Mitochondrial Respiration is Preserved, While Respiratory Protein Organization is Altered in Pressure-Overload Induced Heart Failure

Maria Canellas da Silva, Harry Z. Li, Yuan Liu, Gabriel S. Pena, Sarah Kuzmiak-Glancy.
University of Maryland, College Park, MD.

Heart Failure (HF) is a condition in which the cardiac muscle is unable to adequately pump blood to the body, leading to the inability to perform physical activity and poor quality of life. Mitochondria provide the ATP needed for muscle contraction, and this requires the coordinated uptake of Ca\(^{++}\) and activation of respiration, with the organization of the electron transport chain into super-complexes (SCs) affecting mitochondrial function. **PURPOSE:** The goal was to determine if Ca\(^{++}\) activated respiration of cardiac mitochondria was lower from rats with, compared to those without HF, and identify if HF affects the organization of cardiac mitochondrial respiratory proteins, after controlled perfusion and after ischemia-reperfusion (IR). **METHODS:** Four-week-old Sprague-Dawley rats underwent transverse aortic constriction (TAC) to induce cardiac pressure overload, hypertrophy, and HF. Forty weeks post-surgery, the rats were sacrificed, and mitochondria was isolated from hearts of TAC and SHAM-operated controls. Maximal O\(_2\) consumption rates (J\(_O\)) were measured in the presence of respiration media supplemented with 5uM CaCl\(_2\), fuels, and ADP. Alternatively, mitochondria were isolated from hearts perfused with Krebs-Henseleit buffer immediately or after ischemia and reperfusion. Mitochondria membranes were solubilized at 8g/g digitonin to protein ratio and loaded into a 3-13% gradient BN-PAGE for the analysis of protein complexes and SCs in their native state. Differential expression of mitochondria proteins (A.U.) was analyzed using Image Lab (Biorad) and two-tailed t-tests. **RESULTS:** Maximal calcium-activated J\(_O\) was similar between TAC and SHAM mitochondria (498.2 ± 35.8 vs. 426.9± 40.1nmol/mg/min). TAC and SHAM rats had similar expression of SCs regardless of hearts being perfused (76.2 ± 3.4 vs 71.8 ± 4.5) or exposed to IR (71.9 ± 0.4 vs 63 ± 12.7). The abundance of I+III\(_2\) SC was lower in perfused hearts of TAC rats when compared to control (22.5 ± 0.8 vs 27.6 ± 1.1, p=0.02). While levels of CV expression were observed in TAC vs SHAM with (9.8 ±0.3 vs 12.6 ± 3.3) or without IR (13.2 ± 0.6 vs 14.5 ± 0.3), after IR CV expression was reduced in TAC but maintained in SHAM rats (p=0.02 vs p=0.67). **CONCLUSION:** Despite mitochondria J\(_O\) rates being similar between TAC and SHAM hearts, baseline decreased I+III\(_2\) SC formation and lower CV expression after IR can be implicated in the disease mechanism and prognosis. **SIGNIFICANCE:** Uncovering molecular alterations in mitochondria, potentially involved in HF disease mechanisms, can illuminate new drug targets, while conferring prognostic value and improving care.

**FUNDING:** This work was funded by Dr. Kuzmiak-Glancy’s start up at University of Maryland College Park.