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Disrupted mitochondrial and metabolic plasticity underlie comorbidity between age-related and degenerative disorders as Parkinson disease and Type 2 Diabetes Mellitus

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Mitochondrial oxygen consumption (Seahorse)

Oxygen consumption rates (OCRs) were measured with Agilent Seahorse XF24 Analyzer Mitostress Test (Seahorse Bioscience, www.seahorsebio.com), according to manufacturer's protocol. Briefly, 30,000-35,000 fibroblasts/well were seeded by quadruplicate in customized 24-well Seahorse cell culture plates and kept overnight in 250 µl of either 25mM or 5mM glucose medium. Growth medium was then removed, and wells were washed once with Seahorse XF Base Medium (Seahorse Bioscience) containing 10 mM Glucose, 1 mM Sodium Pyruvate and 1 mM Glutamine. Plates were incubated in this media for 30 min at 37 °C without CO_2. The bioenergetic profile was measured obtaining the OCRs under basal conditions and after the addition of oligomycin, carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP) and rotenone-antimicyn (all reagents from Sigma-Aldrich). OCR values were normalized to total cell protein content and reported as pmol/min*ug protein.

Mitoworking capacity was measured as the ratio between the Basal Respiration and the Maximal respiration.

Despite using identical cell number for all analyses, results were additionally normalized by total protein and mitochondrial content through the CS activity and were expressed as pmol O_2/min*µg protein*CS activity.

Mitochondrial oxygen consumption (Oroboros)

Mitochondrial respiration was measured in parallel by high-resolution respirometry using Oroboros™ Oxygraph-2K® (Innsbruck, Austria) in permeabilized fibroblasts, following manufacturer protocols (Pesta and Gnaiger 2012). Briefly, 1 million of living fibroblasts were obtained and resuspended in ice-cold respiration MiR05 medium (0.5 mM EGTA, 3 mM $MgCl_2$, 60 mM K-lactobionate, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 110 mM sucrose and 0.1% BSA (w/v), pH 7.1). Data recording and analysis were performed using DatLab software v5.1.1.9 (Oroboros Instruments), following manufacturer's protocol. The oxygen consumption was measured using the same procedure as in the Seahorse Mitostress test: Firt measuring the "basal" respiration, followed by complete blockage of Complex V (1uM Oligomycin), titration with sequential doses of 0.5uM FCCP until a maximal respiration was achieved and finally blocking of Complex I and III (to measure non-mitochondrial oxygen consumption) through the addition of 0.05uM rotetone + 0.05uM antimycine.

Despite using identical cell number for all analyses, results were additionally normalized by total protein and mitochondrial content through the CS activity and were expressed as pmol O_2/min*μg protein*CS activity.