Potential Link of High Fat Diet on the Expression of Alzheimer s Disease Related Genes in the Ileal Mucosa of Alzheimer's Disease Model of Rats

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PLAIN LANGUAGE SYNOPSIS

The consumption of high-fat diet caused the differential expression of Alzheimer's disease related genes in WT animals as compared to APP/PS1 overexpressed AD rat model. Identified genes play crucial role in the synaptic transmission and have been suggested to play a role in the development of neurodegenerative diseases.

ABSTRACT

A high-fat diet (HFD) is shown to have a profound effect on the gut and brain, and its consumption is linked to dementia accelerating Alzheimer's disease (AD) pathology. The aim of this study is to assess if HFD has any impact on the expression of AD related genes in the ileal mucosa of the gastrointestinal tract (GIT) and its potential links to the development of AD, using the RT-qPCR gene expression method in both wild-type (WT) and APP/PS1 (Amyloid precursor protein/Presenilin 1) overexpressed AD rat model. Our findings show that HFD significantly increased the mRNA expression of AD related key genes in the ileal tissue. Out of a total of 84 AD related genes, the metabolic consequences of HFD contributed to 52.4% of altered gene expression both WT and AD rats. Among them, WT rats had a higher percentage of AD genes expressed (88.6%) than in AD rats (36.4%). The male rats showed significantly lower (9.09%) expression of those genes in the AD model than in WT (70.5%). Likewise, female rats also showed a similar pattern of lower gene expression in the AD model (32%) than in WT (52.3%). In female WT rats, expression of apoptotic genes such as *App* (amyloid beta precursor protein) and *EP300* (E1A binding protein p300) was markedly increased due to HFD. Additionally, in the HFD fed AD rats there was overexpression of genes like *Bdnf* (Brain derived neurotrophic factor) in females and *Chat* (choline acetyltransferase) in males, which are crucial for synaptic transmission. Earlier it was shown that the mucosal BDNF is in close proximity to the sensory nerve terminals; similarly, *Chat* is present in the cholinergic neurons in the central as well as peripheral nervous systems. The higher percent of AD related genes expressed in WT on a HFD suggests that long-term consumption of HFD may have an impact on the expression of key neurotransmitters. The findings also show that the females are relatively more vulnerable to developing AD while consuming HFD. Studies are ongoing to correlate these gene expression patterns with the localization in the intestinal mucosa and abundance of microbiota in the GIT. This study will aid in the development of novel therapeutics for the prevention of AD.

INTRODUCTION

- Alzheimer's disease (AD) is a progressive neurodegenerative disorder and is characterized by β-amyloid (Aβ) protein plaques and hyper-phosphorylation of tau protein in the form of neurofibrillary tangles (NFTs) in the brain.
- In the literature, several studies have shown close connection between the gut and brain, due to the virtue of secretion of several neurotransmitters, hormones, cytokines, and chemokines factors from the intestine. These secreted molecules are known to communicate with the central nervous system (CNS) through immune, circulatory, and neuroendocrine signaling pathways.
- The brain, on the other hand, can release stress and endocrine factors- which then influence the gastrointestinal health via the "gut-brain axis". A healthy GIT is vital for normal cognitive and emotional processing.
- While genetics and family history are important risk factors for the development and progression of AD, - environment and lifestyle are also key contributing risk factors. One such environment factor is diet, which has emerged as a key influencer of AD development and progression.
- The HFD can have a profound effect on the gut and brain axis, and some of which are also sex dependent.
- Interestingly, studies in animal models revealed that HFD increased cognitive impairment and AD pathologies like inflammation. However, the underlying mechanisms is still not very clear.

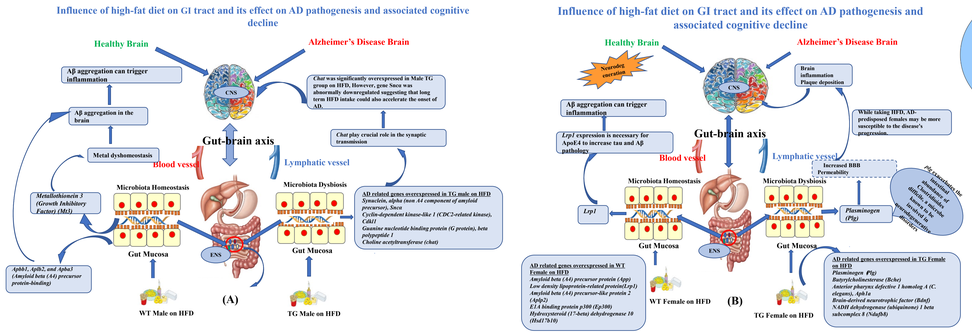


Figure 1: The figure shows possible molecular mechanisms of HFD for signaling of the gut-brain axis in control and disease model of AD. In figure (A), the left side represents the AD related genes overexpressed in healthy intestine (WT males) while consuming HFD, and the right side shows AD related genes overexpressed in the diseased intestine (TG males) fed HFD. The WT male rats had a higher number of genes expressed than the AD rats. It is possible that long-term consumption of HFD may affect the expression of genes associated to Aß generation (Apbb1, Aplb2, and Apba3) and metal dys-homeostasis (Mt3), which are known to be implicated in the pathogenesis of AD. Likewise, In figure (B), the left side represents the AD related genes overexpressed in healthy intestine (WT females) while on HFD, and the right side shows AD related genes overexpressed in the diseased intestine (TG females) fed HFD. It has been speculated that excessive consumption of HFD may compromise the integrity of the intestinal barrier that may damage BBB (leaky gut) resulting from external assault by excessive numbers of Aß-containing lipoproteins from a diet high in saturated fats and cholesterol.

AIM OF THE STUDY

- The aim of this study is to assess if introduction of HFD at an adult age has any impact on expression of AD related genes in WT rats and APP/PS1 overexpressing AD rats. In addition, we aim to determine the molecular mechanisms of HFD on signaling the gut-brain axis.
- The research highlights the impact of HFD on mRNA expression of genes in the ileal mucosa that has been suggested to play a role in the development, and progression of AD-related pathologies in the AD rat model.

MATERIALS AND METHODS

Animal Housing and Care

- Use of animals for this study was approved by the NCTR Animal Care and Use Committee and was done under protocols that were approved by the NCTR institutional animal care and use committee (IACUC).
- Experimental animals consisted of different groups of TgF344-AD transgenic rats along with the wild type (WT) [Fischer 344 (F344)] rats of both sexes. TgF344-AD rats express human APP with the Swedish mutation and human PSEN1 with the Δ exon 9 mutation.
- The TgF344-APP/PS1 rats were PCR genotyped when they were young by using traditional PCR analysis using ear clips to confirm overexpression of APP/PS1 genes. However, after sacrifice of these animals, modified method of K114 as well as Congo Red staining was performed to confirm the presence of amyloid plaques in brain.

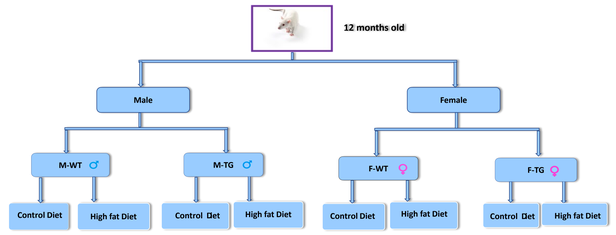
Animal sacrifice

- All the rats in this study received an overdose of Euthasol (100 mg/kg; Vibrac AH, Inc., USA) prior to sacrifice**.** After sacrifice of these animals, modified method of K114 as well as Congo Red staining was performed to confirm the presence of amyloid plaques .

Experimental Design

- At age of 12 months, one set of both WT and TG rats (male and female) were kept on the 6 months on the control diet (CD, 8% energy from fat; Rodent NTP-2000 National Toxicology Program from Zeigler), whereas the other set of animals were fed HFD (45% kcal energy from fat, D12451 Research Diets)
- The animals were sacrificed at 18 months, ileal tissues were collected to assess the gene expression (mRNA) profile of AD related genes.
- The Rat Alzheimer's disease RT^2 profiler™ PCR array profiles the expression of 84 genes important in the development, and progression of Alzheimer's disease (PARN-057ZA).

MATERIALS AND METHODS contd:



- 6 months of HFD diet
- Sacrifice at 18 months age

Figure 2: Schematic diagram of the study design. WT and AD rats were fed a high-fat dietary regime and sacrificed, and their ileal tissues were isolated to study gene expression.

Experimental Procedure

- The RNA was extracted from the tissue samples using the TRIzol reagent.
- Chloroform was added to cell lysate to obtain aqueous phase; an equal proportion of isopropanol was added and incubated at -20°C for overnight to precipitate the RNA.
- All samples containing RNA were treated using the TURBO DNA-free Kit before reverse transcription.
- The purified RNA was converted to cDNA
- The expression of AD related genes was detected by Bio-Rad CFX Opus 384 RT-qPCR detection system.
- The Fold regulation (FR) and *p*-values were exported from the Qiagen Data Analysis web portal were used in data representation. p-values in graphs are depicted by either '*' (p -value ≤ 0.05) or '†' (p -value ≤ 0.005).

RESULTS AND DISCUSSION

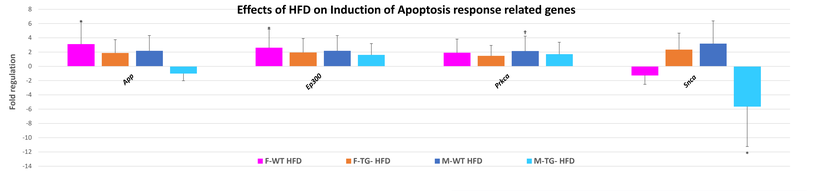
The HFD significantly increased the mRNA expression of genes in ileal tissue

*Figure 3: Venn diagram showing similarity in gene expression between **a)** WT male and female fed HFD, **b)** TG groups male and female fed HFD, **c)** male WT and male TG groups, and **d)** female WT and female TG groups on HFD.*

Figure 4: The animals fed HFD showed significant differences in the mRNA expression of the AD related genes (in several categories) when compared to animals received control diet.

TABLE: FUNCTIONS OF GENES OVEREXPRESSED IN ANIMALS FED WITH HFD

Genes	Wild-type		Experiment Group		Description	Function
	F-WT Hf/ HS Diet	M-WT Hf/ HS Diet	F-TG- Hf/ HS Diet	M-TG- Hf/ HS Diet		
Hsd17b10	x	x	x		Hydroxysteroid (17-beta) dehydrogenase 10	Alzheimer's Disease Genes (Oxidoreductases & Oxidative Stress)
Uqcrc1	x	x	x		Ubiquinol-cytochrome c reductase core protein I	Alzheimer's Disease Genes (Oxidoreductases & Oxidative Stress)
Uqcrc2	x	x	x		Ubiquinol cytochrome c reductase core protein 2	Alzheimer's Disease Genes (Oxidoreductases & Oxidative Stress)
Ctsd	x	x			Cathepsin D	Alzheimer's Disease Genes (Proteases)
Ctsl		x				Alzheimer's Disease Genes (Proteases)
App	x				Amyloid beta (A4) precursor protein	Apoptosis
Ep300	x				E1A binding protein p300	Apoptosis
Prkca		x			Protein kinase C, alpha	Induction of Apoptosis
Snca				x	Synuclein, alpha (non A4 component of amyloid precursor)	Apoptosis (Anti-Apoptotic)
Plg		x		x	Plasminogen	Beta-Amyloid Generation, Oligomerization, Clearance & Degradation
Lrp1	x	x			Low density lipoprotein-related protein 1 (alpha-2-macroglobulin receptor)	Beta-Amyloid Generation, Oligomerization, Clearance & Degradation
Ache		x			Acetylcholinesterase	Beta-Amyloid Generation, Oligomerization, Clearance & Degradation
Bche		x		x	Butyrylcholinesterase	Beta-Amyloid Generation, Oligomerization, Clearance & Degradation
Ide	x	x			Insulin degrading enzyme	Beta-Amyloid Generation, Oligomerization, Clearance & Degradation
Aph1a	x	x		x	Anterior pharynx defective 1 homolog A (C. elegans)	Beta-Amyloid Generation, Oligomerization, Clearance & Degradation
Ikbce2		x			Beta-site APP-cleaving enzyme 2	Beta-Amyloid Generation, Oligomerization, Clearance & Degradation
Mt3		x			Metallothionein 3 (Growth Inhibitory Factor)	Bind or release cellular transition metals
Cdk1	x	x	x		Cyclin-dependent kinase 1	Cell Cycle Genes
Cdkl1	x	x	x	x	Cyclin-dependent kinase-like 1 (CDC2-related kinase)	Cell Cycle Genes
Nictn	x	x			Nicastrin	Cell Signaling Molecules (Notch Signaling)
Mapt		x			Microtubule-associated protein tau	Cytoskeleton Regulators
Pkp4	x	x			Plakophilin 4	Cytoskeleton Regulators
Aplp2		x			Amyloid beta (A4) precursor protein-binding, family A, member 3	G-Protein Coupled Receptor (Intracellular Signaling F)
Gpr43		x			Growth associated protein 43	G-Protein Coupled Receptor (other cell signaling molecules)
Aplp2	x	x			Amyloid beta (A4) precursor-like protein 2	G-Protein Coupled Receptor (signaling)
Gnao1		x			Guanine nucleotide binding protein (G protein), alpha activating activity polypeptide O	G-Protein Coupled Receptor (Signaling)
Gnb1			x	x	Guanine nucleotide binding protein (G protein), beta polypeptide 1	G-Protein Coupled Receptor (Signaling)
Gnb5	x				Guanine nucleotide binding protein (G protein), beta 5	G-Protein Coupled Receptor (Signaling)
Gng10	x		x		Guanine nucleotide binding protein (G protein), gamma 10	G-Protein Coupled Receptor (Signaling)
Gng11		x			Guanine nucleotide binding protein (G protein), gamma 11	G-Protein Coupled Receptor (Signaling)
Gng5		x	x		Guanine nucleotide binding protein (G protein), gamma 5	G-Protein Coupled Receptor (Signaling)
Gng7	x				Guanine nucleotide binding protein (G protein), gamma 5	G-Protein Coupled Receptor (Signaling)
Gngt8		x			Guanine nucleotide binding protein (G protein), gamma 8	G-Protein Coupled Receptor (Signaling)
Ndufb8	x	x	x		NADH dehydrogenase (ubiquinone) 1 beta subcomplex 8	Involved in mitochondrial respiratory chain complex I assembly
Apoa1	x				Apolipoprotein A-I	Lipid & Lipoprotein Metabolism
Serpina3c			x		Serine (or cysteine) peptidase inhibitor, clade A, member 3K	Other Alzheimer's Disease Genes (Protease Inhibitors)
Gsk3b		x			Glycogen synthase kinase 3 beta	Protein Kinase
Insr	x	x			Insulin 2	Protein Kinases
Prkci	x	x			Protein kinase C, iota	Protein Kinases
Apba1	x				Amyloid beta (A4) precursor protein-binding, family A, member 1	Synaptic formation (other Synaptic functions)
Bdnf			x		Brain-derived neurotrophic factor	Synaptic formation (other Synaptic functions)
Chat				x	Choline acetyltransferase	Cholinergic Synaptic transmission)
Apbb1		x			Amyloid beta (A4) precursor protein-binding, family B, member 1 (Fe65)	Transcriptional Regulation
Apbb2	x				Amyloid beta (A4) precursor protein-binding, family B, member 2	Transcriptional Regulation
44	23	31	14	4		
%	52.27	70.45	31.82	9.09		



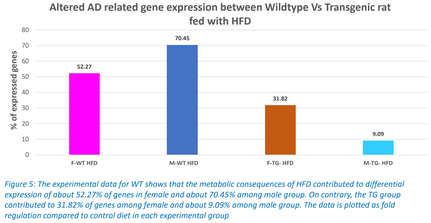


Figure 5: The experimental data for WT shows that the metabolic consequences of HFD contributed to differential expression of about 52.27% of genes in female and about 70.45% among male group. On contrary, the TG group contributed to 31.82% of genes among female and about 9.09% among male group. The data is plotted as fold regulation compared to control diet in each experimental group

Summary Table

Rats	Wildtype		Transgenic	
Genes	Upregulated Genes	Downregulated Genes	Upregulated Genes	Downregulated Genes
Male	31	0	3	1
Female	23	0	13	1

Table: Gene expression trends in male and female groups. The genes that are depicted as upregulated or downregulated, respectively, are those with fold changes larger than 2 or less than -2.

CONCLUSION

- In summary, poor diet and our lifestyle choices have a major effect on the expression of genes that have been suggested to play a role in the development of neurodegenerative diseases such as AD
- This study demonstrated that consumption of HFD for 24 weeks significantly perturbed the expression of AD related genes in the ileal tissue of GIT in both WT and AD rats.
- The WT rats had a higher number of genes expressed than in AD rats suggesting that long-term consumption of HFD may have some impact on the expression of key neurotransmitters that are known to be involved in the pathogenesis of AD.
- Also, the results showed that females are more susceptible to develop AD when consumed HFD. Thus, diet and lifestyle may be an effective approach to lowering AD risk.
- Studies are ongoing to correlate these gene expression patterns with the localization in the intestinal mucosa and abundance of microbiota in the GIT.
- More research is needed to fully understand the critical mechanisms of bi-directional communication between the gut microbiota and the brain.

FUTURE DIRECTIONS

- We are still in the process of measuring inflammatory cytokines in serum and ileum.
- Evaluation of the abundance of microbial population in stool and mucosa samples by 16S rRNA gene sequencing.
- Expression analysis of the cell-cell junction related genes by real time PCR.

FDA SIGNIFICANCE

The outcome of this study will be an effort to discover novel biomarkers and elucidate pathways for the gut-brain axis that may support the development of more effective AD therapies. The research will help, in future, in the development of novel therapeutics for AD and may benefit the FDA's commitment of improving the health of the American public through scientific and regulatory innovation.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare there exist no real or perceived conflict of interest.

***Disclaimer**: The information in this poster represents the opinions of the presenter and does not necessarily represent NCTR's or FDA's position or policy.*