

ORIGINAL RESEARCH

Hippocampal endoplasmic reticulum stress hastens motor and cognitive decline in adult male rats sustainedly exposed to high-sucrose diet.

AUTHORS

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SUPPLEMENTARY INFORMATION

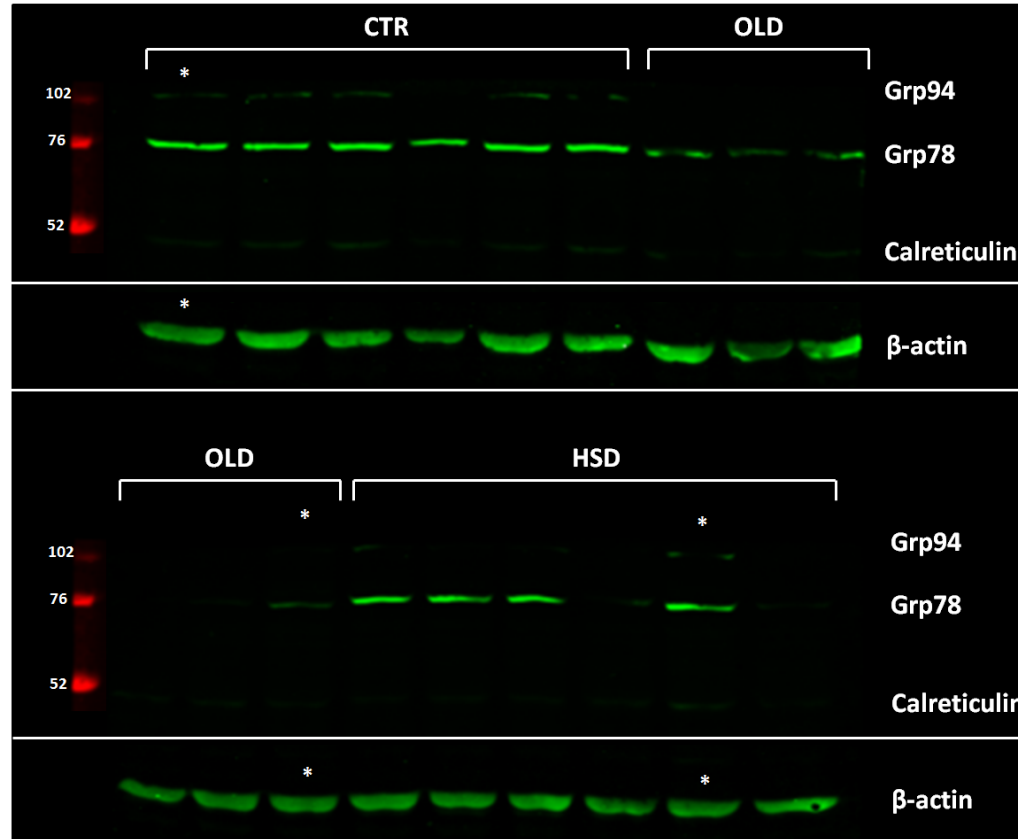
Supplementary Table S1. Primers sequences.

Genes*	Foward primer	Reverse primer	Amplicon	GenBank nº
Gapdh	5'-GAGACAGCCGCATCTTCTTGT-3'	5'-CGACCTTCACCATCTTGTCTATGA-3'	62	NM_017008.4
Ire1α	5'-GGATGTGAGTGACCGAATAGAAAA-3'	5'-TCCAAGTCCGCACGAT-3'	60	NM_001191926.1
Perk	5'-GGCTGGTGAGGGATGGTAAA-3'	5'-TTGGCTGTGTAACCTTGTGTCATCA-3'	64	NM_031599.2
Atf6	5'-TTCTCTGATGGCCGTGCAT-3'	5'-TGAAGATGACCCACAGAACCAA-3'	65	NM_001107196.1
Bdnf	5'-CACTTTTGAGCACGTGATCGA-3'	5'-AGAAGGTTCCGCCCAACG-3'	59	NM_001270630.1
Nrf2	5'-CCCATTGAGGGCTGTGATCT-3'	5'-GCCTTCAGTGTGCTTCTGGTT-3'	60	NM_031789.2
Grp94	5'-TGATGATGAAGCCGCAGTAG-3'	5'-AAGTTCCCAGTCCCACACAG-3'	88	NM_001012197.2
Grp78	5'-AAAGAAGGTCACCCATGCAGTT-3'	5'-GCCGCTGTGCATCATTGA-3'	59	NM_013083.2
Pdi A2	5'-GCACCTGGTCACAGAATTCAAC-3'	5'-TGAGGATCCTGGCTGCAAA-3'	62	NM_001105775.2
Calreticulin	5'-GCCAGACACTGGTGGTACAGTTC-3'	5'-AATATCGACTGTGGGGGCG-3'	60	NM_022399.2
Bcl2	5'-GGGATGCCTTTGTGGAACATATG-3'	5'-CAGCCAGGAGAAATCAAACAGA-3'	62	NM_016993.1
Chop	5'-TGGCACAGCTTGCTGAAGAG-3'	5'-TCAGGCGCTCGATTCCT-3'	54	NM_001109986.1
p53	5'-AAGACCAAGAAGGGCCAGTCTA-3'	5'-CAATGATCAAGAAAGTGGGGC-3'	61	NM_030989.3
p21	5'-CCTCCGCTGGGAACGTT-3'	5'-GCATAACTTCTGCTCAAGCACG-3'	56	NM_031550.1

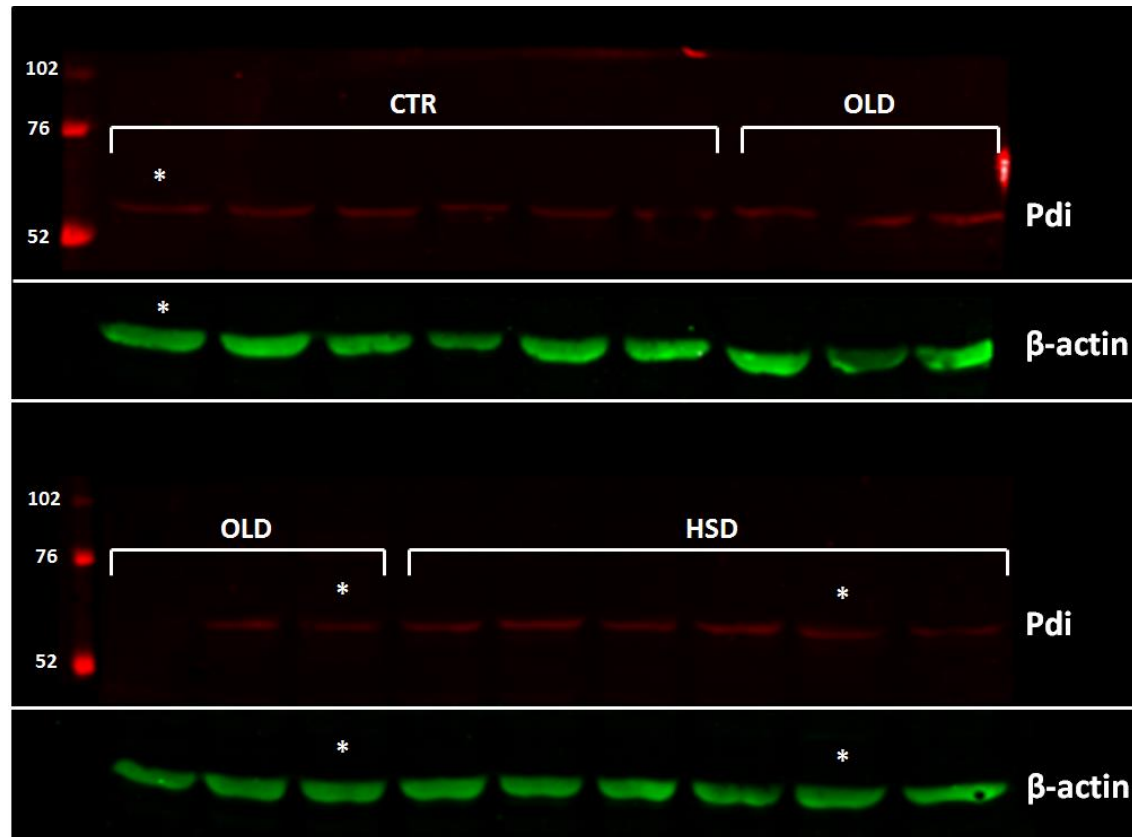
* Gapdh: Glyceraldehyde-3-phosphate dehydrogenase; Ire1α: Inositol-requiring enzyme 1 alpha; Perk: Protein kinase RNA-like ER kinase; Atf6: Activating transcription factor 6; Bdnf: Brain derived neurotrophic factor; Nrf2: Nuclear factor, erythroid derived 2, like 2; Grp94: Glucose regulated protein 94; Grp78: Glucose regulated protein 78; Pdi A2: Protein disulfide isomerase family A, member 2; Calreticulin; Bcl2: B-cell CLL/lymphoma 2; Chop: C/EBP-homologous protein; p53: Tumor protein p53; p21: Cyclin dependent kinase inhibitor 1A.

Supplementary Table S2. Morphometric and biochemical profile of OLD animals.

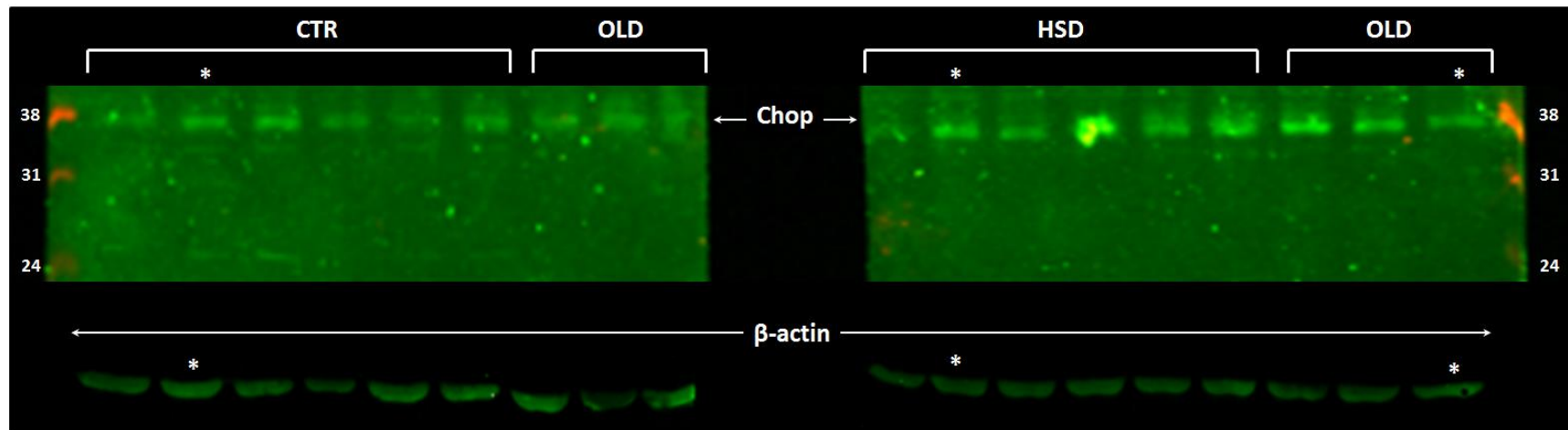
Parameters	OLD group	CTR group	<i>p</i> value
Body weight	572.7 ± 15.78 g	422.4 ± 5.39 g	<0,0001
Fasting glycemia	83.4 ± 2.5 mg/dL	93.1 ± 1.1 mg/dL	0,0075
Fed glycemia	104.3 ± 1.72 mg/dL	102.6 ± 3.4 mg/dL	0,6673
Insulinemia	0.09 ± 0.02 µLU/mL	0.32 ± 0.04 µLU/mL	0,0009
HOMA-B	1.17 ± 0.36	3.12 ± 0.36	0,0050
HOMA-IR	0.0008 ± 0.0002	0.003 ± 0.0003	0,0007
Retroperitoneal fat pad	2.27 ± 0.2 g/100 BW	0.96 ± 0.1 g/100 BW	0,0004
Periepididymal fat pad	2.52 ± 0.22 g/100 BW	1.15 ± 0.09 g/100 BW	0,0003
Mesenteric fat pad	2.66 ± 0.21 g/100 BW	0.75 ± 0.08 g/100 BW	<0,0001



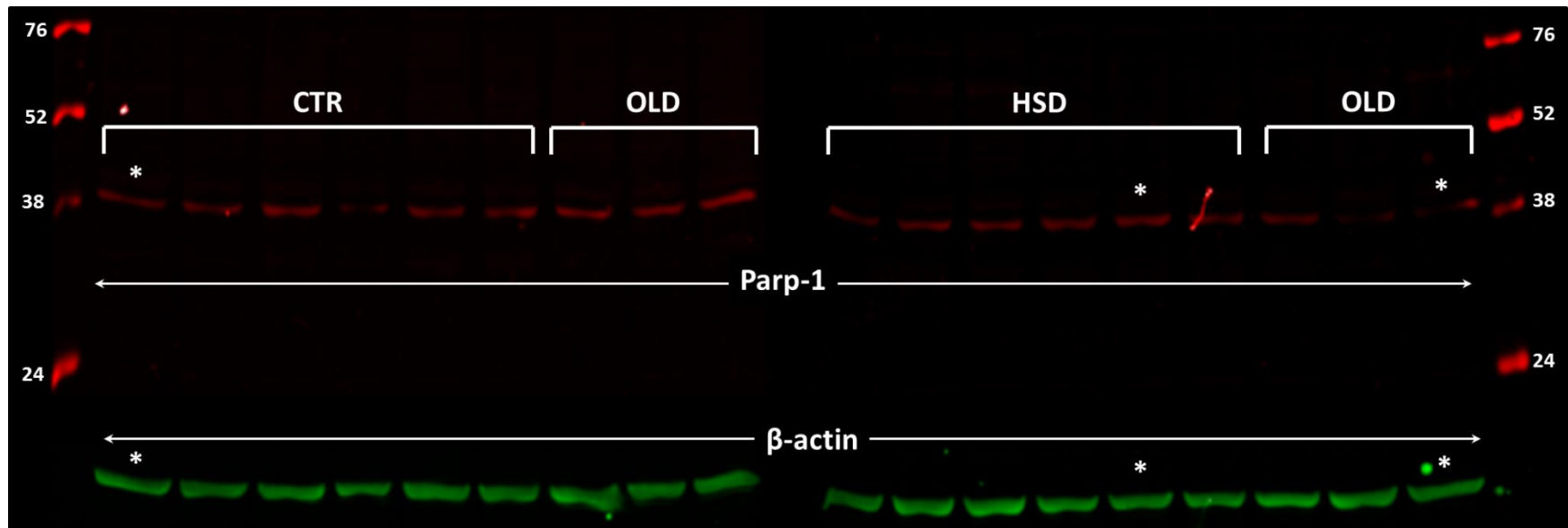
Supplementary Figure S1. Full-length gels/blots for Grp94, Grp78 and Calreticulin. Protein levels of Grp94, Grp78, Calreticulin and β -Actin assessed in hippocampus of rats fed a standard chow (CTR) and high-sucrose diet (HSD) until 6-months-old from weaning, and middle-aged (20-months-old) animals fed a standard chow (OLD). * indicate the bands used as representative in Figures 5J, K and M.



Supplementary Figure S2. Full-length gels/blots for Pdi. Protein levels of Pdi and β -Actin assessed in hippocampus of rats fed a standard chow (CTR) and high-sucrose diet (HSD) until 6-months-old from weaning, and middle-aged (20-months-old) animals fed a standard chow (OLD). * indicate the bands used as representative in Figure 5L.



Supplementary Figure S3. Full-length gels/blots for Chop. Protein levels of Chop and β-Actin assessed in hippocampus of rats fed a standard chow (CTR) and high-sucrose diet (HSD) until 6-months-old from weaning, and middle-aged (20-months-old) animals fed a standard chow (OLD). * indicate the bands used as representative in Figure 6C.



Supplementary Figure S4. Full-length gels/blots for Parp-1. Protein levels of Parp-1 and β -Actin assessed in hippocampus of rats fed a standard chow (CTR) and high-sucrose diet (HSD) until 6-months-old from weaning, and middle-aged (20-months-old) animals fed a standard chow (OLD). * indicate the bands used as representative in Figure 6D.