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

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Charlotte Bonnard, Annie Durand, Simone Peyrol, Emilie Chanseaume, 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*, 2008, 118 (2), pp.789-800. 10.1172/JCI32601 . inserm-00808486v2

HAL Id: inserm-00808486

<https://inserm.hal.science/inserm-00808486v2>

Submitted on 25 Apr 2013

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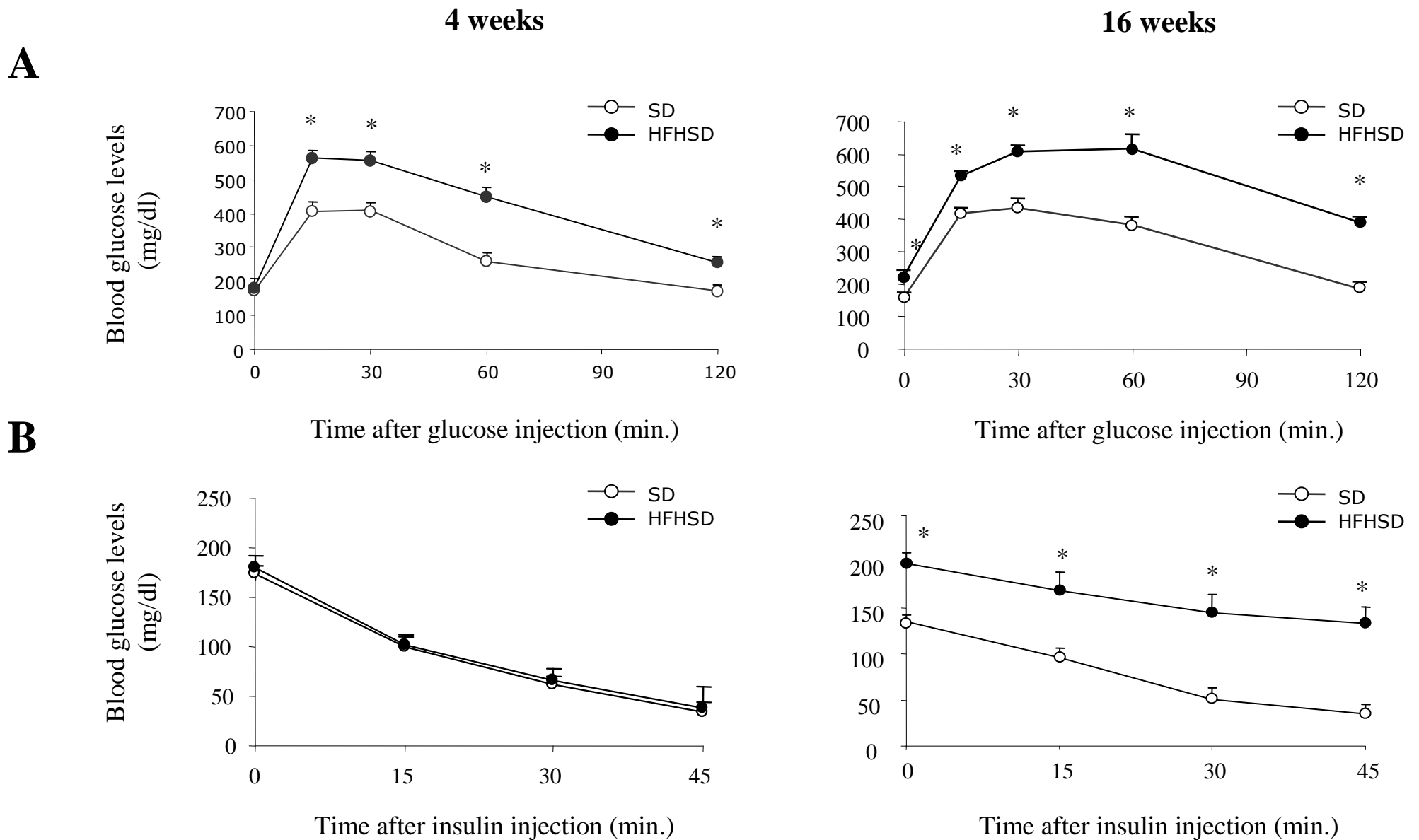


Figure S1: Systemic insulin sensitivity in SD and HFHSD mice. Intraperitoneal glucose (A) and insulin (B) tolerance tests in 6h fasted SD (white circles) and HFHSD (black circles) mice, after 4 (left panel) and 16 (right panel) weeks of diet. Animals were injected intraperitoneally with 2mg/g body weight 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.

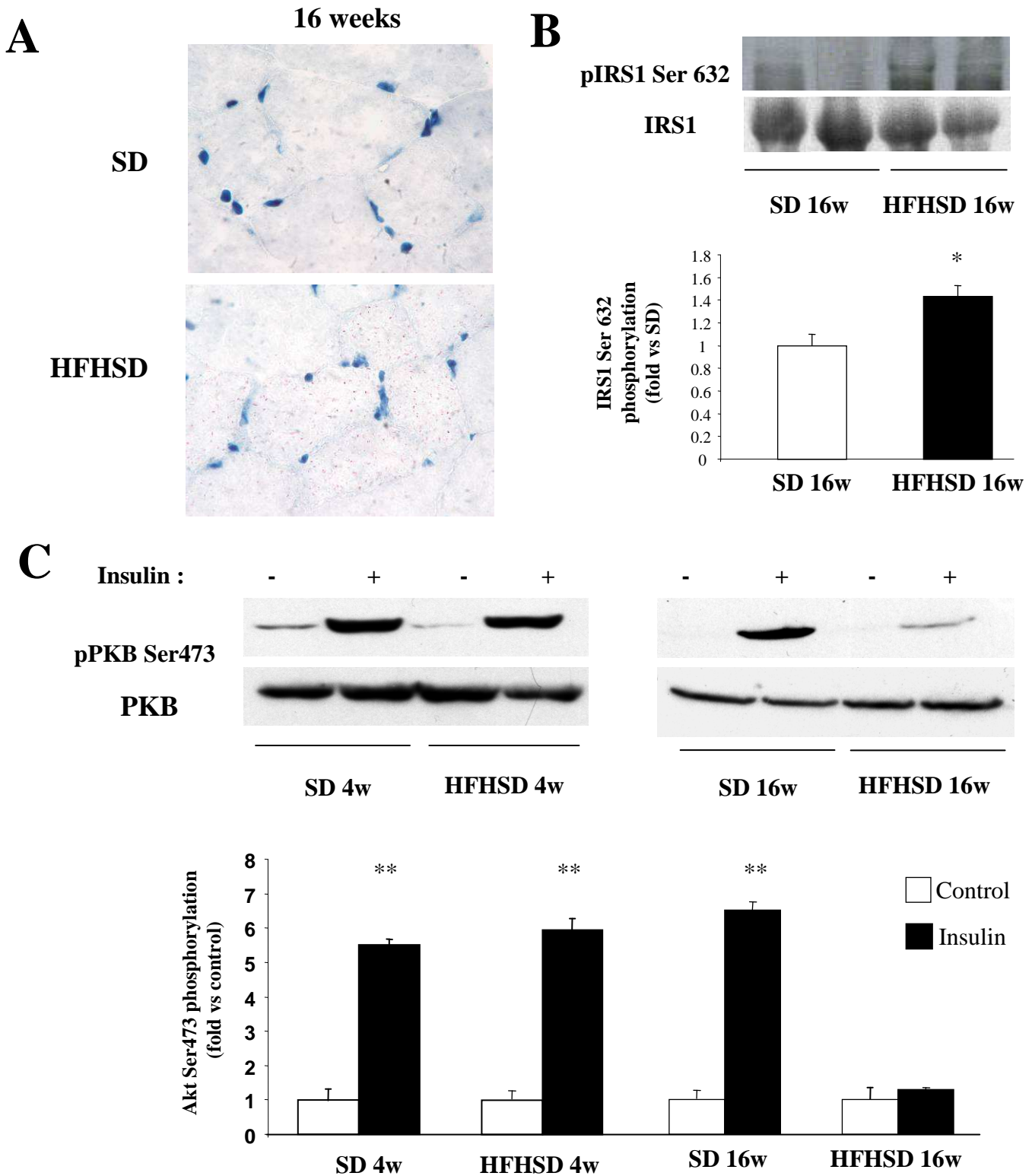


Figure S2: Altered insulin responsiveness in skeletal muscle of 16 week HFHSD mice. A: Oil Red O staining of gastrocnemius muscle from 16 week SD and HFHSD mice. B: Basal IRS1 phosphorylation on serine 632 in gastrocnemius muscle of 16 week SD and HFHSD mice. IRS1 phosphorylation was normalized to total 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). * $p < 0.05$, ** $p < 0.01$.

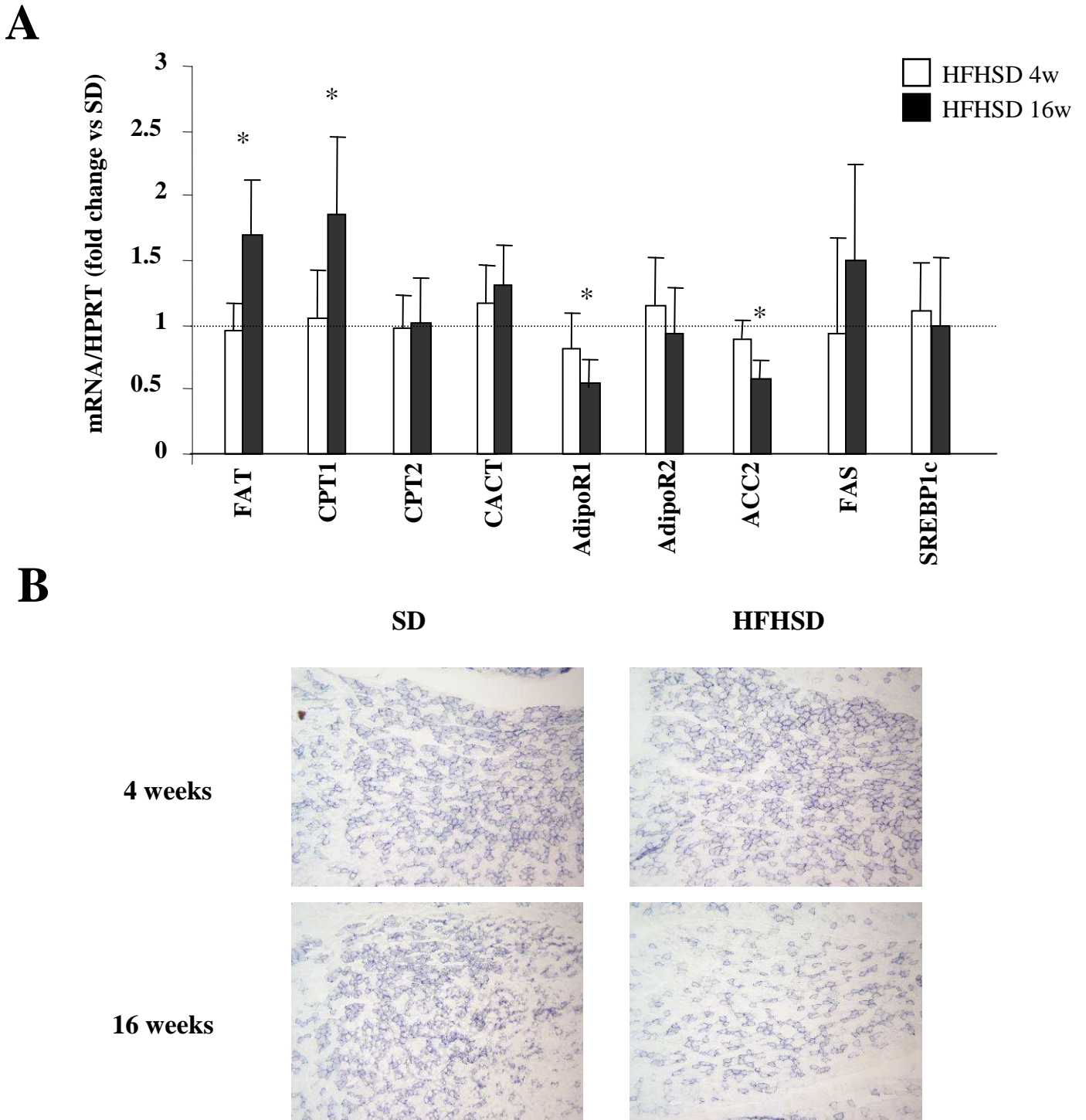


Figure S3: Oxidative and lipid metabolisms in muscle of SD and HFHSD mice. A: mRNA levels of lipid metabolism genes, determined by real-time RT-PCR, in gastrocnemius muscle of SD and HFHSD mice, After 4 and 16 weeks of diet (n=6). Results are expressed relative to SD condition (dotted line). * $p < 0.05$. 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.

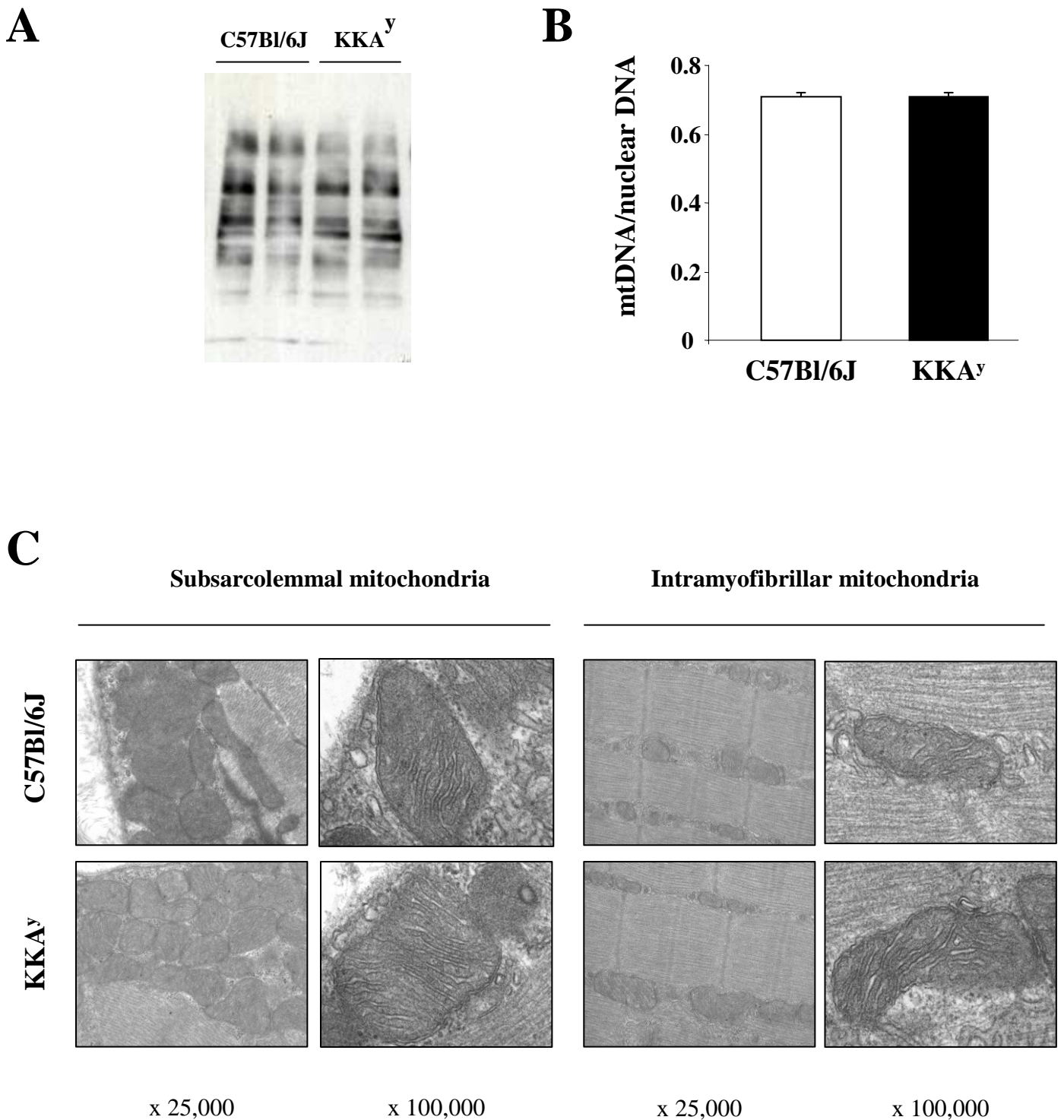


Figure S4: Lack of muscle oxidative stress and mitochondrial alterations in KKA^y mice. A- Immunoblots showing total protein carbonylation in gastrocnemius muscle of C57Bl/6J and KKA^y mice. B- mtDNA levels, determined by real time PCR, in skeletal muscle of C57Bl/6J and KKA^y mice (n=6). mtDNA copy 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.

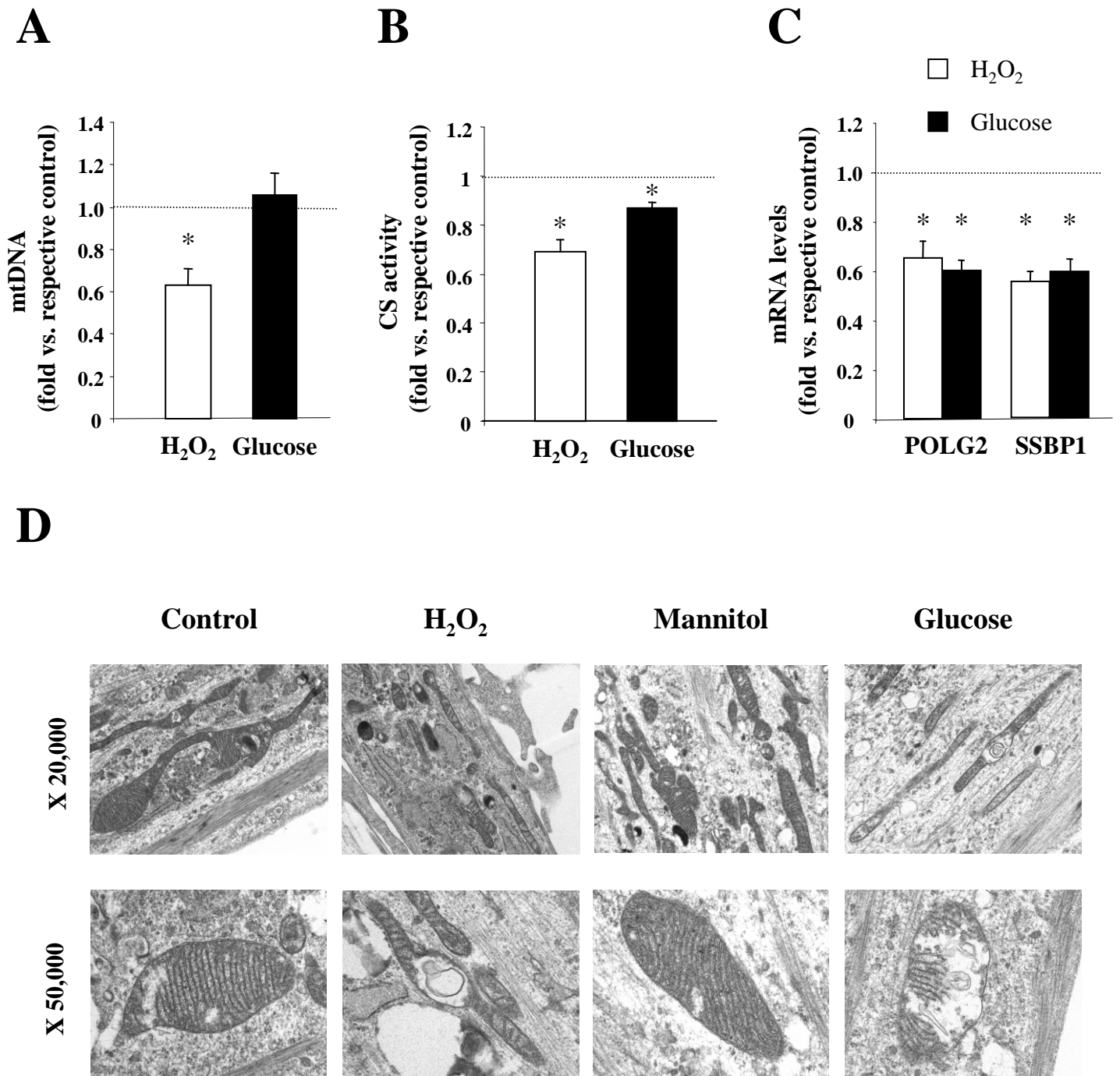


Figure S5: Effects of ROS on mitochondria density, structure and function in human myotubes.
 A: mtDNA copy number from myotubes treated for 96 hours with H₂O₂ (0.1mM) and glucose (25mM).
 B: Citrate synthase (CS) activity measured in total lysates of myotubes treated with H₂O₂ (0.1mM) and glucose (25mM) 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.