

**Supplemental Table S7.** Brain Parameters at Follow-up between Rats with Higher Baseline CRF versus Rats with Lower Baseline CRF

Brain parameter	ND (n=8)		P value	HFD (n=8)		P value
	Lower baseline CRF (n=4)	Higher baseline CRF (n=4)		Lower baseline CRF (n=4)	Higher baseline CRF (n=4)	
Mitochondrial metabolism at week 28						
PGC-1 $\alpha$ protein expression	0.63 $\pm$ 0.04	0.85 $\pm$ 0.06	0.0478	0.45 $\pm$ 0.03	0.50 $\pm$ 0.07	NS
p-AMPK/Total AMPK protein expression	0.96 $\pm$ 0.02	0.87 $\pm$ 0.03	NS	0.90 $\pm$ 0.01	1.02 $\pm$ 0.01	0.0212
Insulin signaling at week 28						
p-IRS/Total IRS expression	0.91 $\pm$ 0.03	0.98 $\pm$ 0.15	NS	0.40 $\pm$ 0.03	0.71 $\pm$ 0.05	0.0491
p-AKT/Total AKT protein expression	0.66 $\pm$ 0.14	0.67 $\pm$ 0.18	NS	0.76 $\pm$ 0.05	0.94 $\pm$ 0.03	0.0495
Mitochondrial dynamics at week 28						
MFN1 protein expression	0.68 $\pm$ 0.04	0.87 $\pm$ 0.05	0.0212	0.62 $\pm$ 0.01	0.75 $\pm$ 0.01	0.0453
MFN2 protein expression	0.75 $\pm$ 0.02	0.99 $\pm$ 0.11	0.0353	0.69 $\pm$ 0.06	0.75 $\pm$ 0.05	NS
OPA1 protein expression	0.82 $\pm$ 0.12	0.72 $\pm$ 0.12	NS	0.70 $\pm$ 0.02	0.65 $\pm$ 0.04	NS
p-DRP1 <sup>ser616</sup> /total DRP1 protein expression	0.85 $\pm$ 0.04	0.82 $\pm$ 0.10	NS	0.92 $\pm$ 0.09	0.88 $\pm$ 0.03	NS
Mitophagy at week 28						
PINK1/Parkin protein expression	0.75 $\pm$ 0.08	0.82 $\pm$ 0.04	NS	1.27 $\pm$ 0.03	1.43 $\pm$ 0.08	0.0468
Autophagy at week 28						
Beclin-1 protein expression	0.94 $\pm$ 0.03	1.15 $\pm$ 0.02	0.0498	0.68 $\pm$ 0.01	0.82 $\pm$ 0.02	0.0462
LC3-II protein expression	0.91 $\pm$ 0.02	1.07 $\pm$ 0.02	0.0214	0.59 $\pm$ 0.03	0.79 $\pm$ 0.03	0.0451
p62 protein expression	0.95 $\pm$ 0.06	0.99 $\pm$ 0.04	NS	0.81 $\pm$ 0.01	0.77 $\pm$ 0.02	NS
Apoptosis at week 28						
Bax/Bcl-2 protein expression	0.69 $\pm$ 0.07	0.75 $\pm$ 0.01	NS	1.29 $\pm$ 0.07	1.25 $\pm$ 0.03	NS
Cleaved caspase 3/Procaspase 3 protein expression	0.63 $\pm$ 0.04	0.39 $\pm$ 0.05	0.0331	0.99 $\pm$ 0.09	0.90 $\pm$ 0.17	NS
Antioxidant at week 28						
GPX4 protein expression	0.92 $\pm$ 0.04	1.27 $\pm$ 0.05	0.0212	0.76 $\pm$ 0.01	0.88 $\pm$ 0.01	0.0375
SOD2 protein expression	0.90 $\pm$ 0.01	1.19 $\pm$ 0.06	0.0385	0.71 $\pm$ 0.04	0.76 $\pm$ 0.04	NS
Oxidative stress at week 28						
ROS level, arbitrary unit	121.83 $\pm$ 9.55	79.58 $\pm$ 7.61	0.0499	408.17 $\pm$ 22.98	214.83 $\pm$ 19.39	0.0472
MDA level, mmol/g protein	246.00 $\pm$ 34.33	163.12 $\pm$ 25.38	0.0431	408.50 $\pm$ 28.38	389.24 $\pm$ 35.45	NS
Inflammation at week 28						
TNF- $\alpha$ protein expression	0.64 $\pm$ 0.12	0.72 $\pm$ 0.03	NS	0.76 $\pm$ 0.01	0.78 $\pm$ 0.02	NS
p-NF $\kappa$ B/Total NF $\kappa$ B protein expression	1.00 $\pm$ 0.14	0.52 $\pm$ 0.02	0.0496	0.88 $\pm$ 0.04	0.85 $\pm$ 0.03	NS
Blood brain barrier at week 28						
Claudin-5 protein expression	0.95 $\pm$ 0.07	1.00 $\pm$ 0.10	NS	0.45 $\pm$ 0.01	0.73 $\pm$ 0.03	0.0457
Synapse at week 28						
Synaptophysin protein expression	0.90 $\pm$ 0.02	1.05 $\pm$ 0.05	0.0487	0.62 $\pm$ 0.01	0.84 $\pm$ 0.02	0.0055
PSD-95 protein expression	0.95 $\pm$ 0.06	0.87 $\pm$ 0.06	NS	0.90 $\pm$ 0.05	0.85 $\pm$ 0.01	NS
Neurogenesis at week 28						
p-TrkB/Total TrkB protein expression	0.65 $\pm$ 0.01	0.99 $\pm$ 0.07	0.0493	0.46 $\pm$ 0.02	0.62 $\pm$ 0.06	0.0286
BDNF protein expression	0.90 $\pm$ 0.14	0.94 $\pm$ 0.11	NS	0.43 $\pm$ 0.10	0.81 $\pm$ 0.11	0.0332
DCX protein expression	0.77 $\pm$ 0.03	0.84 $\pm$ 0.07	NS	0.94 $\pm$ 0.05	0.88 $\pm$ 0.06	NS

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Supplemental Table S7. Continued

Brain parameter	ND (n=8)		P value	HFD (n=8)		P value
	Lower baseline CRF (n=4)	Higher baseline CRF (n=4)		Lower baseline CRF (n=4)	Higher baseline CRF (n=4)	
Alzheimer's disease marker at week 28						
p-Tau/Total Tau protein expression	1.10±0.05	1.21±0.15	NS	1.55±0.03	1.35±0.03	0.0265
APP protein expression	0.89±0.07	0.93±0.04	NS	1.25±0.01	1.12±0.04	0.0498
Aβ protein expression	0.49±0.10	0.39±0.09	NS	0.68±0.05	0.55±0.11	NS
BACE-1 protein expression	0.83±0.10	0.94±0.10	NS	1.23±0.05	1.17±0.07	NS
Anti-aging marker at week 28						
sRAGE protein expression	0.87±0.08	0.87±0.04	NS	0.69±0.02	0.86±0.05	0.0345
Behavior at week 28						
Anxiety-like behavior	39.37±10.02	6.19±1.82	0.0215	25.75±16.35	26.77±4.75	NS
%Preference index of novel location	54.55±3.43	59.05±2.30	NS	28.24±0.23	39.00±1.83	0.0172
%Preference index of novel recognition	58.61±1.52	51.97±0.78	NS	37.93±1.93	39.72±2.06	NS

Values are expressed as mean ± standard error of the mean (n=8 per group). Week 12, baseline; Week 28, follow-up. All protein expressions are normalized to the expression of housekeeping proteins.

CRF, cardiorespiratory fitness; ND, normal diet-fed rats; HFD, high-fat diet-fed rats; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator-1α; p-AMPK, phosphorylated-activated protein kinase; AMPK, activated protein kinase; NS, no significance; p-IRS, phosphorylated-insulin receptor substrate 1; IRS, insulin receptor substrate 1; MFN1, mitofusin 1; MFN2, mitofusin 2; OPA1, optic atrophy 1; p-DRP1<sup>ser616</sup>, phosphorylated-dynamin-related at serine<sup>616</sup>; DRP1, dynamin-related protein 1; PINK1, PTEN-induced kinase 1; LC3-II, light chain 3-II; Bax/Bcl, Bcl-2-associated X protein/B-cell lymphoma; GPX4, glutathione peroxidase 4; SOD2, superoxide dismutase 2; ROS, reactive oxygen species; MDA, malondialdehyde; TNF-α, tumor necrosis factor-α; p-NFκB, phosphorylated-nuclear factor kappa-light-chain-enhancer of activated B cells; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; PSD-95, postsynaptic density protein 95; p-TrkB, phosphorylated-tropomyosin receptor kinase B; TrkB, tropomyosin receptor kinase B; BDNF, brain-derived neurotrophic factor; DCX, doublecortin; p-Tau, phosphorylated -tau; APP, amyloid-beta precursor protein; Aβ, amyloid β; BACE-1, beta-site amyloid precursor protein cleaving enzyme 1; sRAGE, soluble-receptor for advanced glycation end product.