

Supplemental Table S6. Cardiac Parameters at Follow-up between Rats with Higher Baseline CRF versus Rats with Lower Baseline CRF

Heart 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 function at week 28						
Mitochondrial ROS level, arbitrary unit	874.67±49.50	559.78±43.07	0.0346	614.16±70.36	358.56±35.04	NS (0.0669)
Mitochondrial membrane potential change	1.27±0.01	1.05±0.12	NS	1.24±0.12	1.76±0.09	0.0124
Normalized mitochondrial absorbance at λ 540 nm	0.94±0.01	0.91±0.03	NS	0.88±0.01	0.96±0.01	NS (0.0690)
Mitochondrial metabolism at week 28						
CPT1 protein expression	0.41±0.05	0.39±0.04	NS	0.52±0.03	0.68±0.01	NS (0.0727)
PGC-1α protein expression	0.59±0.03	0.71±0.01	0.0494	0.71±0.05	0.76±0.06	NS
p-AMPK/Total AMPK protein expression	1.96±0.40	2.77±0.55	NS	1.56±0.21	1.27±0.05	NS
Insulin signaling at week 28						
p-IRS/Total IRS protein expression	0.51±0.01	0.65±0.03	NS (0.0871)	0.43±0.01	0.46±0.02	NS
p-AKT/Total AKT protein expression	0.91±0.08	0.95±0.02	NS	0.69±0.02	0.79±0.02	0.0347
Mitochondrial respiration at week 28						
State 3 respiration rate, pmol/min	51.92±4.94	123.10±3.41	0.0109	236.84±16.40	332.37±20.19	0.0461
State 4 respiration rate, pmol/min	19.62±5.60	23.05±8.56	NS	28.57±5.23	57.89±4.26	0.0201
Oxygen consumption rate	2.29±0.81	5.99±0.49	0.0467	5.23±0.10	8.12±0.06	NS (0.0807)
Complex I protein expression	0.33±0.03	0.29±0.02	NS	0.20±0.03	0.18±0.03	NS
Complex II protein expression	1.57±0.02	2.03±0.07	0.0437	1.48±0.03	1.68±0.05	0.0453
Complex III protein expression	1.17±0.05	1.08±0.09	NS	0.91±0.10	0.79±0.07	NS
Complex IV protein expression	0.85±0.05	0.93±0.08	NS	0.56±9.93	0.59±0.05	NS
Complex V protein expression	1.10±0.03	1.37±0.09	NS (0.0898)	1.16±0.03	1.40±0.05	0.0378
Mitochondrial dynamics at week 28						
MFN1 protein expression	0.73±0.03	0.87±0.01	0.0377	0.73±0.01	1.00±0.04	0.0448
MFN2 protein expression	1.67±0.06	1.90±0.07	NS (0.0806)	1.64±0.02	1.89±0.03	0.0312
OPA1 protein expression	0.58±0.02	0.62±0.05	NS	0.83±0.05	0.88±0.04	NS
Mitochondrial DRP1 protein expression	0.90±0.03	0.40±0.05	0.0351	0.87±0.01	0.45±0.03	0.0279
Cytosolic p-DRP1 ^{ser616} /total DRP1 protein expression	0.97±0.02	0.77±0.01	NS (0.0722)	0.87±0.02	0.87±0.03	NS
Mitophagy at week 28						
Mitochondrial PINK1/Parkin protein expression	2.05±0.21	2.33±0.46	NS	1.12±0.12	2.55±0.42	0.0482
Cytosolic PINK1/Parkin protein expression	0.26±0.01	0.37±0.03	NS (0.0874)	0.82±0.03	0.81±0.02	NS
Autophagy at week 28						
Beclin-1 protein expression	0.66±0.09	0.72±0.05	NS	0.75±0.01	0.97±0.04	NS (0.0738)
LC3-II protein expression	0.89±0.11	0.87±0.04	NS	1.07±0.07	1.38±0.02	NS (0.0675)
p62 protein expression	0.88±0.07	0.82±0.05	NS	1.35±0.19	1.27±0.10	NS
Apoptosis at week 28						
Cytosolic/Mitochondrial cytochrome c protein expression	0.79±0.07	0.46±0.02	0.0163	0.72±0.02	0.51±0.03	0.0373
Bax/Bcl-2 protein expression	0.69±0.05	0.71±0.05	NS	0.66±0.02	0.58±0.01	0.0388
Cleaved caspase 3/Procaspase 3 protein expression	0.53±0.04	0.49±0.06	NS	0.50±0.07	0.43±0.05	NS

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Supplemental Table S6. Continued

Heart 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)	
Antioxidant at week 28						
GPX4 protein expression	0.55±0.09	0.59±0.04	NS	0.57±0.02	0.77±0.02	0.0101
SOD2 protein expression	1.71±0.12	1.71±0.13	NS	1.28±0.03	1.60±0.09	NS (0.0713)
Lipid peroxidation at week 28						
MDA level, mmol/g protein	329.76±27.29	283.27±21.99	NS	323.27±39.87	250.32±21.31	NS
Inflammation at week 28						
TNF- α protein expression	0.57±0.05	0.48±0.05	NS	1.01±0.01	0.68±0.03	0.0239
p-NF κ B/Total NF κ B protein expression	2.06±0.25	1.72±0.23	NS	1.68±0.05	1.70±0.07	NS
Anti-aging marker at week 28						
sRAGE protein expression	1.16±0.02	1.21±0.06	NS	0.89±0.09	0.92±0.06	NS
Cardiac function at week 28						
LVEF, %	79.88±2.47	89.43±1.73	NS	78.60±0.35	88.10±1.48	0.0480
%FS	49.26±1.20	52.75±1.28	NS	42.45±0.32	52.10±2.12	NS (0.0724)
E/A ratio	1.24±0.08	1.19±0.07	NS	1.32±0.05	1.36±0.03	NS
LF/HF ratio	0.16±0.02	0.13±0.01	NS	0.28±0.06	0.23±0.04	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; ROS, reactive oxygen species; NS, no significance; CPT1, carnitine palmitoyltransferase 1; PGC-1 α , peroxisome proliferator-activated receptor gamma coactivator-1 α ; p-AMPK, phosphorylated-activated protein kinase; AMPK, activated protein kinase; p-IRS, phosphorylated-insulin receptor substrate 1; IRS, insulin receptor substrate 1; MFN1, mitofusin 1; MFN2, mitofusin 2; OPA1, optic atrophy 1; DRP1, dynamin-related protein 1; p-DRP1^{ser616}, phosphorylated-dynamin-related at serine⁶¹⁶; 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; MDA, malondialdehyde; TNF- α , tumor necrosis factor- α ; p-NF κ B, phosphorylated-nuclear factor kappa-light-chain-enhancer of activated B cells; NF κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; sRAGE, soluble-receptor for advanced glycation end product; LVEF, left ventricular ejection fraction; FS, fractional shortening; E/A, early to late ventricular filling velocity; LF/HF, lower frequency/high frequency.