Unbiased proteomic analysis reveals dietary choline deficiency induces changes to neurodegeneration-relevant pathways in the 3xTg-AD mouse model of Alzheimer's disease

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INTRODUCTION

Alzheimer's Disease (AD) is the most common cause of dementia.

There is an urgent need for mechanistic insight into **modifiable environmental risk factors** to offset disease.

Today

More than **6 million** Americans have AD.

AD costs the US **$355 billion** annually.

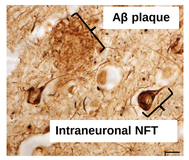
More than **11 million Americans** provide unpaid care, valued at nearly **$257 billion**.

By 2050

~13 million patients projected in the US by 2050 with projected annual costs up to **~$1.1 trillion**.

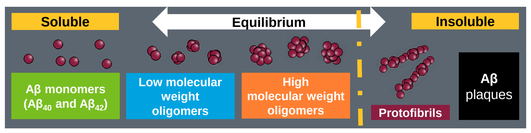
Alzheimer's statistics from: Alzheimer's Association Facts and Figures (https://www.alz.org/alzheimers-dementia/facts-figures)

Pathological Characteristics of AD



- Extra-neuronal plaques of Amyloid Beta (Aβ)
- Intra-neuronal neurofibrillary tangles (NFTs) of hyperphosphorylated tau
- Neuroinflammation and cell death

$A\beta_{40}$ and $A\beta_{42}$ are the main Aβ isoforms that combine to form oligomers and insoluble plaques.

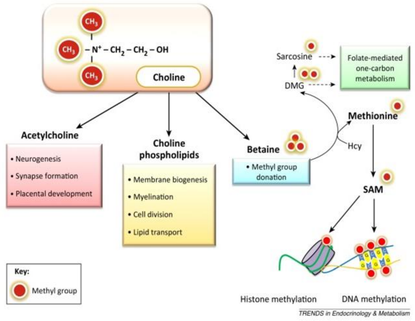


Eventually, neurons degenerate, leading to **memory loss**, decline in executive functioning, and **changes to mood, behavior and personality**.

There is no cure.

AD Pathology and Histology Image from Winblad et al – *Lancet Neurol* 2016; 15:455–532
Soluble and insoluble $A\beta_{40-42}$: Yang, et al – *J Neurosci* 2017; 37(1):152-163

Choline plays an integral role in diverse neurodegeneration-relevant pathways such as acetylcholine biosynthesis, myelination, synapse formation, neurogenesis, and epigenetic modification.



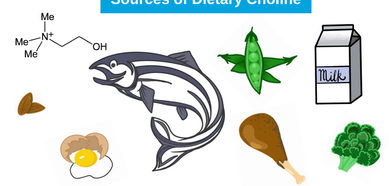
Dietary Choline

The dietary nutrient **choline** is found in a variety of foods.

Adequate intake values were established in 1998.

Recent reports suggest **90% of Americans do not meet** these requirements, which may affect cognition.

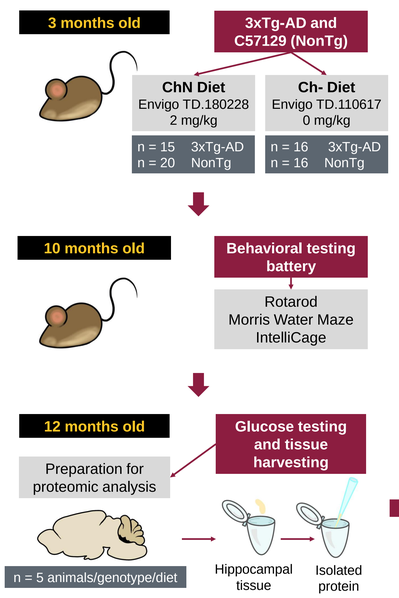
Sources of Dietary Choline



Choline metabolism: Jiang, West and Caudill - *Trends Endocrinol Metab* 2014; 25(5):263-73
Adequate intake in Americans: Wallace et al - *Nutr. Today* 2018; 53:240–253
Inadequate choline and cognition: Liu et al – *Behav Neurology* 2021; 2021(296225):1-11
Dietary choline sources from Table 2 of Zeisal and da Costa - *Nutr Rev.* 2009; 67(11):615-23

Inadequate dietary choline may contribute to an **increase in AD pathology**.

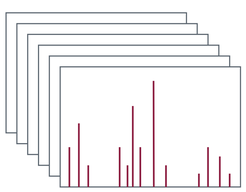
METHODS



3xTg-AD Mouse Model

Human mutations	Aβ plaques	Behavior phenotype	Tau pathology
APPSw PSEN1M146V MAPTP301L	Aβ plaques begin at 6 months, widespread by 12 months.	Cognitive impairment at 6 months of age.	Neurofibrillary tangles widespread by 12 months.

3xTg-AD Mode Genetics and Behavior: Alzheimer's Forum Research Models, 3xTg (https://www.alzforum.org/research-models/3xtg)
Development of plaques: Oddo et al – *Neuron* 2003; 39:409-421.
Development of tau pathology: Oh et al – *Int J Alz Dis* 2010; 2010(78102):1-25



Label-free quantification (LFQ) was performed using high-resolution mass spectrometry.

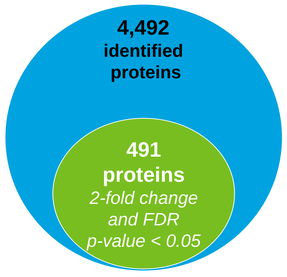
Determines differentially abundant proteins across experimental groups.

Gene Set Enrichment Analysis (GSEA; hypergeometric test) using STRING (v11.0)

1. Analyzed number of proteins in each network annotated with a particular pathway term.
2. Measured the strength, or how large the enrichment effect was, using:
   - the number of proteins in the network that were annotated with the term, and
   - the number of expected proteins annotated with this term in random network of the same size.
3. Addressed significance of enrichment with Benjamini–Hochberg correction for multiple comparisons.

RESULTS

Choline deficiency induced protein alterations in **3xTg-AD mice**



n = 5 per diet, 2 technical replicates

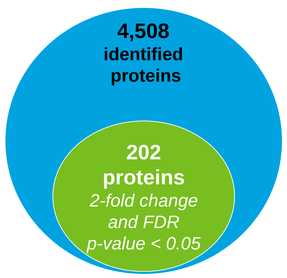
348 proteins downregulated

Description	Functional Enrichment Analysis	Count in Network	Strength	FDR
Mitochondrial RNA processing	Gene Ontology: Biological Process	3 of 13	1.2	0.0342
Cellular response to insulin-like growth factor stimulus	Gene Ontology: Biological Process	3 of 14	1.17	0.0381
Negative regulation of blood vessel endothelial cell migration	Gene Ontology: Biological Process	4 of 32	0.94	0.038
Scaffold protein binding	Gene Ontology: Molecular Function	7 of 70	0.84	0.0035
Tau and MAP protein, tubulin-binding repeat	Protein Domains (Pfam Database)	3 of 3	1.84	0.0266

143 proteins upregulated

Description	Functional Enrichment Analysis	Count in Network	Strength	FDR
Intermediate filament organization	Gene Ontology: Biological Process	4 of 23	1.43	0.0054
Intermediate filament cytoskeleton organization	Gene Ontology: Biological Process	5 of 41	1.27	0.0031
Cellular response to calcium ion	Gene Ontology: Biological Process	5 of 66	1.07	0.0129
Axon ensheathment	Gene Ontology: Biological Process	5 of 97	0.9	0.0485
Calmodulin binding	Gene Ontology: Molecular Function	8 of 183	0.83	0.0031
Cytoskeletal protein binding	Gene Ontology: Molecular Function	18 of 877	0.5	0.0021
Myelin proteolipid protein PLP, conserved site	Protein Domains & Features (InterPro Database)	2 of 3	2.01	0.0268
Myelin proteolipid protein PLP	Protein Domains & Features (InterPro Database)	2 of 3	2.01	0.0268

Choline deficiency also induced protein alterations in **NonTg mice**



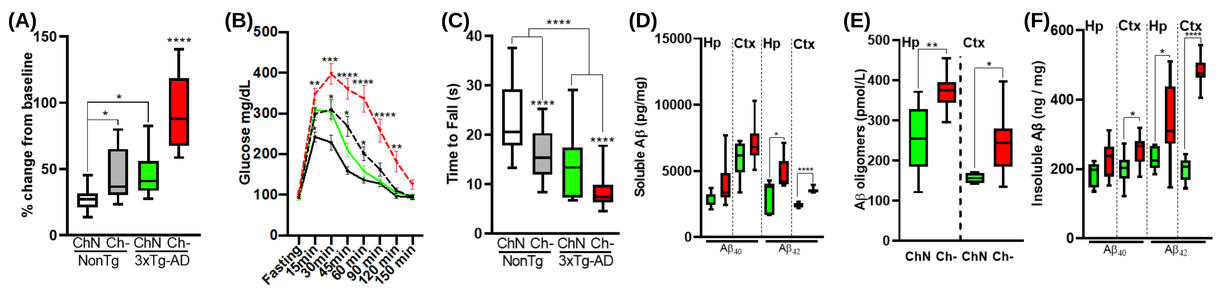
n = 5 per diet, 2 technical replicates

109 proteins downregulated

Description	Functional Enrichment Analysis	Count in Network	Strength	FDR
Generation of neurons	Gene Ontology: Biological Process	20 of 1538	0.44	0.0454
Nervous system development	Gene Ontology: Biological Process	25 of 2181	0.39	0.0454
Metabolism	Reactome Pathway Analysis	15 of 1346	0.38	0.037
Metabolic pathways	KEGG Pathway Analysis	16 of 1296	0.42	0.0438

93 proteins upregulated

Description	Functional Enrichment Analysis	Count in Network	Strength	FDR
Axon ensheathment	Gene Ontology: Biological Process	4 of 97	1.02	0.0451
Autophagy	Gene Ontology: Biological Process	6 of 192	0.9	0.0278
Exocytosis	Gene Ontology: Biological Process	6 of 202	0.88	0.0278
Vesicle-mediated transport	Gene Ontology: Biological Process	17 of 1020	0.63	0.001
Structural constituent of myelin sheath	Gene Ontology: Molecular Function	2 of 10	1.7	0.0184



NonTg ChN
NonTg Ch-
3xTg-AD ChN
3xTg-AD Ch-

(A) Choline deficiency leads to weight gain in NonTg and 3xTg-AD mice: % weight change from baseline.
(B) Choline deficiency impairs glucose tolerance in NonTg and 3xTg-AD mice: Glucose levels after 16 hrs fasting and subsequent 2.0g/kg glucose injection.
(C) Choline deficiency impairs motor function in NonTg and 3xTg-AD mice: Ch- mice show decreased time to fall in the rotarod task.
(D-F) Choline deficiency elevates soluble, oligomeric, and insoluble $A\beta_{40-42}$: (D) Soluble $A\beta_{40-42}$ levels in Cortex (Ctx) and Hippocampus (Hp) of 3xTg-AD mice. (E) Toxic soluble oligomer levels in the Hp and Ctx of 3xTg-AD mice. (F) Insoluble $A\beta_{40-42}$ levels in the Hp and Ctx of 3xTg-AD mice.

Data are means ± SE. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

CONCLUSIONS

Collectively, these results highlight that **inadequate dietary choline alters AD-relevant biochemical pathways** in the hippocampus of NonTg mice as well as in 3xTg-AD mice and may **increase pathologies seen in AD**.

Scan the QR code to read more about this project and other ongoing research within the Velazquez Lab of Neurodegenerative Research!