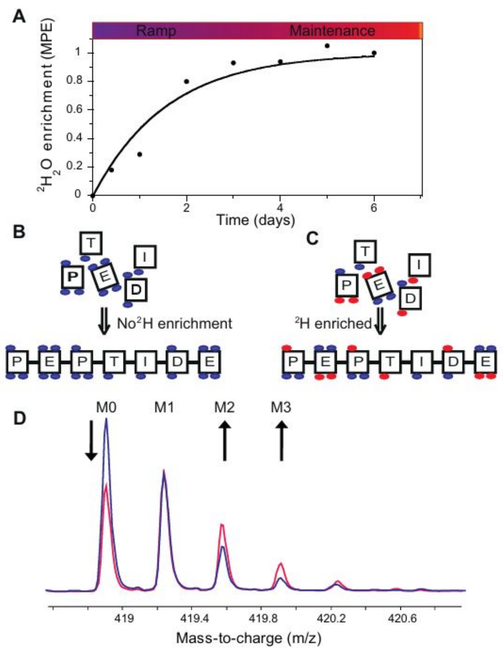


Figure 1 - Samples taken over different timepoints allow us to measure ^2H_2O (D_2O) enrichment over time (A). Peptides from later time points will have more D_2O labeling (B, C). As a sample becomes more labeled, the isotopic distribution will shift (D). The purple peaks are unlabeled and the red peaks are labeled.

N-Value Calculations

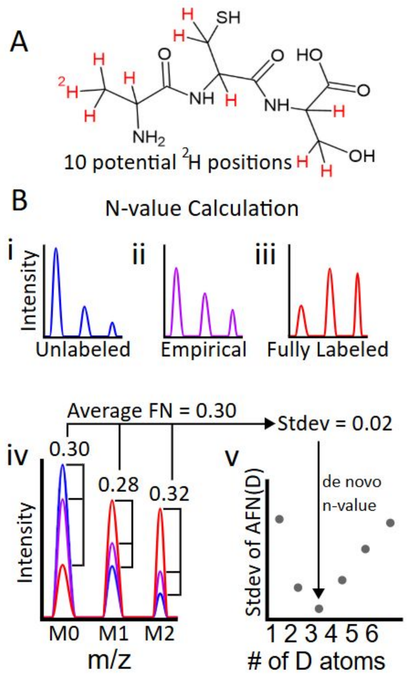


Figure 2 - A molecule will have a number of locations where hydrogen can be replaced by deuterium, marked in red (A). Subtracting a theoretical fully labeled distribution (iii) from the unlabeled distribution (i) gives the maximum abundance of a molecule that can be new in the population. The percent of that maximum abundance that is empirically measured (ii) is called fraction new (FN). The theoretical distribution with the lowest standard deviation is selected to represent the n-value for that molecule (iv) (3).

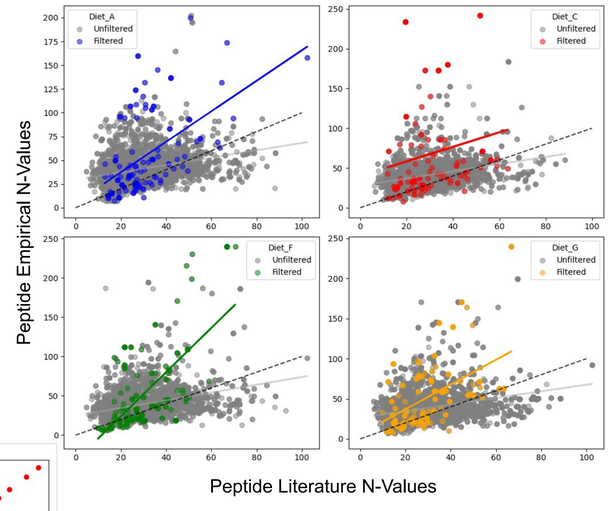
Empirical vs Literature Amino Acid N-Values

Amino Acid	LP AL N-Values	LP CR N-Values	HP AL N-Values	HP CR N-Values	Literature N-Values	Max Labeling Sites
Phenylalanine	8	8	8	8	0.47	8
Histidine	5	2.56	5	0	2.15	5
Isoleucine	6.51	0	4.18	0	0.8	10
Lysine	0	9	4.82	8.13	0.3	9
Leucine	3.38	10	8.23	10	0.7	10
Methionine	3.77	0	0	7.11	1.02	8
Threonine	0	5	0	1.22	0.21	5
Valine	7.83	8	0.74	2.5	0.79	8
Tryptophan	4.5	2.11	1.43	3.25	0.06	8
Average Essential AA Change	**3.61**	**4.24**	**2.88**	**3.75**		
Alanine	4	0	4	4	3.95	4
Cysteine	0	3	0	0	1.67	3
Aspartic Acid	3	3	0	0	2.5	3
Glutamic Acid	4.43	0	5	3.11	4.18	5
Glycine	2	2	0	0	1.84	2
Asparagine	0	3	1.47	0	1.15	3
Proline	0	7	0	0	1.57	7
Glutamine	4.68	0	5	5	3.62	5
Arginine	5.29	1.35	0	7	3.4	7
Serine	3	0	3	1.66	1.96	3
Tyrosine	7	7	7	6.19	0.5	7
Average Non-Essential AA Change	**0.64**	**0**	**-0.08**	**0.06**		
Combined Average AA Change	**1.98**	**1.91**	**1.25**	**1.72**		

Figure 3 - Estimated amino acid n-values using a least-squares solution for the rectangular, overdetermined matrix of peptides (rows) and amino acids (columns)(4) given the empirical n-values. The max labeling sites column represents the number of potential labeling locations for each amino acid.

Noise Reduction and Quality Control

Figure 4 (right) - Filtering out noise based on n-value standard deviations (<=0.05) and intensity (top 75%). Empirical n-values would ideally be similar to the literature n-values and would follow a y=x trend (dashed line). The grey points and linear fit show the peptide data before filtering. Colored points and linear fit represent the results of the filters and show mixed results in matching the y=x trend (4).



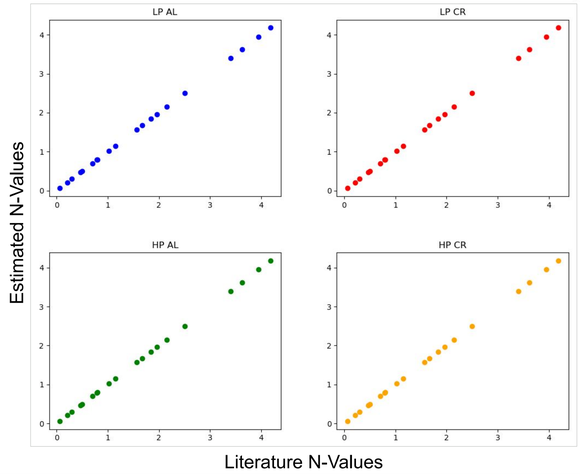
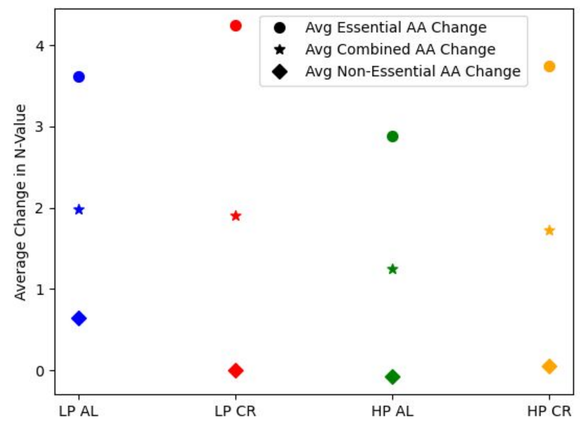


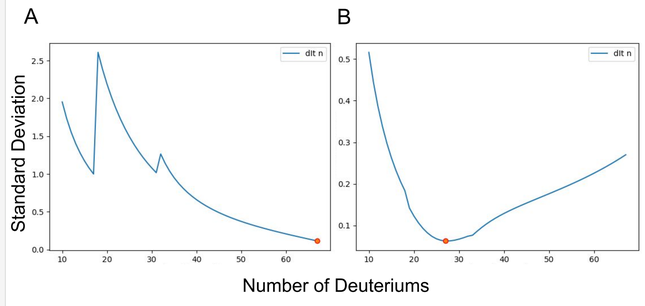
Figure 5 (left) - For each diet, we used the same matrix constructed of peptides (rows) and amino acids (columns) and calculated the least-squares solution using the literature n-values. For each diet, the least-squares solution gave estimated amino acid n-values (y-axis) that matched the literature n-values (x-axis)(4).

Results

- Filtering by n-value standard deviation and intensity moderately decreased noise, but there were outliers that have high empirical n-values with low standard deviations (**Figure 4**).
- **Figure 6 (below)** We predicted there would be less variance in essential amino acid n-values since they are always obtained through diet. However, our analysis showed essential amino acid n-value changes were higher than non-essential amino acid changes.



- Better criteria for filtering out noisy peptide data would decrease confounding factors that affect the accuracy of the least-squares matrix solution and increase the confidence of our empirical amino acid n-values.
- **Figure 7 (below)** plots the n-value standard deviation over the number of deuteriums, and shows a potential source of noise. **Graph A** has a few local minima, but also has an infinitely decreasing curve after ~35 deuteriums. This predicts an extremely high n-value that is likely inaccurate. **Graph B** shows an ideal curve with a single minima.
- Our current n-value method can only predicts integer n-values. We hypothesize by expanding the calculation to at least one decimal point would decrease noise.



References

1. DeuteRater: https://github.com/JC-Price/DeuteRater
2. Price, J. C., et al. (2012). "Measurement of human plasma proteome dynamics with (2)H(2)O and liquid chromatography tandem mass spectrometry." Anal Biochem 420(1): 73-83.
3. Carson, R. H., et al. (2017). "Imaging regiospecific lipid turnover in mouse brain with desorption electrospray ionization mass spectrometry." J Lipid Res 58(9): 1884-1892.
4. Amino Acid N-Value Calculations: https://github.com/StarLicker/Amino_Acid_N_Value_Calculator

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