Dietary choline deficiency throughout adulthood induces systems-wide dysfunction and increases Alzheimer's disease risk across several pathogenic axes

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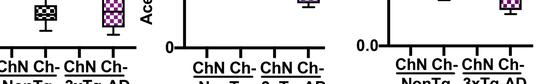
Background

- Alzheimer's disease (AD) is a widespread and costly disease, necessitating insight into modifiable risk factors, such as diet, to reduce disease incidence.[1]
- Choline plays key roles in body and brain function.[2]
- The phosphatidylethanolamine N-methyltransferase (PEMT) enzyme in the liver produces choline endogenously, but is insufficient for healthy metabolic function, necessitating dietary intake.[3]
- Choline supplementation above the adequate daily intake (ADI) has been shown to reduce AD pathology.[4]
- Low choline intake is associated with incidence of AD and dementia prevalence[5]
- However, ~90% of Americans do not reach the ADI. [6]
- ***Here, we investigate the role of choline deficiency on AD pathogenesis.***

Experimental Timeline and Groups

Genotype	Diet	n/group
NonTg	ChN	20
3xTg-AD	ChN	15
NonTg	Ch-	18
3xTG-AD	Ch-	16

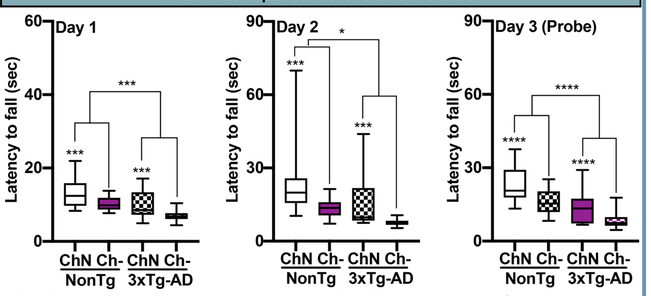
- ChN mice: standard laboratory chow diet with normal choline levels (ChN; 2.0g/kg), based on the human ADI
- Ch- mice: laboratory chow choline deficient (Ch-; 0.0g/kg) diet
- All other dietary values were identical between diets
- Experimental diets were started at 3 months



Choline and acetylcholine (ACh) were decreased in Ch- diet groups. No significant differences in liver PEMT across groups, illustrating effects were due to diet regimen

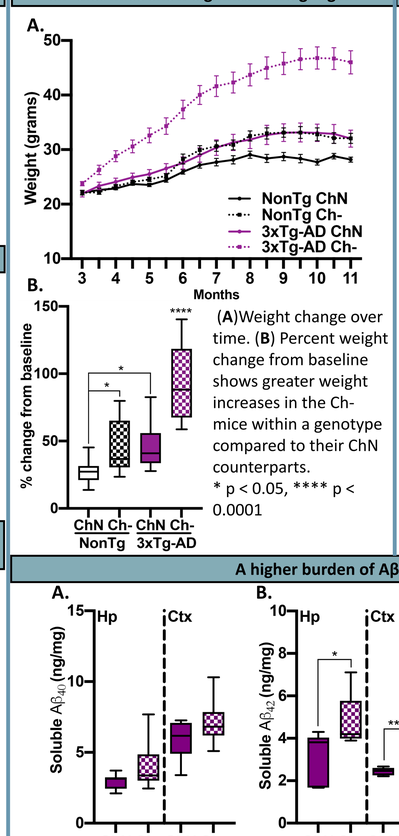
Plasma choline (**A**) and ACh (**B**) were decreased in both Ch- groups. (**C**) Liver PEMT levels were similar between all groups. Choline levels in the Hippocampus (**D**, Hp) and Cortex (**E**, Ctx) were reduced in the Ch- groups. **** p < 0.0001

A Ch- diet impaired motor skill on rotarod



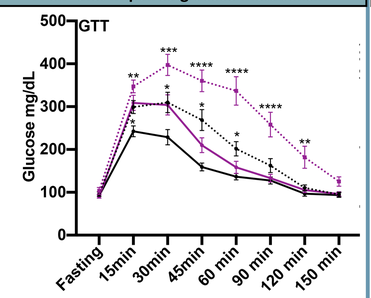
On all days of rotarod testing, 3xTg-AD mice fell off the rotating rod (i.e., lower latency to fall) than non-Tg mice. Within each genotype, the Ch- mice had lower latency to fall than their ChN counterparts.

Ch- diet mice show significant weight gain



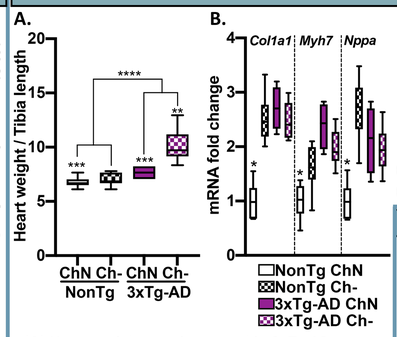
(**A**)Weight change over time. (**B**) Percent weight change from baseline shows greater weight increases in the Ch- mice within a genotype compared to their ChN counterparts.
* p < 0.05, **** p < 0.0001

A Ch- diet impaired glucose metabolism

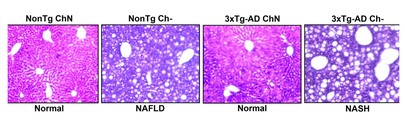


3xTg-AD mice show higher glucose levels than NonTg mice. Ch- mice had higher glucose levels than the ChN mice. We also found a significant genotype by diet by time interaction and post hoc analysis revealed that 3xTg-AD Ch- mice had significantly higher glucose levels from the 15 through 150-minute time points compared to their ChN counterparts (p < 0.05). NonTg Ch- mice had higher glucose levels than their ChN counterparts at the 15 through 60 min time points (p < 0.05). ** p < 0.01, *** p < 0.0001, **** p < 0.0001

Ch- diet induced cardiac and liver pathology



(**A**) Heart weight was increased in 3xTg-AD compared to NonTg mice. Within each genotype, Ch- mice had larger hearts than ChN. 3xTg-AD Ch- mice showed significantly higher heart weight than all other groups. (**B**) *Col1a1, Myh7, and Nppa*, genes whose increased expression is associated with cardiac pathology are elevated in NonTg Ch- and both 3xTg-AD groups compared to Non-Tg ChN. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001

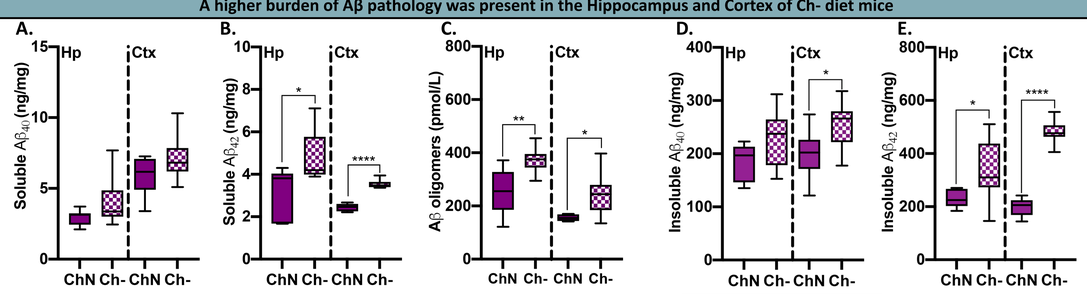


D.

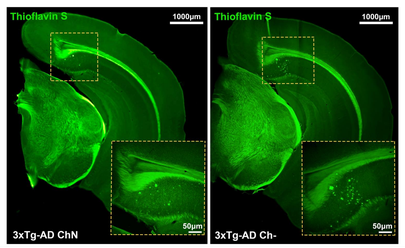
Genotype	Diet	n/group	Normal	NAFLD	NASH
NonTG	ChN	4	4	0	0
3xTG-AD	ChN	4	3	1	0
Non-Tg	Ch-	4	0	4	0
3xTG-AD	Ch-	4	0	1	3

(**C**) Representative liver images from each group. (**D**) Table reporting non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) in each group.

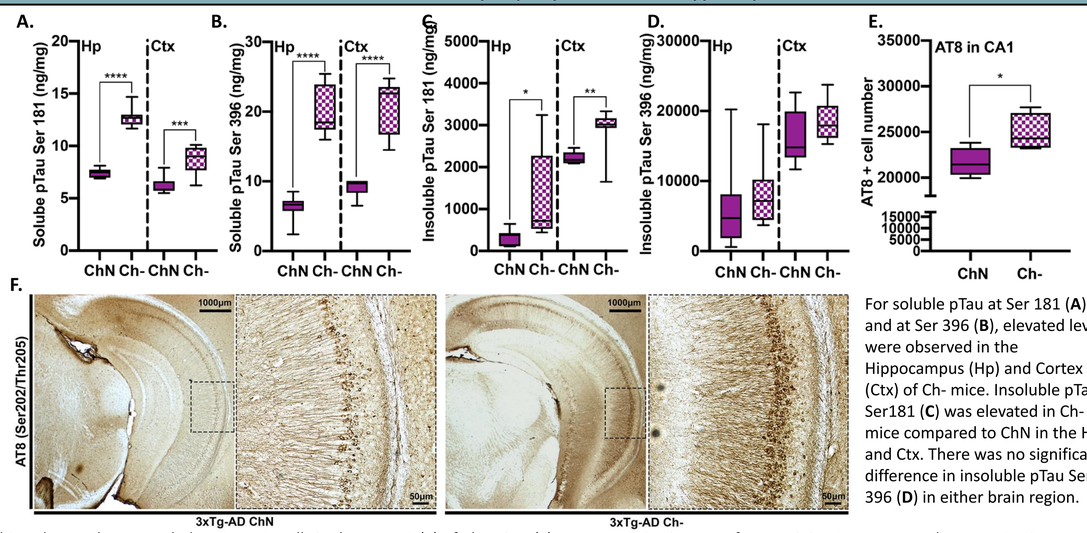
A higher burden of Aβ pathology was present in the Hippocampus and Cortex of Ch- diet mice



For soluble Aβ, (**A**) Aβ40 wasn't significantly different in the diet groups in either the hippocampus (Hp) or cortex (Ctx), but (**B**) Aβ42 was elevated in Ch- mice in both the Hp and Ctx. (**C**) Aβ oligomers were elevated in the Hp and Ctx of Ch- mice. (**D**) Insoluble Aβ40 was elevated in the Ctx of Ch- mice, but not in the Hp. (**E**) Insoluble Aβ42 was increased in the Hp and Ctx of Ch- mice. (**F**) Thioflavin S staining illustrates Aβ plaques in the Hp and Ctx. Note, only 3xTg-AD mice were used here because NonTg mice do not develop pathology. * p < 0.05, ** p < 0.01, **** p < 0.0001



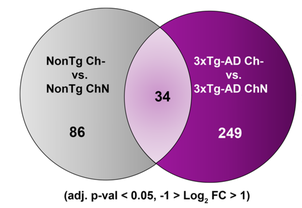
Ch- diet mice exhibited increased phosphorylated tau in the Hippocampus and Cortex



For soluble pTau at Ser 181 (**A**) and at Ser 396 (**B**), elevated levels were observed in the Hippocampus (Hp) and Cortex (Ctx) of Ch- mice. Insoluble pTau Ser181 (**C**) was elevated in Ch- mice compared to ChN in the Hp and Ctx. There was no significant difference in insoluble pTau Ser 396 (**D**) in either brain region.

Unbiased stereology revealed more AT8+ cells in the Hp CA1 (**E**) of Ch- mice. (**F**) Representative images of AT8 staining CA1. Note, only 3xTg-AD mice were used for tau pathology. * p < 0.05, ** p < 0.01, **** p < 0.0001

A Ch- diet alters hippocampal protein networks in both NonTg and 3xTg-AD mice



B. NonTg Ch- vs. NonTg ChN

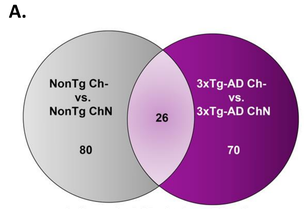
GO-term	Description	Count in network	Strength	FDR
Biological Process				
GO:0045861	Negative regulation of proteolysis	9 of 361	0.83	0.0189
GO:1901564	Organonitrogen compound metabolic process	35 of 4475	0.32	0.0112
GO:0006807	Nitrogen compound metabolic process	42 of 5878	0.28	0.0100
GO:0044237	Cellular metabolic process	45 of 6445	0.27	0.0077
GO:0008152	Metabolic process	50 of 7331	0.26	0.0059
GO:0071704	Organic substance metabolic process	46 of 6824	0.25	0.0100
GO:0044238	Primary metabolic process	42 of 6369	0.24	0.0429
GO:0009987	Cellular process	71 of 13330	0.15	0.0059

C. 3xTg-AD Ch- vs. 3xTg-AD ChN

GO-term	Description	Count in network	Strength	FDR
Biological Process				
GO:0045109	Intermediate filament organization	6 of 25	1.33	0.00057
GO:0035728	Response to hepatocyte growth factor	4 of 21	1.23	0.0198
GO:0045104	Intermediate filament cytoskeleton organization	8 of 46	1.19	0.00018
GO:0018149	Peptide cross-linking	4 of 24	1.18	0.0278
GO:1903539	Protein localization to postsynaptic membrane	6 of 43	1.10	0.0035
GO:0099072	Regulation of postsynaptic membrane neurotran..	8 of 62	1.06	0.00057
GO:0099637	Neurotransmitter receptor transport	5 of 39	1.06	0.0160
GO:0035418	Protein localization to synapse	8 of 75	0.98	0.0012
GO:0007215	Glutamate receptor signaling pathway	5 of 47	0.98	0.0292
GO:0097120	Receptor localization to synapse	5 of 50	0.95	0.0354
GO:0090630	Activation of gtpase activity	8 of 90	0.90	0.0030
GO:0098876	Vesicle-mediated transport to the plasma membr..	6 of 83	0.81	0.0439
GO:0050772	Positive regulation of axonogenesis	7 of 104	0.78	0.0273
GO:2000300	Regulation of synaptic vesicle exocytosis	7 of 108	0.77	0.0313
GO:0048167	Regulation of synaptic plasticity	14 of 221	0.76	0.00028

Liquid chromatography tandem mass spectrometry followed by label free quantification identified (adj. p-val < 0.05, -1 > Log_2 FC > 1) 86 differentially abundant proteins between NonTg Ch- and NonTg ChN, and 249 differentially abundant proteins between 3xTg-AD Ch- and 3xTg-AD ChN. (**A**) 34 proteins were identified as differentially abundant due to Ch- in both NonTg and 3xTg-AD. (**B, C**) NonTg ChN vs. NonTg Ch- and 3xTg-AD ChN vs. 3xTg- AD Ch- gene ontology (GO) biological process classification analyses. (for C, the top 15 biological processes are displayed based on strength of prediction).

A Ch- diet alters plasma protein networks in both NonTg and 3xTg-AD mice



B. NonTg Ch- vs.NonTg ChN

GO-term	Description	Count in network	Strength	FDR
Biological Process				
GO:0006957	Complement activation, alternative..	3 of 10	2.1	0.0111
GO:0006000	Fructose metabolic process	3 of 11	2.06	0.0111
GO:0019835	Cytolysis	5 of 24	1.95	8.68e-05
GO:0006953	Acute-phase response	4 of 40	1.63	0.0111
GO:0002526	Acute inflammatory response	5 of 76	1.45	0.0085
GO:0016064	Immunoglobulin mediated immune..	4 of 80	1.33	0.0435
GO:0002443	Leukocyte mediated immunity	5 of 173	1.09	0.0472
GO:0002250	Adaptive immune response	6 of 280	0.96	0.0472
GO:0010038	Response to metal ion	7 of 374	0.9	0.0344
GO:0010035	Response to inorganic substance	9 of 556	0.84	0.0111
GO:0043603	Cellular amide metabolic process	9 of 689	0.74	0.0348
GO:0006952	Defense response	13 of 1133	0.69	0.0085
GO:0051707	Response to other organism	12 of 1145	0.65	0.0190

C. 3xTg-AD Ch- vs. 3xTg-AD ChN

GO-term	Description	Count in network	Strength	FDR
Molecular Function				
GO:0015288	Porin activity	2 of 5	2.25	0.0307
GO:0043559	Insulin binding	2 of 6	2.17	0.0375
GO:0051082	Unfolded protein binding	5 of 82	1.43	0.00084
GO:0008144	Drug binding	4 of 116	1.18	0.0390
GO:0042802	Identical protein binding	21 of 1962	0.67	1.56e-06
GO:0036094	Small molecule binding	22 of 2406	0.61	3.78e-06
GO:0000166	Nucleotide binding	16 of 2021	0.54	0.0028
GO:0043168	Anion binding	20 of 2678	0.52	0.00040
GO:0005515	Protein binding	36 of 6764	0.37	3.78e-06
GO:1901363	Heterocyclic compound binding	24 of 4771	0.35	0.0122
GO:0097159	Organic cyclic compound binding	24 of 4858	0.34	0.0149
GO:0005488	Binding	43 of 11199	0.23	0.00015

Liquid chromatography tandem mass spectrometry followed by label free quantification (adj. p-val < 0.05, -1 > Log_2 FC > 1) identified 80 differentially abundant proteins between NonTg Ch- and NonTg ChN plasma, and 70 differentially abundant proteins between 3xTg-AD ChN- and 3xTg-AD ChN plasma. (**A**) 26 proteins were commonly identified as differentially abundant due to Ch- in both NonTg and 3xTg-AD plasma. (**B,C**) NonTg ChN vs. NonTg Ch- gene ontology (GO) biological process classification analysis and 3xTg-AD ChN vs. 3xTg-AD Ch- GO molecular function classification analysis.

Conclusions

- We showed that dietary choline deficiency (Ch-) throughout adulthood negatively impacts cellular and molecular function across a variety of biological systems.
- Our measurements in plasma and brain tissue confirmed that Ch- resulted in significantly lower levels of choline. Ch- led to motor impairments, weight gain, impaired glucose metabolism, cardiac pathology, and hepatic disease.
- In the brain, we found that Ch- increased Aβ and pathological tau markers in both the hippocampus and cortex, two areas affected in AD.[7]
- Our proteomic analysis of the hippocampus revealed that Ch- altered the expression of proteins networks related to AD pathogenesis including microtubule function and postsynaptic membrane regulation. Our plasma proteomic analysis, we found dysregulation of inflammatory-response and insulin-signaling related proteins, which corresponds to our physiological observations as well as well-known risk factors that link the peripheral system with the aging brain.

Together, these data illustrate the importance of adequate dietary choline intake to promote healthy aging across multiple bodily systems and show that Ch- poses significant health risks for a large majority of the American population.

Acknowledgements

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Data Availability

Proteomic data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository.

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