the temporal relationship between the time of apparently complete mitochondrial inner membrane potential ($\Delta\Psi_m$) depolarization and the onset of sarcolemmal permeabilization (SP) during I/R using confocal imaging of the mitochondrial fluorophore TMRM and the SP indicator YO-PRO1. Whole rabbit hearts (n=6) were subjected to 60-minutes global ischemia and 180-minutes reperfusion (37°C). In some hearts the electromechanical uncoupler 2,3-Butanedione monoxime (20 mM) or temporary perfusion with high [K^+] (20 mM) was used to abolish motion during imaging. Two or three day-old cultured neonatal rat ventricular cardiomyocyte monolayers (NRVCM, n=3) were subjected to 30-minutes simulated ischemia (including near-anoxia, pH=6.5, $[K^+]_o = 8.8$ mM and [deoxyglucose] = 11 mM, 37°C) and 60-minutes reperfusion. In hearts and NRVCMs the relatively uniform $\Delta\Psi_m$ loss occurred at 31.2 ± 5.6 and 18.3 ± 0.6 minutes of ischemia, respectively, amid absence of SP events. $\Delta\Psi_m$ recovered to a variable degree upon reperfusion in both models, followed by a secondary and heterogeneous $\Delta\Psi_m$ loss in individual myocytes. Total of 7 myocytes from whole hearts and 6 myocytes from NRVCMs were tracked throughout the entire period of reperfusion to accurately determine the delay between $\Delta\Psi_m$ loss and the earliest detectable SP (Δt). Δt varied between −5.3 and +14.5 minutes in the heart myocytes and between −3.0 and +19.0 minutes in NRVCM myocytes. Excluding outlier cells with exceedingly large positive Δts, the mean ± standard deviation of Δt became 0.8 ± 4.0 minutes in hearts and 0.2 ± 3.2 minutes in NRVCMs. Conclusions: (1) critical $\Delta\Psi_m$ loss upon reperfusion typically overlaps in time with the emergence of sarcolemmal pores of at least 1 nm diameter; (2) 2-3 day-old NRVCM qualitatively recapitulate the critical I/R events observed in whole hearts.

944-Pos Board B699

Beta-Hydroxybutyrate Improves Cardiac Excitation-Contraction Coupling (ECC) and Mitochondrial Function in Type-2 Diabetic Hearts

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The risk of cardiovascular disease (CVD) and mortality in type-2 diabetic patients is twice as high as in age-matched healthy subjects. However, the mechanisms linking type-2 diabetes with CVD remain poorly understood. We studied changes in ECC and mitochondrial function in control and diabetic (db/db) mice. We found no major changes in ECC (Ca transients, cell shortening, sarcoplasmic reticulum Ca^{2+} load) under basal conditions despite decreased heart-to-body weight. However, under stress conditions (beta-adrenergic stimulation) ECC was significantly impaired, and increased ROS generation and a shift of the redox environment towards oxidation were observed. Furthermore mitochondrial Ca^{2+} uptake was increased leading to enhanced mPTP opening. Mitochondrial respiration monitored using the Seahorse extracellular flux analyzer was decreased in diabetic hearts. We determined that despite elevated blood glucose levels (395 vs 157 mg/dl in control) and obesity (BMI was 5.50 vs 3.10 in control), blood levels of beta-hydroxybutyrate (a ketone body formed in the liver) remained unchanged (821 vs 784 µM in control). Ketone body oxidation becomes a significant contributor to overall energy metabolism in extrahepatic tissues under numerous physiological conditions (neonatal period, starvation, post-exercise, low carbohydrate diet), when circulating ketone body concentrations increase from micromolar levels in normal fed state to 7 mM, and can rise to ~20 mM under pathological conditions like diabetic ketoacidosis. We evaluated the effect of elevated beta-hydroxybutyrate levels on ECC and mitochondrial function in diabetic hearts. Exposure to 2-10 mM beta-hydroxybutyrate alone or in the presence of low concentrations of pyruvate (0.1-1 mM) improved cardiac ECC, preserved the redox environment, decreased ROS generation and mPTP opening with no effect on mitochondrial Ca^{2+} uptake. Therefore, these data suggest that type-2 diabetes patients potentially could benefit from ketogenic (low carbohydrate) diet which increases beta-hydroxybutyrate generation.

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Metabolic Inflexibility of Malonyl CoA Decarboxylase (MCD) Knockout Mice Leads to Cardiac Remodelling and High Mortality During Peri-Weaning Period

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MCD inhibition shifts metabolism from fatty acid towards glucose oxidation, which has therapeutic potential for obesity and myocardial ischemic injury. However, ~40% of patients with MCD deficiency develop cardiomyopathy during infancy. The aim of this study was to clarify the early life link between MCD deficiency, cardiac dysfunction and to identify any underlying systemic and cardiac metabolic perturbations. MCD knockout mice (-/-) obtained by heterozygous breeding exhibited non-Mendelian genotype ratios (31% fewer MCD-/-) with deaths clustered prior to weaning at 21 days. Further deaths occurred, such that only n=10 MCD-/- survived >50 days after which no further increased mortality was observed. Immediately prior to weaning (18 days) MCD-/- mice had lower body weights ($P<0.01$), elevated body fat ($P<0.01$), hepatic steatosis ($P<0.01$) and glycogen depletion ($P<0.01$) compared to WT littermates. MCD-/- plasma was hyperketonemic, hyperlipidemic, had 60% lower lactate levels and elevated markers of cellular damage (creatine kinase and lactate dehydrogenase $P<0.05$). MCD-/- hearts exhibited hypertrophy (heart weight:body weight $P<0.001$), impaired ejection fraction ($P<0.05$) and were energetically compromised (32% lower total adenine nucleotide pool $P<0.05$). However differences between WT and MCD-/- converged with age, suggesting that, in surviving MCD-/- mice, early cardiac dysfunction resolves over time. These observations indicate normalisation of the MCD-/- metabolic phenotype and improved cardiac efficiency when switched from a high-fat diet (representative of suckling) to a low-fat post-weaning diet, independent of any developmental changes. Thus, MCD-/- mice consistently exhibited cardiac dysfunction and severe metabolic perturbations while on maternal milk high-fat, low carbohydrate diet and these gradually resolved post-weaning. This suggests that cardiac insufficiency is a common feature of MCD deficiency but that severity is dependent on composition of dietary substrates.

946-Pos Board B701

Anti- and Pro-Apoptotic Bcl2 Proteins Distribution and Metabolic Profile in Human Aorta Endothelial Cells Before and After Hyp-PDT

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Deregulation of apoptosis can contribute to diverse pathologic processes. Understanding its regulatory mechanisms in endothelial cells (ECs) has great importance for the development of novel therapy strategies for cancer and cardiovascular pathologies. An oxidative stress with the generation of radical oxygen species (ROS) is a common mechanism causing ECs' dysfunction and apoptosis. The generation of ROS can be triggered by various stimuli including photodynamic therapy (PDT). The molecular mechanisms underlying PDT, and specifically Hypericin PDT (HypPDT), are not completely understood, although it has been shown that the sub-cellular Hyp localization and distribution determine which signaling pathway will lead to cell death. Cell responses to HypPDT are highly dependent on the Hyp intracellular localization and accumulation. The mechanisms by which ROS cause or regulate ECs apoptosis typically include receptor activation, caspase activation, Bcl-2 family proteins, and mitochondrial dysfunction. We were particularly interested in Bcl-2 family proteins and investigated their role in apoptosis of ECs triggered by HypPDT. In the present work, we show that the presence of Hyp itself has an effect on the distribution of Bcl2 family members. Presence of Hyp triggers translocation of Bax into mitochondria, and translocation of Bax and Bcl2 into nuclei in ECs. Further, HypPDT results in oxidative stress via mitochondrial superoxide production and primarily in necrotic type of death in HAEC cells. HAEC cell respiratory control is similar in control cells and in cells after 24 hrs incubation with Hyp without irradiation, suggesting that even though Hyp presence resulted in an increased number of apoptotic cells, the mitochondria function was not affected. However, HypPDT resulted in diminished OCR, indicating that HypPDT oxidative stress abolished mitochondria function in intact cells.

Synthetic Biology

947-Pos Board B702

Reconstitution of Protein Oscillations in Micro Compartments

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Dynamic protein oscillations spatially regulate the localization of the division site in the bacterium Escherichia coli. The pole-to-pole oscillations of Min proteins result on time-average in a nonhomogeneous protein concentration profile with the highest concentration at the cell poles, where cell division is inhibited.
Aiming at understanding spatial organization during cell division on the scale of individual molecules we reconstituted a system of self-organizing Min proteins in artificial micro compartments in a bottom-up approach. Using a devise