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Uncoupling Protein-2 Expression and Effects on Mitochondrial Membrane Potential and Oxidant Stress in Heart Tissue

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Abstract

Myocardial uncoupling protein (UCP)-2 is increased with chronic peroxisome proliferator-activated receptor γ (PPAR γ) stimulation but the effect on membrane potential and superoxide is unclear. Wild type (WT) and UCP-2 knock-out (KO) mice were given a 3-week diet of control (C) or the PPAR γ agonist pioglitazone (50 μ g/gram-chow per day) (PIO). In isolated mitochondria, UCP-2 content by Western blots, membrane potential ($\Delta\Psi_m$) by tetraphenylphosphonium (TPP) and relative superoxide levels by dihydroethidium (DHE) were measured. Oxygen respiration was determined at baseline and following 10 minutes anoxia-reoxygenation. PIO induced a 2-fold increase in UCP-2 and nuclear-bound PGC1 α in WT mice with no UCP-2 expression in KO mice. Mitochondrial $\Delta\Psi_m$ from WT mice on C and PIO diets was -166 ± 4 mv and -147 ± 6 mV respectively ($P<0.05$) and were lower than UCP-2 KO mice on C and PIO (-180 ± 4 and -180 ± 4 mv respectively; $P<0.05$). Maximal complex III inhibitable superoxide from WT mice on C and PIO diets was 22.5 ± 1.3 and 17.8 ± 1.1 AU respectively ($P<0.05$) and were lower than UCP-2 KO on C and PIO (32.9 ± 2.3 and 29.2 ± 1.9 AU respectively; $P<0.05$). Post-anoxia, the respiratory control index (RCI) in mitochondria from WT mice with and without PIO was 2.5 ± 0.3 and 2.4 ± 0.2 respectively and exceeded that of UCP-2 KO mice on C and PIO (1.2 ± 0.1 and 1.4 ± 0.1 respectively ($P<0.05$). In summary, chronic PPAR γ stimulation leads to depolarization of the inner membrane and reduced superoxide of isolated heart mitochondria, which was critically dependent upon increased expression of UCP-2. UCP-2 expression affords resistance to brief anoxia-reoxygenation.

Mitochondrial sources of reactive oxygen species are a fundamental cause of oxidant damage to the heart following ischemia-reperfusion and accordingly, have generated enormous interest in the signaling pathways of preconditioned myocardium (1, 2). A potentially important modifiable approach for promoting cellular protection against oxidant stress is a slight degree of depolarization of the inner membrane of mitochondria (3–5).

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Depolarization of the inner mitochondrial membrane potential ($-\Delta\Psi_m$) can be induced by a number of sources including a proton leak from uncoupling protein (UCP) (6). Increased UCP-2 expression reduces oxidant tissue damage in a variety of animal models (7–10) and has been shown to extend the lifespan of mutant mice that lacking superoxide dismutase-2 (11). Within the intact animal, UCP-2 expression is increased in hearts exposed to brief ischemia and reperfusion and protects against cell death by an anti-oxidant pathway (12). In chronic hibernating swine heart tissue, we have previously shown that UCP-2 content is increased in the chronically ischemic myocardial regions and is protective against brief anoxia-reoxygenation in isolated mitochondria (13).

In light of these considerations, we investigated the cardioprotective effects of upregulation of UCP-2 in heart mitochondria, by chronic administration of the peroxisome proliferator-activator receptor- γ (PPAR γ) agonist pioglitazone (14). Specifically, we tested the hypothesis that upregulation of UCP-2 expression in isolated heart mitochondria reduces membrane potential and maximal superoxide, while imparting resistance to brief anoxia-reoxygenation. For this purpose, we studied the effects of pioglitazone not only in wild type mice but also in mice with genetic disruption of the UCP-2 gene (15).

METHODS

The present study was performed under the guidance of the animal care committees at the Minneapolis VA Medical Center and University of Minnesota and conforms to *Guide for the care and use of laboratory animals* published by the US National Institutes of Health (NIH publication No 85-23, 1996).

Mouse Models

Dr. Bradford Lowell kindly donated the transgenic mice with targeted disruption of the UCP-2 gene (15). Animals were transferred to our institution and maintained under the supervision and guidelines, as specified by a breeders' protocol. Wild type (WT) mice and UCP-2 knock-out (KO) littermates were given either control diet or daily supplements of the PPAR- γ agonist pioglitazone (50 μ g/gram chow) for 3 weeks (16).

Sacrifice and Isolation of Mouse Heart Mitochondria

Mice (n=30 per group) were euthanized by cervical dislocation and the heart was removed via a midline sternotomy. Hearts were placed in iced mitochondrial isolation buffer (MIB) at pH 7.15, containing 50 mM Sucrose, 200 mM Mannitol, 1 mM EGTA, 5mM KH_2PO_4 , 5 mM MOPS and 0.1 % Fatty acid free BSA. Myocardial tissue was minced and homogenized in the MIB buffer in a glass homogenizer with a teflon pestle and centrifuged at 750 g for 10 minutes in sorvall centrifuge tubes at 4° C. The supernatant was centrifuged twice, each at $8,000 \times g$ for ten minutes.

Mitochondrial Respiration

Mitochondria were suspended in respiration buffer (MRB) comprised of 110 mM Sucrose, 0.5 mM EGTA, 3 mM MgCl_2 , 70 mM KCl, 10 mM KH_2PO_4 , 20 mM Taurine, 20 mM HEPES and 0.1% fatty acid free BSA. They were placed into the respiration chamber equipped with an oxygen electrode to measure oxygen concentrations at 30°C. Once steady state was achieved, state 2 respiration was determined by the rate constant for oxygen consumption in the presence of Complex I (10 mM glutamate and 5 mM malate) and Complex II (10 mM succinate) substrates. Once the oxygen curve stabilized, 5 mM ADP was given and the rate constant for oxygen consumption was determined for state 3 respiration. The respiratory control index (RCI) was calculated by the ratio of state 3 to state 2 respiration and only samples demonstrating adequate control were considered for

additional studies (17). To test the effects of deactivation of UCP-2 in isolated mitochondrial samples, mitochondrial respiration was repeated by incubating isolates for 5 to 10 minutes in the presence of GDP (1 mM), as previously described(13).

A separate group of mitochondria were used for anoxia-reoxygenation experiments, by a method that has been previously described (18). Briefly, substrates were added to the isolated mitochondria in the polarograph, using the same respiration buffer as described above, and respiration was continued so that all of the oxygen as well as the small amount of endogenous ADP under state 2 conditions were consumed. After 10 min of anoxia, reoxygenation was allowed by opening the chamber to equilibrate with ambient oxygen and when steady state was achieved, respiration was reanalyzed (13, 18).

UCP-2 Expression, Superoxide and Membrane Potential

UCP-2 content was determined from mitochondrial membranes by Western analysis. After loading 10% SDS-PAGE gels with equivalent amounts of mitochondrial protein (20 μ g), immunoblots were performed by standard techniques and use of primary antibodies for UCP-2 (Santa Cruz Biotechnology, Santa Cruz CA). Western blots were analyzed in a densitometer and the results are expressed in arbitrary units (A.U.). PGC1- α expression was determined from nuclear fractions prepared following the NE-PER Nuclear and Cytoplasmic protocol from Pierce Biotechnology (Rockford, Illinois). Nuclear membrane samples were supplemented with HALTS protease inhibitors (Pierce Biotechnology, Rockford, Illinois) and incubated with a primary PGC1- α antibody (Santa Cruz Biotechnology, Santa Cruz CA) and secondary antibody at a dilution of 1–2000.

Relative superoxide levels were measured by incubating 1mg of mitochondrial protein with 250 μ M DNA, and 50 mM dihydroethidium and maximal levels were determined by incubating with 100 μ g Antimycin A to inhibit complex III respiration. Following incubation at 37° C for 30 minutes, the spectra, or endpoint fluorescence, was determined using a fluorimeter (Jasco Instruments FB 6200, Halifax, Nova Scotia). Spectra were collected between 540 nm and 650 nm (peak emission of 590 nm) at an excitation wavelength at 530 nm (19). Membrane potential ($\Delta\Psi_m$) was estimated by using tetraphenyl phosphonium (TPP) and a TPP ion selective electrode. A combination of complex I and complex II substrates were used at the time that the membrane potential measurements were obtained, to mimic the conditions obtained during the respiration studies. TPP is a lipophilic cation that is accumulated in mitochondria following the Nernst equation and a model is described to correct for probe binding to the membranes (20). Briefly, the voltage between the TPP ion selective electrode and a reference electrode was measured using a pH meter. TPP was added to the incubation medium (110 mM KCl, 5mM K₂HPO₄, 5mM succinate, 5mM pyruvate and 10mM MOPS at pH 7.15) in 5 sequential additions to a final concentration of 2 μ M. This serves as a calibration procedure for the TPP electrode. Mitochondria (1 mg) were then added and the accumulation fraction (R_c) determined by measuring the amount of TPP in the incubation medium. To estimate redox state from isolated mitochondrial samples, total glutathione (GSSG + GSH) and oxidized glutathione (GSSG) species in isolated mitochondria were measured using an enzymatic recycling method, using glutathione reductase, for the quantification of GSH (Cayman Chemical Company). Briefly, the assay involves the conversion of GSSG to GSH and the sulfhydryl group of GSH reacts with DTNB (5,5'-dithio-bis-2-nitrobenzoic acid) and produces TNB (5-thio-2-nitrobenzoic acid), this assay measures total glutathione. The conversion of DTNB to TNB is measured at 405 nm. To measure oxidized glutathione (GSSG), prior to performing the enzymatic assay, the reduced glutathione (GSH) is derivatized with 2-vinylpyridine (10 mM final concentration) for 60 minutes at room temperature. The mitochondrial content for each sample is normalized by protein content as measured by the bicinchoninic acid (BCA) method.

Statistics

Data are expressed as means and standard error of the mean (SEM). Intergroup differences were considered significant at the $P < 0.05$ level, based on ANOVA and a post-hoc test using Fishers Least Square Differences (LSD).

RESULTS

Chronic PPAR γ Stimulation and Myocardial Uncoupling Protein-2

In WT mice given either chronic PPAR γ stimulation with dietary supplementation of pioglitazone (PIO) or a regular diet for 3 weeks, there was no effect on total body weight (25.4 ± 0.6 versus 25.1 ± 0.3 grams respectively; NS). An 18.3% increase in liver weight was noted with PIO however (1.23 ± 0.03 grams versus 1.04 ± 0.02 grams; $P < 0.01$) along with a comparable increase in liver-to-body weight ratios (4.84 ± 0.07 versus 4.02 ± 0.12 ; $P < 0.01$). Similar findings were noted in the UCP-2 KO mice given PIO. In isolated heart mitochondria from the WT mice given pioglitazone, UCP-2 content was increased 2.2 ± 0.4 -fold compared with mitochondria from WT littermates on a regular diet. An equivalent increase in PGC-1 α content was also noted from the nuclear-bound membranes in WT mice given pioglitazone, compared with WT mice on a regular diet (Figure 1). Negligible expression in UCP-2 content was noted in UCP-2 KO mice, either on a control diet or with pioglitazone.

Mitochondrial Membrane Potential and Myocardial Uncoupling Protein-2

Mitochondrial inner membrane potential estimates from excised heart tissue were lower in WT mice given pioglitazone with increased UCP-2 content, compared with WT mice on a regular diet (-146.6 ± 6.0 mV versus -166.4 ± 4.2 mV; $P < 0.01$). In mitochondria from UCP-2 KO mouse heart tissue, the membrane potential was increased relative to both WT groups, in both diets (Table 1). State 2 respiration from isolated cardiac mitochondria from the WT mice fed pioglitazone was higher than that of WT mice on a regular diet and greater than mitochondria isolated from the UCP-2 KO mouse hearts on both diets. The relationship between state 2 respiration and inner mitochondrial membrane potential is shown in Figure 2, and demonstrates increased state 2 respiration, commensurate with the decrease in membrane potential in the WT mice. These findings are consistent with a proton leak induced by enhanced expression of UCP-2 in response to chronic PPAR γ stimulation with pioglitazone. In parallel studies from tissue extracted from WT mice on a pioglitazone diet ($N=6$), addition of GDP (1 mM) restored the membrane potential and state 2 respiration to levels observed in WT mice on a regular diet, consistent with UCP-2 as the cause of the proton leak (Figure 3).

Mitochondrial Membrane Potential and Reactive Oxygen Species

Basal superoxide levels, expressed in fluorescent units (FU), are shown in Table 1, and were not statistically different between groups. Following inhibition of complex III during incubation with antimycin A, maximal superoxide levels were 23 ± 1 FU in WT mice on a control diet and 18 ± 1 FU in WT mice following pioglitazone ($P < 0.01$). The measured superoxide from isolated mitochondria from UCP-2 KO mouse hearts on control and Pio supplemented diets was 33 ± 2 and 29 ± 2 FU respectively (NS), and both were higher than the WT groups ($P < 0.05$). The inverse relationship between superoxide and membrane potential is demonstrated in Figure 4, in the groups of mice with differentially expressed UCP-2 mitochondrial protein.

Oxidant Conditions and Uncoupling Protein-2 Expression

The redox state was measured by the glutathione redox couple (GSH/GSSG) in isolated mitochondria during basal conditions and was similar in all groups (Figure 5). The ratio of GSH to GSSG in mitochondria from WT mice on regular and pioglitazone diets was 8 ± 4 and 12 ± 7 respectively and did not differ from UCP-2 KO mice on either diet. Despite no differences at baseline, mitochondria from WT hearts exposed to brief anoxia-reoxygenation demonstrated higher state 3 respiration and a greater respiratory control index (RCI) compared with UCP-2 KO mice (Figure 6), consistent with stress-resistance in the presence of UCP-2 expression. In post-anoxic mitochondrial samples, the maximal superoxide level in WT mouse hearts on control and pioglitazone diets was 34 ± 3 and 26 ± 3 FU respectively and each were lower than the levels in UCP-2 hearts on control and pioglitazone diets (53 ± 4 and 48 ± 4 FU; $P < 0.01$). Similarly, the post-anoxic membrane potential in WT mouse hearts on control and pioglitazone diets was -180 ± 5 and -165 ± 5 mV respectively and both were lower than the measurement in UCP-2 hearts on control and pioglitazone diets (-194 ± 3 and -194 ± 10 mV FU respectively; $P < 0.01$). These data support the notion that UCP-2 expression is protective following anoxia-reoxygenation, although no additional protection was afforded by treatment with pioglitazone.

DISCUSSION

The principal finding of the present study is that uncoupling protein-2 in isolated cardiac mitochondria is increased in response to chronic PPAR γ stimulation with dietary supplementation of pioglitazone and results in a depolarization of the inner membrane potential and lower levels of maximal superoxide levels. Compared with mice that have genetic disruption of the UCP-2 gene, enhanced UCP-2 expression leads to stress resistance of isolated mitochondria, as noted by higher state 3 respiration and a greater respiratory control index following brief anoxia-reoxygenation. These findings support the concept that UCP-2 expression can be increased by chronic PPAR γ stimulation and is protective against ROS mediated pathways.

Mitochondrial sources of reactive oxygen species have been viewed as a fundamental cause of oxidant damage to the heart during ischemia and reperfusion and may underlie key mechanisms related to cardioprotection within preconditioned myocardium (2). As such, a slight degree of depolarization within the inner membrane of mitochondria might play a protective role, by attenuating the accumulation of reactive oxygen species (3–5). A proton leak across the inner membrane of mitochondria can be generated by several sources, including activation of UCP (5, 6). In the present study, increased UCP-2 expression had a relative increase in state 2 respiration with a relative decrease in the membrane potential, which is consistent with uncoupling (21). The uncoupling was attenuated with addition of GDP to isolated mitochondrial heart samples by inhibition of the proton leak through the uncoupling proteins, supporting the findings that UCP-2 was the source of the proton leak.

In our study involving heart tissue from wild type mice, an increase in UCP-2 expression in response to chronic PPAR γ stimulation lead to further depolarization of the inner membrane with a reduction in maximal superoxide levels during complex III inhibition. This inverse relationship between UCP-2 expression and superoxide is consistent with previous studies that relate the inner membrane potential of mitochondria with concentrations of reactive oxygen species. In fact, the relationship observed in the present study is very similar to that seen from isolated rat heart mitochondria, with a logarithmic increase in superoxide levels relative to the degree of hyperpolarization of the inner membrane potential (3). There are several experimental observations in various animal models that also suggest that UCP-2 plays an important role as an antioxidant. In hearts extracted from rats that have undergone a vigorous exercise protocol, UCP-2 expression was increased nearly 3-fold and was

associated with a decrease in both the mitochondrial inner membrane potential and concentrations of reactive oxygen species (22). In rats that have been exposed to a brief ischemic preconditioning protocol and studied 24 hours later in the second window of protection, UCP-2 expression was shown to be increased and protected against necrosis during a sustained period of ischemia (12). In a swine model of chronic myocardial ischemia with hibernating myocardial regions that are void of significant necrosis, UCP-2 expression is increased and protects against ex vivo anoxia-reoxygenation in isolated mitochondria (13). Finally, in addition to serving as an antioxidant role in heart tissue, UCP-2 expression in mice during conditions with increased reactive oxygen species has a favorable effect on lifespan (11).

In the present study, using a mouse model with genetic disruption of UCP-2 in heart tissue, the effects of UCP-2 expression on relative superoxide levels, inner membrane potential and resistance to anoxia-reoxygenation were compared. In wild type mice, chronic PPAR γ stimulation with dietary supplementation of pioglitazone increased UCP-2 expression two-fold, an effect that has previously been shown to be dependent upon reactive oxygen species (14). The depolarization of mitochondria from isolated mitochondria with increased UCP-2 expression was associated with a nearly 2-fold reduction in maximal superoxide levels, when compared with heart mitochondria from UCP-2 KO mice. Despite the fact that there were no differences in the redox state during basal conditions, preserved state 3 respiration and the respiratory control index following transient anoxia indicates protection against generation of reactive oxygen species during reoxygenation. Pioglitazone has been shown to increase resistance to oxidant damage in several animal models, presumably by either reducing oxidant damage or preventing mitochondrial death pathways (23–25). Although the degree of anoxic insult in the present study was severe enough to induce marked differences in state 3 respiration between WT and KO mouse hearts, it may not have been severe enough to discriminate additional protection in those WT hearts that received pioglitazone versus control diets. Alternatively, it is possible that a normal level of UCP-2 expression is sufficient for adequate protection to anoxia-reoxygenation in isolated mitochondria, as demonstrated in these studies.

Antimycin A was used to inhibit complex III from isolated mitochondria and resulted in an increase level of superoxide generation above basal levels. These data are consistent with our previous findings from isolated mitochondria in swine hearts, as measured by chemiluminescence as well as those results from isolated rat heart mitochondria (26). Although this paradoxical release of superoxide in the presence of complex III inhibition with antimycin A may be dose dependent, occurring at only lower doses, it has not been observed with either myxothiazol or stigmatellin. This has suggested that the site of inhibition of the cytochrome with different inhibitors may lead to spatial differences in superoxide release (26).

In summary, these data demonstrate in isolated heart tissue from mouse hearts, that UCP-2 expression can be increased with chronic PPAR γ stimulation with dietary supplementation of pioglitazone. The effect of increased UCP-2 within the inner membrane leads to slight depolarization of the inner membrane potential and reduced maximal superoxide levels. The stress-resistance against brief anoxia-reoxygenation in isolated mitochondria demonstrates an important anti-oxidant role for UCP-2 in heart tissue, which needs at least a normal level of expression to become effective. Although the beneficial effects of UCP-2 expression as an antioxidant are evident in models of ischemia and reperfusion, the long-term effects of a proton leak with depolarization of the inner membrane on maximal energy production in hibernating hearts remain unclear. As we have previously speculated, the heart may adapt to offset reactive oxygen species during repetitive-supply demand mismatch, but at the expense of maximal energy production and the potential for heart failure (27). This may be

particularly important related to the STAT3 regulation of mitochondrial protein expression function, as demonstrate by a reduction in STAT3 and associated cardiomyopathy (28, 29). In addition, UCP-2 activation may have a negative impact on excitation-contraction, and promote heart failure independent of mitochondrial pathways (30). Clearly, more experiment evidence is needed to understand the chronic effects of altered expression of mitochondrial electron transport proteins, on cardiac function and the failing heart.

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References

1. Chen Q, Camara A, Stowe D, Hoppel C, Lesnefsky E. Modulation of electron transport protects cardiac mitochondria and decreases myocardial injury during ischemia and reperfusion. *Am J Physiol Cell Physiol*. 2007; 292:C137–47. [PubMed: 16971498]
2. Halestrap A, Clarke S, Khaliulin I. The role of mitochondria in protection of the heart by preconditioning. *Biochim Biophys Acta*. 2007; 1767:1007–1031. [PubMed: 17631856]
3. Korshunov S, Skulachev V, Starkov A. High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. *FEBS Letters*. 1997; 416:15–18. [PubMed: 9369223]
4. Kowaltowski A, Castilho R, Vercesi A. Mitochondrial permeability transition and oxidative stress. *FEBS Letters*. 2001; 495:12–15. [PubMed: 11322939]
5. Brookes P. Mitochondrial H(+) leak and ROS generation: an odd couple. *Free Radic Biol Med*. 2005; 38:12–23. [PubMed: 15589367]
6. Nadtochiy S, Tompkins A, Brookes P. Different mechanisms of mitochondrial proton leak in ischaemia/reperfusion injury and preconditioning: implications for pathology and cardioprotection. *Biochem J*. 2006; 395:611–618. [PubMed: 16436046]
7. Kowaltowski A, Costa A, Vercesi A. Activation of the potato plant uncoupling mitochondrial protein inhibits reactive oxygen species generation by the respiratory chain. *FEBS Letters*. 1998; 425:213–216. [PubMed: 9559650]
8. Mattiason G, Shamloo M, Gido G, Mathi K, Tomasevic G, Yi S, Warder C, Castilho R, Melcher T, Gonzalez-Zulueta M, Nikolich K, Wieloch T. Uncoupling protein-2 prevents neuronal death and diminishes brain dysfunction after stroke and brain trauma. *Nature Medicine*. 2003; 9:1062–68.
9. Kizaki T, Suzuki K, Hitomi Y, Taniguchi N, Saitoh D, Watanabe K, Onoe K, Day N, Good R, HO. Uncoupling protein 2 plays an important role in nitric oxide production of lipopolysaccharide-stimulated macrophages. *PNAS*. 2002; 99:9392–7. [PubMed: 12089332]
10. Teshima Y, Akao M, Jones S, Marban E. Uncoupling protein-2 overexpression inhibits mitochondrial death pathway in cardiomyocytes. *Circ Res*. 2003; 93:192–200. [PubMed: 12855674]
11. Andrews Z, Horvath T. Uncoupling protein-2 regulates lifespan in mice. *Am J Physiol*. 2009; 296:E621–E627.
12. McLeod C, Aziz A, Hoyt R, McCoy P, Sack M. Uncoupling proteins 2 and 3 function in concert to augment tolerance to cardiac ischemia. *J Biol Chem*. 2005; 280:33470–33476. [PubMed: 16079144]
13. McFalls E, Sluiter W, Schoonderwoerd K, Manintveld O, Lamers J, Bezstarosti B, van Beusekom H, Sikora J, Ward H, Merkus D, Duncker D. Mitochondrial adaptations within chronically ischemic swine myocardium. *J Mol Cell Cardiol*. 2006; 41:980–988. [PubMed: 16926020]
14. Chan S, Wu C, Kay L, Ho Y, Chang A, Chan J. Transcriptional upregulation of mitochondrial uncoupling protein 2 protects against oxidative stress-associated neurogenic hypertension. *Circ Res*. 2009; 105:886–896. [PubMed: 19762685]

15. Krauss S, Zhang C, Scorrano L, Dalgaard L, St-Pierre J, Grey S, Lowell B. Superoxide-mediated activation of uncoupling protein 2 causes pancreatic B cell dysfunction. *J Clin Invest.* 2003; 112:1831–1842. [PubMed: 14679178]
16. Rodriguez W, Joshua I, Falcone J, Tyagi S. Pioglitazone prevents cardiac remodeling in high-fat, high-calorie-induced type 2 diabetes mellitus. *Am J Physiol.* 2006; 291:81–7.
17. St Pierre J, Buckingham J, Roebuck S, Brand M. Topology of superoxide production from different sites in the mitochondrial electron transport chain. *J Biol Chem.* 2002; 277:44784–90. [PubMed: 12237311]
18. Ozcan C, Bienengraeber M, Dzeja P, Terzic A. Potassium channel openers protect cardiac mitochondria by attenuating oxidant stress at reoxygenation. *Am J Physiol.* 2002; 282:H531–H539.
19. Zhao H, Kalivend S, Zhang H, Joseph J, Nithipatikom K, Vasquez-Vivar J, Kalyanarman B. Superoxide reacts with hydroethidine but forms a fluorescent product that is distinctly different from ethidium: Potential implications in intracellular fluorescence detection of superoxide. *Free Radic Biol Med.* 2003; 34:1359–1368. [PubMed: 12757846]
20. Rottenberg H. Membrane potential and surface potential in mitochondria: Uptake and binding of lipophilic cations. *J Membrane Biology.* 1984; 81:127–38.
21. Lambert A, Brand M. Superoxide production by NADH: Ubiquinone oxidoreductase (complex I) depends upon pH gradient across mitochondrial inner membrane. *Biochem J.* 2004; 392:511–517. [PubMed: 15175007]
22. Bo H, Jiang N, Ma G, Zhang G, Cao D, Wen L, Liu S, Zhang Y. Regulation of mitochondrial uncoupling respiration during exercise in rat heart: Role of reactive oxygen species (ROS) and uncoupling protein 2. *Free Radic Biol Med.* 2008; 44:1373–1381. [PubMed: 18226608]
23. Honda T, Kaikita K, Tsujita K, Hayasaki T, Matsukawa M, Fuchigami S, Sugiyama S, Sakashita N, Ogawa H, Takeya M. Pioglitazone, a peroxisome proliferator-activated receptor-gamma agonist, attenuates myocardial ischemia-reperfusion injury in mice with metabolic disorders. *J Mol Cell Cardiol.* 2008; 44:915–926. [PubMed: 18436235]
24. Li J, Lang M, Mao X, Tian L, Feng Y. Antiapoptosis and mitochondrial effect of pioglitazone preconditioning in the ischemic/reperfused heart of rat. *Cardiovasc Drugs Ther.* 2008; 22:283–91. [PubMed: 18470603]
25. Yang H, Deleuze S, Zuo Y, Potthoff S, Ma L, Fogo A. The PPARgamma agonist pioglitazone ameliorates aging-related progressive renal injury. *J Am Soc Nephrol.* 2009; 20:2380–2388. [PubMed: 19797472]
26. Raha S, McEachern G, Mynt T, Robinson B. Superoxides from mitochondrial complex III: the role of manganese superoxide dismutase. *Free Radical Biology & Medicine.* 2000; 29:170–180. [PubMed: 10980405]
27. Kelly R, Sluiter W, McFalls E. Myocardial Hibernation: Is the Program to Survive a Pathway to Failure? *Circ Res.* 2008; 102:3–5. [PubMed: 18174469]
28. Boengler K, Hilfiker-Kleiner D, Drexler H, Heusch G, Schulz R. The myocardial JAK/STAT pathway: From protection to failure. *Pharmacology & Therapeutics.* 2008; 120:172–185.
29. Qiu H, Lizano P, Laure L, Sui X, Rashed E, Park J, Hong C, Gao S, Holle E, Morin D, Dhar S, Wagner T, Berdeaux A, Tian B, Vatner S, DePre C. H11 kinase/heat shock protein 22 deletion impairs both nuclear and mitochondrial functions of STAT3 and accelerates the transition into heart failure on cardiac overload. *Circulation.* 2011; 124:406–415. [PubMed: 21747053]
30. Turner J, Gaspers L, Wang G, Thomas A. Uncoupling protein-2 modulates myocardial excitation-contraction coupling. *Circ Res.* 2010; 106:730–738. [PubMed: 20056920]

Figure 1.

Chronic peroxisome proliferator-activator receptor γ (PPAR γ) stimulation was provided with daily pioglitazone (Pio) in the diet for 3 weeks of wild type (WT) and uncoupling protein (UCP)-2 knock-out (KO) mice. Pioglitazone increased (A) UCP-2 content in mitochondria from excised hearts of wild type mice, with no expression noted in UCP-2 KO mice and (B) PGC1- α in the nuclear fraction of WT mice.

Figure 2.

The relationship between state 2 respiration and inner membrane potential is shown in isolated mitochondria from heart tissue extracted from UCP-2 KO mice and WT mice with or without chronic PPAR γ stimulation with dietary supplementation of pioglitazone. The presence of a proton leak across the inner membrane induced by UCP-2 is associated with a higher state 2 respiration relative to the lower membrane potential, in mice with increased expression of UCP-2. Data are expressed as means and SEM bars.

Figure 3.

In parallel studies, GDP (1 mM) was added to mitochondrial samples from heart tissue extracted from WT mice fed pioglitazone (Pio) leading to a reduction in both the (A) membrane potential and (B) state 2 respiration. The findings are consistent with the increased UCP-2 content as the cause of the depolarization in isolated mitochondria. N=6/group; *P<0.05. Grouped data from all WT mice on a control (C) diet are shown for comparison.

Figure 4.

The relationship between maximal superoxide levels during inhibition of complex III with antimycin A and inner membrane potential is shown in isolated mitochondria from heart tissue extracted from UCP-2 KO mice and WT mice with or without pioglitazone. The presence of a proton leak across the inner membrane induced by over-expression of UCP-2 is associated with lower levels of superoxide, in mice with increased expression of UCP-2. Data are expressed as means and SEM bars.

Figure 5.

The redox state from isolated mitochondria was compared in WT mice with and without pioglitazone (Pio) and UCP-2 KO mouse hearts and demonstrated no differences under basal conditions between reduced (GSH) and total glutathione (GSH + GSSG) concentrations. GSH/GSSG in mitochondria from WT mice on regular and pioglitazone diets were 7.7 ± 3.6 and 12.5 ± 7.3 respectively and did not differ from UCP-2 KO mice with either diet (NS). Data are expressed as means and SEM.

Figure 6.

In isolated mitochondria exposed to 10 minutes anoxia-reoxygenation, (A) state 3 respiration and (B) the respiratory control index (RCI) were higher in hearts extracted from WT mice on control (C) and Pioglitazone (Pio) diet compared with UCP-2 KO mice on either C or Pio (* $P < 0.05$ versus WT + Pio).

Effects of chronic administration of PPAR- γ stimulation with dietary supplementation of pioglitazone on isolated heart mitochondria at baseline from wild type (WT) compared with WT and UCP-2 knock-out (KO) littermates on a regular diet.

Table 1

	Membrane Potential (mv)	State 2 (nmol/min/mg)	State 3 (nmol/min/mg)	RCI (State 3/2)	Superoxide (FU)
WT+C	-166 $\pm$ 4	48 $\pm$ 5	159 $\pm$ 13	3.6 $\pm$ 0.2	16 $\pm$ 2
WT+Pio	-147 $\pm$ 6 [*]	68 $\pm$ 8 [*]	192 $\pm$ 18	3.5 $\pm$ 0.5	13 $\pm$ 1
KO+C	-180 $\pm$ 4 ^{*^}	39 $\pm$ 4 [^]	160 $\pm$ 15	4.8 $\pm$ 1.0	16 $\pm$ 1
KO+Pio	-182 $\pm$ 3 ^{*^}	49 $\pm$ 6 [^]	156 $\pm$ 14	4.1 $\pm$ 0.4	16 $\pm$ 2

N=30 per group; Data expressed as means and SEM; Pio (Dietary supplementation for 3 weeks with pioglitazone (50 μ g/gram of chow); Respiratory control index (RCI; state 3/state 2 respiration).

^{*} P<0.05 vs WT+C;

[^] P<0.05 vs WT+Pio; FU (fluorescent units).