

SUPPLEMENTAL MATERIAL

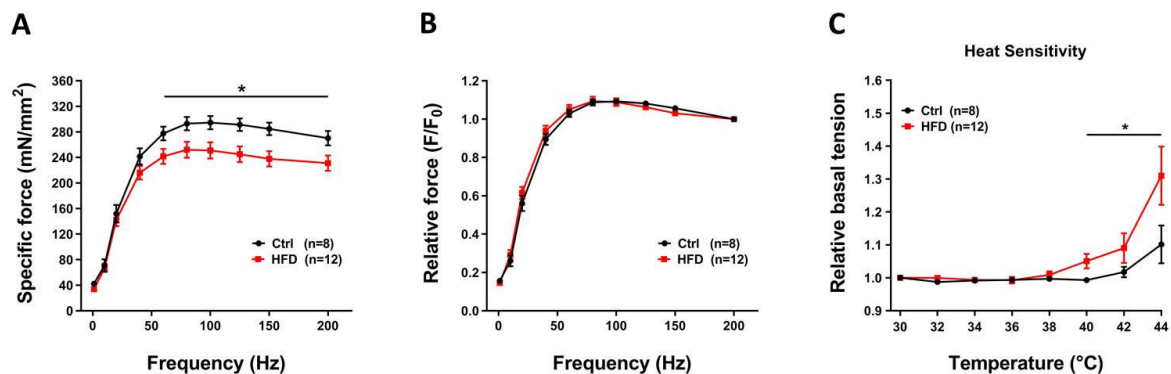


Figure S1. In-vitro specific force and temperature dependence of basal tension in Soleus muscles. (A and B) Force frequency (1-200Hz) curve of specific force (panel A) and relative force normalized to 200Hz (panel B) in isolated Soleus muscles. (C) Relative basal tension (normalized to 30°C) during exposure to increasing temperature (from 30 to 44°C). Data are shown as mean \pm SEM (* $p < 0.05$), as evaluated two-way ANOVA followed by Tukey's post-hoc test. n = number of Soleus muscles tested.

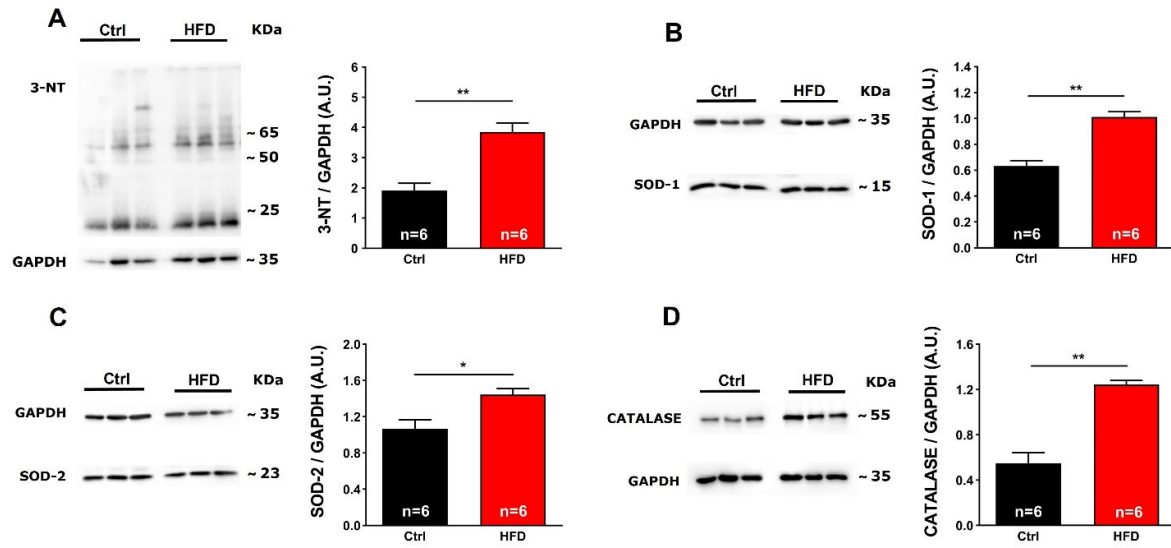


Figure S2. Markers of oxidative and nitrosilative stress in Soleus muscles. Representative immunoblots (left) and relative band densities normalized to GAPDH levels (right) of 3-NT (panel **A**), SOD-1 (panel **B**), SOD-2 (panel **C**), and Catalase (panel **D**) in Soleus muscle homogenates. Data are shown as mean \pm SEM (* p <0.05 and ** p <0.01), as evaluated by two-tailed unpaired Student's t -test. n = number of mice tested.

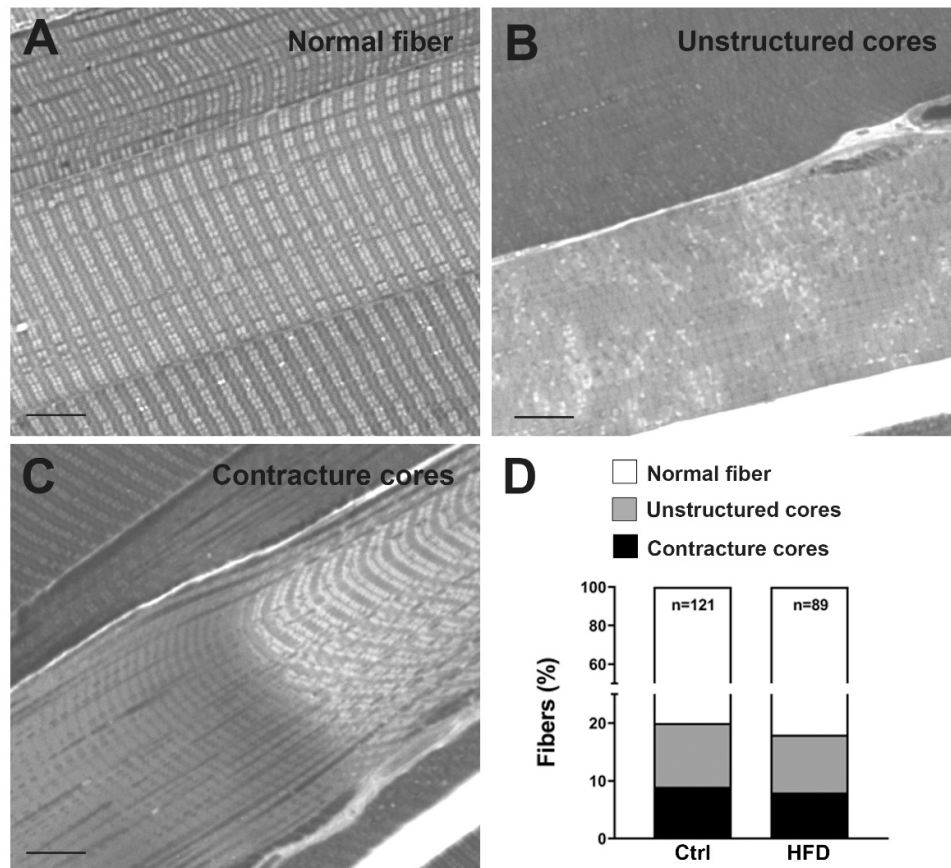


Figure S3. Muscle damage after the heat stress protocol evaluated by histological analysis. Quantitative analysis (panel **D**) in histological sections of EDL fibers, which were classified in three groups: presenting normal appearance (as in panel **A**), containing areas with loss of striation (as in panel **B**), or finally containing contracture cores (as in panel **C**). n = number of EDL fibers analyzed.

Table S1. Energetic and nutrients composition of the diets administrated to Ctrl and HFD mice.

(Ctrl Diet)		
Metabolized energy		
Content	Value (Kcal/Kg)	Percentage (%)
Fat	3.780	70
Protein	842	16
Carbonhydrates	766	14

Crude nutrients and moisture		
Content	Value (mg/Kg)	Percentage (%)
Moisture	44.328	4.4
Crude Ash	61.481	6.1
Crude Fibre	47.350	4.7
Crude Fat	420.018	42.0
Crude Protein	210.545	21.1
Nitrogenfree Extractives	216.278	21.7

(HF Diet)		
Metabolized energy		
Content	Value (Kcal/Kg)	Percentage (%)
Fat	358	10
Protein	828	24
Carbonhydrates	2.329	66

Crude nutrients and moisture		
Content	Value (mg/Kg)	Percentage (%)
Moisture	79.090	7.9
Crude Ash	43274	4.3
Crude Fibre	30.829	3.1
Crude Fat	39.725	4.0
Crude Protein	206.900	20.7
Nitrogenfree Extractives	600.183	60

Table S2. Manufacturer and catalogue number by material reagent used.

CHEMICAL/REAGENT	MANUFACTURER	CATALOGUE NUMBER
2-mercaptoethanol	Sigma-Aldrich, USA	M3148
Anti-3-NT primary antibody	Merck Millipore, Italy	05-233
Anti-Catalase primary antibody	Santa Cruz Biotechnology Inc., USA	SC-271803
Anti-GAPDH primary antibody	OriGene Technologies Inc., USA	TA802519
Anti-SOD1 primary antibody	Santa Cruz Biotechnology Inc., USA	SC-11407
Anti-SOD2 primary antibody	Santa Cruz Biotechnology, Inc., USA	SC-30080
BCA quantification kit	ThermoFisher scientific, USA	23225
CaCl ₂	Sigma-Aldrich, USA	C8106
Caffeine	Sigma-Aldrich, USA	C0750
Calcium colorimetric assay kit	Sigma-Aldrich, USA	MAK022
Creatine Kinase colorimetric assay kit	Sigma-Aldrich, USA	MAK116-1KT
DPX mounting medium for histology	Sigma-Aldrich, USA	06522
EDTA	Sigma-Aldrich, USA	ED2SS
Enhanced chemiluminescent liquid	Perkin-Elmer, USA	NEL104001EA
Epoxy resin (Epon 812)	Electron Microscopy Sciences, USA	14120
Glucose	Sigma-Aldrich, USA	G8270
Glutaraldehyde	Histo-line Laboratories, Italy	18428-10
HEPES	Sigma-Aldrich, USA	H3375
Horseradish peroxidase conjugated secondary antibodies (anti-mouse)	Merck Millipore, USA	401253
Horseradish peroxidase conjugated secondary antibodies (anti-rabbit)	Merck Millipore, USA	401393
KCl	Sigma-Aldrich, USA	P9333
MgSO ₄	Sigma-Aldrich, USA	M7506
NaCaCO	Electron microscopy sciences, USA	12300
NaCl	Sigma-Aldrich, USA	S7653
NaH ₂ PO ₄	Sigma-Aldrich, USA	71505
Non-fat dry milk	EuroClone, Italy	APA08300500
OsO ₄	Electron microscopy sciences, USA	19151
Potassium turbidimetric assay kit	MyBioSource, USA	MBS2540590
Protease inhibitors cocktail	Roche, Germany	11836153001
SDS	Bio-Rad, USA	1610301
Sodium Borate Tetra	Electron microscopy sciences, USA	21130
Sodium Deoxycholate	Sigma-Aldrich, USA	D6750
Toluidine blue O	Electron microscopy sciences, USA	22050
TRIS HCl	Sigma-Aldrich, USA	T3253
Triton-X	Sigma-Aldrich, USA	T8787
Tween 20	Sigma-Aldrich, USA	P1379