Induction of brain insulin resistance and Alzheimer's molecular changes by Western diet

Anna Mietelska-Porowska[1] [†], Justyna Domańska[1] [†], Andrew Want[1], Angelika Więckowska-Gacek[1], Dominik Chutorański[1], Maciej Koperski[1], Urszula Wojda[1,*]

[1] Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland
      Laboratory of Preclinical Testing of Higher Standard;
[*] Correspondence: u.wojda@nencki.edu.pl; Tel.: +48 225892578, UW
[†] These authors contributed equally to this work.

Figure S1: WD-derived insulin signaling impairment on AD biomarkers, analyzed for extreme values for each individual in all experimental and age groups (paragraph 2.3).
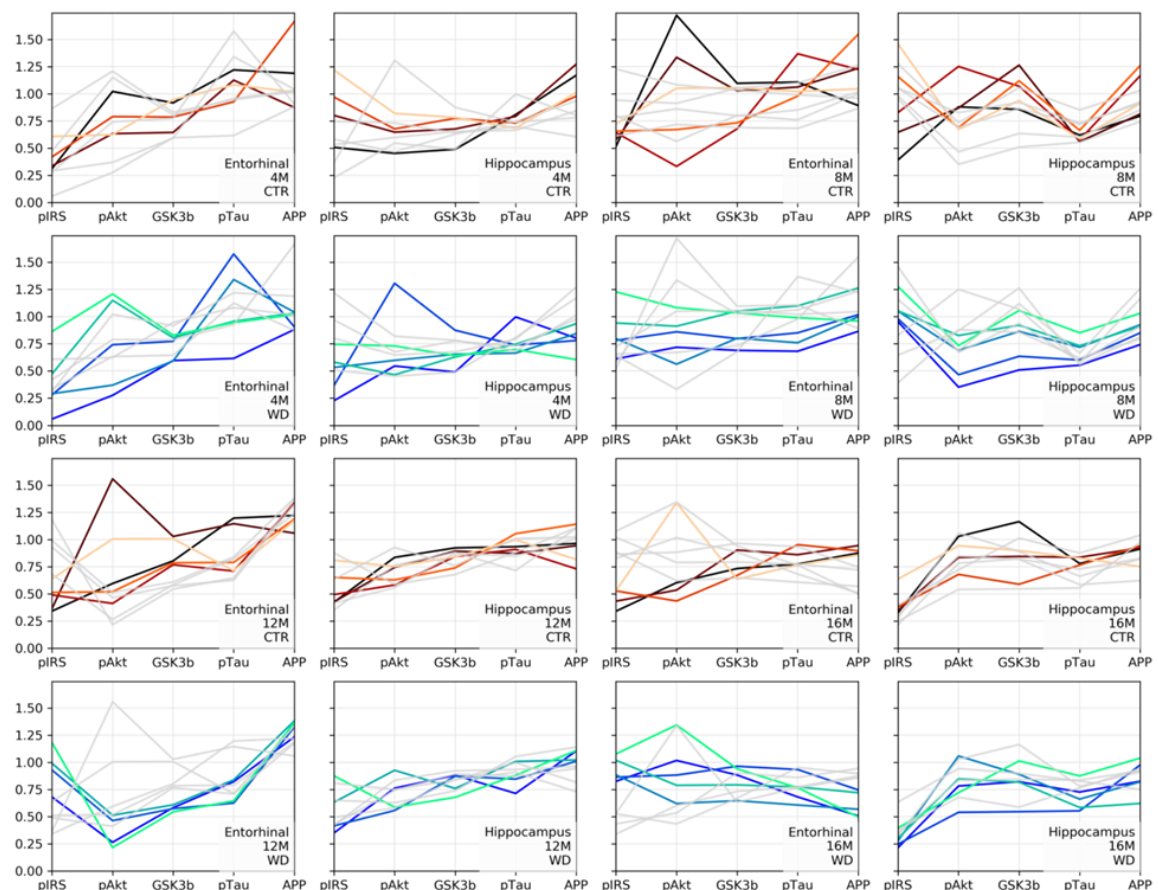
Table S1: Completed statistical data – analysis of peripheral markers in C57BL/6 mice (paragraph 2.1)

Figure number: Analyzed parameter	Age groups	Statistical test	Significance	p-Value	Value of F (DFn, DFd)
Fig.1. Glucose	ALL	2way ANOVA	Interaction: ns	Interaction: 0.2284	Interaction: $F_{(3,28)}$ = 1.530
			Age: ns	Age: 0.1503	Age: $F_{(3,28)}$ = 1.914
			Diet: **	Diet: 0.0077	Diet: $F_{(1,28)}$ = 8.255
Fig.1. Glucose	4M vs. 16M WD WD	Tukey's post-hoc	*	0.0404	-
Fig.1. Insulin	ALL	2way ANOVA	Interaction: ns	Interaction: 0.3169	Interaction: $F_{(3,28)}$ = 1.231
			Age: ns	Age: 0.2901	Age: $F_{(3,28)}$ = 1.312
			Diet: **	Diet: 0.0094	Diet: $F_{(1,28)}$ = 7.787
Fig.1. HOMA-IR	ALL	2way ANOVA	Interaction: ns	Interaction: 0.1056	Interaction: $F_{(3,28)}$ = 2.240
			Age: ns	Age: 0.2196	Age: $F_{(3,28)}$ = 1.567
			Diet:**	Diet:0.0026	Diet: $F_{(1,28)}$ = 10.98
Fig.2. p-IRS-1 (Ser616) Entorhinal cortex	ALL	2way ANOVA	Interaction: *	Interaction: 0.0165	Interaction: $F_{(3,29)}$ = 4.023
			Age: **	Age: 0.0010	Age: $F_{(3,29)}$ = 7.104
			Diet: ****	Diet: <0.0001	Diet: $F_{(1,29)}$ = 25.18
Fig.2. p-IRS-1 (Ser616) Entorhinal cortex	4M vs. 8M WD WD	Tukey's post-hoc	**	0.0042	-
Fig.2. p-IRS-1 (Ser616) Entorhinal cortex	4M vs. 12M WD WD	Tukey's post-hoc	**	0.0016	-
Fig.2. p-IRS-1 (Ser616) Entorhinal cortex	4M vs. 16M WD WD	Tukey's post-hoc	***	0.0010	-
Fig.2. p-IRS-1 (Ser616) Entorhinal cortex	12M vs. 12M CTR WD	Tukey's post-hoc	**	0.0084	-
Fig.2. p-IRS-1 (Ser616) Entorhinal cortex	16M vs. 16M CTR WD	Tukey's post-hoc	**	0.0081	-
Fig.2. p-Akt (Ser473) Entorhinal cortex	ALL	2way ANOVA	Interaction: ns	Interaction: 0.3047	Interaction: $F_{(3,29)}$ = 1.265
			Age: ns	Age: 0.2769	Age: $F_{(3,29)}$ = 1.352
			Diet: ns	Diet: 0.3506	Diet: $F_{(1,29)}$ = 0.899

*p<0.05, **p<0.01

Table S2: Completed statistical data – analysis of brain markers in C57BL/6 mice (paragraph 2.2)

Figure number: Analyzed parameter	Age groups	Statistical test	Significance	p-Value	Value of F (DFn, DFd)
Fig.2: p-GSK-3β (Ser9) Entorhinal cortex	ALL	2way ANOVA	Interaction: * Age: ns Diet: ns	Interaction:0.0297 Age: 0.0705 Diet: 0.0733	Interaction: $F_{(3,29)}$ = 3.439 Age: $F_{(3,29)}$ = 2.608 Diet: $F_{(1,29)}$ = 3.454
Fig.2. p-GSK-3β (Ser9) Entorhinal cortex	12M vs. 12M CTR WD	Tukey's post-hoc	*	0.0491	-
Fig.2. p-Tau (Thr231) Entorhinal cortex	ALL	2way ANOVA	Interaction: ns Age: * Diet: ns	Interaction: 0.6246 Age: 0.0123 Diet: 0.0726	Interaction: $F_{(3,29)}$ = 0.5931 Age: $F_{(3,29)}$ = 4.324 Diet: $F_{(1,29)}$ = 3.471
Fig.4. p-IRS-1 (Ser616) Hippocampus	ALL	2way ANOVA	Interaction: ns Age: **** Diet: ns	Interaction: 0.0926 Age: <0.0001 Diet: 0.2727	Interaction: $F_{(3,29)}$ = 2.354 Age: $F_{(3,29)}$ = 11.97 Diet: $F_{(1,29)}$ = 1.250
Fig.3. p-IRS-1 (Ser616) Hippocampus	4M vs. 8M WD WD	Tukey's post-hoc	*	0.0108	-
Fig.3. p-IRS-1 (Ser616) Hippocampus	8M vs. 16M WD WD	Tukey's post-hoc	***	0.0003	-
Fig.3. p-Akt (Ser473) Hippocampus	ALL	2way ANOVA	Interaction: ns Age: ns Diet: ns	Interaction: 0.3352 Age: 0.4925 Diet: 0.3518	Interaction: $F_{(3,29)}$ = 1.178 Age: $F_{(3,29)}$ = 0.822 Diet: $F_{(1,29)}$ = 0.895
Fig.3. p-GSK-3β (Ser9) Hippocampus	ALL	2way ANOVA	Interaction: ns Age: * Diet: ns	Interaction: 0.3954 Age: 0.0170 Diet: 0.0871	Interaction: $F_{(3,29)}$ = 1.026 Age: $F_{(3,29)}$ = 3.993 Diet: $F_{(1,29)}$ = 3.137
Fig.3. p-GSK-3β (Ser9) Hippocampus	4M vs. 8M CTR CTR	Tukey's post-hoc	*	0.0372	-
Fig.3. p-Tau (Thr231) Hippocampus	ALL	2way ANOVA	Interaction: ns Age: **** Diet: ns	Interaction: 0.0936 Age: <0.0001 Diet: 0.4117	Interaction: $F_{(3,29)}$ = 2.344 Age: $F_{(3,29)}$ = 11.47 Diet: $F_{(1,29)}$ = 0.694
Fig.3. p-Tau (Thr231) Hippocampus	8M vs. 12M CTR CTR	Tukey's post-hoc	****	<0.0001	-

*p<0.05, ***p<0.001, ****p<0.0001

Table S3: Completed statistical data – analysis of brain markers in C57BL/6 mice (paragraph 2.2)

Figure number: Analyzed parameter	Age groups	Statistical test	Significance	p-Value	Value of F (DFn, DFd)
Fig.4. APP Entorhinal cortex	ALL	2way ANOVA	Interaction: ns Age: **** Diet: *	Interaction: 0.0687 Age: <0.0001 Diet: 0.0234	Interaction: $F_{(3,29)}$ = 2.634 Age: $F_{(3,29)}$ = 14.49 Diet: $F_{(1,29)}$ = 5.727
Fig.4. APP Entorhinal cortex	4M vs. 16M WD WD	Tukey's post-hoc	*	0.0314	-
Fig.4. APP Entorhinal cortex	8M vs. 16M WD WD	Tukey's post-hoc	*	0.0113	-
Fig.4. APP Entorhinal cortex	12M vs. 16M WD WD	Tukey's post-hoc	****	<0.0001	-
Fig.4. APP Hippocampus	ALL	2way ANOVA	Interaction: * Age: ns Diet: ns	Interaction: 0.0164 Age: 0.3531 Diet: 0.1239	Interaction: $F_{(3,29)}$ = 4.031 Age: $F_{(3,29)}$ = 1.130 Diet: $F_{(1,29)}$ = 2.512
Fig.4. APP Hippocampus	4M vs. 4M CTR WD	Tukey's post-hoc	*	0.0424	-

*p<0.05, ****p<0.0001

Table S4: Completed statistical data – comparable analysis of brain markers in Tg2576 (APPswe) mice (paragraph 2.4)

Figure number: Analyzed parameter	Age groups	Statistical test	Significance	p-Value	Value of F (DFn, DFd)
Fig.6A. p-IRS-1 (Ser616) Entorhinal cortex	ALL	2way ANOVA	Interaction: ns Age: ** Diet: *	Interaction: 0.3039 Age: 0.0050 Diet: 0.0122	Interaction: $F_{(2,19)}$ = 1.269 Age: $F_{(2,19)}$ = 7.094 Diet: $F_{(1,19)}$ = 7.663
Fig.6A. p-Tau (Thr231) Entorhinal cortex	ALL	2way ANOVA	Interaction: ns Age: *** Diet: **	Interaction: 0.1109 Age: 0.0004 Diet: 0.0032	Interaction: $F_{(2,19)}$ = 2.474 Age: $F_{(2,19)}$ = 12.31 Diet: $F_{(1,19)}$ = 11.35
Fig.6A. p-Tau (Thr231) Entorhinal cortex	4M vs. 4M CTR WD	Tukey's post-hoc	*	0.0284	-
Fig.6A. p-Tau (Thr231) Entorhinal cortex	4M vs. 8M CTR CTR	Tukey's post-hoc	**	0.0048	-
Fig.6A. p-Tau (Thr231) Entorhinal cortex	4M vs. 12M CTR CTR	Tukey's post-hoc	*	0.0194	-
Fig.6A. APP Entorhinal cortex	ALL	2way ANOVA	Interaction: ns Age: ns Diet: ns	Interaction: 0.4321 Age: 0.1534 Diet: 0.2377	Interaction: $F_{(2,19)}$ = 0.8774 Age: $F_{(2,19)}$ = 2.073 Diet: $F_{(1,19)}$ = 1.486
Fig.6B. p-IRS-1 (Ser616) Hippocampus	ALL	2way ANOVA	Interaction: ns Age: ns Diet: ns	Interaction: 0.3754 Age: 0.0504 Diet: 0.2780	Interaction: $F_{(2,21)}$ = 1.027 Age: $F_{(2,21)}$ = 3.456 Diet: $F_{(1,21)}$ = 1.240
Fig.6B. p-Tau (Thr231) Hippocampus	ALL	2way ANOVA	Interaction: ns Age: ns Diet: ns	Interaction: 0.1660 Age: 0.9990 Diet: 0.6162	Interaction: $F_{(2,19)}$ = 1.977 Age: $F_{(2,19)}$ = 0.001 Diet: $F_{(1,19)}$ = 0.26
Fig.6B. APP Hippocampus	ALL	2way ANOVA	Interaction: * Age: *** Diet: ns	Interaction: 0.0272 Age: 0.0009 Diet: 0.0710	Interaction: $F_{(2,20)}$ = 4.338 Age: $F_{(2,20)}$ = 10.24 Diet: $F_{(1,20)}$ = 3.635
Fig.6B. APP Hippocampus	4M vs. 4M CTR WD	Tukey's post-hoc	*	0.0277	-
Fig.6B. APP Hippocampus	4M vs. 8M CTR CTR	Tukey's post-hoc	**	0.0059	-
Fig.6B. APP Hippocampus	4M vs. 12M CTR CTR	Tukey's post-hoc	**	0.0018	-

*p<0.05, **p<0.01