Published in final edited form as:

Circulation. 2011 August 16; 124(7): 806–813. doi:10.1161/CIRCULATIONAHA.110.003418.

The mitochondrial transporter ABC-me (ABCB10) is a novel gene required for cardiac recovery after ischemia-reperfusion

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Abstract

Background—Oxidative stress and mitochondrial dysfunction are central mediators of cardiac dysfunction following ischemia-reperfusion. ABC-me (ABCB10/mABC2) is a mitochondrial transporter highly induced during erythroid differentiation and predominantly expressed in bone marrow, liver and heart. However, ABC-me function in heart is unknown. Several lines of evidence demonstrate that the yeast orthologue of ABC-me protects from increased oxidative stress. Therefore, ABC-me is a potential modulator of the outcome of ischemia-reperfusion in the heart.

Methods and results—Mice harboring one functional allele of ABC-me (ABC-me +/-) were generated by replacing ABC-me exons 2 and 3 by a neomycin resistance cassette. Cardiac function was assessed using Langendorff perfusion and echocardiography. Under basal conditions, ABC-me +/- mice had normal heart structure, hemodynamic function, mitochondrial respiration and oxidative status. However, following ischemia-reperfusion, the recovery of hemodynamic function was reduced by 50% in ABC-me +/- hearts due to impairments in both systolic and diastolic function. This reduction was associated with impaired mitochondrial bioenergetic function and with oxidative damage to both mitochondrial lipids and the sarcoplasmic reticulum calcium ATPase (SERCA) after reperfusion. Treatment of ABC-me +/- hearts with the superoxide dismutase/catalase mimetic EUK-207 prevented oxidative damage to mitochondria and SERCA, and restored mitochondrial and cardiac function to wild type levels after reperfusion.

Conclusions—Inactivation of one allele of ABC-me increases the susceptibility to oxidative stress induced by ischemia-reperfusion, leading to increased oxidative damage to mitochondria

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Disclosures: The authors have no conflicts of interest.

and SERCA, and to impaired functional recovery. Thus, ABC-me is a novel gene that determines the ability to tolerate cardiac ischemia-reperfusion.

Keywords

ischemia-reperfusion; oxidative stress; ABC-me; ABCB10; mABC2

Introduction

Acute coronary occlusion leading to cardiac ischemia-reperfusion is a major cause of mortality in western societies. Multiple lines of evidence demonstrate that increased oxidative stress and mitochondrial dysfunction are key mediators of the cardiac dysfunction induced by ischemia-reperfusion [1-15]. After ischemia-reperfusion, oxidative stress may impair mitochondrial electron transport [2, 4, 8-10], leading to decreased mitochondrial ATP synthesis and respiration and thereby contributing to diastolic stiffness and contractile dysfunction [8-10, 16]. Conversely, the sudden increase in mitochondrial respiration during early reperfusion and the impairment in electron transport chain activity may increase the production of reactive oxygen species and contribute to oxidative damage [4, 5, 8, 12, 17]. Furthermore, the opening of the mitochondrial transition pore and mitochondrial fission promote cardiac myocyte death after ischemia-reperfusion [7, 11, 18].

The central role that oxidative stress plays in the myocardial recovery from ischemia-reperfusion has been well illustrated. Targeting antioxidants to mitochondria or overexpression of global and mitochondrial antioxidant enzymes (such as catalase, superoxide dismutase 1/sod1 or superoxide dismutase 2/sod2) protects and improves the recovery from myocardial ischemia-reperfusion injury [1, 6, 14, 19, 20]. In contrast, partial loss-of-function of antioxidant enzymes (such as mitochondrial sod2) worsens the recovery from ischemia-reperfusion [3]. In the latter model, impaired recovery is independent of changes in cell viability, suggesting that oxidative stress leads to functional impairment of the contractile machinery and/or mitochondria (and the concomitant bioenergetic defect) [3]. In this regard, inactivation of SERCA by sulfonic acid oxidation of the thiol on cysteine 674 is increased after ischemia-reperfusion, impairing calcium handling and contractility in cardiomyocytes [21, 22]. In addition, increased mitochondrial lipid oxidation has been shown to be one of the main alterations decreasing the electron transport chain activity after ischemia-reperfusion [10]. Therefore, any novel gene that protects mitochondrial and/or contractile function from oxidative damage is a potential modulator of cardiac recovery after ischemia-reperfusion.

ABC-me (ATP binding cassette mitochondrial erythroid, ABCB10 or mABC2) was first discovered in the erythroid tissue, where it is induced during erythroid differentiation [23]. ABC-me is a mitochondrial exporter of unknown substrate/s located in the inner mitochondrial membrane, with its nucleotide binding domain in the matrix [23, 24]. In erythroid cell lines, ABC-me has been shown to regulate hemoglobin synthesis and stabilize Mitoferrin 1 (Mfrn1), a mitochondrial iron importer [23, 25]. While cardiac tissue is one of the main sites of ABC-me expression, its function in the heart has not been studied [23].

Therefore, it is likely that ABC-me, in such non-erythroid tissues, plays a role not directly related to hemoglobinization.

Studies of ABC-me orthologues suggest that ABC-me may alter the capacity of cells to handle oxidative stress [26]. In this study, we tested the hypothesis that ABC-me plays a role in the protection of mitochondrial and contractile functions from increased oxidative stress induced by ischemia-reperfusion in the heart. We found that hearts from mice harboring only one functional allele of ABC-me (ABC-me +/-) show impaired hemodynamic recovery, increased oxidative damage of both mitochondrial lipids and SERCA and exhibit severe mitochondrial dysfunction after ischemia-reperfusion. These defects were selectively restored in ABC-me +/- hearts by treatment with a superoxide dismutase/catalase mimetic.

Methods

Animals—ABC-me +/- mice were generated by a gene targeted KO strategy through Lexicon Genetics (ABC-me exons 2 and 3 were replaced by a neomycin resistance cassette) on a C57BL6/129SvEvBrd mixed background, and backcrossed up to four generations onto C57BL6 background (Supplementary Figure 1). Three month-old male or female wild type and ABC-me +/- littermates were used (inactivation of one allele of ABC-me did not cause gender specific differences in the cardiac phenotype). Genotyping was performed by PCR of tail lysates. All procedures and experiments were carried out according to institutional guidelines for animal care (IACUC #14856) at Boston University in compliance with United States Public Health service regulations.

Ex-vivo Langendorff preparations and EUK-207 treatment

Mice were heparinized (100 U, intraperitoneally) and anesthetized by sodium pentobarbital (150 mg/kg, intraperitoneally). The heart was excised and perfused at a constant pressure of 80 mm Hg at 37°C as previously described [27]. The perfusate was equilibrated with 95% O_2 and 5% CO_2 (pH 7.4) and contained the following (in mmol/L): NaCl (118), $NaHCO_3$ (25), KCl (5.3), $CaCl_2$ (1.8), $MgSO_4$ (1.2), glucose (10) and pyruvate (0.5). Hearts were paced at 7.5 Hz, maintaining a constant heart rate of 450 bpm throughout the protocol. A water-filled balloon was inserted into the left ventricle (LV) to record ventricular pressure. After stabilization, a LV pressure–volume (P–V) relationship was obtained by stepwise increases of the balloon volumes, until the maximum LV developed pressure was reached for each heart. In a separate cohort, hearts were perfused (while continuously recording LV function) with either vehicle (initial concentration, 4% Dextrose) or EUK 207 (50 μM final concentration in perfusate) for 20 min, followed by 2 min wash out, 10 minutes of no-flow global ischemia and then reperfused during 20 min (maintaining and controlling temperature at 37 °C during the procedure). For ATP measurements, beating hearts were freeze-clamped with Wollenberger tongs that were pre-cooled in liquid nitrogen. EUK-207 was custom synthesized by Dalton Chemical Laboratories (Toronto), as previously described [28]. Purity was greater than 98 % as assessed by HPLC. Identity was confirmed by NMR spectroscopy of the ligand and by elemental analysis and mass spectrometry of the Mn-ligand complex as we have described previously for salen Mn or Mn porphyrin SOD/catalase mimetics [29]. The dose selected was based on protective activity reported previously in tissue culture [29, 30], as

well as on that of another salen Mn complex, EUK-8, in an isolated perfused rat heart model [31].

Echocardiography and tissue Doppler were performed as described [32]. See supplementary information on-line.

Mitochondria isolation

Hearts were incubated and minced in ice-cold fiber relaxation buffer (during approximately 10 minutes; KCl 100 mM, EGTA 5 mM, HEPES 5 mM adjusted with KOH to pH 7.4; to help releasing intermyofibrillar mitochondria) and they were homogenized in 2 ml of HES buffer (HEPES 5 mM, EDTA 1 mM, Sucrose 0.25M, pH 7.4 adjusted with KOH 1M) using a glass dounce homogenizer (20 strokes with loose pestle, 20 strokes tight pestle). The homogenate was centrifuged at 500×g for 10 minutes at 4 °C (pellet discarded and supernatant recentrifuged at 500×g). The supernatant was centrifuged at 9000×g for 15 minutes at 4 °C and the mitochondrial pellet was re-suspended in 100-200 µl of HES buffer with 0.2% of BSA fatty acid-free. Protein was quantified using BCA (Pierce) and the value of protein measured in HES-BSA 0.2% buffer alone was subtracted.

Mitochondrial oxygen consumption measurements

Isolated mitochondria (20-40 µg in HES-BSA 0.2% buffer per well, n=3-4 replicates per mouse) were loaded in a V7 24-well Seahorse plate on ice and 440 µl of ice cold mitochondrial assay buffer (MAS: Sucrose 70 mM, Mannitol 220 mM, KH_2PO_4 5 mM, $MgCl_2$ 5 mM, HEPES 2 mM, EGTA 1 mM, BSA fatty acid-free 0.2 %, pH 7.4 adjusted with KOH 1 M) + 50 µl of MAS buffer with 10× substrates (complex II: succinate 50 mM + rotenone 20 µM; complex I : pyruvate + malate, 50 mM each) were added on top. The four sequential injection ports of the Seahorse cartridge contained (in MAS solution and adjusted to pH 7.4): A: 50 µl 10× substrate and ADP 2.5 mM; B: 55 µl Oligomycin 20 µM; C: 60 µl 2,4-dinitrophenol 1 mM; D: 65 µl Antimycin 40 µM. Oxygen consumption rates were monitored in real time after the injection. State III was determined after port A injection, State IV after port B and uncoupled respiration rates after port C. Antimycin A was used as a control, as it blocks ETC oxygen consumption. See supplementary information on-line and www.shirihai-lab.org for more details.

Western blot and protein carbonylation—SDS-PAGE and transfer was performed as described [33]. See supplementary information on-line.

Measurements of superoxide in isolated mitochondria using Mitosox (Invitrogen)—This assay was adapted from Johnson-Caldwell et al [34] and performed in a 96-well microplate reader in a 200 µl reaction volume. The slope of Mitosox fluorescence increase was determined between the first 5-30 minutes after addition of Mitosox on isolated mitochondria at 37°C.

ATP measurements—The concentrations of ATP were measured spectrophotometrically in neutralized perchloric acid filtrates, as previously described [35], and normalized against

total protein contents. ATP synthesis rates were measured in respiring mitochondrial fractions using the ATP Kit CLS II (Roche); see Supplementary information on-line.

Histology to detect SERCA oxidation—This methodology was performed as described [21].

Lipid oxidation measurements by TBARS—Isolated mitochondria (100 μg) were solubilized with SDS and nmols of TBARS/mg protein were measured using Cell Biolabs kit and following manufacturer's indications, based on previously reported methodologies (among other references) [10].

Statistical analysis—Non-parametric (unpaired, two-tailed) tests were used: Mann-Whitney U for n≥ 4 and a test based on Chebyshev's Inequality for n=3. Statistically significant differences and exact p values are stated in the Figure legends.

Results

ABC-me +/- hearts have normal hemodynamic and mitochondrial function under basal conditions

ABC-me -/- mice were found to be embryonic lethal (data not shown). ABC-me +/- mice did not show any apparent phenotype under basal conditions and their fertility was similar to that of wild type mice. No differences in body weight, heart weight or the heart weight/body weight ratio were observed between wild type and ABC-me +/- mice (Table 1). Cardiac function was assessed by *ex-vivo* Langendorff preparation, echocardiography and tissue Doppler. Systolic and end-diastolic pressure-volume curves, and developed pressure were similar over a range of LV volumes in wild type and ABC-me +/- hearts (Figure 1A). Echocardiography showed that LV end-diastolic volume, end-systolic volume, wall thickness, mass index and fractional shortening were similar in wild type and ABC-me +/- hearts (Table 1). Tissue Doppler also revealed similar diastolic function (Figure 1B and Table 1).

Consistent with normal hemodynamic function, the rate of mitochondrial oxygen consumption measured in the presence of substrates that drive respiration through complex I or II was also normal in ABC-me +/- hearts (Figure 2A and 2B). This was also confirmed by the lack of differences in mitochondrial structure visualized by confocal microscopy (data not shown) or in maximal respiratory capacity and respiration linked to ATP synthesis measured with the Seahorse XF24 in intact cardiomyocytes isolated from adult ABC-me +/- and wild type mice (Supplementary Figure 2). In addition, under basal conditions there was no evidence for increased oxidative stress in ABC-me +/- hearts, as reflected by protein carbonylation levels (Figure 2C), mitochondrial superoxide production (Figure 2D), the upregulation of sod2 and catalase protein (Figure 2E) or the lack of significant detection of oxidized SERCA (Figure 3F).

ABC-me +/- hearts show impaired hemodynamic recovery and increased oxidative stress after ischemia-reperfusion

To study the role of ABC-me in the recovery from ischemia-reperfusion, Langendorff preparations of wild type and ABC-me +/- hearts were subjected to 10 minutes of global ischemia followed by 20 minutes of reperfusion. In wild type hearts, there was a 55 % recovery of developed pressure after 20 minutes of reperfusion (Figure 3A and D). In contrast, in ABC-me +/- hearts the recovery of developed pressure was markedly reduced to 27 % (Figure 3A and D), in association with an increase in diastolic pressure (Figure 3B) and a small decrease in systolic pressure (Figure 3C). In order to test whether an alteration in the oxidative status was responsible for this impaired recovery, we measured lipid oxidation in isolated mitochondria. We observed an increase of oxidized lipids in isolated mitochondria from ABC-me +/- hearts after reperfusion (Figure 3E). In addition, we studied SERCA oxidation, as it is a protein modification linking impaired oxidative status and altered diastolic function after reperfusion [21, 22]. We detected a marked increase of oxidized SERCA in sections of ABC-me +/- hearts after reperfusion, using an antibody that specifically detects sulfonyc acid oxidation of the thiol on SERCA cysteine 674 (Figure 3F). This data was confirmed by decreased BIAM labeling of SERCA (measure of total free thiol groups) in lysates from ABC-me +/- hearts after reperfusion (Supplementary Figure 3).

A superoxide dismutase/catalase mimetic (EUK-207) rescues contractile and mitochondrial function after ischemia-reperfusion in ABCme +/- hearts

To test to which extent increased oxidative stress mediates the reduced recovery from ischemia-reperfusion in ABC-me +/- hearts, 20 minutes prior to the start of ischemia, we perfused hearts with EUK-207, a catalytic antioxidant with catalase and superoxide dismutase activities [28-30, 36]. EUK-207 pre-treatment completely prevented the impairment in recovery of hemodynamic function in ABC-me +/- hearts caused by ischemia-reperfusion (Figure 3). EUK-207 normalized end-diastolic pressure in ABC-me +/- hearts to the absolute values observed in wild type hearts (Figure 3B). Of note, EUK-207 increased developed and systolic pressures in both ABC-me +/- hearts and wild type hearts to similar absolute levels that exceeded that of untreated wild type hearts (Figure 3A, C and D). Importantly, the degree of developed pressure recovery in ABC-me +/- hearts treated with EUK-207 was greater (from 27% to 90% = 3.3 fold increase) than in wild type hearts (from 55% to 82% = 1.5 fold increase) (Figure 3D), demonstrating the selectivity of the rescue by EUK-207 in ABC-me +/- hearts.

To further address the mechanism leading to impaired ischemia-reperfusion recovery and confirm the relevance of increased mitochondrial lipid oxidation in ABC-me +/- hearts, we measured mitochondrial respiration rates after 20 minutes of reperfusion. Respiration measurements were made in state III (respiration linked to maximal ATP synthesis rate), state IV (respiration not-linked to ATP-synthesis) and uncoupled respiration (by adding 2,4-dinitrophenol, DNP, which uncouples oxygen consumption from ATP synthesis). Ischemia-reperfusion selectively decreased mitochondrial respiration rates in all states and under DNP in ABC-me +/- hearts when compared to wild type hearts, suggesting impairment of the electron transport chain (Figure 4A). This impairment also caused a marked decrease in mitochondrial ATP synthesis rates (Figure 4B). Of note, pre-treatment with EUK-207

completely prevented the decrease in mitochondrial respiration caused by ischemia-reperfusion in ABC-me +/- hearts, but had no effects on mitochondrial respiration in wild type hearts after reperfusion (Figure 4A). This lack of EUK-207 effects in wild type mitochondrial respiration demonstrated again the specificity of the mitochondrial defect in ABC-me +/- hearts after reperfusion and the selectivity of its correction by EUK-207 treatment. The correction of mitochondrial respiration by EUK-207 was accompanied by complete restoration of mitochondrial ATP synthesis rates in ABC-me +/- hearts (Figure 4B). This was associated with a marked increase in total ATP levels (Figure 4C). Furthermore, EUK-207 treatment prevented the increase of both SERCA and mitochondrial lipids oxidation (Figure 3E, F). In addition, this increased oxidative damage also caused some necrosis in ABC-me +/- hearts (detected as triphenyl-tetrazolium chloride unstained areas within viable tissue), which was also prevented by EUK-207 (data not shown).

Discussion

We describe, for the first time, the phenotype of the ABC-me +/- mouse model and a role for the mitochondrial transporter ABC-me in the recovery of cardiac function after ischemia-reperfusion. This conclusion is supported by the impaired recovery of hemodynamic function after ischemia-reperfusion in mice having inactivation of one allele of ABC-me. Hemodynamic dysfunction was due to abnormalities in diastolic pressure and contraction, both of which are ATP-dependent and thus reliant on normal mitochondrial oxidative phosphorylation [8, 16]. In this regard, we show an impaired oxidative status in mitochondria from ABC-me +/- hearts after reperfusion. This is demonstrated by the increase in mitochondrial lipid oxidation and the concomitant decrease in mitochondrial respiration and ATP synthesis rates in ABC-me +/- hearts after reperfusion. These results are in line with several reports showing that lipid oxidation decreases mitochondrial respiration [8-10]. Furthermore, we also observe an increase in SERCA oxidation in ABC-me +/- hearts, corroborating a lack of protection from oxidative stress induced by ischemia-reperfusion. In addition, this increased oxidative damage can also explain the detection of some necrotic areas in ABC-me +/- hearts. Therefore, increased oxidative stress is sufficient to explain the hemodynamic dysfunction in ABC-me +/- hearts after ischemia-reperfusion.

In order to confirm that the alteration in the oxidative status was sufficient to cause the impaired recovery from ischemia-reperfusion in ABC-me +/- hearts, these were perfused with EUK-207, a catalytic antioxidant with catalase and superoxide dismutase activities [28-30, 36]. EUK-207 treatment prevented the appearance of necrotic areas and the increase in oxidation of mitochondrial lipids and SERCA. Furthermore, the functional alterations in mitochondria and contractility of ABC-me +/- hearts were completely and selectively prevented by perfusion with EUK-207 shortly before the onset of ischemia. The protective effect of this superoxide dismutase/catalase mimetic suggests that inactivation of one allele of ABC-me decreases the ability of the cardiac myocyte to protect mitochondrial and contractile function from oxidative stress in the setting of ischemia-reperfusion. Consistent with this line of evidence, the ABC-me yeast orthologue (Mdl1p, 42% amino-acid sequence similarity) protects from increased oxidative stress caused by iron accumulation in mitochondria triggered by ATM1 deletion [26]. Also consistent with our observation, partial loss of sod2 (mitochondrial superoxide dismutase), but not of sod1

(cytosolic), causes a worsening of the recovery from ischemia-reperfusion that is similar to that observed with ABC-me +/- hearts [3].

We selected EUK-207 for these experiments because it exerts both superoxide dismutase and catalase activities. Similar pre-treatments with a predominant superoxide dismutase mimetic (e.g. MnTBAP in cardiac ischemia-reperfusion) have been shown to increase accumulation of hydrogen peroxide and impair recovery after ischemia-reperfusion [6]. On the other hand, when MnTBAP was administered with catalase, recovery after ischemia-reperfusion was improved compared to hearts treated with catalase alone or untreated hearts [6]. Thus, it is likely that increasing both superoxide dismutase and catalase activities, shortly before the onset of ischemia, is a better strategy to improve functional recovery from ischemia-reperfusion than acute treatments predominantly increasing either superoxide dismutase or catalase activities.

In contrast to the impaired response to ischemia-reperfusion, under basal conditions ABC-me +/- hearts have normal mitochondrial respiration and hemodynamic function. Under basal conditions, there was also no evidence of increased oxidative stress as reflected by protein carbonylation, up-regulation of sod2 and catalase protein expression, the production of mitochondrial superoxide or detection of oxidized SERCA. This finding further suggests that the impaired response to ischemia-reperfusion is not due to an increase in basal oxidative stress. The lack of a basal cardiac phenotype is similar to observations in sod2 +/- mice [3].

A possible explanation for impaired mitochondrial oxidative status after ischemia-reperfusion is that partial loss of ABC-me affects heme synthesis and/or mitochondrial iron import in heart, as ABC-me overexpression increases hemoglobinization and stabilizes the iron importer Mitoferrin-1 in erythroid cell lines [23, 25]. Indeed, alterations in iron and/or in heme homeostasis are widely known to trigger mitochondrial dysfunction and/or oxidative stress. For example, the mouse heart model of Friedrich's ataxia shows dramatic defects in mitochondrial iron homeostasis and is associated with a severe cardiac phenotype characterized by dilated cardiomyopathy at 4.5-9 weeks of age that is prevented by iron chelation [37]. In this model, there is a marked decrease in the level of the complex II subunit Sdha [37]. However, in marked contrast, ABC-me +/- hearts have normal basal structure and function, and do not develop dilated cardiomyopathy or changes in Sdha or Mitoferrin 1 levels (Supplementary Figure 4). Thus, it is unlikely that inactivation of one allele of ABC-me causes important defects in iron and/or heme homeostasis in heart under basal conditions.

Another potential explanation is that ABC-me transport activity is increased by ischemia-reperfusion and mediates protection from oxidative stress either by a) preventing the accumulation of molecules that would increase oxidative stress inside the mitochondria or b) stimulating and/or enabling a cellular antioxidant response.

This study has potential clinical implications. First, these findings raise the possibility that genetic variations (mutations and/or polymorphisms) and/or drugs inactivating ABC-me may decrease tolerance for ischemia-reperfusion in humans. Second, this study demonstrates

that pre-treatment with antioxidants could be used to restore normal tolerance for ischemia-reperfusion in subjects showing genetic variations in ABC-me or to prevent the toxicity of drugs that could inactivate ABC-me.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are thankful to Dani Dagan for comments on the manuscript, Richard A. Cohen for SERCA antibodies, Janice M. Weinberg for advice on statistics, Wuhbet Abraham for technical assistance and to the rest of the members in the Shirihai lab.

Sources of Funding. This work was supported by NIH Grants R01 HL071629-03 and R01DK074778 to O.S.S.; HL-061639 and HL-064750 to W.S.C.. M.L. is a recipient of a post-doctoral fellowship from Fundación Ramón Areces.

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Clinical Perspective

This study reports that the mitochondrial transporter ABC-me (ABCB10) plays an essential role in the recovery of cardiac function after ischemia-reperfusion. This is demonstrated by the impaired recovery of hemodynamic function (reduced by half) after ischemia-reperfusion in mice having inactivation of one allele of ABC-me (ABC-me +/-).

Hemodynamic dysfunction was due to abnormalities in diastolic pressure and contraction, both of which are energy dependent (in form of ATP) and thus reliant on normal mitochondrial bioenergetics. In this regard, ABC-me inactivation caused increased oxidative damage to mitochondria, which decreased their ability to synthesize ATP. In addition, the sarcoplasmic reticulum calcium ATPase (SERCA) was also damaged by oxidation, contributing to the diastolic dysfunction observed in ABC-me +/- hearts after ischemia-reperfusion. Thus, decreased protection from oxidative damage induced by ischemia-reperfusion causes the impaired recovery of hemodynamic function in ABC-me +/- hearts. This is further demonstrated by the restoration of all the defects described in ABC-me +/- hearts by an antioxidant pre-treatment with a superoxide dismutase/catalase mimetic (EUK-207). The potential clinical relevance of these findings is emphasized by two different aspects: 1) ABC-me is a transporter and might be subject to competitive inhibition by drugs 2) Genetic variations (mutations/polymorphisms in the gene or the promoter) partially inactivating ABC-me may determine the ability of an individual to tolerate ischemia-reperfusion. These aspects are of potential clinical relevance, as they could predict the outcome from ischemia-reperfusion or determine selection of the appropriate treatments of individuals harboring a partial inactivation.

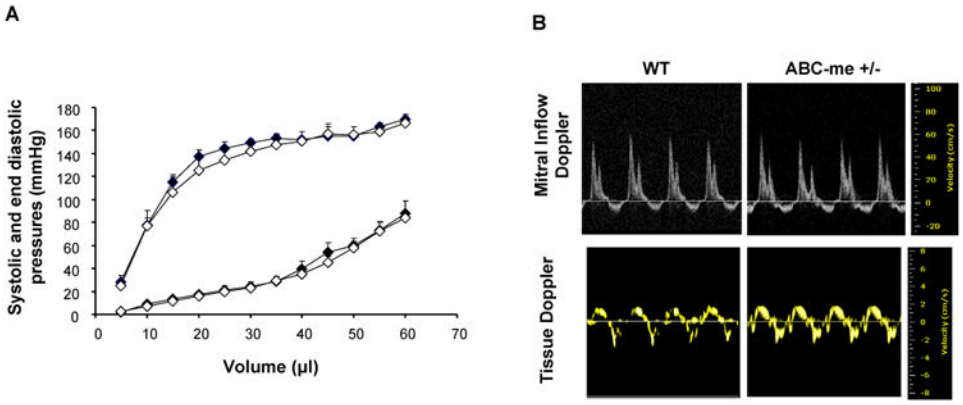

Figure 1. ABC-me +/- hearts have normal structure and function

A) Left ventricular pressure–volume relationships in isolated Langendorff perfused wild type (black symbols) and ABC-me +/- hearts (open symbols) (n=7). Upper traces represent systolic pressure and lower traces represent end diastolic pressure (EDP). **B)** Representative tracings of mitral inflow and tissue Doppler analysis of wild type and ABC-me +/- hearts. For values see table 1.

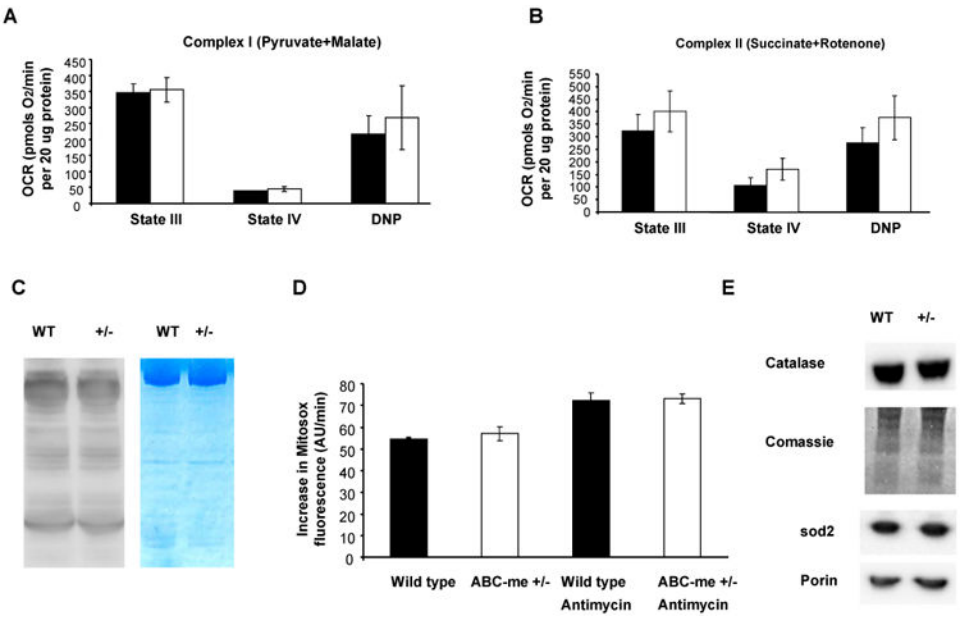

Figure 2. ABC-me +/- hearts have normal mitochondrial function and oxidative status under basal conditions

A) & **B)** Oxygen consumption rates (OCR) of isolated mitochondria (20μg) from wild type and ABC-me +/- hearts after Langendorff (n=4). State III was induced by incubating mitochondria with 0.25 mM ADP, State IV by 2 μM Oligomycin and uncoupled respiration by 100 μM 2,4-dinitrophenol (DNP). Respiration was driven by **A)** complex I (5mM pyruvate and 5 mM malate) or **B)** complex II (5 mM succinate and 2 μM rotenone). **C)** Representative Western blot analysis from total lysates of wild type (WT) and ABC-me +/- (+/-) hearts (n=7) to detect carbonylated proteins (the comassie blue staining of the transferred gel was used as a loading control). **D)** Mitochondrial superoxide production rates were addressed by monitoring the linear increase on Mitosox fluorescence in 96-well plate with 5-10 μg of mitochondria from wild type and ABC-me +/- hearts. Antimycin A was used as a positive control, as it increases superoxide production (black bars, wild type, open bars, ABC-me +/-, n=6). **E)** Representative Western blot analysis from total lysates of wild type (WT) and ABC-me +/- (+/-) hearts (n=7) to detect mitochondrial superoxide dismutase (sod2) and catalase. The comassie blue staining of the transferred gels and Porin were used as loading controls. Data are shown as mean with ± SEM.

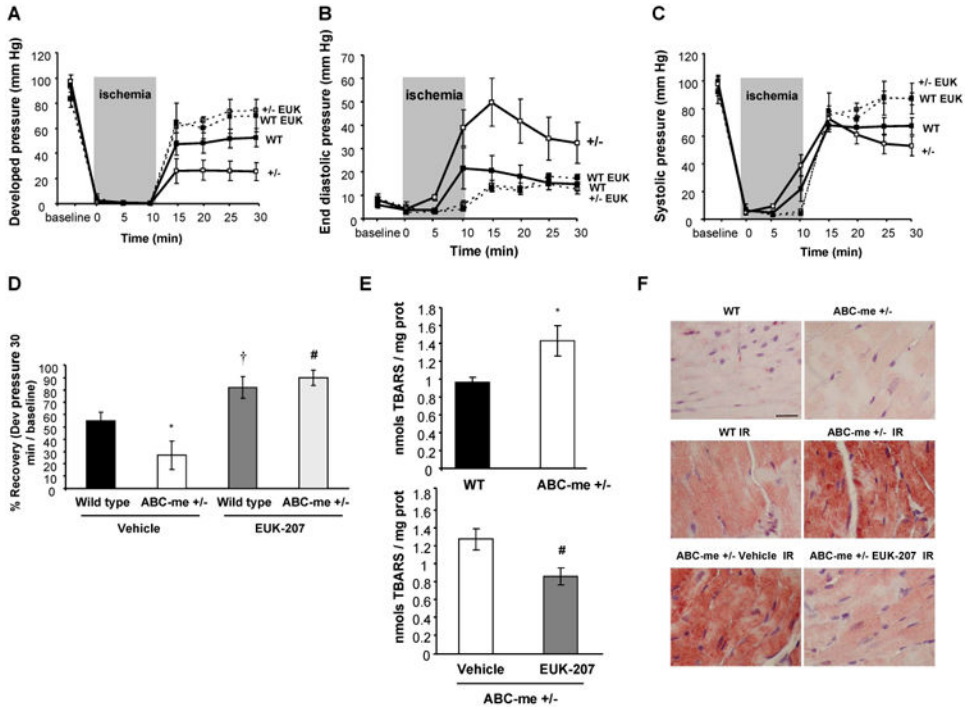

Figure 3. ABC-me +/- hearts have increased oxidative damage and impaired recovery after ischemia-reperfusion, which is specifically rescued by the superoxide dismutase / catalase mimetic, EUK-207

Continuous recording of left ventricular pressure (baseline and during ischemia-reperfusion) was performed in isolated Langendorff perfused hearts. **A)** Developed pressure (difference between systolic and diastolic pressures, mmHg) **B)** End diastolic pressure and **C)** Systolic pressure of wild type (black symbols) and ABC-me +/- hearts (open symbols) (n=7) pre-treated (20 minutes before ischemia) with vehicle (continuous line) or 50 μM EUK-207 (dashed line). **D)** Percentage of recovery of contractile function (ratio of Developed Pressure at the end of reperfusion vs. baseline) of wild type and ABC-me +/- hearts (n=7) pre-treated with vehicle or 50 μM EUK-207. *, p=0.02, wild type vs. ABC-me +/-; #, p=0.002, vehicle ABC-me +/- vs. EUK-207 ABC-me +/-. †, p=0.03, wild type vs. wild type EUK-207. **E)** Quantification of lipid oxidation by TBARS in isolated mitochondria from wild type and ABC-me +/- hearts after ischemia-reperfusion, or ABC-me +/- hearts pre-treated with EUK-207 or with vehicle after ischemia-reperfusion (n=5). All data are shown as mean with SEM. *, p= 0.0079, wild type vs. ABC-me +/-; #, p= 0.03, vehicle +/- vs. EUK-207. **F)** Representative images of heart sections immunostained with an antibody detecting sulfonylated SERCA on cysteine 674 from wild-type (WT) and ABC-me +/- hearts under basal conditions, after ischemia reperfusion (I-R) and pre-treated with EUK-207 or vehicle after ischemia-reperfusion. Scale bar, 25 μm.

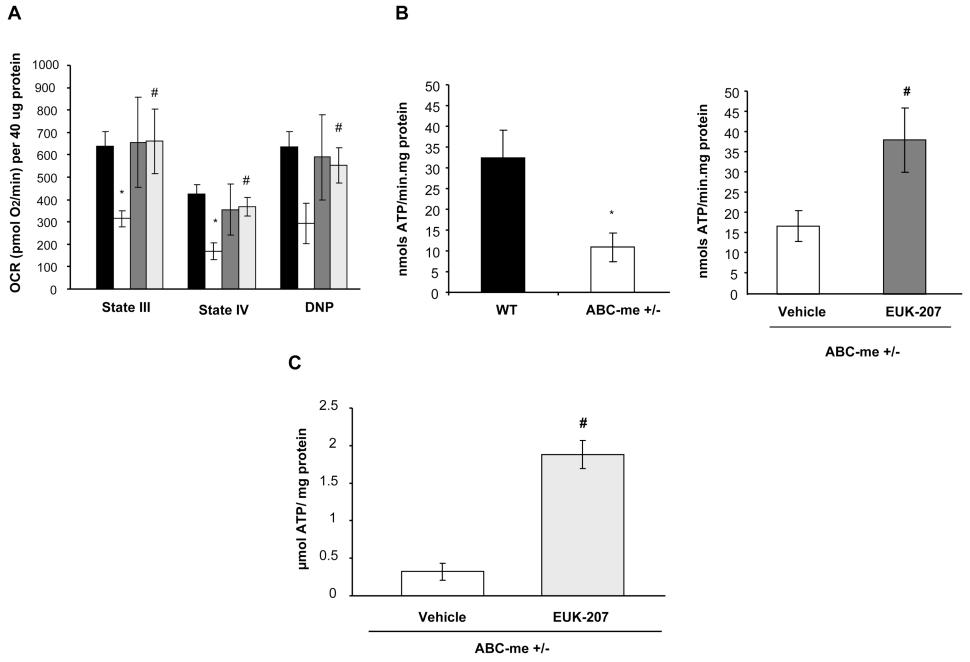

Figure 4. EUK-207 restores mitochondrial function and ATP levels in ABC-me +/- hearts after ischemia-reperfusion

A) Oxygen consumption rates (OCR) driven by complex II after ischemia-reperfusion (5 mM succinate + 2 μM rotenone) of isolated mitochondria (40μg) from vehicle and EUK-207 (50 μM) wild type (black, vehicle; dark grey, EUK-207) and ABC-me +/- pre-treated hearts (white, vehicle; light grey, EUK-207) (n=3). Data are shown as mean with ± SEM. State III was induced by incubating mitochondria with 0.25 mM ADP, State IV by 2 μM Oligomycin and uncoupled respiration by 100 μM 2,4-dinitrophenol (DNP). For detailed respirometry procedure see supplementary material. Data are shown as mean with ± SEM. *, p≤0.01, vehicle wild type vs. vehicle- ABC-me +/- hearts; #, p≤0.04, vehicle vs. EUK-207 ABC-me +/- hearts. **B)** Measurement of ATP synthesis rates in isolated mitochondria (nmols synthesized per minute and milligram of protein) from wild type (WT) and ABC-me +/- hearts after ischemia-reperfusion (n=5) or ABC-me +/- vehicle or EUK-207 (50 μM) after ischemia-reperfusion (n=5). Data are shown as mean with ± SEM. *, p=0.016, wild type vs. ABC-me +/-; #, p=0.03, vehicle vs. EUK-207 pre-treated ABC-me +/- hearts. **C)** Measurement of total ATP content after ischemia-reperfusion (μmols of nucleotide per mg of total protein) in freeze-clamped ABC-me +/- pre-treated with EUK-207 or with vehicle (n=4). Data are shown as mean with ± SEM. #, p=0.03.

Table 1

Heart weight, echocardiographic dimensions and Doppler analysis.

Dimension (units)	Wild type	ABC-me +/-	p-value
BW (g)	22.8 ± 2.2	21.2 ± 2.4	0.349
HW (mg)	125 ± 13	112 ± 8	0.917
HW/BW	5.3 ± 0.2	5.4 ± 0.3	0.686
IVSTh (mm)	0.60 ± 0.1	0.63 ± 0.1	>0.12
PWTh (mm)	0.9 ± 0.1	0.9 ± 0.1	>0.12
LVEDD (mm)	3.1 ± 0.2	3.1 ± 0.1	>0.12
LVESD (mm)	1.3 ± 0.1	1.2 ± 0.1	>0.12
LVMI (mg/g)	2.8 ± 0.3	2.7 ± 0.2	>0.12
FS (%)	59.6 ± 1.6	60.9 ± 2.6	>0.12
HR (bpm)	372 ± 5	371 ± 4	>0.12
E/A	1.63 ± 0.06	1.65 ± 0.03	>0.12
Em/Am	1.32 ± 0.04	1.31 ± 0.03	>0.12
E/Em	20.6 ± 2.1	21.9 ± 2.7	>0.12

BW, Body weight; HW, Heart weight; IVSTh, Interventricular septal thickness in diastole; PWTh, Posterior wall thickness in diastole; LVEDD, LV end-diastolic diameter; LVESD, LV end-systolic diameter; LVMI, LV mass index; FS, Fractional Shortening; HR, heart rate; E (early) and A (late) diastolic velocities, m (mitral). Data are shown as mean ± SEM; n = 3-6 per group.