

Supplementary Material

Tetrahydrobiopterin Improves Recognition Memory in the Triple-Transgenic Mouse Model of Alzheimer's Disease, Without Altering Amyloid- β and Tau Pathologies

Supplementary Table 1. Composition of control and high-fat diets

Composition	Diets	
	Control	High fat
Proteins (%<i>, w/w</i>)	20.3	27.4
Carbohydrates (%<i>, w/w</i>)	66	25.3
Fats (%<i>, w/w</i>)	5	35.1
Calories per diet weight (kcal/g)	3.9	5.3
Ingredients (g/kg)		
Casein	200	200
Corn starch	150	25
Maltodextrin 10	0	100
Sucrose	500	52.5
Cellulose, BW200	50	50
Corn oil	30	0
Safflower oil	0	125
Lard	0	135
Soybean oil	10	0
Canola oil	10	0
Minerals (S19101)	35	35
Vitamins (V15908)	10	10
Choline bitartrate	2	2
Cholesterol, USP	0.6	3
Fatty acids (g/kg)*		
18:2n-6	21.6	100.8
20:4n-6	0	0.3
22:4n-6	0	0.2
Total n-6 PUFA	21.6	101.3
18:3n-3	1.4	1.7
20:5n-3	0	0
22:5n-3	0	0.1
22:6n-3	0	0
Total n-3 PUFA	1.4	1.8
16:1n-7	0.1	2.1
18:1n-9	14.9	57
Total MUFA	15	59
14:0	0.1	1.6
16:0	4.8	31.2
18:0	1.2	16.4
Total SFA	6.1	49.2
Total fatty acids	44.1	211.3

PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids

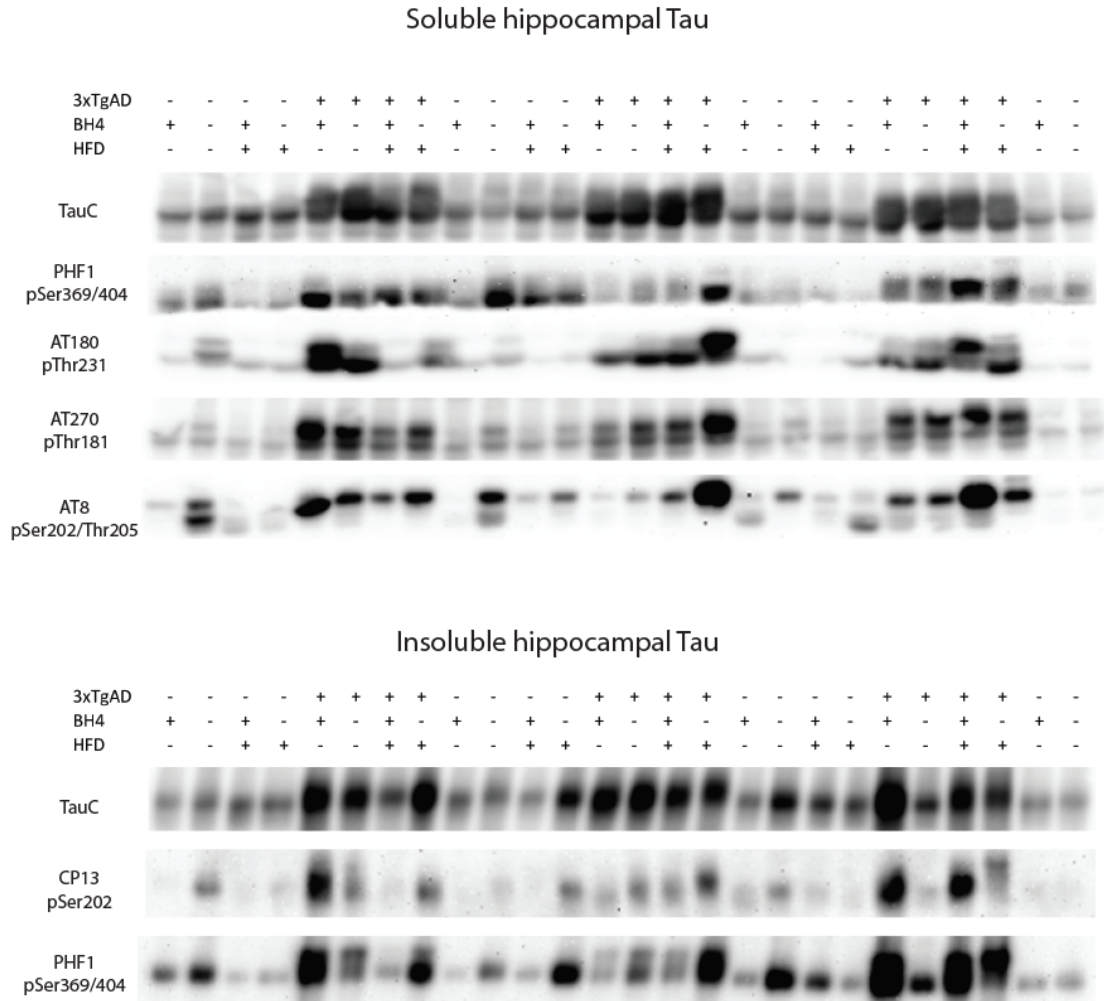
Supplementary Table 2. Primary antibodies used for western blot analyses.

Antibody	Clone	Specificity	Host	Dilution	Source
TauC	Polyclonal	Tau, C-term region	Rabbit	1/10000	Dako (Burlington, ON, Canada)
PHF1	Monoclonal	Tau, phosphorylated at Ser-369 and Ser-404	Mouse	1/5000	Generous gift from Peter Davies
AT180	Monoclonal	Tau, phosphorylated at Thr-231	Mouse	1/1000	Pierce Endogen Inc. (Rockford, IL, USA)
AT270	Monoclonal	Tau, phosphorylated at Thr-181	Mouse	1/1000	Pierce Endogen Inc. (Rockford, IL, USA)
AT8	Monoclonal	Tau, phosphorylated at Ser-202 and Thr-205	Mouse	1/1000	Pierce Endogen Inc. (Rockford, IL, USA)
CP13	Monoclonal	Tau, phosphorylated at Ser-202	Mouse	1/700	Generous gift from Peter Davies
eNOS	Monoclonal	eNOS, C-term region	Mouse	1/1000	Abcam (Cambridge, MA, USA)
iNOS	Monoclonal	iNOS, residues surrounding Gly-1133	Rabbit	1/1000	Cell Signaling (Danvers, MA, USA)
nNOS	Monoclonal	nNOS, C-term region	Rabbit	1/1000	Abcam
COX2	Polyclonal	/	Mouse	1/1000	Cayman Chemical (Ann Arbor, MI, USA)
SOD1	Polyclonal	/	Rabbit	1/1000	Millipore (Burlington, MA, USA)
GFAP	Polyclonal	/	Rabbit	1/1000	Abcam (Cambridge, MA, USA)

Supplementary Table 3. Statistical comparisons for measures of A β peptides by ELISA in the parietotemporal cortex.

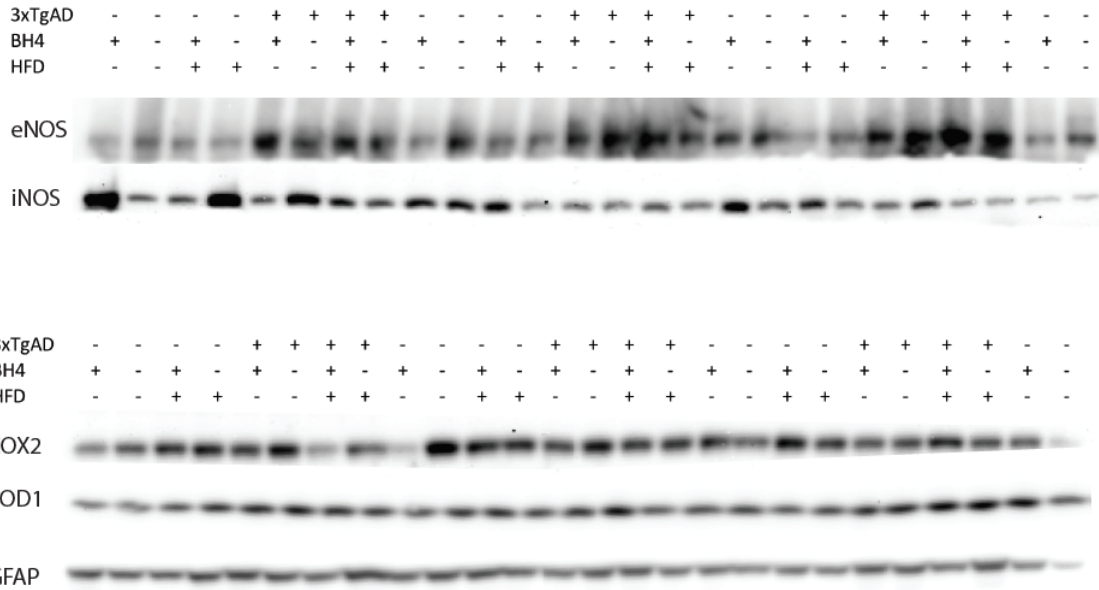
Aβ peptides	Sex	Diet	Treatment	Interactions
Soluble A β_{40} fg/ μ g proteins	p<0.001	p=0.021	p=0.865	Diet x Sex p=0.031
Soluble A β_{42} fg/ μ g proteins	p=0.001	p=0.330	p=0.545	-
Insoluble A β_{40} fg/mg tissue	p=0.007	0.3794	p=0.629	-
Insoluble A β_{42} fg/mg tissue	p=0.002	p=0.622	p=0.721	-
Insoluble A β_{42} /A β_{40}	p=0.014	p=0.719	p=0.425	-
Soluble A β_{42} /A β_{40}	p=0.019	p=0.298	p=0.627	-
Ins/sol A β_{40}	p=0.554	p=0.515	p=0.483	-
Ins/sol A β_{42}	p=0.002	p=0.974	p=0.452	-

Supplementary Figure 1. Western blot of soluble and insoluble hippocampal Tau antibodies. Total Tau (TauC) and phosphorylated tau at Ser369/Ser404 (PHF1), Thr231 (AT180), Thr181 (AT270), Ser202/Thr205 (AT8), and Ser202 (CP13) antibodies on soluble and insoluble fractions.



3xTg-AD, triple transgenic (+) or Non-transgenic (-) mice; HFD, High-fat diet (+) or Control diet (-); BH4, tetrahydrobiopterin (+) or saline vehicle (-) injected

Supplementary Figure 2. Western blot of hippocampal protein level related to inflammatory markers. Nitric oxide synthases (NOS) markers as endothelial (eNOS) or inducible (iNOS) NO synthase, cyclooxygenase 2 (COX2), glial fibrillary acidic protein (GFAP) and superoxide dismutase (SOD1) levels were studied in hippocampus.



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