qPCR was ~39, 50 and 10%, respectively. Each siRNA decreased its corresponding VDAC mRNA by ~90% without affecting other isoforms. After VDAC1/2/3 knockdown, TMRM fluorescence was 58, 41 and 21%, respectively, compared to 100% after non-target siRNA. In control cells, Ncz decreased TMRM fluorescence by 70%, and Ptx increased fluorescence by 51%. After knockdown of VDAC1/2/3, Ncz decreased TMRM fluorescence by 25, 22 and 9%, respectively, whereas Ptx increased fluorescence by 26, 28, and 27% . **CONCLUSION**: Each of the three VDAC isoforms contributes to maintenance of $\Delta\Psi$ in HepG2 cells. After knockdown, the relative response to changes of free tubulin was attenuated. These results are consistent with the conclusion that free tubulin dynamically regulates $\Delta\Psi$ in HepG2 cells by interacting with VDAC.

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Aldehyde Products of Ethanol Oxidation and Oxidative Stress Suppress Ureagenic but not Basal Respiration of Cultured Hepatocytes

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BACKGROUND: Recently we demonstrated that ethanol suppresses ureagenic respiration of cultured hepatocytes (*Biophys. J.* 2008; **94**:535a). Our **AIM** here was to determine the roles of acetaldehyde (AcAld), a toxic intermediate of ethanol oxidation, malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), the aldehyde products of lipid peroxidation, in suppression of ureagenesis. **METHODS**: Overnight cultured hepatocytes were treated with ureagenic substrates (in mM: 3 NH_4Cl, 5 L-ornithine, 5 Na-lactate) in the presence and absence of AcAld (0-1000 μM), MDA (0-1000 μM) and 4-HNE (0-1000 μM). To prevent evaporation of volatile aldehydes, KRH was covered with mineral oil. Respiration was measured with a Seahorse XF24 Extracellular Flux Analyzer, and aldehyde concentration determined from NADH reduction in ALDH reaction. **RESULTS:** Addition of ureagenic substrates nearly doubled respiration of hepatocytes. Ethanol (0 - 200 mM) dose dependently inhibited ureagenic respiration of hepatocytes by up to 50%. Inhibition of ADH, cytochrome P4502E1 and catalase with 4-methylpyrazole, trans-dichloroethylene and 3-amino-1,2,4-triazole, respectively, partially abrogated the inhibitory effect of ethanol. By contrast, ALDH inhibition with phenethyl isothiocyanate (PITC, 10 μM), augmented ethanol-suppressed ureagenic respiration by an extra 32%, although PITC by itself had no effect. When added directly, AcAld, MDA and 4-HNE, dose-dependently suppressed ureagenic respiration with an apparent K_i from Dixon plots of 200, 5 and 80 μM, respectively. AcAld and 4-HNE, but not MDA, inhibited uncoupled (150 μM of 2,4-dinitrophenol) but not basal respiration of hepatocytes. **CONCLUSIONS:** 1) Suppression of ureagenic respiration by ethanol is mediated by acetaldehyde, the major intermediate of ethanol oxidation. 2) Malondialdehyde and 4-hydroxynonenal, aldehydes generated during lipid peroxidation, also suppress ureagenic respiration of cultured hepatocytes. Based on our earlier work (*ABB* 2009;**481**:226-233), these aldehyde-dependent changes may be the consequence of closure of voltage dependent anion channels (VDAC).

2493-Pos Board B479

Homeostasis of Mitochondrial Calcium in Alcoholic Liver Diseases

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The production of reactive oxygen species (ROS) and oxidative stress are thought to be important pathological factors in onset and development of alcoholic liver diseases (ALD). Elevated level of ROS has previously been shown to alter calcium homeostasis by changing the activity of calcium-release and calcium-uptake mechanisms in the ER and mitochondria. In these studies, we investigated the effects of chronic ethanol consumption on calcium content and calcium handling in the mitochondria. Hepatocytes from ethanol-fed and their paired-fed littermates were cultured in the absence of alcohol for 1 to 18 hrs then loaded with fura-2/AM. Cells were washed into calcium free buffer then treated with the mitochondrial uncoupler, carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP), plus oligomycin, an inhibitor of F1FO-ATPase, to induce calcium release from mitochondrial matrix. The resultant increase in cytosolic calcium was measured with fura-2. The data show that FCCP-induces more calcium release in hepatocytes isolated from alcohol-fed rats compared to controls. The differences in FCCP-releasable calcium content was observed in both overnight-cultured cells and in freshly plated hepatocytes suggesting that chronic alcohol exposure causes a long lasting increase mitochondrial matrix calcium levels. The rates of mitochondrial calcium-uptake and efflux were determined in fresh-isolated and digitonin-permeabilized cells using extramitochondrial fura-6 FF free acid. The data indicate that the rates of mitochondrial calcium uptake were significantly faster in alcoholics compared to controls. The rates of mitochondrial calcium efflux were not significantly different when the matrix calcium content was normalized between control and alcoholics. These data indicate that long-term alcohol exposure induces adaptive changes in the mitochondrial calcium-uptake mechanisms that lead to chronically elevated levels of calcium in the mitochondrial matrix. This adaptive process is predicted to increase mitochondrial ROS production and thereby contribute to the pathogenesis of ALD.

2494-Pos Board B480

A Novel Assay of Muscle Energy Dynamics in Mechanically Loaded Enzymatically Isolated Adult Mammalian Skeletal Myocytes

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Current methods and tools available to study bioenergetics in adult mammalian skeletal muscle are mostly limited to whole organ or fiber bundle preparations. However, single cell preparations remain attractive for studying mechanics and bioenergetics, because they are free from connective tissue and endothelium, allow for consistent and uniform oxygen and substrate availability and can be investigated using real-time imaging methods at high temporal and spatial resolution. The majority of research on single muscle cells is conducted in mechanically unloaded conditions in which the rate of ATP consumption is low. ATP production is generally matched to consumption and due to both cytosolic glycolysis and mitochondrial oxidative phosphorylation.
Investigations of mechanically loaded muscle cells that may consume ATP at "normal" rates provide us with a preparation that enables us to explore issues related to mitochondrial function, ROS production as will as functional Ca^{2+} signaling and contractile performance. However, doing experiments with mechanically loaded single cells requires technically challenging manually dissection methods. We have recently developed an improvement that enables us to use enzymatically dissociated skeletal myocytes. Using MyoTak, a tenacious biological adhesive, we have been able to attach dissociated skeletal myocytes to both force transducer and length controller units. The myocytes thus prepared can be readily imaged using an inverted microscope fluorescence imaging system in confocal, widefield and multiphoton systems. We will show results that use this system to examine the force produced in enzymatically isolated mammalian skeletal myocytes with isometric contractions elicited by field stimulation, NADPH auto-fluorescence, ROS (reactive oxygen species) production during contraction.

2495-Pos Board B481

Mechanism of Reactive Oxygen Species Generation in Cardiac Mitochondria a Computational Approach

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The generation of mitochondrial reactive oxygen species (ROS) across the electron transport chain has been implicated in various diseases like aging, diabetes, neurodegenerative diseases and cellular injury during ischemia reperfusion. To better understand the regulation of ROS production, we present a mechanistic model of ROS production based on principles of reaction kinetics. The mathematical model includes a sequential process of ROS generation through various reaction intermediates. The model describes the redox reactions occurring at protein complexes I, III and IV that involve transfer of electrons across the respiratory complexes and is coupled to translocation of protons from the matrix space to the inner mitochondrial membrane. The steady state production of superoxide anion ($O_2^{\cdot-}$) by complex I and III is based on chemical kinetics model of electron transfer from NADH to cytochrome c1 through various intermediates. The electron transport system fluxes for complex IV is based on thermodynamically balanced biophysical model described by Beard et.al. The components of TCA cycle and mechanisms controlling Ca^{2+} regulation in cardiac energy metabolism is based on model proposed by Jafri et.al. The computational model reproduces qualitatively the experimental data on rate of superoxide production by complex I and it is observed that rate of $O_2^{\cdot-}$ is directly proportional to the complex I and O_2 concentrations. The model simulates changes in mitochondrial rate of $O_2^{\cdot-}$ production with respect to mitochondrial membrane potential in complex III and suggests that there is a significant rise in production of $O_2^{\cdot-}$ radical with increase in membrane potential through an increase in the relative concentration of ubisemiquinone forms. Additional studies explore how ROS production is regulated by other physiological and pathological conditions.

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Calcium Activation of Oxidative Phosphorylation in Skeletal Muscle Mitochondria

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Skeletal muscle work rate can rise ~100-fold above rest. Thus, the ability of myocytes to maintain homeostasis is severely challenged during exercise. This challenge may be partially met through calcium activation of both energy supply and demand. This study focused on energy supply by examining calcium activation of oxidative phosphorylation in skeletal muscle mitochondria. Mitochondrial membrane potential ($\Delta\Psi$), NAD/NADH ratio, and cytosolic