into account. All the inhibitors used were considered irreversible and noncompetitive under these conditions. The inhibitors used were rotenone for Complex I of the respiratory chain, antimycin A for Complex III, cyanide for Complex IV, carboxyatractyloside for ATP/ADP transporter, oligomycin for ATP synthase and mersalyl for Pi transporter. For mitochondrial CK inhibition dinitrofluorobenzene was used. In this way, flux control coefficients were found for all components described.

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17P.3 Respiratory coupling in the mitochondrial electron transport chain in RAW 264.7 cells

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Respiratory coupling, defined as the ability of mitochondria to increase oxygen consumption (VO_2) in the face of increased cytosolic ATP demand, was studied in intact RAW cells using oligomycin to model low ATP demand and FCCP to model increases in ATP demand. VO_2 was measured using respirometry, the oxidation changes of the cytochromes of the electron transport chain were monitored using multi-wavelength spectroscopy and NAD(P)H oxidation changes were measured using fluorescence spectroscopy. The mitochondrial membrane potential ($\Delta\psi$) and redox potential (E_h) of the ubiquinone pool (UQ) were calculated from the redox poise of the b_H and b_L centers of the bc_1 complex using a model that takes into account their redox cooperativity. After inhibition with oligomycin, the VO_2 response to FCCP could be split into two regions. At low concentrations of FCCP (0–150 nM) VO_2 increased 11-fold with increasing FCCP (positive respiratory coupling) whereas at high concentrations of FCCP (200–500 nM), VO_2 decreased with increasing FCCP (negative respiratory coupling). Over the positive respiratory coupling range, $\Delta\psi$ decreased from 175 ± 3 to 135 ± 10 mV (mean $\pm$ SD, $n = 6$). The heme *a* center of cytochrome oxidase reduced from 90 ± 2 to $84 \pm 2\%$ oxidized. Contrary to the results from isolated mitochondria, cytochrome *c* initially reduced from $76 \pm 2\%$ to $72 \pm 2\%$ oxidized (0–100 nM) and then reoxidized back to $76 \pm 2\%$ oxidized at 150 nM FCCP. The oxidation state of heme b_H was almost independent of FCCP at about 70% oxidized whereas b_L oxidized from $32 \pm 2\%$ to $61 \pm 7\%$ oxidized. However, the calculated E_h of b_H increased from 22 ± 5 mV to 45 ± 6 mV due to the redox cooperativity between b_H and b_L and the E_h of UQ increased from 66 ± 5 mV to 74 ± 5 mV. Preliminary results showed that NADH varied from almost fully reduced to almost fully oxidized. All the cytochromes and NADH became highly oxidized in the negative respiratory coupling range. The results suggest that cytochrome oxidase is almost entirely responsible for respiratory coupling in RAW cells and that it responds to the decline in $\Delta\psi$ by increasing its turnover by 11-fold as $\Delta\psi$ decreases from 175 to 135 mV at a constant cytochrome *c* E_h of 290 mV. The E_h of UQ and NADH increase due to the declining energy requirement of the proton pumps in the bc_1 complex and complex I respectively.

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17P.4 Increased expression of the cardiac ubiquitin ligase MuRF1 alters mitochondrial bioenergetic capacity *in vivo*

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Muscle ring finger protein 1 (MuRF1) is a muscle-specific RING-finger-dependent ubiquitin ligase that regulates the development of cardiac hypertrophy, PPARα-mediated fatty acid metabolism, and creatine kinase activity. We have identified these mechanisms using a model of constitutive over-expression of cardiac MuRF1 (MuRF1 Tg^+) and ablation of MuRF1 (*MuRF1*$^-$). At baseline, MuRF1 Tg^+ hearts exhibit a 15% reduction in fractional shortening and a 20% reduction in LV wall thickness compared to strain-matched wild type controls. *MuRF1*$^-$ hearts do not have a discernable phenotype from wild type controls. Microarray analysis of the MuRF1 Tg^+ hearts revealed that genes involved in mitochondrial oxidative phosphorylation are significantly decreased, including ATP50, NDUFB8, NDUFS6, NDFS7, NDUFV2, and SDHA. Here, we investigated the effects of MuRF1 Tg^+ and *MuRF1*$^-$ on cardiac mitochondrial bioenergetic capacity. Cardiac mitochondria from MuRF1 Tg^+ and *MuRF1*$^-$ were isolated by differential centrifugation and respiration parameters were measured using a Clarke-type oxygen electrode employing a combination of malate (M), pyruvate (P), palmitoyl-l-carnitine (CP) and succinate as oxidative substrates. Oligomycin-insensitive basal proton leak was augmented in the MuRF1 Tg^+ cardiac mitochondria by $40 \pm 2.4\%$ ($n = 5$; $p < 0.05$ vs. wild-type control) in the presence of M + P. The respiratory control index (RCI), a marker of mitochondrial viability, was increased in the *MuRF1*$^-$ mice by $48 \pm 4.5\%$ ($n = 5$; $p < 0.05$ vs. wild-type control) when succinate was employed as an oxidative substrate. To determine how MuRF1 expression might affect mitochondrial number, we assayed the mitochondrial genes Cytb1, C01, Ndl by RT-PCR. We found that mitochondrial DNA content in the MuRF1 Tg^+ and *MuRF1*$^-$ hearts were not significantly different compared to the wild-type controls, respectively. Our data demonstrate that increased cardiac MuRF1 expression impairs mitochondrial oxidative phosphorylation, possibly as a result of increased proton leak. The changes observed in oxidative phosphorylation genes in the MuRF1 Tg^+ hearts suggest transcription as a more specific regulation of oxidative phosphorylation and not mitochondrial number.

References

[1] Willis *et al.* (2009) *Circ. Res.* **105:** 80–88.
[2] Willis *et al.* (2007) *Circ. Res.* **100:** 456–459.

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