THE EFFECT OF NERVE GROWTH FACTOR ADMINISTRATION ON GUT MICROBIOTA HOMEOSTASIS IN AN OBESE, TYPE 2 DIABETES MELLITUS, AND ALZHEIMER'S DISEASE MOUSE MODEL

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Introduction

■ High-Fat High-Sugar Western Diet

   – *Obesity*

      ■ Body Mass Index (BMI)

■ Type II Diabetes Mellitus (T2DM)

   – *Sustained high blood sugar levels*

   – *Pancreatic beta cell dysfunction*

■ Alzheimer's Disease (AD)

   – *Neurodegenerative disorder*

   – *Progressively greater cognitive decline*

   – ***Nerve Growth Factor (NGF)***

Weight Categories Based on BMI

Under Weight	Healthy Weight	Overweight	Obesity	Severe Obesity
<18.5	18.5-24.9	25.0-29.9	30.0-39.9	>40

→ **Microbiome**

Microbiome

- **Microbiome:** Diverse gene collection of 10-100 trillion microbial cells with majority in GI tract
  - *Protective barrier*
  - *Regulator of metabolism*
  - *Inflammation*
- Dysbiosis leads to chronic diseases
  - *Diet-induced obesity*
  - *Associations with impaired NGF signaling*
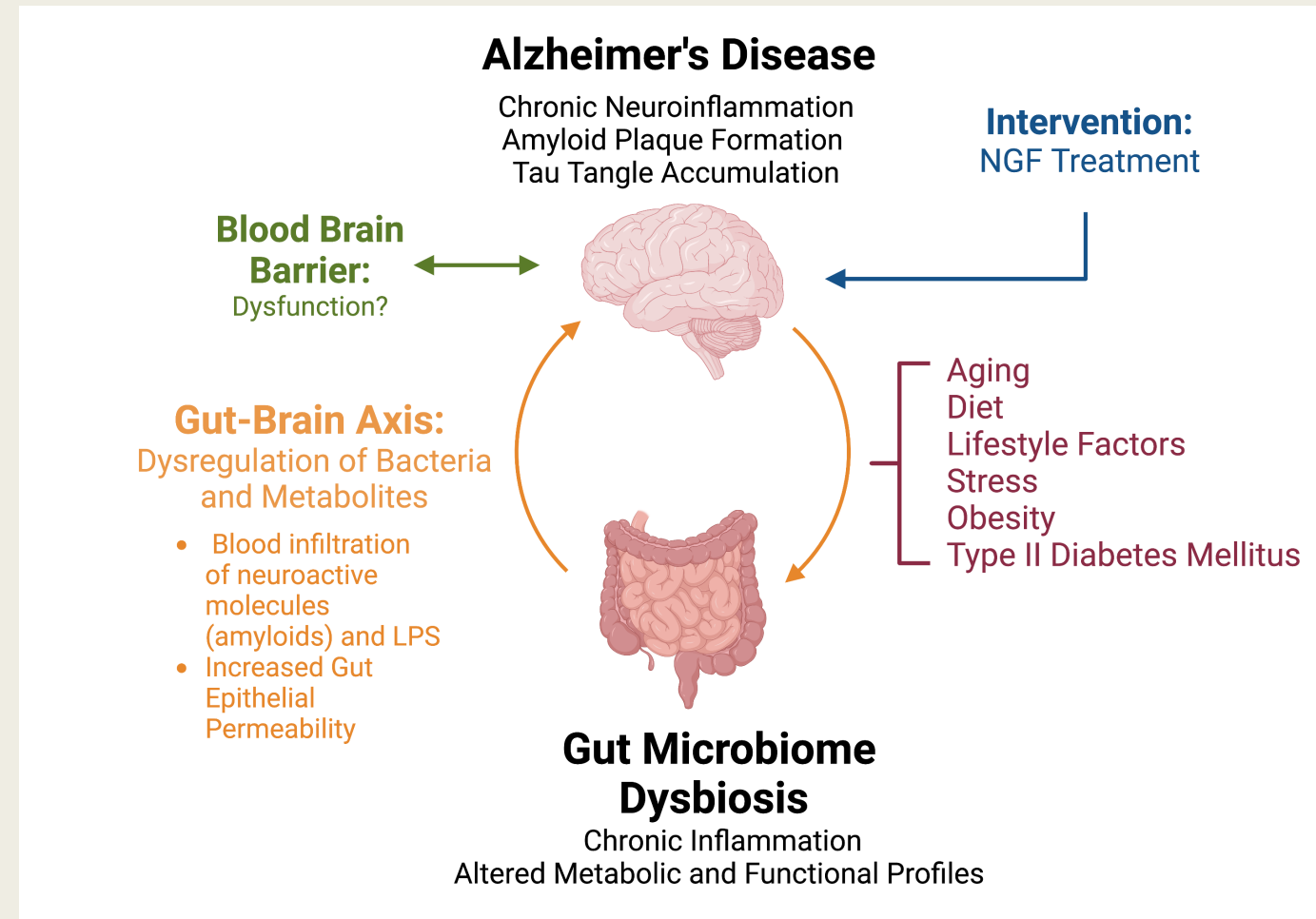
Central Question

- Does NGF administration restore microbiota homeostasis in an obese, T2DM, and AD mouse model via reducing pro-inflammatory and increasing anti-inflammatory microbial taxa and altering their proportions?

Timeline and Treatment Groups

| High-Fat/High-Sugar "Western Diet" | → | Obesity | → | Diabetes Alzheimer's Disease |

Dysregulation of gut microbiome = increased inflammation

NGF → regulation? = decreased inflammation

			Day	Week												
Group #	Group Name	Treatment	0	1	2	3	4	5	6	7	8	9	10	11	12	13
1	Lean Control + PBS	Chow Diet	Groups Separated base on Diet (chow or HFWD)								Groups 5 + 6: I.P. Injection with STZ at 40 mg/kg b.w.	Groups 2, 4, + 6: intranasal NGF (0.1 mg/mL PBS)		Insulin Tolerance Test	Overnight Fasting and Sacrifice of Animals. Fecal and Intestinal Sample Collection	
2	Lean Control + NGF	Chow Diet and Treatment														
3	HFWD	High Fat/High Sugar (Western) Diet														
4	HFWD + NGF	High Fat/High Sugar (Western) Diet and Treatment														
5	HFWD + STZ + PBS	Diabetes Model: High Fat/High Sugar (Western) Diet and STZ														
6	HFWD + STZ + NGF	Diabetes Model with Treatment: High Fat/High Sugar (Western) Diet, STZ, + NGF														
															Metabolic Cages	

Timeline and Treatment Groups
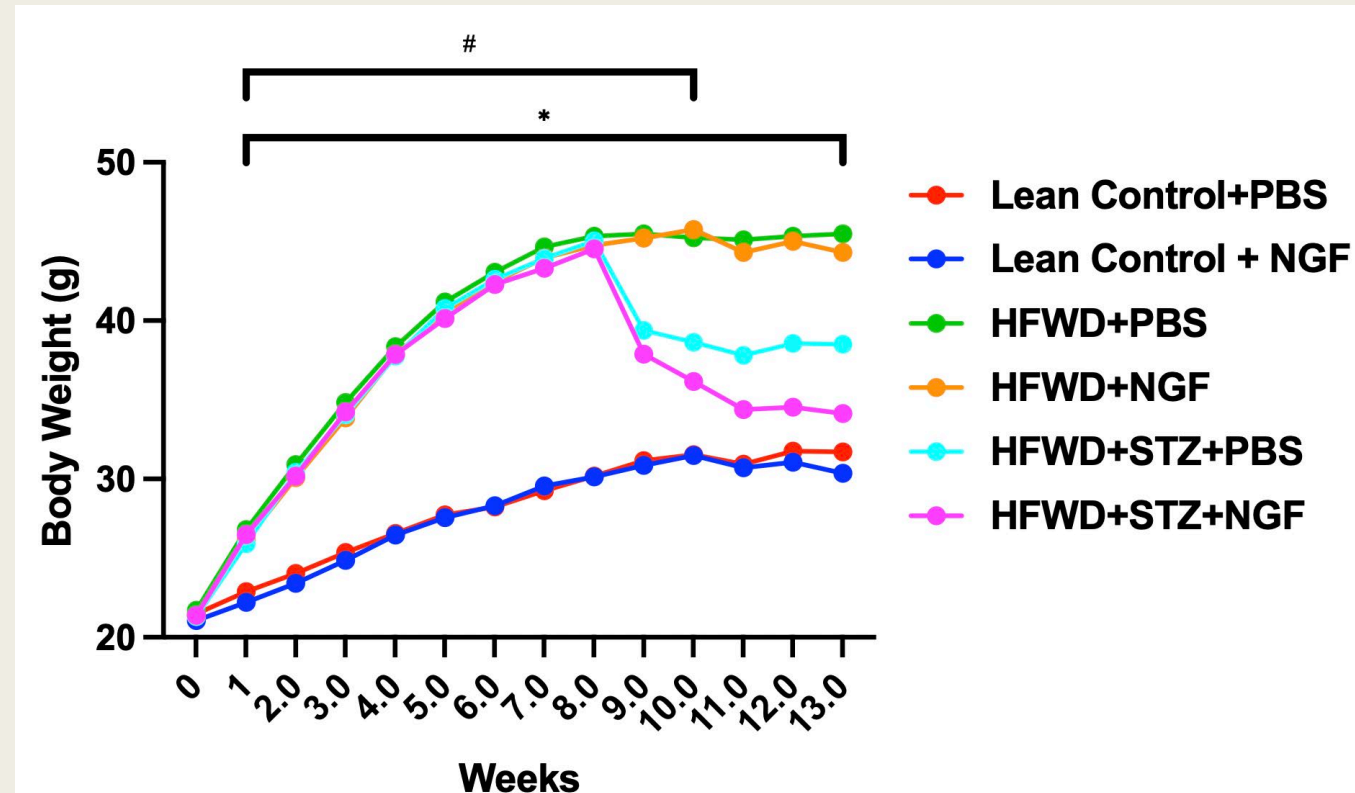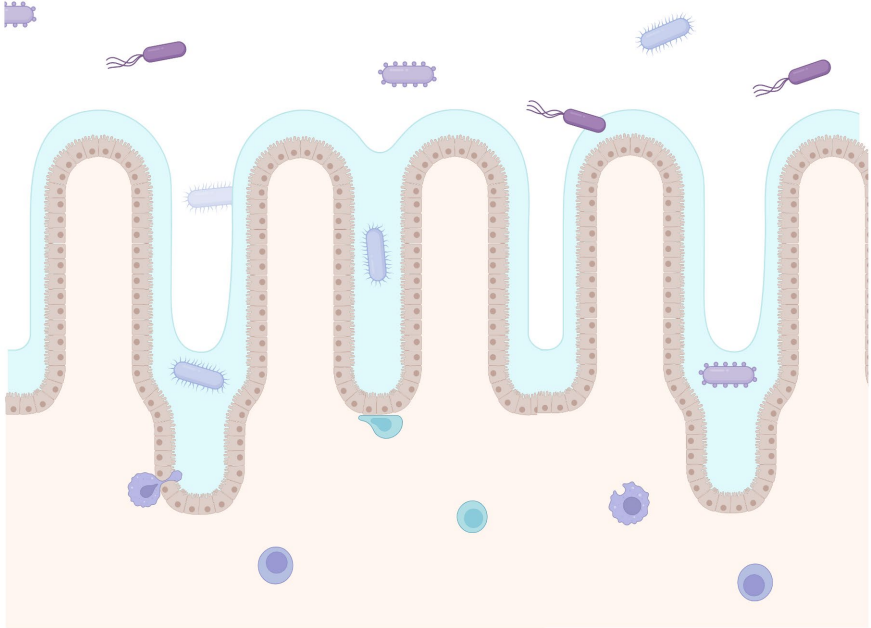
Results: Body Weight

Figure. Significant differences between groups 1 and 3 as well as groups 1 and 5 from weeks 1 to end of week 13 ($P < 0.05$) as marked by "*". Significant differences between groups 1 and 6 from weeks one through week 10 ($P < 0.05$) as marked by "#".
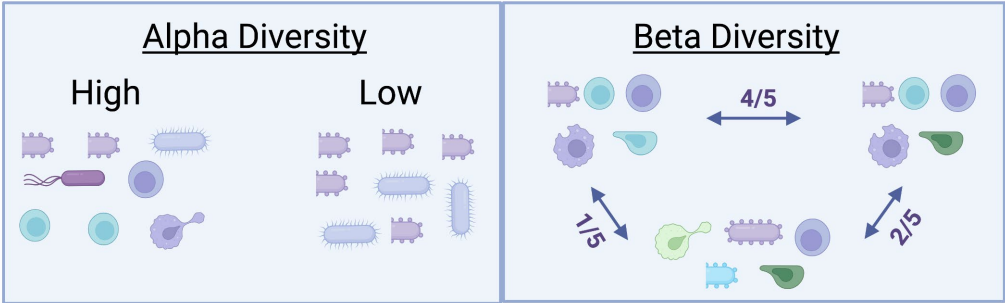
Methods: 16s rRNA Analysis
α-diversity & β-diversity

α-diversity:
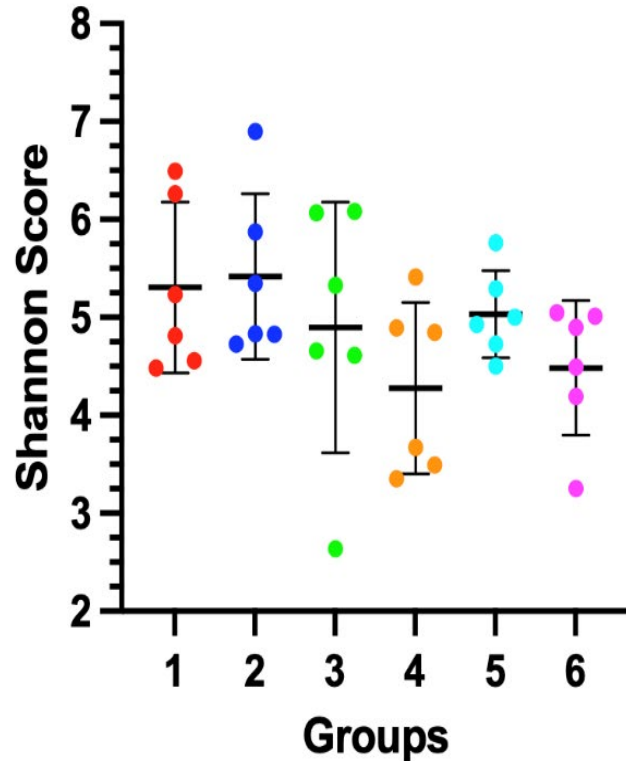the mean diversity of species within a sample

β-diversity:
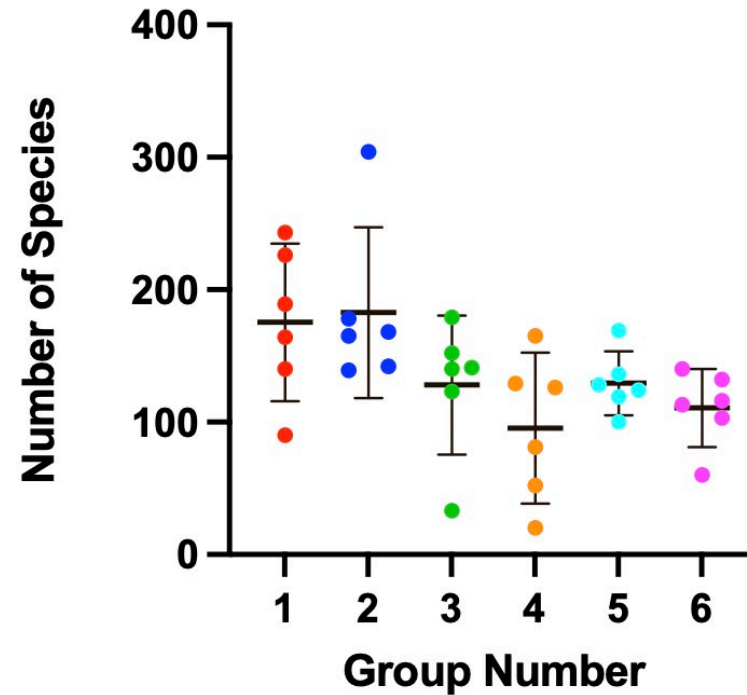Diversity similarity (or distance) between groups

Results: α-diversity

Mean and Standard Deviation of Shannon Index

Descriptive statistics for calculated Shannon values. Mean and standard deviations.

Observed Number of Species

Means with 95% confidence intervals of Observed Number of Species

Results: Relative Abundances

| Relative Abundances (Family Level) Groups 1, 2, and 6		1	2	6	P	FDR_P
Family	Identification	1	2	6	P	FDR_P
L5	Lachnospiraceae	0.048	0.049	0.012	0.052	0.256
L5	Clostridiaceae	0.001	0.001	0.000	0.054	0.256
L5	Peptococcaceae	0.000	0.000	0.000	0.059	0.267

> Treatment groups (3-6) contained significantly more pro-inflammatory and less anti-inflammatory bacteria compared to control groups (1-2)

Relative Abundances (Family Level) Groups 1, 3, and 5		1	3	5	P	FDR_P
Family	Identification	1	3	5	P	FDR_P
L5	Family XIII	0.002	0.000	0.000	0.004	0.114
L5	Muribaculaceae	0.126	0.026	0.016	0.006	0.114
L5	Prevotellaceae	0.031	0.000	0.000	0.008	0.114
L5	Marinifilaceae	0.004	0.000	0.000	0.012	0.114
L5	Burkholderiaceae	0.001	0.002	0.000	0.012	0.114
L5	Streptococcaceae	0.011	0.043	0.065	0.020	0.156
L5	Desulfovibrionaceae	0.009	0.020	0.020	0.023	0.156
L5	Rikenellaceae	0.011	0.003	0.003	0.030	0.173
L5	Bifidobacteriaceae	0.014	0.067	0.111	0.033	0.173
L5	Erysipelotrichaceae	0.042	0.201	0.178	0.041	0.192

Relative Abundances (Family Level) Groups 2, 3, and 4		2	3	4	P	FDR_P
Family	Identification	2	3	4	P	FDR_P
L5	Prevotellaceae	0.025	0.000	0.000	0.001	0.021
L5	Rikenellaceae	0.010	0.003	0.001	0.001	0.021
L5	Muribaculaceae	0.120	0.026	0.011	0.002	0.026
L5	Streptococcaceae	0.000	0.043	0.044	0.003	0.026
L5	Erysipelotrichaceae	0.002	0.201	0.273	0.003	0.026
L5	Marinifilaceae	0.003	0.000	0.000	0.004	0.031
L5	Burkholderiaceae	0.001	0.002	0.000	0.005	0.031
L5	Bifidobacteriaceae	0.000	0.067	0.071	0.005	0.031
L5	Tannerellaceae	0.000	0.028	0.001	0.013	0.066
L5	Family XIII	0.001	0.000	0.000	0.017	0.079
L5	Clostridiaceae 1	0.001	0.000	0.000	0.022	0.093
L5	Enterococcaceae	0.002	0.007	0.014	0.026	0.100
L5	Desulfovibrionaceae	0.008	0.020	0.013	0.028	0.100
L5	Bacteroidaceae	0.020	0.029	0.007	0.036	0.122
L5	Lachnospiraceae	0.460	0.270	0.185	0.046	0.145

Results: β-diversity

| Group Comparison	BC	W	UW
I. 1_2_6	0.001**	0.002**	0.001**
II. 1_3_5	0.001**	0.003**	0.002**
III. 2_3_4	0.001**	0.001**	0.001**
IV. 4_6	0.042*	0.07	0.29
V. 5_6	0.092	0.028*	0.609
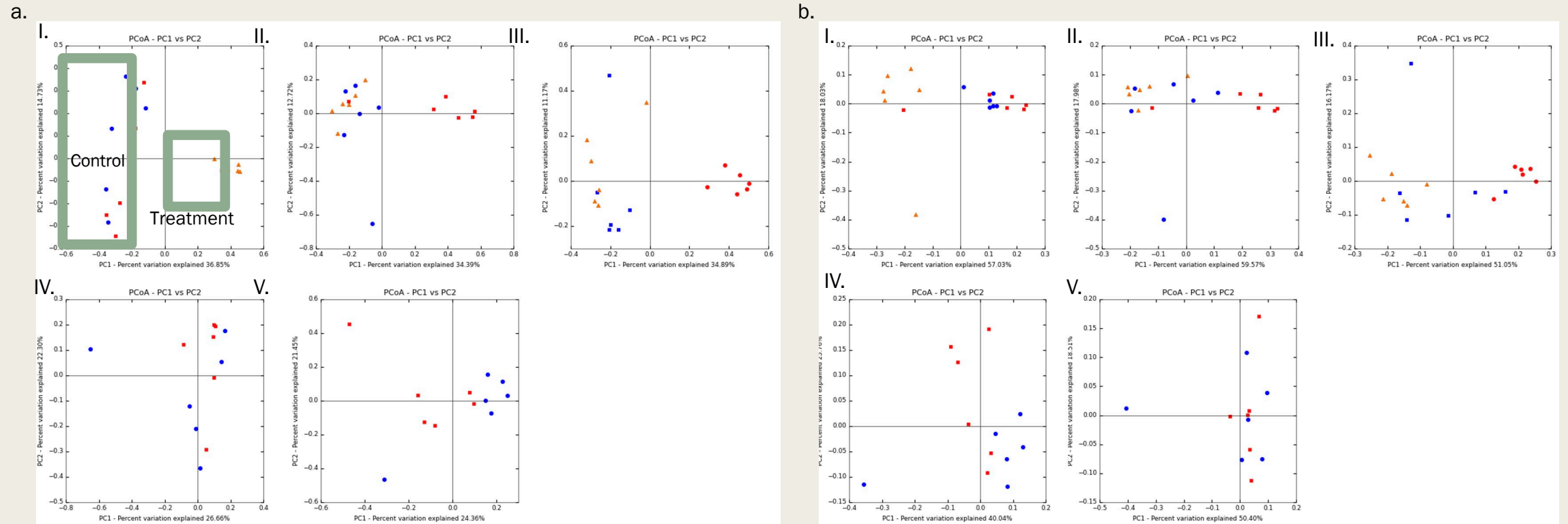
Kruksal-Wallis values 2D visualization for (**a**) Bray-Curtis Dissimilarities comparisons between groups. (**b**) Weighted Uni-Frac.

Conclusion + Discussion

- Diet significantly impacted gut microbiome composition

- Obese and T2D Groups more similar to one another than to the lean control groups

- Limitations:

  - *Only male mice*

  - *Study length*

  - *More frequent sampling needed at key timepoints in the study*

- Future Work

  - *Analysis of selected genes to investigate potential disturbances to the intestinal barrier, potentially correlating with dysbiosis of gut-brain axis*

Acknowledgements

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  - *My brother, Dr. Andrew Moeller*
  - *Ramsay & Julia*
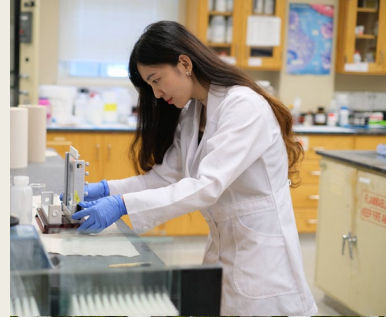
QUESTIONS?

AD + Nerve Growth Factor (NGF)

- NGF: growth, survival, apoptosis of neurons within mammals

  - *Regulator of synthesis and secretion of insulin*

- In pre-clinical AD, Impaired signaling associated

- In AD, proNGF malfunctions, dysregulation of cortical cholinergic synapses and basal forebrain cholinergic cell bodies

Molecular Pathways Linking Insulin Resistance and Neurodegeneration

Desai G….. Babu JR. Journal of Alzheimer's disease 2014; (42): 47-56.
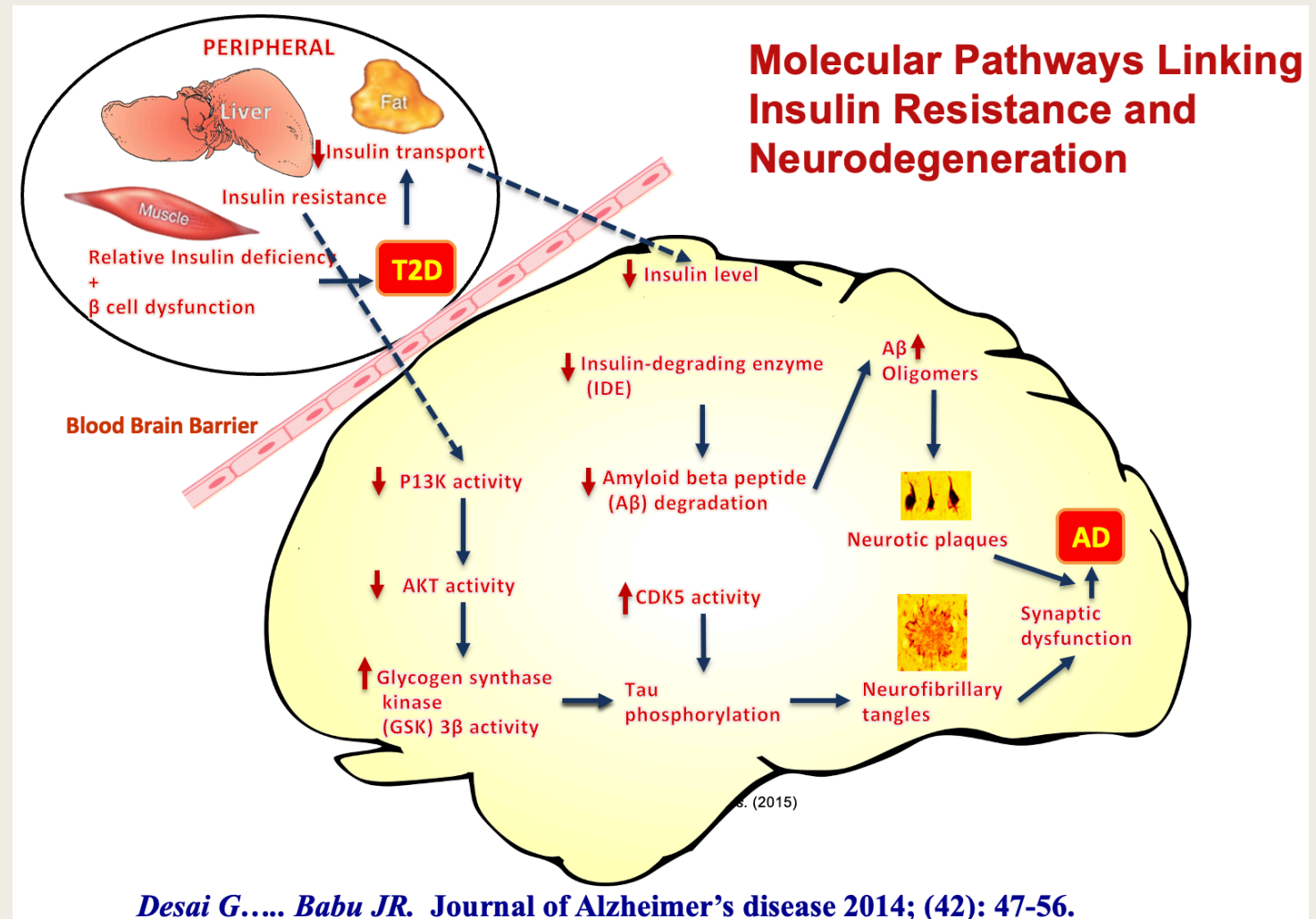
Body Weight Averages

Week		Lean Control + PBS			Lean Control + NGF			HFWD + PBS			HFWD + NGF			HFWD + STZ + PBS			HFWD + STZ + NGF		
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Week	0	21.49	1.089		21.05	1.141		21.71	1.377		21.33	0.833		21.30	1.050		21.40	0.636	
	1	22.9	1.208		22.21	1.289		26.81	1.876		26.15	1.806		25.91	2.073		26.49	1.315	
	2	24.04	1.406		23.42	1.433		30.92	1.950		30.08	2.192		30.42	2.899		30.19	1.947	
	3	25.36	1.592		24.87	1.514		34.85	1.822		33.87	2.800		34.05	2.967		34.23	2.055	
	4	26.56	1.677		26.47	1.651		38.36	1.442		37.83	2.617		37.77	2.795		37.85	2.087	
	5	27.73	1.991		27.56	1.287		41.19	1.402		40.57	2.596		40.76	2.775		40.15	2.330	
	6	28.24	2.111	N = 10	28.31	1.567	N = 10	43.07	1.434	N = 11	42.45	2.626	N = 10	42.62	2.756	N = 11	42.28	2.257	N = 10
	7	29.26	2.095		29.57	1.413		44.65	1.519		43.95	2.269		43.94	2.279		43.32	2.478	
	8	30.18	2.283		30.15	1.571		45.33	1.160		44.77	2.335		45.04	2.538		44.54	2.213	
	9	31.16	2.531		30.85	1.497		45.49	1.559		45.22	1.677		39.38	2.791		37.88	2.246	
	10	31.53	2.653		31.49	1.574		45.23	4.079		45.75	1.697		38.64	3.262		36.16	3.612	
	11	30.93	2.552		30.71	1.490		45.10	1.847		44.32	2.509		37.81	3.397		34.38	4.247	
	12	31.76	2.576		31.07	1.402		45.34	2.614		45.02	1.937		38.57	3.953		34.55	4.997	
	13	31.72	2.756		30.37	1.835		45.48	3.012		44.31	2.164		38.50	4.449		34.14	5.319	

Comparison 1 vs 3

	1 (Mean)	SD	N	3 (Mean)	SD	N	Significance (P Value)	
0	21.49	1.089		21.71	1.377		0.9983	ns
1	22.9	1.208		26.81	1.876		0.0003	***
2	24.04	1.406		30.92	1.950		<0.0001	****
3	25.36	1.592		34.85	1.822		<0.0001	****
4	26.56	1.677		38.36	1.442		<0.0001	****
5	27.73	1.991		41.19	1.402		<0.0001	****
6	28.24	2.111	N = 10	43.07	1.434	N = 11	<0.0001	****
7	29.26	2.095		44.65	1.519		<0.0001	****
8	30.18	2.283		45.33	1.160		<0.0001	****
9	31.16	2.531		45.49	1.559		<0.0001	****
10	31.53	2.653		45.23	4.079		<0.0001	****
11	30.93	2.552		45.10	1.847		<0.0001	****
12	31.76	2.576		45.34	2.614		<0.0001	****
13	31.72	2.756		45.48	3.012		<0.0001	****

Comparison 1 vs 5

	1 (Mean)	SD	N	5 (Mean)	SD	N	Significance (P Value)	
0	21.49	1.089		21.30	1.050		0.9983	ns
1	22.90	1.208		25.91	2.073		0.0086	**
2	24.04	1.406		30.42	2.899		0.0001	***
3	25.36	1.592		34.05	2.967		<0.0001	****
4	26.56	1.677		37.77	2.795		<0.0001	****
5	27.73	1.991		40.76	2.775		<0.0001	****
6	28.24	2.111	N = 10	42.62	2.756	N = 11	<0.0001	****
7	29.26	2.095		43.94	2.279		<0.0001	****
8	30.18	2.283		45.04	2.538		<0.0001	****
9	31.16	2.531		39.38	2.791		<0.0001	****
10	31.53	2.653		38.64	3.262		0.0003	***
11	30.93	2.552		37.81	3.397		0.0006	***
12	31.76	2.576		38.57	3.953		0.0022	**
13	31.72	2.756		38.50	4.449		0.0062	**

Comparison 1 vs 6

	1 (Mean)	SD	N	6 (Mean)	SD	N	Significance (P Value)	
0	21.49	1.089		21.40	0.636		0.9999	ns
1	22.9	1.208		26.49	1.315		<0.0001	****
2	24.04	1.406		30.19	1.947		<0.0001	****
3	25.36	1.592		34.23	2.055		<0.0001	****
4	26.56	1.677		37.85	2.087		<0.0001	****
5	27.73	1.991		40.15	2.330		<0.0001	****
6	28.24	2.111	N = 10	42.28	2.257	N = 10	<0.0001	****
7	29.26	2.095		43.32	2.478		<0.0001	****
8	30.18	2.283		44.54	2.213		<0.0001	****
9	31.16	2.531		37.88	2.246		<0.0001	****
10	31.53	2.653		36.16	3.612		0.0448	*
11	30.93	2.552		34.38	4.247		0.2931	ns
12	31.76	2.576		34.55	4.997		0.6298	ns
13	31.72	2.756		34.14	5.319		0.7919	ns

Comparison 5 vs 6

	5 (Mean)	SD	N	6 (Mean)	SD	N	Significance (P Value)	
0	21.30	1.050		21.40	0.636		0.9998	
1	25.91	2.073		26.49	1.315		0.9684	
2	30.42	2.899		30.19	1.947		>0.9999	
3	34.05	2.967		34.23	2.055		>0.9999	
4	37.77	2.795		37.85	2.087		>0.9999	
5	40.76	2.775		40.15	2.330		0.9931	
6	42.62	2.756	N = 11	42.28	2.257	N = 10	0.9996	ns
7	43.94	2.279		43.32	2.478		0.9904	
8	45.04	2.538		44.54	2.213		0.9964	
9	39.38	2.791		37.88	2.246		0.7469	
10	38.64	3.262		36.16	3.612		0.5828	
11	37.81	3.397		34.38	4.247		0.3658	
12	38.57	3.953		34.55	4.997		0.365	
13	38.50	4.449		34.14	5.319		0.3669	

Glucose Tolerance Test

Legend:
- Lean Control
- Lean Control + NGF
- HFWD + PBS
- HFWD + NGF
- HFWD + STZ + PBS
- HFWD + STZ + NGF
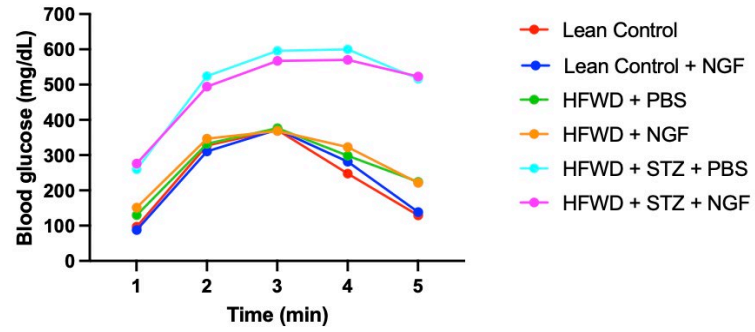
GTT Area Under the Curve

*

ITT Area Under the Curve

*

ITT

Legend:
- Lean Control+PBS
- Lean Control + NGF
- HFWD+PBS
- HFWD+NGF
- HFWD+STZ+PBS
- HFWD+STZ+NGF
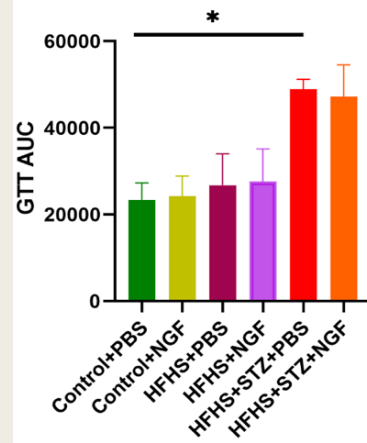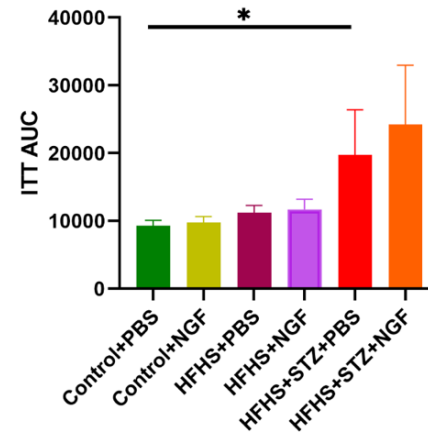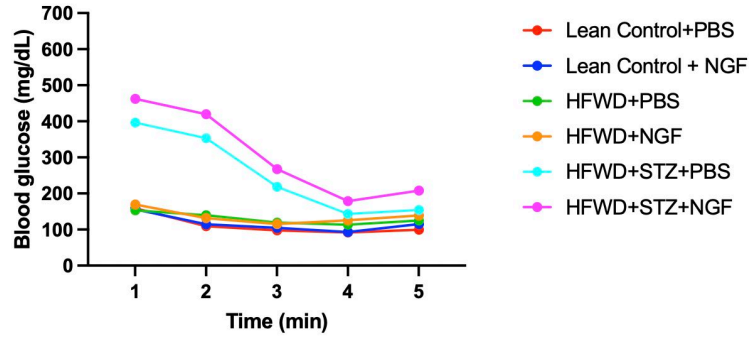
Relative Abundances (Family Level) Groups 4 and 6					
Family	Identification	4	6	P	FDR_P
L5	Burkholderiaceae	0.000	0.002	0.007	0.348
L5	Rikenellaceae	0.001	0.003	0.036	0.586
L5	Lactobacillaceae	0.218	0.105	0.037	0.586

Relative Abundances (Family Level) Groups 5 and 6					
Family	Identification	5	6	P	FDR_P
L5	Burkholderiaceae	5.17E-05	0.0019	0.0326	0.9475