



Mitochondrial dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice.

Charlotte Bonnard, Annie Durand, Simone Peyrol, Emilie Chanseume, Marie-Agnes Chauvin, Béatrice Morio, Hubert Vidal, Jennifer Rieusset

► To cite this version:

Charlotte Bonnard, Annie Durand, Simone Peyrol, Emilie Chanseume, Marie-Agnes Chauvin, et al.. Mitochondrial dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice.. *Journal of Clinical Investigation*, American Society for Clinical Investigation, 2008, 118 (2), pp.789-800. <10.1172/JCI32601>. <inserm-00808486v2>

HAL Id: inserm-00808486

<http://www.hal.inserm.fr/inserm-00808486v2>

Submitted on 25 Apr 2013

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

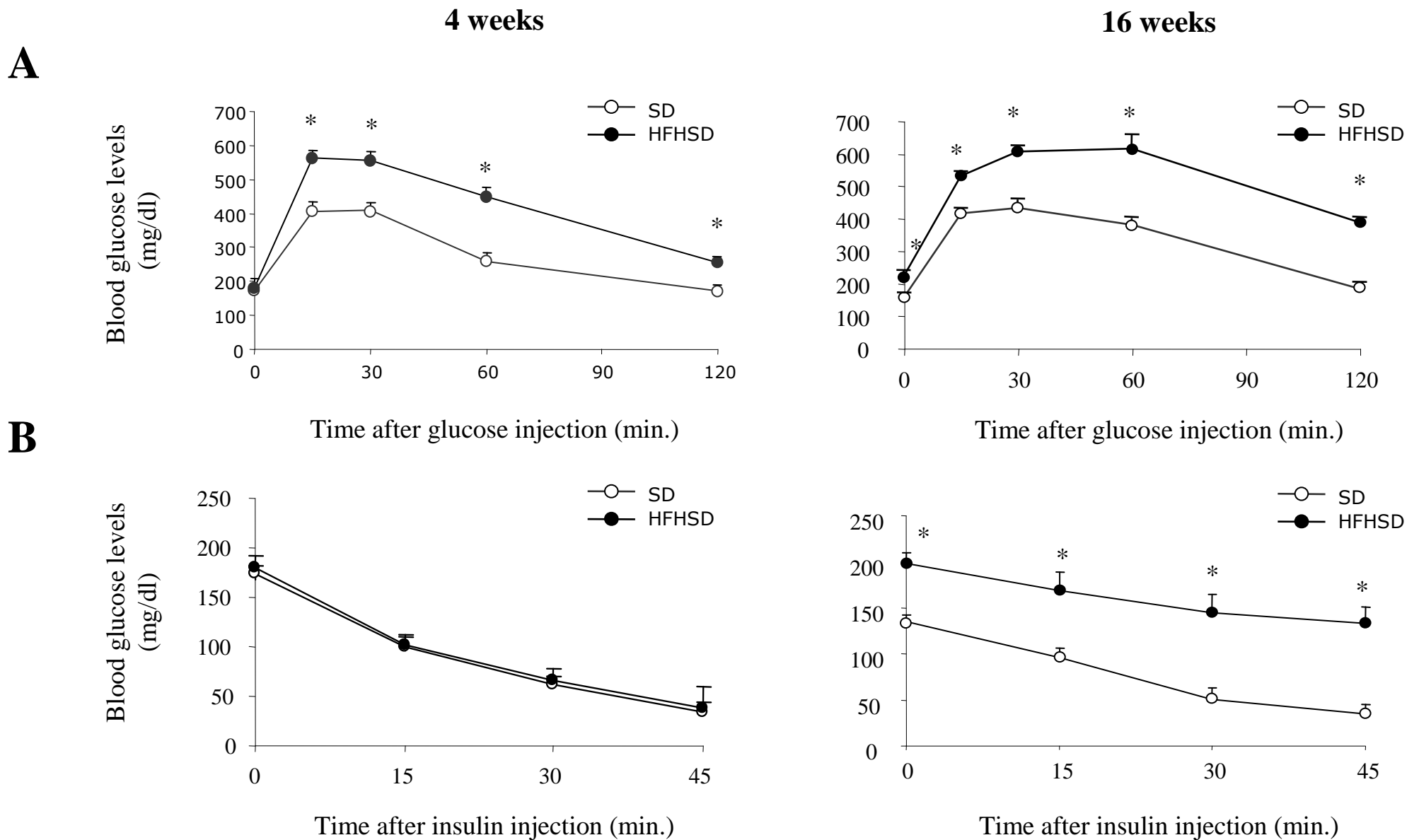


Figure S1: Systemic insulin sensitivity in SD and HFHSD mice. Intraperitoneal glucose (A) and insulin (B) tolerance tests in 6h fasted SD (white circles) and HFHSD (black circles) mice, after 4 (left panel) and 16 (right panel) weeks of diet. Animals were injected intraperitoneally with 2mg/g body weight of glucose or 0.75mU/g body weight of insulin. Data represent the means \pm sem of 6-16 mice. * $p < 0.01$ vs SD.

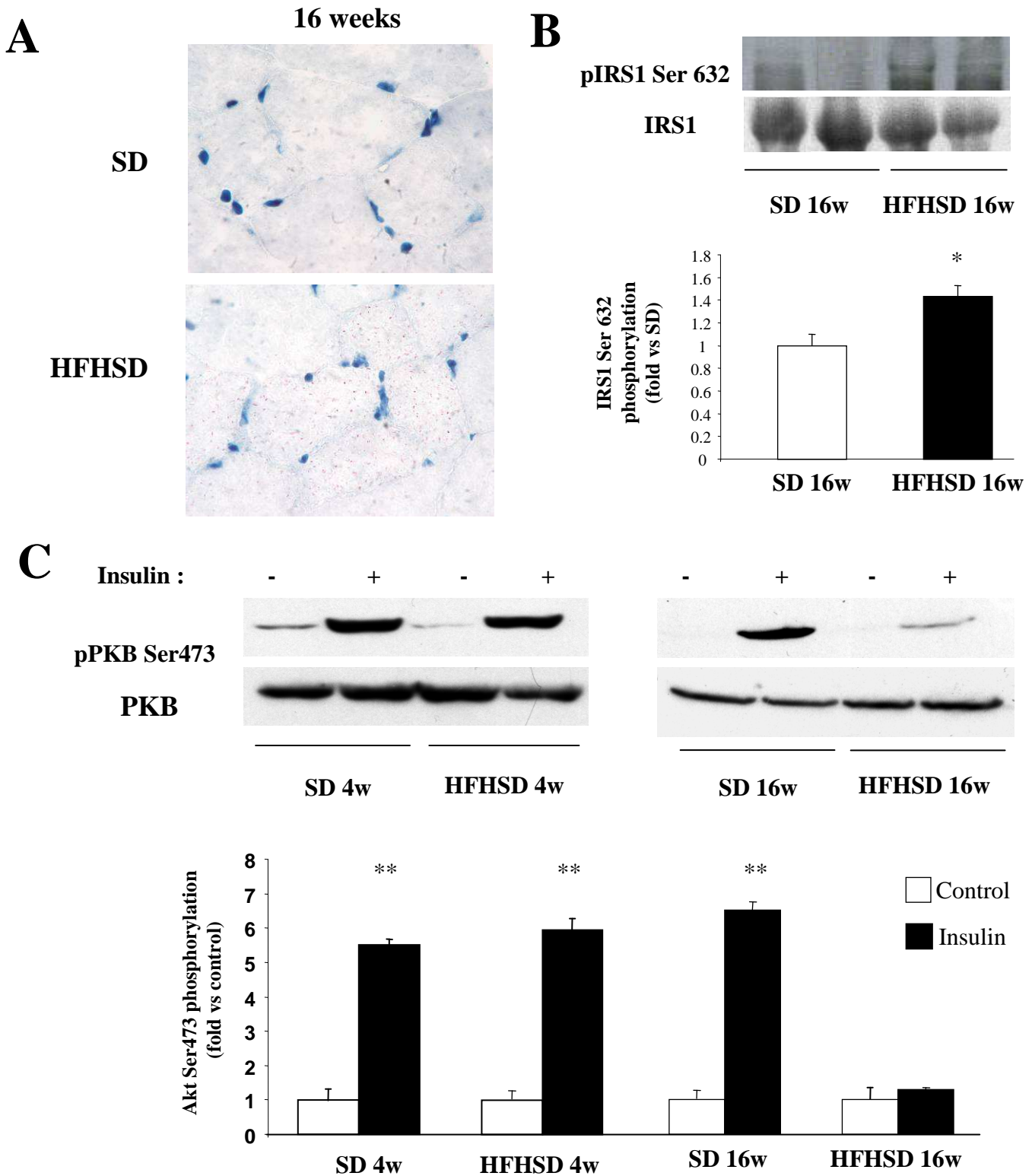


Figure S2: Altered insulin responsiveness in skeletal muscle of 16 week HFHSD mice. A: Oil Red O staining of gastrocnemius muscle from 16 week SD and HFHSD mice. B: Basal IRS1 phosphorylation on serine 632 in gastrocnemius muscle of 16 week SD and HFHSD mice. IRS1 phosphorylation was normalized to total IRS1 protein expression. C: Insulin-stimulated Akt phosphorylation on serine 473, measured on muscle fragments incubated ex vivo in the absence or in the presence of insulin (10^{-7} M) for 15 minutes. Akt phosphorylation was normalized to total Akt protein expression. Results are expressed as fold increase over insulin-free basal conditions ($n=3$). * $p<0.05$, ** $p<0.01$.

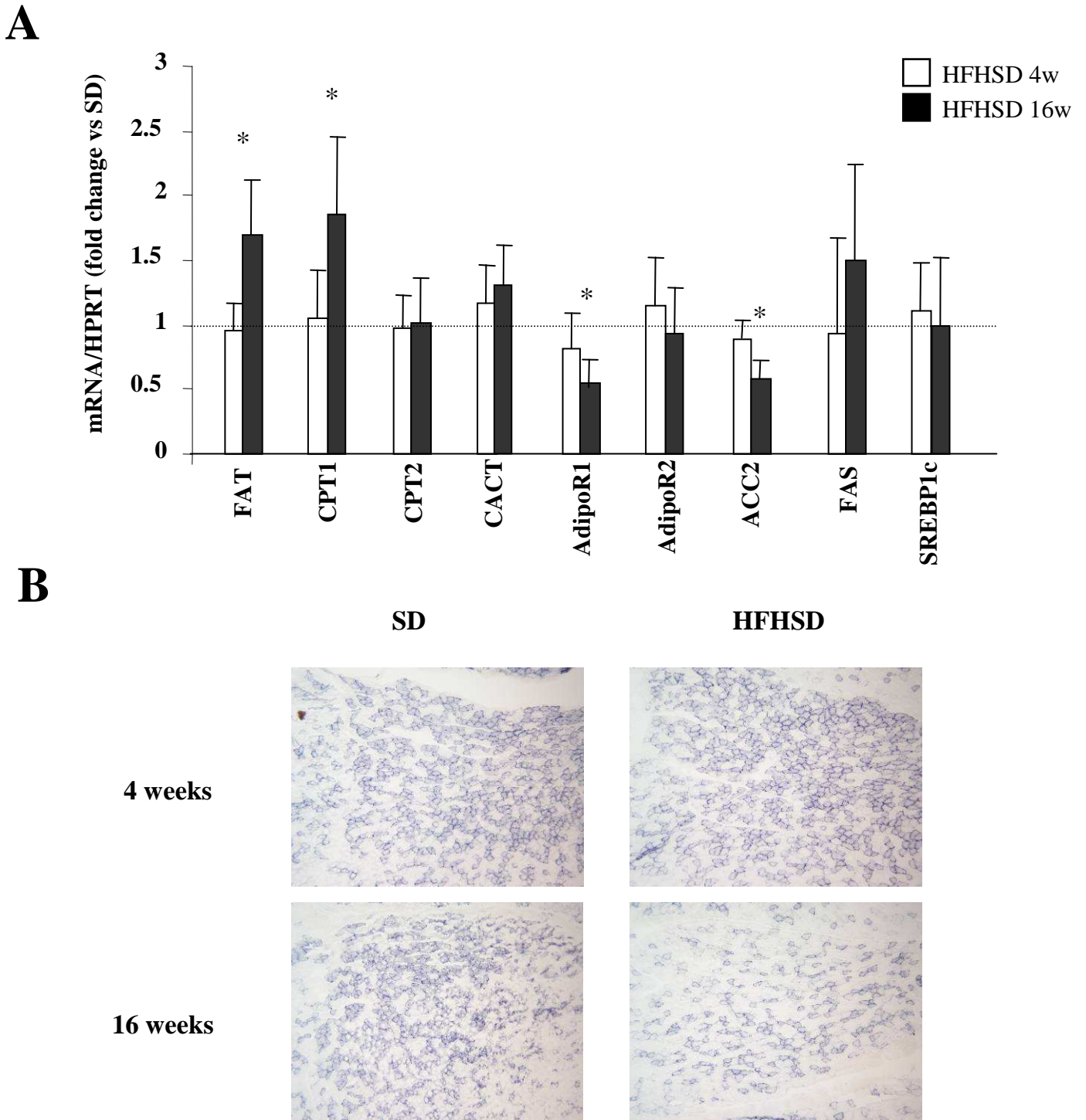


Figure S3: Oxidative and lipid metabolisms in muscle of SD and HFHSD mice. A: mRNA levels of lipid metabolism genes, determined by real-time RT-PCR, in gastrocnemius muscle of SD and HFHSD mice, After 4 and 16 weeks of diet (n=6). Results are expressed relative to SD condition (dotted line). * p< 0.05. B: Succinate dehydrogenase staining of gastrocnemius muscle from 4 and 16 week SD and HFHSD mice. Images have been taken in the deep gastrocnemius muscle of mice, which has a higher proportion of slow twitch fibers.

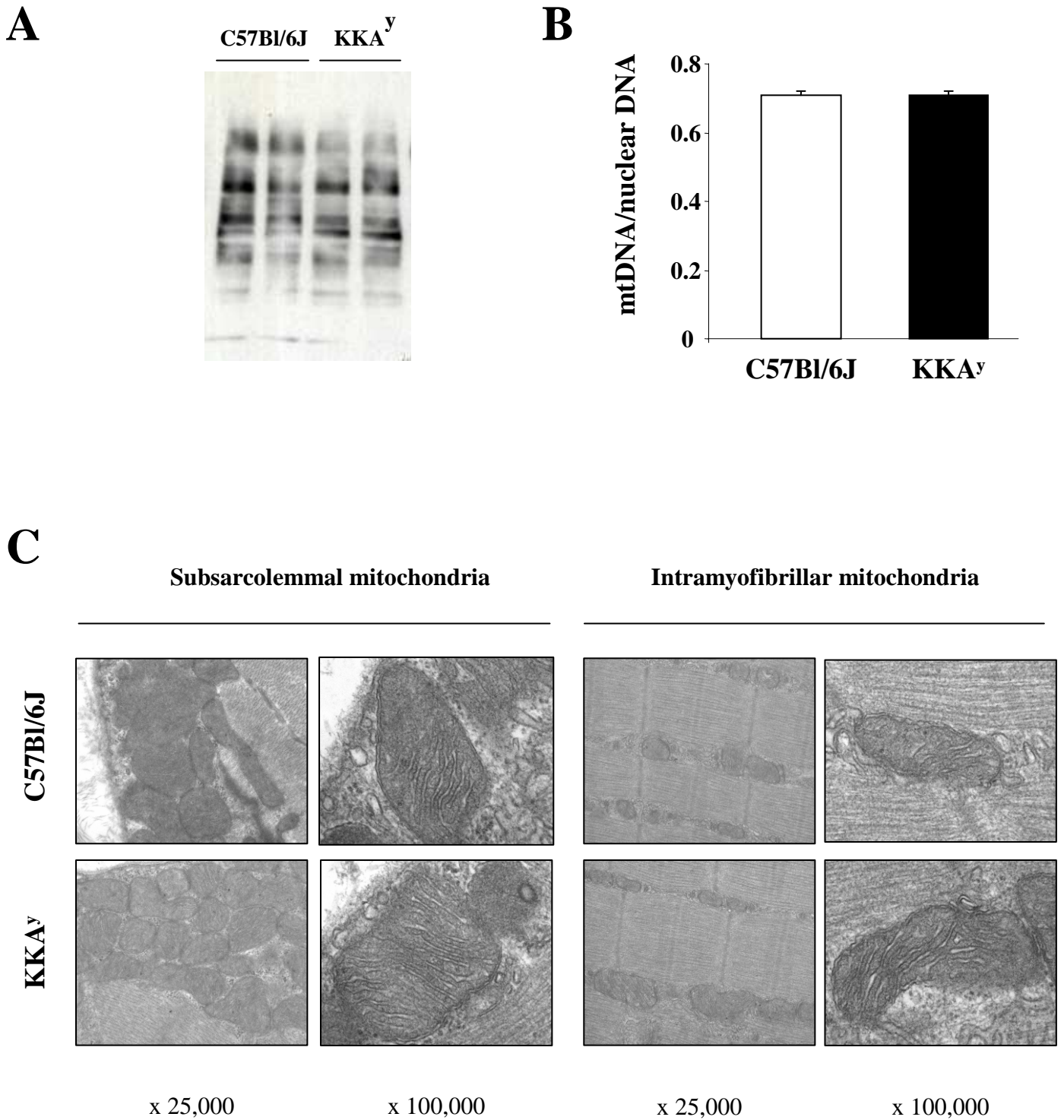


Figure S4: Lack of muscle oxidative stress and mitochondrial alterations in KKA^y mice. A- Immunoblots showing total protein carbonylation in gastrocnemius muscle of C57Bl/6J and KKA^y mice. B- mtDNA levels, determined by real time PCR, in skeletal muscle of C57Bl/6J and KKA^y mice (n=6). mtDNA copy number was calculated as the ratio of COX1 to cyclophilin A. C- Transmission electronic microscopy images (magnification x25,000 and x100,000) of subsarcolemmal and intermyofibrillar mitochondria in gastrocnemius muscle of C57Bl/6J and KKA^y mice.

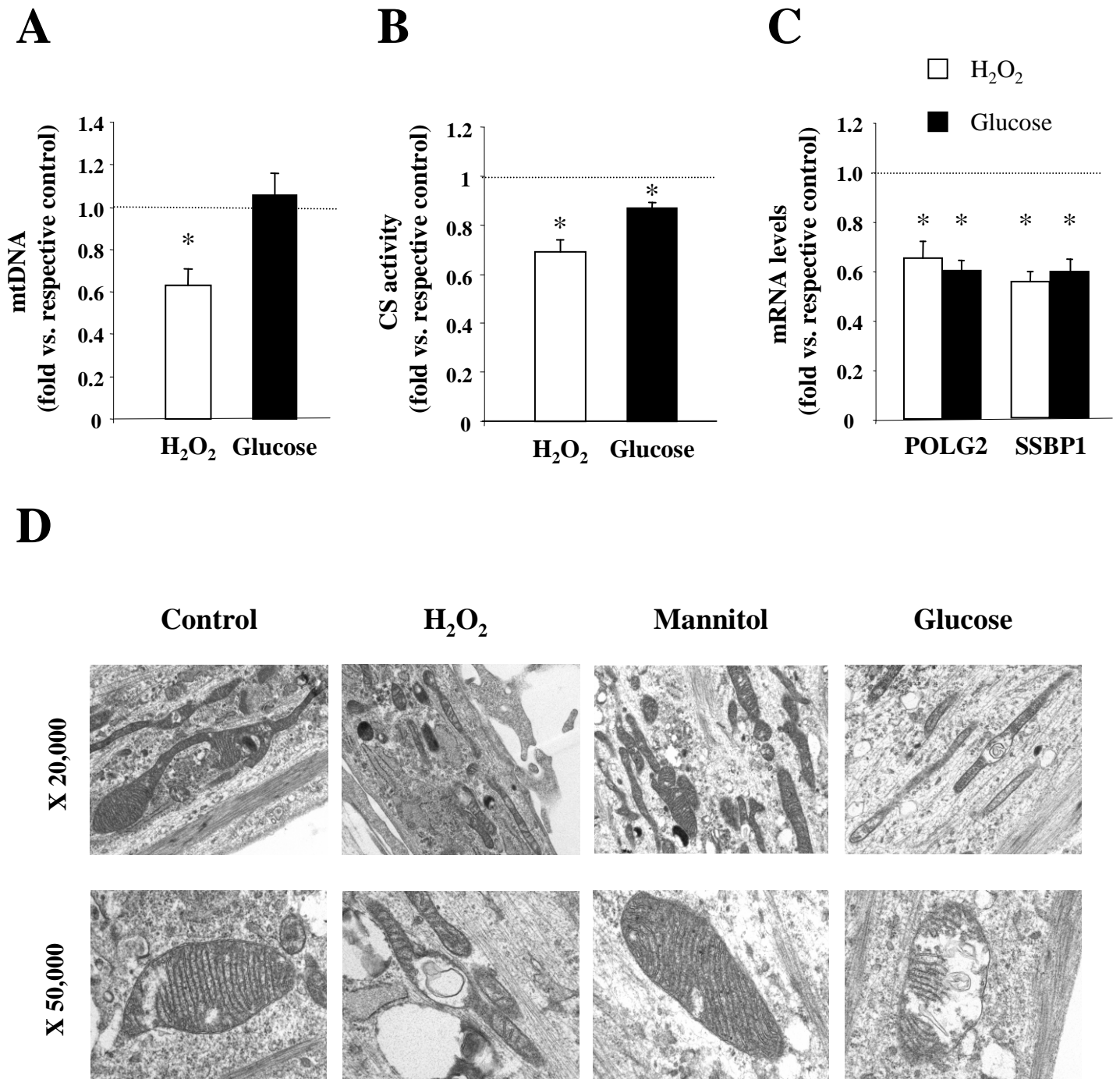


Figure S5: Effects of ROS on mitochondria density, structure and function in human myotubes.
 A: mtDNA copy number from myotubes treated for 96 hours with H₂O₂ (0.1mM) and glucose (25mM).
 B: Citrate synthase (CS) activity measured in total lysates of myotubes treated with H₂O₂ (0.1mM) and glucose (25mM) for 96 hours. C: mRNA levels of POLG2 and SSBP1 genes, determined by real-time RT-PCR, in H₂O₂ and glucose-treated myotubes for 96 hours. D- Transmission electronic microscopy images (magnification x 20,000 and x 50,000) of mitochondria in human myotubes treated or not with H₂O₂ and glucose for 96 hours. Mannitol (25mM) is added as control for glucose treatment. All results are expressed relative to untreated cells (dotted line) (n=3 in triplicate). * p<0.05.

Table S1 : Metabolic characteristics of age-matched C57Bl/6J control and KKA^y mice.

	C57Bl/6J	KKA^y
Body weight (g)	22.7 ± 0.5	28 ± 0.8 ^{**}
Fat weight (g)	0.39 ± 0.02	0.81 ± 0.07 ^{**}
Glucose (mg/dl)	166.8 ± 6.3	304.3 ± 46 ^{**}
Insulin (ng/ml)	0.51 ± 0.04	3.83 ± 1.2 [*]
TG (g/l)	0.85 ± 0.05	3.29 ± 0.4 ^{**}
FFA (mM)	0.1 ± 0.02	0.14 ± 0.01
H ₂ O ₂ (μM)	61.9 ± 7	85.4 ± 4.8 [*]

Data represent the means ± sem of 10 mice per group.

* p<0.05, ** p<0.001 vs the control mice.