mitochondrial redox response to increased work. Isolated cardiomyocytes were field stimulated and fractional shortening simultaneously recorded with epi-fluorescence measurements of NAD(P)H and FAD. Cells were paced at 0.5Hz and the stimulation frequency step increased to 1Hz, 2Hz and 3Hz in order to increase work intensity. NAD(P)H was excited at 340nm (fluorescence collected 455-480nm) and FAD was excited at 430nm (fluorescence collected 505-600nm).
Increasing the stimulation frequency from 0.5Hz to 2Hz and 3Hz, but not 1Hz, resulted in a decrease in NAD(P)H fluorescence and an increase in FAD fluorescence, indicating oxidation of the cell environment. Reducing work intensity back to 0.5Hz pacing led to immediate recovery of metabolite fluorescence. Addition of 2mM NaCN established a completely reduced mitochondrial environment, leading to NAD(P)H fluorescence increasing to a maximum and FAD fluorescence decreasing to a minimum. Subsequent step increase in stimulation to 3Hz caused no change in NAD(P)H or FAD fluorescence. Treatment with 2μM FCCP established a completely oxidised state, resulting in NAD(P)H fluorescence falling to a minimum and FAD fluorescence increasing to a maximum. Pacing at 3Hz in this state again led to no change in metabolite fluorescence, confirming the response to increased work was mitochondrial in origin. Increasing stimulation frequency to 3Hz in the presence of the movement uncoupler cytochalasin D, minimising cell contraction, also led to no change in NAD(P)H or FAD fluorescence, thus confirming that contractile work was the cause of the change in mitochondrial redox state.
In conclusion, the response to increased work intensity in cardiomyocytes is oxidation of the cell, suggesting that the mitochondria are initially unable to maintain NAD(P)H/$FADH_2$ supply in order to cope with increased metabolic demand.

1246-Pos Board B90
Trimetazidine Effects On The Mitochondrial Metabolism During Rabbit Heart Failure
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Background: We have previously shown that the anti-ischemic agent trimetazidine (TMZ) rescued $[Ca^{2+}]_i$ transient and mechanical alternans in ventricular myocytes from rabbits with non-ischemic heart failure (HF), induced by combined aortic insufficiency and stenosis. The cardioprotective action of TMZ has been linked to the inhibition of free fatty acid (FFA) oxidation, however the underlying mechanism remains poorly defined. The aim of this study was to determine whether the plasma levels of FFA (total ($[FFA]_{tot}$) and unbound to albumin ($[FFA]_u$)) are elevated in rabbit HF and whether TMZ affects mitochondrial metabolism. **Methods and Results:** We found that both $[FFA]_{tot}$ and $[FFA]_u$ were significantly elevated in HF rabbits. $[FFA]_u$ increased 4-times during HF (from 13 ± 4 to 53 ± 7 nM) while the $[FFA]_{tot}$ increased only two-fold (from 58 ± 16 to 121 ± 29 μM), demonstrating that $[FFA]_u$ is a reliable biomarker of HF. Furthermore, using TMRM fluorescence confocal microscopy and a Strathkelvin micro volume precision respirometry system, we determined that mitochondrial complex II activity was significantly elevated (+72%) during HF, while complex I activity was decreased (-90%). Cell treatment with TMZ had no effects on the complex I activity in control (+6%), while it increased (+26%) the activity of complex I under HF conditions. Moreover, TMZ reversed complex II activity in HF myocytes (-55%), while it had no effect on complex II activity in control cells (-10%). The oxidation of palmitoylcarnitine, the upstream substrate for FFA oxidation, was decreased 32% by TMZ, while TMZ had no effect on complex IV activity. Furthermore, FADH-mediated auto-fluorescence levels were significantly elevated in HF myocytes treated with TMZ. **Conclusion:** TMZ suppresses the elevated activity of mitochondrial complex II while it increases the depressed activity of complex I in rabbit HF, and therefore it preserves metabolic reserve of the cell.

1247-Pos Board B91
Mitochondrial Dynamics In Heart Cells: Very Low Amplitude High Frequency Fluctuations In Adult Cardiomyocytes And Flow Motion In Non-beating Hl-1 Cells
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The arrangement and movements of mitochondria were quantitatively studied in adult rat cardiomyocytes and in the cultured continuously dividing non beating NB HL-1 cells with differentiated cardiac phenotype.
Mitochondria were stained using fluorescent dye MitoTrackerGreen, a dye associated with inner membrane of mitochondria, and studied by fluorescent confocal microscopy. Imaging during different time intervals made it possible to visualize the 2-dimensional movements and dynamics of cardiac mitochondria.
In adult cardiac cells mitochondria were always arranged very regularly in a crystal-like manner and did not show any changes in their position during 30 min of low speed scanning. However, high speed scanning (pixel dwell time 3 ms, time interval between images 400 ms) revealed very rapid fluctuations of the positions of fluorescence centers which followed the pattern of a random walk movement within the limits of the internal space of mitochondria, most probably due to transitions between condensed and orthodox configurational states of matrix and inner membrane as a result of functioning of transmembrane metabolite carriers. No evidence for mitochondrial fusion or fission was found in adult cardiomyocytes.
In contrast, in NB HL-1 cells, mitochondria were arranged as a dense tubular network, in permanent fusion, fission and displacement with high velocity around 90 nm/s.
The differences observed are related to specific structural organization of the cells, and most probably due to differences in mitochondria-cytoskeleton organization. Intracellular local restrictions of diffusion of adenine nucleotides and metabolic feedback regulation of respiration via phosphotransfer networks are also different in these cells.

1248-Pos Board B92
Cgp-37157 Abrogates The Adverse Effect Of Ouabain On Mitochondrial Energetics
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Cardiac glycosides have been used to treat heart failure for more than 200 years and their major effect is to inhibit the Na^+/K^+ pump. Inhibition of the pump on the sarcolemma of cardiac myocytes elevates intracellular Na^+ ($[Na^+]_i$), resulting in a positive inotropic effect by increasing Ca^{2+} load. However, our previous work demonstrated that elevated $[Na^+]_i$ impairs mitochondrial energetics by blunting mitochondrial Ca^{2+} ($[Ca^{2+}]_m$) accumulation. Moreover, we showed that CGP-37157, an inhibitor of $[Ca^{2+}]_m$ efflux, restored $[Ca^{2+}]_m$ accumulation and improved mitochondrial energetics. Here, we investigated the effects of ouabain with or without CGP-37157 on $[Na^+]_i$ and NADH production in isolated cardiomyocytes and examined the effects on hemodynamics and Oxygen consumption (mVO_2) in whole hearts. Application of ouabain to isolated myocytes elevated $[Na^+]_i$ in a dose-dependent way. During 1 Hz stimulation, the NADH/NAD+ redox potential in ouabain treated myocytes was decreased significantly, whereas NADH levels were well maintained in the presence of CGP-37157. In whole-heart studies, ouabain increased LVDP, +dP/dt, and -dP/dt, and addition of CGP-37157 further increased +dP/dt and -dP/dt. When isoproterenol was employed to increase cardiac work, LVDP was not increased, but +dP/dt and -dP/dt were increased by 57% and 52%, respectively, in hearts without concomitant CGP-37157 treatment. In isoproterenol-treated hearts also exposed to CGP-37157, LVDP increased by 30%, and +dP/dt and -dP/dt were increased by 75% and 53%, respectively. Whole heart mVO_2 increased by 18% after ouabain treatment and by 25% after isoproterenol administration compared to baseline. With concomitant CGP-37157 treatment, ouabain increased mVO_2 by 32% and isoproterenol increased mVO_2 by 53%. Our findings revealed an adverse effect of the glycoside on mitochondrial energetics and indicate that CGP-37157 can prevent this impairment. In addition, inotropic responses to both ouabain and isoproterenol were enhanced in the presence of CGP-37157.

1249-Pos Board B93
Mitochondrial Energetics During Transients Following Substrate And Ca2+ Additions. Modeling And Experimental Studies
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Ionic equilibria are known to be dramatically altered in failing hearts, as well as during and after ischemic injury. Ion transport across the mitochondrial inner membrane has been shown to modulate the energetic performance of mitochondria. Consequently, it is critical to thoroughly understand the interrelationship between ion fluxes and energetics. With this aim in mind, here we continue to develop our computational model of mitochondrial energetics to account for pH regulation, Na+/H+ cotransport, and the Pi carrier, and study their effects on mitochondrial energy production and Ca2+ handling mediated by the Ca2+