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Folic Acid Deficiency and Methylation in 3 Neurodegenerative Mouse Models: DSP4-Alzheimer's, Down's Syndrome, and Aged

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Summer Undergraduate Research in Biology

Introduction

Folate is a vitamin that is normally found in leafy green vegetables, oranges, legumes, and peanuts. Once folate is absorbed into the body, this water-soluble vitamin is primarily used in the folate cycle. The folate cycle is multipurpose, indeed, folate is converted into an active form called 5-methyltetrahydrofolate, which donates a methyl group to homocysteine to make methionine. Methionine is also important because it a necessary to synthesize DNA. Methionine converts to S-adenosylmethionine (SAM), which is a cofactor for DNA methyltransferases.

DNA methyltransferases are necessary for methylation. Methylation is when a methyltransferase adds a methyl group to a DNA strand at a CpG site (Figure, Folate Cycle). Methylation is an important process because it can inactivate certain genes, and when changes in methylation occur, it can potentially affect a person's risk of developing neurodegenerative diseases such as Down Syndrome (DS), Alzheimer's disease (AD), and in the process of aging. DNMT3L is a DNA methyltransferase that, when paired with DNMT3a or DNMT3b, causes methylation. DNMT3L will only be present when there is methylation, as it has no other function.

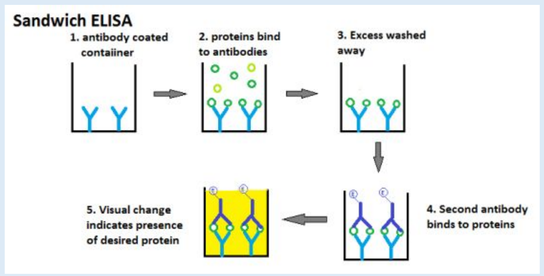
The presence of DNMT3L was measured in the livers and hearts of mice treated with a neurotoxin, DSP_4 to simulate Alzheimer's disease, in Ts65Dn, Down's Syndrome mice and, in aged CBl57/J6 mice. The purpose of this experiment was to determine how a dietary folic acid deficiency affects the amount of methylation. A quantitative determination of DNMT3L, 5-MTHFR, SAMe, and methylation were completed to examine the folate cycle among 3 neurodegenerative mouse models (Alzheimer's Disease, Down Syndrome, and Aging). The results descriptively establish the importance of dietary folic acid in the metabolism of folate during conditions of neurodegeneration.

Hypothesis

Folic Acid Deficiency will decrease presence of DNMT3L with consequent hypomethylation.

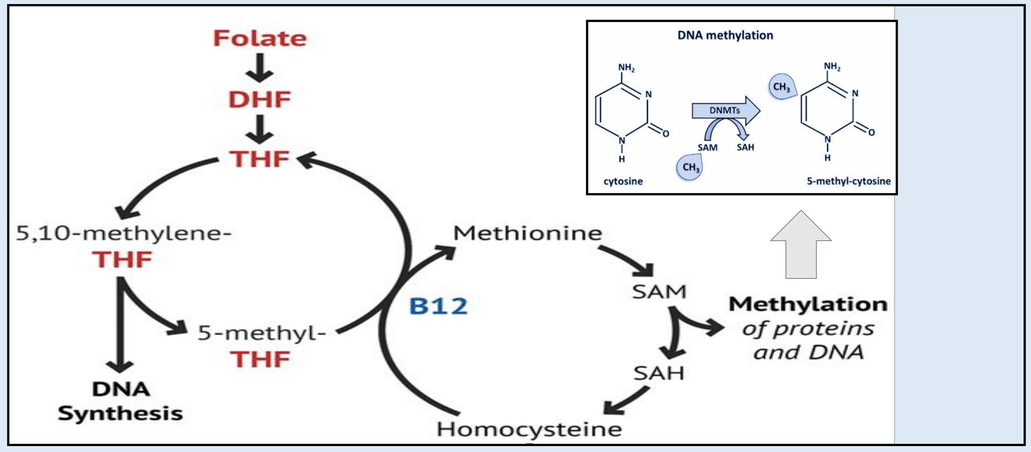
Methods and Analysis

Both liver and heart samples collected from 3 experiments over an eight year span were used to analyze the presence of DNMT3L by conducting an enzyme-linked immunosorbent assay (ELISA). To prepare the samples, they were thawed, ground with phosphate buffered saline (PBS), and used for the DNMT3L ELISA. The sandwich ELISA, used antibodies to detect the antigen as shown in Figure below. After ELISA procedure, a plate reader analyzed absorbance, and, a standard curve was created to calculate the concentration.



Excel was used to find the standard curve and the best fit line to find the unknown concentrations (pg/mL) of DNMT3L. After grouping the randomized samples by neurodegenerative condition (e.g. Folic Acid Deficient DSP_4 Mice), an average concentration was used to develop the bar graphs (Figures 1-4). T-tests calculated significance ($P<0.05$) between different conditions.

Folate Cycle Metabolites, DNMT3L, and Methylation Pathways



Results

KEY

NFC- Normal Folate Acid Control
FAD- Folic Acid Deficient
DS- Down's Syndrome
DSP4- Alzheimer's Disease, model

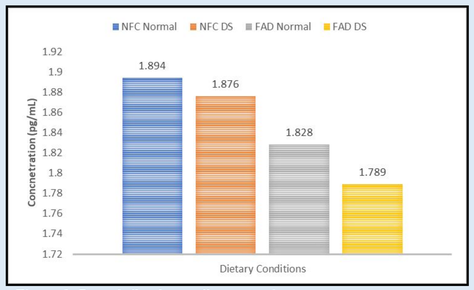


Figure 1. Down's Syndrome Concentrations
Average concentration for the dietary conditions in the Down's Syndrome and control livers. No significant difference (NSD).

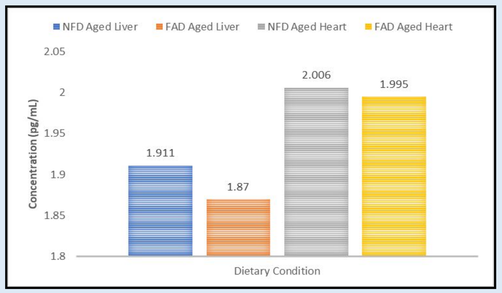


Figure 2. Aged Liver and Heart Concentrations
Average concentrations for aged liver and hearts. Significance between NFD and FAD Liver ($p<0.06$)

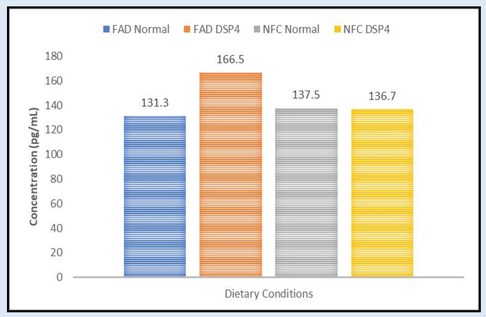


Figure 3. Alzheimer's Hearts Concentrations
Average concentrations in the DSP4/Alzheimer's hearts. No significance difference (NSD).

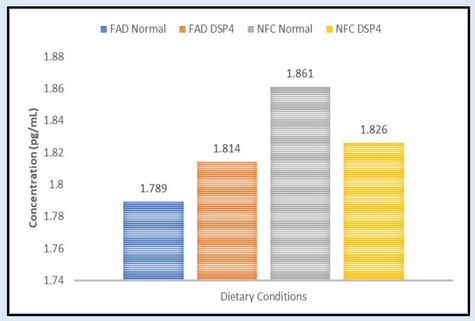


Figure 4. Alzheimer's Livers Concentrations
Average concentrations in the DSP4/Alzheimer's livers. No significance difference (NSD).

Results continued

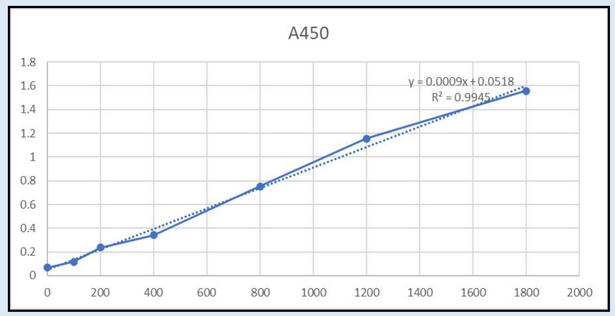


Figure 5. Standard Curve
Standard curve calculated from ELISA absorbance raw data, with linear equation and r-squared value

Conclusions

In the results section, many Figures had similar concentrations in each dietary grouping. There was no significance found when calculating the T-test. When comparing the Folic Acid Deficient Down's Syndrome liver to the Folic Acid Deficient normal liver, the p-value was around 0.06 and was approaching significance. There was significance calculated between the aged liver and heart. The significance shows that the metabolites are proportionately distributed between tissues when standardized by weight. Although there is NSD, the figures illustrate, a trend toward a higher concentration of DNMT3L in mice with a normal folic acid diet, and the Alzheimer's hearts. This data can be used as starting point to test for DNMT3L in other disease types as well as other tissue types. Follow up research could be done to find out why there was higher concentration in the Alzheimer's hearts. There could also be an experiment done on Down's Syndrome hearts and that data can be compared to this data on Down's Syndrome livers for significance. Overall, this experiment was successful in showing us more information on the effect of folic acid deficiency in the methylation pathway.

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