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Dysregulation of cardiac mitochondrial aldehyde dehydrogenase 2: Studies in dogs with chronic heart failure

Ramesh C. Gupta,
Vinita Singh-Gupta,
Kristina J. Szekely,
Kefei Zhang,
David E. Lanfear,
Hani N. Sabbah*

Department of Medicine, Division of Cardiovascular Medicine, Henry Ford Hospital, 2799 West Grand Boulevard, Detroit, MI 48202, USA

Henry Ford Health & Michigan State University Health Science, USA

Abstract

Mitochondrial (MITO) dysfunction occurs in the failing heart and contributes to worsening of heart failure (HF). Reduced aldehyde dehydrogenase 2 (ALDH2) in left ventricular (LV) myocardium of diabetic hearts has been implicated in MITO dysfunction through accumulation of toxic aldehydes including and elevated levels of 4-hydroxy-2-nonenal (4HNE). This study examined whether dysregulation of MITO ALDH2 (mALDH2) occurs in mitochondria of the failing LV and is associated with increased levels of 4HNE.

LV tissue from 7 HF and 7 normal (NL) dogs was obtained. Protein quantification of total mitochondrial ALDH2 (t-mALDH2), phosphorylated mALDH2 (p-mALDH2), total MITO protein kinase c epsilon (t-mPKCε), phosphorylated mPKCε (p-mPKCε) was performed by Western blotting, and total mALDH2 enzymatic activity was measured. Protein adducts of 4HNE-MITO and 4HNE-mALDH2 were also measured in MITO fraction by Western Blotting.

Protein level of t-mALDH2 was decreased in HF compared with NL dogs (0.63 ± 0.07 vs 1.17 ± 0.08, $p < 0.05$) as did mALDH2 enzymatic activity (51.39 ± 3 vs. 107.66 ± 4 nmol NADH/min/mg, $p < 0.05$). Phosphorylated-mALDH2 and p-mPKCε were unchanged. 4HNE-MITO proteins adduct levels increased in HF compared with NL (2.45 ± 0.08 vs 1.30 ± 0.03 du, $p < 0.05$) as did adduct levels of 4HNE-mALDH2 (1.60 ± 0.20 vs 0.39 ± 0.08, $p < 0.05$). In

*Corresponding author at: Department of Medicine, Division of Cardiovascular Medicine, Henry Ford Hospital, 2799 West Grand Boulevard, Detroit, MI 48202, USA. hsabbah1@hfhs.org (H.N. Sabbah).

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Ramesh C. Gupta: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Vinita Singh-Gupta:** Methodology, Investigation. **Kristina J. Szekely:** Methodology. **Kefei Zhang:** Methodology. **David E. Lanfear:** Writing – review & editing. **Hani N. Sabbah:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization.

isolated failing cardiomyocytes (CM) exposure to 4HNE decreased mALDH2 activity, increased ROS and 4HNE-ALDH2 adducts, and worsened MITO function. Stimulation of mALDH2 activity with ALDA-1 in isolated HF CMs compared to NL CMs improved ADP-stimulated respiration and maximal ATP synthesis to a greater extant (+47 % and +89 %, respectively).

Down-regulation of mALDH2 protein levels and activity occurs in HF and contributes to MITO dysfunction and is likely caused by accumulation of 4HNE-mALDH2 adduct. Increasing mALDH2 activity (via ALDA-1) improved MITO function in failing CMs.

Keywords

Heart failure; aldehydes; Aldehyde dehydrogenase; Mitochondria; Protein kinase

1. Introduction

Even in the presence of highly effective modern guideline directed medical therapy, heart failure with reduced ejection fraction (HFrEF) carries a substantial residual risk for adverse outcomes and continues to be a major cause of morbidity and mortality worldwide [1]. HFrEF is associated with functional abnormalities of cardiac mitochondria that include not only reduced energy production but also reduced activity of dehydrogenase enzymes which are crucial metabolic enzymes for detoxification of reactive oxygen species, resulting in increased oxidative stress [2,3].

There are 19 neighboring genes in the aldehyde dehydrogenase (ALDH) family that play key roles in oxidation and detoxification of reactive aldehydes/acetaldehydes (to acetic acid) in a wide range of organs and cell types. All ALDH genes are encoded in nuclei, but five of the ALDH isozymes reside in and function as mitochondrial ALDH [4,5]. Mitochondrial (MITO) aldehyde dehydrogenase 2 (mALDH2) is an important enzyme that is involved in detoxification of 4-hydroxy-2-nonenal (4HNE) and other reactive aldehydes that accumulate during ischemia and reperfusion [6,7]. During oxidative stress, the reactive oxygen species (ROS) induced 4HNE readily binds to cysteine, histidine, and lysine residues by means of Michael's reaction and inhibits the functions of important proteins and enzymes [7]. If not sufficiently processed by mALDH2. 4HNE accumulation in mitochondria results in adduct formation on mALDH2 and works as a potent inhibitor of the enzyme [8,9], potentially creating a self-amplifying mechanism of mitochondrial dysfunction.

The protein kinase C (PKC) signaling pathway is an important regulator of mALDH2, which can be phosphorylated (and thus activated) by PKC epsilon (PKCε) [4,10]. PKCε translocates to mitochondria in response to oxidative stress and stimulates a series of cardioprotective events. Co-immunoprecipitation by anti-PKCε or with anti-ALDH2 antibodies confirms their association in cardiac mitochondria [10]. Finally, in addition to its dehydrogenase activity, ALDH2 has an esterase activity that catalyzes the conversion of nitroglycerin (glyceryl trinitrate [GTN]) to 1,2 glyceryl dinitrate (1,2-GDN), and consequently mediates bioactivation of GTN [11]. Accumulation of GTN (similar to such as 4HNE), due to reduced enzymatic activity, could adversely impact MITO function in the failing myocardium.

Although the basic biochemistry of mALDH2 seems well understood, it is not sufficiently studied in the setting of the failing heart, where many of these mechanisms may be amplified or dysregulated compared to normal state. The current experiments examined changes in mALDH2 protein levels and activity, mALDH2 phosphorylation state and PKCε levels in isolated mitochondria from left ventricular (LV) myocardium of dogs with chronic HF. The study also examined the cause and the effect relationship between 4HNE and mALDH2 activity and the consequence on overall MITO function.

2. Methods

2.1. Procurement of LV tissue

The current study was performed using LV tissue obtained from 7 male dogs with HF produced by multiple, sequential intracoronary microembolization as previously described [12]. The choice of male dogs was based on previous observations that at that heart mitochondrial function vary significantly between male and female [13]. Briefly, coronary microembolization with latex microspheres (~90 μm in diameter) were performed 1 week apart and were discontinued when LV ejection fraction was 30 % to 35 %. Microembolizations result in cumulative loss of viable myocardium and, as such, the model is synonymous with ischemic cardiomyopathy in humans. LV tissue was also obtained from 7 normal (NL) male dogs for comparison. All tissue samples were obtained immediately upon removal of the heart and were rapidly frozen in liquid nitrogen and stored at −70 °C until used. The study was approved by Henry Ford Health System Institutional Animal Care and Use Committee and conformed to the National Institute of Health “Guide and Care for Use of Laboratory Animals.”

2.2. Isolation of mitochondria

2.2.1. Isolation of mitochondria from fresh tissue—Mitochondria from fresh LV tissue were isolated by differential centrifugation as previously described [14] with slight modifications. Approximately 2.5 g LV tissue was washed with buffer-1 (100 mM KCl, 50 mM MOPS, 5 mM $MgSO_4 \cdot 7H_2O$, 1 mM EGTA, pH adjusted to 7.4) followed by mincing tissue in small pieces by scissors. Minced tissue was homogenized in 10 ml buffer-1 containing 2 mg/ml BSA with polytron and then with a glass Dounce homogenizer with loose pestle. The homogenate was centrifuged at 500*g* for 10 min at 4 °C. The pellet was discarded and the supernatant recentrifuged at 9000*g* for 15 min at 4 °C. The pellet containing mitochondria was resuspended in 2.5 ml KME buffer (100 mM KCl, 50 mM MOPS, 0.5 mM EGTA, pH 7.4) and recentrifuged at 9000*g* for 15 min. The final resulting pellet was resuspended in 0.3 ml KME buffer.

2.2.2. Isolation of mitochondrial fractions from frozen tissue—Mitochondrial fractions were isolated from 1 g of frozen LV tissue by first pulverizing in liquid nitrogen followed by directly homogenizing in Buffer-1 containing BSA and protease and phosphatase inhibitors purchased from Sigma. The rest of the procedure was similar to that used in fresh LV tissue. Protein concentration in the mitochondrial pellet was determined by Lowry’s method using Bovine Serum Albumin as a standard. LV tissue was not subjected to proteases prior to isolation of mitochondria and the population was

mostly subsarcolemmal mitochondria. The yield of isolated mitochondria was about 3 mg/g tissue. Mitochondrial functional parameters were normalized to porin, a protein exclusively localized to mitochondria and is unchanged in the failing heart [14,15].

2.3. mALDH2 enzymatic activity

Enzymatic Activity Assay Kit for ALDH2 (GenMed Scientifics Inc., Wilmington, DE, USA) was used to measure mALDH2 activity according to manufacturer's specifications. Enzymatic activity was measured at 25 °C in 1 ml reaction system containing 33 mM sodium pyrophosphate (pH 8.8), 0.8 mM NAD^+, 15 μM propionaldehyde, and 0.1 ml mitochondrial fractions isolated from frozen LV tissue. Reduced nicotinamide-adenine dinucleotide phosphate (NADH) production was determined spectrophotometrically by monitoring the alterations in absorbance intensity at 340 nm every 30 s for 5 min. Activity of mALDH2 was expressed as nmols NADH/min/mg protein.

2.4. Isolation of phosphorylated MITO fractions

Phosphorylated mALDH2 (p-mALDH2) and phosphorylated PKCε (p-mPKCε) were quantified in phosphorylated MITO fractions whereas total mALDH2 (t-mALDH2) and total mPKCε (t-mPKCε) were quantified in MITO fractions. Mitochondrial fractions isolated from frozen LV tissue were phosphorylated as described by Nene et al. [10] and the extract of phosphorylated mitochondrial fractions was prepared using 1× extraction buffer supplied by a kit (ab115348) of mitochondrial aldehyde dehydrogenase activity (Abcam Biotechnology company, Cambridge, United Kingdom). Approximately 150 μg MITO extracts were separated using a phosphoprotein enrichment kit according to the supplier's instructions (BD Biosciences, Singapore). During the isolation process, proteins that carry a phosphate group on any amino acid including serine, threonine, or tyrosine, are selectively bound by the resin, and are eluted from the column.

2.5. Quantitation of 4HNE adduct formation with MITO and ALDH2 proteins

To quantify 4HNE adduct formation with MITO proteins (4HNE-MITO), approximately 5 μg MITO extract prepared from frozen LV tissue, was subjected to Western blotting using 4HNE primary antibody. For 4HNE-mALDH2 adduct formation, approximately 20 μg of MITO proteins from 5 dogs were incubated separately with 10 μl 4HNE primary antibody overnight at 4 °C. The immunoprecipitants were collected and subjected to Western blotting using a primary antibody of ALDH2.

2.6. Exposure of isolated mitochondria to 4HNE

Under normal physiological conditions, 4HNE is produced in a concentration range of 0.1–3.0 μM and 10 μM to 5 mM under oxidative stress in membranes [16] as in heart failure. To examine the effect of 4HNE on mALDH2 activity, mitochondria were isolated from fresh LV myocardium of 4 dogs with HF and incubated at 37 °C for 1 h in the absence and presence of 3 μM and 30 μM 4HNE (dissolved in ethanol, Millipore Sigma, St. Louis, MO) as described previously [16–18]. Final concentration of ethanol in the assay was 0.4 %. mALDH2 activity and 4HNE-mALDH2 adduct formation were determined in treated mitochondria as described above. In the same 4 dogs, reactive oxygen species

(ROS) in isolated mitochondria were measured using The OxiSelect™ In Vitro ROS/RNS Assay Kit (Cell Biolabs, Inc., San Diego, CA) also in the presence and absence of 4HNE. The assay employs a proprietary quenched fluorogenic probe, dichlorodihydrofluorescin DiOxyQ (DCFH-DiOxyQ) and the fluorescence intensity is proportional to the total ROS levels within the sample. Controls without mitochondria were performed and the values were subtracted from the those obtained with mitochondria. Using H_2O_2 standard, ROS levels determined in mitochondria and expressed in nmoles H_2O_2/mg protein.

The 4HNE treated mitochondria were also used to evaluate MITO function using XFe96 analyzer (Seahorse Biosciences, Chicopee, MA), as previously described [17,18]. Briefly, after baseline oxygen consumption rate (OCR) measurements (nmols/min/mg), adenosine diphosphate (ADP, 10 μM), oligomycin (1 μg/ml), FCCP (1 μM), and antimycin A and rotenone (10 μM each) were injected consecutively through Seahorse Flux Pak cartridges. ADP was used to calculate ADP-stimulated respiration, oligomycin was used to measure ATP linked OCR, FCCP was used to determine maximal MITO respiratory capacity.

2.7. Treatment of isolated cardiomyocytes with activator of mALDH2

Cardiomyocytes (CM) were freshly isolated from the LV free wall of 7 HF dogs and 4 NL dogs as previously described [19]. Isolated CM were incubated for 1 h in the absence and presence of the ALDH2 activator Alda-1 (10 μM, dissolved in DMSO, Sigma Aldrich, St. Louis, MO). DMSO concentration in the treatment was present 0.1 %. After incubation, ADP-stimulated MITO respiration and maximal ATP synthesis were determined as described previously [15] and expressed as nAtom O/min/mg protein and nmoles/min/mg protein, respectively.

2.8. Western immunoblotting

Phosphorylated mALDH2, t-mALDH2, p-mPKCε, t-mPKCε, 4HNE-MITO-proteins adducts, and 4HNE-ALDH2 adducts were determined by Western blotting. SDS extracts were prepared from MITO and phosphorylated MITO extracts obtained from frozen LV tissue and subjected to Western blotting as described previously [15,19,20]. After separating proteins on 4 % to 20 % SDS-PAGE and transferring on PVDF membrane, blots were treated with specific primary antibodies followed by the corresponding secondary antibodies coupled with horseradish peroxidase. The bands on PVDF membrane were developed by chemiluminescence (ChemiDoc™ Touch Imaging System, Bio-Rad, Philadelphia, PA, USA). The band intensity was quantified using an imaging densitometer (Bio-Rad Model GS-670), expressed as densitometric units (du) and normalized to porin. In all cases, it was ensured that the antibody was present in excess over the antigen and the density of each protein band was in the linear scale. Primary antibody of ALDH2 and porin (Abcam, Cambridge, MA, USA); 4HNE (Alpha Diagnostic, San Antonio, TX, USA) and PKCε (BD Biosciences, San Jose, CA, USA) were purchased. All antibodies used in this study were validated by the manufacturer. The validation was repeated in our laboratories prior to use in the study using dog cardiac tissue and blocking peptides to ensure that each antibody produced a dominant band of the expected molecular weight for the target protein.

2.9. Statistical analysis

Comparisons of all measures between HF and NL dogs were made using a t-statistic for two means with statistical significance set at $p < 0.05$. Comparisons of all measures in experiments examining the effects of exposure to multiple concentration of 4HNE were performed using repeated measures analysis of variance (ANOVA) with alpha set at 0.05. If significance was attained, pairwise comparisons were made using the Student Neuman-Keuls test with $p < 0.05$ considered significant. All data are reported as means ± standard error of the mean (SEM).

3. Results

Dogs with HF used in this study had an average LV ejection fraction, a measure of LV systolic function determined by ventriculography, of 33 % with a range of 30 % to 35 % and a fractional area of shortening, also a measure of LV systolic function determined from 2-dimensional echocardiography of 34 % with a range of 29 to 36 %.

3.1. Activity and protein level of mALDH2 are decreased in HF

Protein level of mALDH2 was significantly reduced in HF dogs compared with NL dogs (0.49 ± 0.02 vs 1.16 ± 0.1 du, $p < 0.05$) without any changes in the porin levels (0.24 ± 0.01 vs 0.26 ± 0.02 du) (Fig. 1A and B). Upon normalization of mALDH2 to porin, a significant reduction of approximately 43 % was seen in HF dogs compared with NL dogs (2.05 ± 0.12 vs 4.72 ± 0.59, $p < 0.05$) (Fig. 1B).

A statistically significant ~2-fold decrease of mALDH2 activity was observed in HF dogs compared with NL dogs (51.01 ± 3 vs 108.21 ± 4 nmol NADH/min/mg, $p < 0.05$) (Fig. 1B).

3.2. p-mPKCε does not regulate mALDH2 activity

Phosphorylated mALDH2 and t-mALDH2 levels were reduced in HF dogs compared to NL dogs. However, when p-mALDH2 was normalized to t-mALDH2, the reduction was eliminated (0.40 ± 0.06 vs 0.39 ± 0.06) (Fig. 2). Since PKCε upon phosphorylation is translocated to mitochondria and phosphorylates mALDH2 [21,22], we examined p-mPKCε and t-mPKCε levels in phosphorylated MITO and in extracts of MITO fractions. Even though protein level of PKCε was reduced in both MITO fractions, normalization of p-mPKCε to t-mPKCε showed no significant changes between HF and NL dogs (0.77 ± 0.14 vs 0.82 ± 0.15) (Fig. 2).

3.3. 4HNE-MITO and 4HNE-mALDH2 adducts levels are increased in HF

Protein adducts of 4HNE-MITO were significantly increased in HF dogs compared to NL dogs (0.82 ± 0.04 vs 0.36 ± 0.01 du, $p < 0{\cdot}005$; Fig. 3A). Protein adducts of 4HNE-ALDH2 when normalized to t-mALDH2 were also significantly elevated in HF dogs compared to NL dogs (1.48 ± 0.18 vs 0.38 ± 0.08, $p < 0.05$; Fig. 3B).

3.4. Exposure to 4HNE decreases mALDH2 activity and increases adduct formation

Exposure of isolated mitochondria from dogs with HF to 4HNE at concentration of 3 μM had no effect on mALDH2 activity or on 4HNE-mALDH2 adducts. In contrast, exposure

to 4HNE at concentrations of 30 μM resulted in a significant decrease of mALDH2 activity and an increase in 4HNE-mALDH2 adduct formation (Fig. 4). Exposure of isolated mitochondria to 3 μM 4HNE had no effect on ROS formation. In contrast, exposure to 4HNE at concentration of 30 μM significantly increased ROS formation (Fig. 4).

3.5. Exposure to 4HNE worsens MITO function

Exposure of isolated mitochondria from HF dogs to 3 μM 4HNE had no significant effects on any of the MITO energetic measures. In contrast, exposure of isolated mitochondria to 30 μM 4HNE resulted in significant reduction of ADP-stimulated respiration, significant reduction of maximal respiratory capacity and significant reduction of ATP-linked respiration (Fig. 5).

3.6. Activation of mALDH2 with ALDA-1 improves MITO function in isolated cardiomyocytes

Treatment of isolated CM from NL dogs with ALDA-1 had no effect on MITO function (Fig. 6). In contrast, treatment of isolated CM from HF dogs with ALDA-1 significantly improved MITO ADP-stimulated respiration and MITO maximal rate ATP synthesis (Fig. 6).

4. Discussion

Results of the present study indicate that mALDH2 activity and protein levels are decreased and ROS levels are increased in mitochondria of the failing LV. These changes were independent of mLADH2 phosphorylation by mPKCε. The reduced mALDH2 activity resulted in increased levels of 4HNE, an indication of increased MITO oxidative stress. The accumulation of 4HNE-MITO adducts and 4HNE-mALDH2 adducts likely compromised MITO function. It has previously been shown that MITO proteins have reactive cysteine residues that tend to bind to 4HNE resulting in the formation of 4HNE-MITO proteins adducts in inner MITO membrane electron transport chain complexes leading to MITO dysfunction [16,21–23]. In the present study, treatment of isolated cardiomyocytes from dogs with HF with the ALDH2 activator ALDA-1, improved MITO function as evidenced by increased ADP-stimulated respiration and maximal rate of ATP synthesis. This observation is new and lends important support for the idea that ALDH2 dysfunction is a key pathophysiologic pathway in HF and could be a target of intervention.

Mitochondrial ALDH2 has emerged as a key cardioprotective enzyme due to its efficiency in eliminating toxic aldehydes by catalyzing their oxidation to non-reactive acids [2,4,5]. Experimental approaches using either pharmacological activation or genetic overexpression of mALDH2 have shown that improved detoxification of reactive aldehydes, such as 4HNE, is protective against acute ischemia-reperfusion injury [21], nitroglycerine tolerance [24] and alcoholic cardiomyopathy [25]. More recently, mALDH2 has been associated with remote preconditioning in humans [26] and metabolic remodeling-related cardioprotection in patients with congenital heart disease [27]. To date, however, the role of mALDH2 in HF has not been fully examined. Studies in transgenic mice have shown that knockout of

ALDH2 results in worsening of LV systolic function in doxorubicin-induced HF that was partially reversed by ALDH2 viral transfection [25].

Elevated levels of 4HNE are known to trigger subcellular organelle damage in the myocardium in a variety of cardiovascular diseases [28]. Increased levels of 4HNE have been reported in humans with post-myocardial infarction cardiomyopathy and in rodents with streptozotocin-induced type 1 and type 2 diabetic hearts [29,30]. Decreased MITO complex II respiration was also reported in diabetic hearts in the presence of elevated 4HNE levels [31]. In LV myocardium of dogs with HF, we previously showed that MITO dysfunction was associated with a significant elevation of 4HNE levels compared to NL dogs [15]. Increased levels of 4HNE in that study was also associated with increased peroxidation of cardiolipin, a key phospholipid of the inner MITO membrane, the residence site of the electron transport chain [15]. In the present study, we showed that increased 4HNE in mitochondria of failing LV myocardium forms adducts with many MITO proteins and subsequently identified one of these as mALDH2. Increased 4HNE-mALDH2 adducts in mitochondria of failing heart is likely to mediate further worsening of mALDH2 activity. In the present study, exposure of HF mitochondria to 4HNE resulted in further reduction of mLADH2 activity and further worsening of MITO function. Therefore, the observed reduction of mALDH2 activity could also be due, in part, to increased 4HNE levels through negative feedback as shown the schematic diagram in Fig. 7.

Previous studies have shown that ROS formation is markedly increased in LV myocardium of dogs with HF [32,33]. In the present study, we showed increased ROS levels in isolated mitochondria from LV of HF dogs after exposure to 4HNE. Increased ROS levels could, in part, be responsible for reduced mALDH2 activity and increased 4HNE levels (Fig. 7). Accumulation of toxic aldehydes derived from catabolism of monoamines have also been shown to alter MITO membrane potential [33].

Activity of mALDH2 can also be regulated by mALDH2 phosphorylation via PKCε [10]. Hence reduced mALDH2 activity observed in this study could also be due to its reduced phosphorylation. Phosphorylation of mALDH2 at serine-279 by PKCε was shown to be critical to activation of mALDH2 [10]. In addition to phosphorylating mALDH2, PKCε has also been implicated in contributing to cardioprotection [23,34,35], including 1) opening of MITO K_{ATP} channels, 2) preventing the opening of MITO permeability transition pore, 3) promoting an active 26S proteasome and 4) increasing cytochrome *c* activity. In the present study, we examined the phosphorylation of mPKCε and mALDH2 in MITO fractions and showed that t-mPKCε, p-mPKCε, t-mALDH2, and p-mALDH2 protein levels reduced in MITO fraction of HF dogs compared with NL dogs. However, upon normalization of p-mPKCε to t-mPKCε and of p-mALDH2 to t-mALDH2 did not significantly change in failing hearts compared to NL. These results suggest that the observed reduction of mALDH2 activity in the failing heart is independent of its phosphorylation by PKCε.

Results of this study indicate that mALDH2 activity and protein levels are reduced in LV mitochondria from dogs with chronic HF. These maladaptations are independent of mALDH2 phosphorylation by mPKCε. The dysregulation of mALDH2 is associated with increased level of 4HNE potentially leading to increased MITO oxidative stress.

Furthermore, the accumulation of 4HNE likely forms adducts with mALDH2 and other MITO proteins that further compromise overall MITO function. The results from our study suggest that the dysregulation of mALDH2 is associated with increased level of 4HNE even though a direct cause and effect relationship cannot be fully confirmed without further research. The existence of abnormalities of mALDH2 in HF and the potential adverse impact this can have on MITO function and, consequently, on overall LV function, warrants consideration of use of mALDH2 activators as therapeutic options for the treatment of HF.

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Declaration of competing interest

H.N.S. has current research grants from the Stealth BioTherapeutics, Inc., Impulse Dynamics, Inc. and Novartis and is a consultant to Stealth BioTherapeutics and Novartis. DL has done research with Akros, Astra Zeneca, Lilly, Novartis, Pfizer, Illumina, Somalogic, Janssen and has served as a consultant to Abbott Laboratories, Astra Zeneca, Beckman-Coulter, and Otsuka. R.C.G., V.S.-G., KZ, and KJS have no conflicts of interest to disclose.

References

[1]. Savarese G, Lund LH. Global public health burden of heart failure. Card Fail Rev 2017;3:7–11. [PubMed: 28785469]

[2]. Chen CH, Budas GR, Churchill EN, Disatnik MH, Hurley TD, Mochly-Rosen D. Activation of aldehyde dehydrogenase-2 reduces ischemic damage to the heart. Science 2008;321:1493–5. [PubMed: 18787169]

[3]. Marchitti SA, Brocker C, Stagos D, Vasiliou V. Non-P450 aldehyde oxidizing enzymes: the aldehyde dehydrogenase superfamily. Expert Opin Drug Metab Toxicol 2008;4:697–720. [PubMed: 18611112]

[4]. Churchill EN, Disatnik MH, Mochly-Rosen D. Time-dependent and ethanol-induced cardiac protection from ischemia mediated by mitochondrial translocation of εPKC and activation of aldehyde dehydrogenase 2. J Mol Cell Cardiol 2009;46:278–84. [PubMed: 18983847]

[5]. Doser TA, Turdi S, Thomas DP, Epstein PN, Li SY, Ren J. Transgenic overexpression of aldehyde dehydrogenase-2 rescues chronic alcohol intake-induced myocardial hypertrophy and contractile dysfunction. Circulation 2009;119:1941–9. [PubMed: 19332462]

[6]. Castro JP, Jung T, Grune T, Siems W. 4-Hydroxynonenal (HNE) modified proteins in metabolic diseases. Free Radic Biol Med 2017;111:309–15. [PubMed: 27815191]

[7]. Perlman DH, Bauer SM, Ashrafian H, Bryan NS, Garcia-Saura MF, Lim CC, et al. Mechanistic insights into nitrite-induced cardioprotection using an integrated metabolomic/proteomic approach. Circ Res 2009;104:796–804. [PubMed: 19229060]

[8]. Chen CH, Sun L, Mochly-Rosen D. Mitochondrial aldehyde dehydrogenase and cardiac diseases. Cardiovasc Res 2010;88:51–7. [PubMed: 20558439]

[9]. Doorn JA, Hurley TD, Petersen DR. Inhibition of human mitochondrial aldehyde dehydrogenase by 4-hydroxynon-2-enal and 4-oxonon-2-enal. Chem Res Toxicol 2006;19:102–10. [PubMed: 16411662]

[10]. Nene A, Chen CH, Disatnik MH, Cruz L, Mochly-Rosen D. Aldehyde dehydrogenase 2 activation and coevolution of its εPKC-mediated phosphorylation sites. J Biomed Sci 2017;24:3. [PubMed: 28056995]

[11]. Chen Z, Stamler JS. Bioactivation of nitroglycerin by the mitochondrial aldehyde dehydrogenase. Trends Cardiovasc Med 2006;16:259–65. [PubMed: 17055381]

[12]. Sabbah HN, Stein PD, Kono T, Gheorghiade M, Levine TB, Jafri S, et al. A canine model of chronic heart failure produced by multiple sequential coronary microembolizations. Am J Physiol 1991;260:H1379–84. [PubMed: 1826414]

[13]. Cao Y, Vergnes L, Wang YC, Pan C, Krishnan KC, Moore TM, et al. Sex differences in heart mitochondria regulate diastolic dysfunction. Nat Commun 2022;13:3850. [PubMed: 35787630]

[14]. Rosca MG, Vazquez EJ, Kerner J, Parland W, Chandler MP, Stanley W, et al. Cardiac mitochondria in heart failure: decrease in respirasomes and oxidative phosphorylation. Cardiovasc Res 2008;80:30–9. [PubMed: 18710878]

[15]. Sabbah HN, Gupta RC, Kohli S, Wang M, Hachem S, Zhang K. Chronic therapy with elamipretide (MTP-131), a novel mitochondria-targeting peptide, improves left ventricular and mitochondrial function in dogs with advanced heart failure. Circ Heart Fail 2016;9:e002206. [PubMed: 26839394]

[16]. Sheeran Freya L, Pepe Salvatore. Post-translational modifications and dysfunction of mitochondrial enzymes in human heart failure. Am J Physiol Endocrinol Metab 2016;311:E449–60. [PubMed: 27406740]

[17]. Galam L, Failla A, Soundararajan R, Lockey RF, Kolliputi N. 4-Hydroxynonenal regulates mitochondrial function in human small airway epithelial cells. Oncotarget 2015;6:41508–21. [PubMed: 26484418]

[18]. Sun A, Cheng Y, Zhang Y, Zhang Q, Wang S, Tian S, et al. Aldehyde dehydrogenase 2 ameliorates doxorubicin-induced myocardial dysfunction through detoxification of 4-HNE and suppression of autophagy. J Mol Cell Cardiol 2014;71:92–104. [PubMed: 24434637]

[19]. Sharov VG, Todor AV, Imai M, Sabbah HN. Inhibition of mitochondrial permeability transition pores by cyclosporine a improves cytochrome c oxidase function and increases rate of ATP synthesis in failing cardiomyocytes. Heart Fail Rev 2005;10:305–10. [PubMed: 16583179]

[20]. Rastogi S, Mishra S, Zacà V, Alesh I, Gupta RC, Goldstein S, et al. Effect of long-term monotherapy with the aldosterone receptor blocker eplerenone on cytoskeletal proteins and matrix metalloproteinases in dogs with heart failure. Cardiovasc Drugs Ther 2007;21:415–22. [PubMed: 17940859]

[21]. Gomes KMS, Bechara LRG, Lima VM, Ribeiro MAC, Campos JC, Dourado PM, et al. Aldehydic load and aldehyde dehydrogenase 2 profile during the progression of post-myocardial infarction cardiomyopathy: benefits of Alda-1. Int J Cardiol 2015; 179:129–38. [PubMed: 25464432]

[22]. Guo D, Nguyen T, Ogbi M, Tawfik H, Ma G, Yu Q, et al. Protein kinase C-epsilon coimmunoprecipitates with cytochrome oxidase subunit IV and is associated with improved cytochrome-c oxidase activity and cardioprotection. Am J Physiol Heart Circ Physiol 2007;293:H2219–30. [PubMed: 17660387]

[23]. Kaludercic N, Carpi A, Nagayama T, Sivakumaran V, Zhu G, Lai EW, et al. Monoamine oxidase B prompts mitochondrial and cardiac dysfunction in pressure overloaded hearts. Antioxid Redox Signal 2014;20:267–80. [PubMed: 23581564]

[24]. Ferreira JC, Mochly-Rosen D. Nitroglycerin use in myocardial infarction patients. Circ J 2012;76:15–21. [PubMed: 22040938]

[25]. Ma H, Yu L, Byra EA, Hu N, Kitagawa K, Nakayama KI, et al. Aldehyde dehydrogenase 2 knockout accentuates ethanol-induced cardiac depression: role of protein phosphatases. J Mol Cell Cardiol 2010;49:322–9. [PubMed: 20362583]

[26]. Contractor H, Støttrup NB, Cunnington C, Manlhiot C, Diesch J, Ormerod JOM, et al. Aldehyde dehydrogenase-2 inhibition blocks remote preconditioning in experimental and human models. Basic Res Cardiol 2013;108:343. [PubMed: 23525499]

[27]. Zhang H, Gong DX, Zhang YJ, Li SJ, Hu S. Effect of mitochondrial aldehyde dehydrogenase-2 genotype on cardioprotection in patients with congenital heart disease. Eur Heart J 2012;33:1606–14. [PubMed: 22507973]

[28]. Mali VR, Palaniyandi SS. Regulation and therapeutic strategies of 4-hydroxy-2-nonenal metabolism in heart disease. Free Radic Res 2014;48:251–63. [PubMed: 24237196]

[29]. Deshpande M, Mali VR, Pan G, Xu J, Yang XP, Thandavarayan RA, et al. Increased 4-hydroxy-2-nonenal-induced proteasome dysfunction is correlated with cardiac damage in streptozotocin-injected rats with isoproterenol infusion. Cell Biochem Funct 2016;34:334–42. [PubMed: 27273517]

[30]. Mali VR, Ning R, Chen J, Yang XP, Xu J, Palaniyandi SS. Impairment of aldehyde dehydrogenase-2 by 4-hydroxy-2-nonenal adduct formation and cardiomyocyte hypertrophy in mice fed a high-fat diet and injected with low-dose streptozotocin. Exp Biol Med (Maywood) 2014;239:610–8. [PubMed: 24651616]

[31]. Lashin OM, Szweda PA, Szweda LI, Romani AM. Decreased complex II respiration and HNE-modified SDH subunit in diabetic heart. Free Radic Biol Med 2006;40: 886–96. [PubMed: 16520240]

[32]. Sabbah HN. Targeting mitochondrial dysfunction in the treatment of heart failure. Expert Rev Cardiovasc Ther 2016;14:305–13.

[33]. Roede JR, Jones DP. Reactive species and mitochondrial dysfunction: mechanistic significance of 4-hydroxynonenal. Environ Mol Mutagen 2010;51:380–90. [PubMed: 20544880]

[34]. Baines CP, Song CX, Zheng YT, Wang GW, Zhang J, Wang OL, et al. Protein kinase C epsilon interacts with and inhibits the permeability transition pore in cardiac mitochondria. Circ Res 2003;92:873–80. [PubMed: 12663490]

[35]. Singh RM, Cummings E, Pantos C, Singh J. Protein kinase C and cardiac dysfunction: a review. Heart Fail Rev 2017;22:843–59. [PubMed: 28702857]

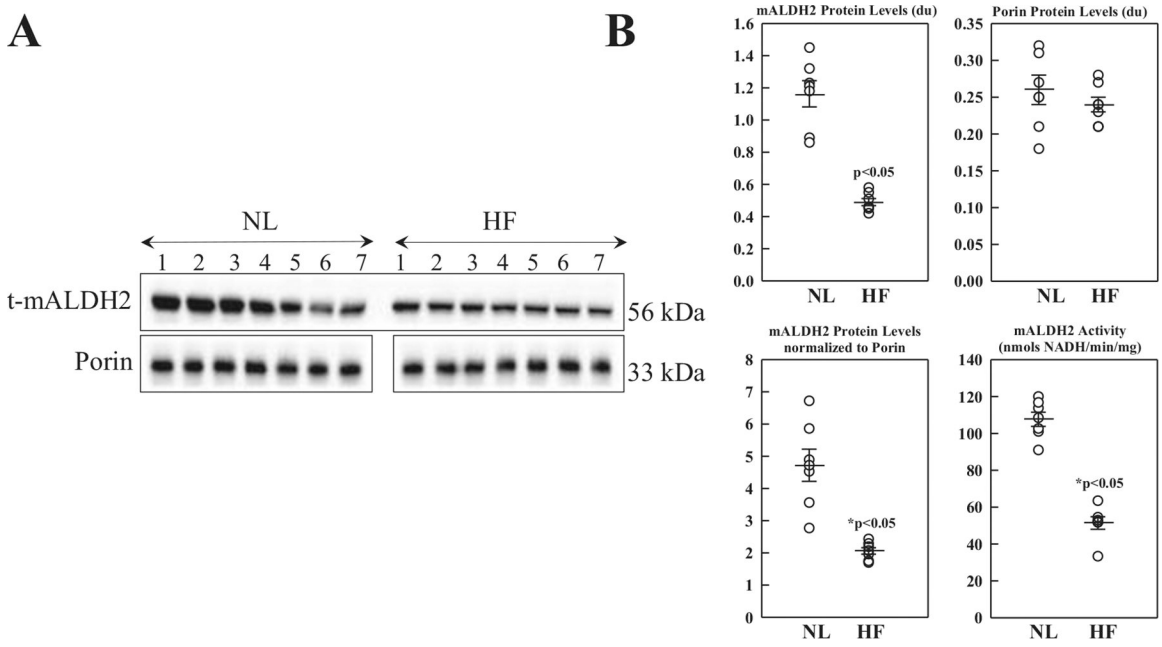

Fig. 1.

A: Western blots of total mitochondrial aldehyde dehydrogenase 2 (t-mALDH2) protein bands (56 kDa, top)) and porin protein bands (33 kDa, bottom) in mitochondria isolated from frozen left ventricular myocardium of normal (NL) dogs ($n = 7$) and dogs with chronic heart failure (HF, n = 7)). B: Vertical point plots with mean ± SEM depicting mitochondrial aldehyde dehydrogenase 2 (mALDH2) protein levels (top left), porin protein levels (top right), mALDH2 protein levels normalized to porin (bottom left) and activity of mALDH2 in mitochondria isolated from frozen left ventricular myocardium of normal (NL) dogs (n = 7) and dogs with chronic heart failure (HF) (n = 7). Data are presented as mean ± SEM with *$p \leq 0.05$ vs. indicated group. p-values are based on a t-statistic for two means.

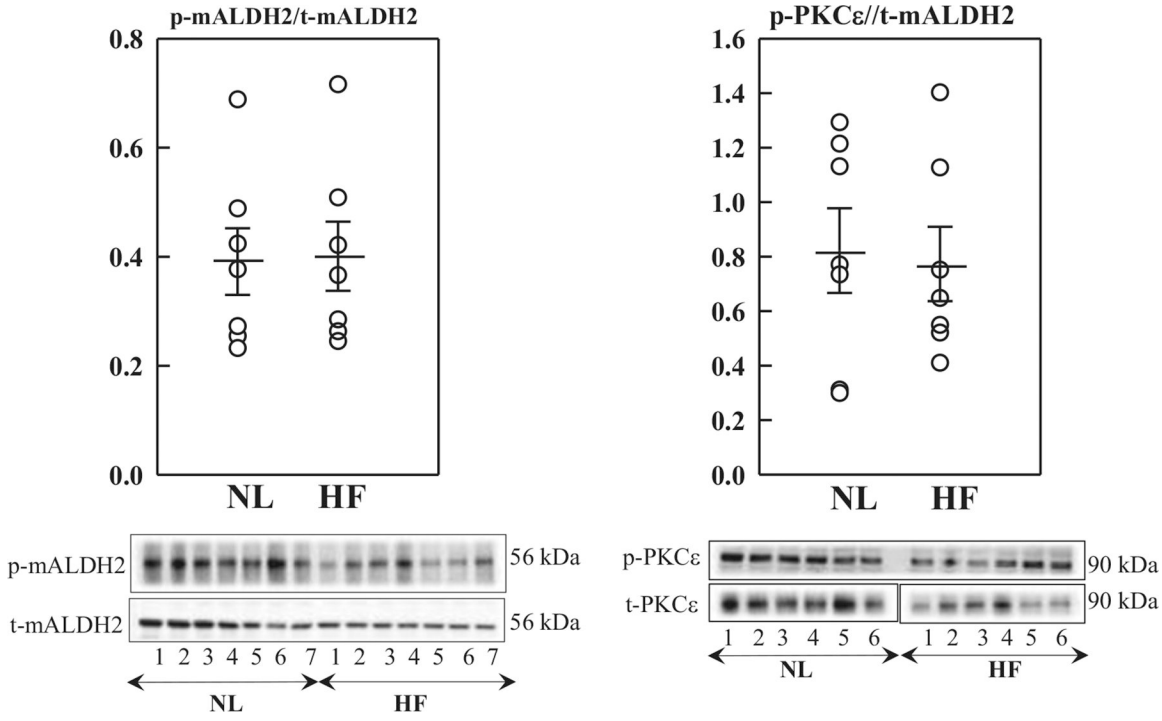

Fig. 2.

Left: Vertical point plots with mean ± SEM depicting the ratio of phosphorylated mitochondrial aldehyde dehydrogenase 2 (p-mALDH2) to total mitochondrial aldehyde 2 (t-mALDH2) in isolated mitochondria from frozen left ventricular myocardium of normal dogs (NL, n = 7) and dogs with chronic heart failure (HF, n = 7). Below are Western blots of p-mALDH2 (56 kDa) and total mitochondrial aldehyde dehydrogenase 2 (t-mALDH2) (33 kDa) from isolated phosphorylated mitochondria of left ventricular myocardium of 7 NL dogs and 7 HF dogs. Right: Vertical point plots with mean ± SEM depicting the ratio of phosphorylated mitochondrial protein kinase C-epsilon (p-mPKCε) to total mitochondrial protein kinase C-epsilon (t-mPKCε) in isolated mitochondria from frozen left ventricular myocardium of normal dogs (NL, $n = 6$) and dogs with chronic heart failure (HF, n = 6). Below are Western blots of p-mPKCε and t-mPKCε from isolated mitochondria of left ventricular myocardium of 6 NL dogs and 6 HF dogs. *P*-values are based on a t-statistic for two means.

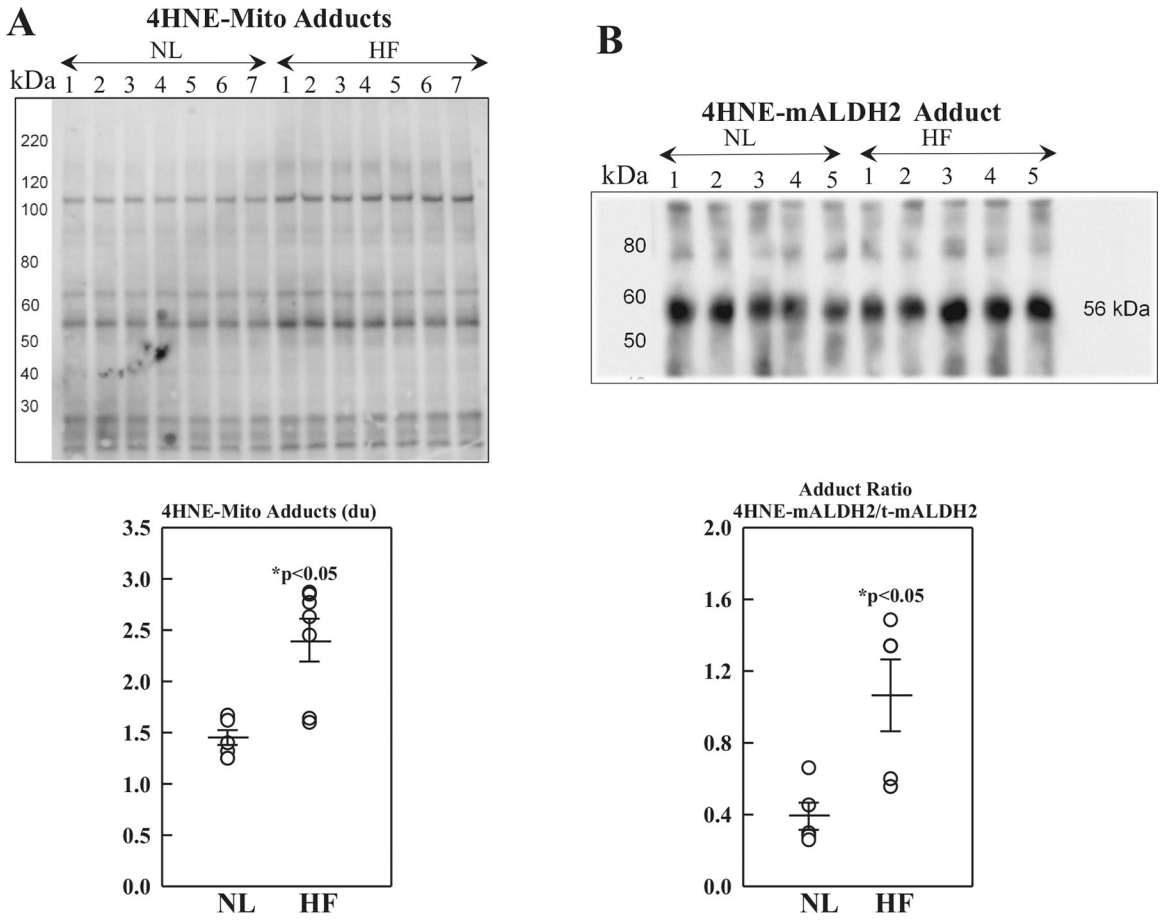

Fig. 3.

A. Left (top): Western blot of 4-hydroxy-2-nonenal (4HNE) adducts to mitochondria (4HNE-MITO) from the frozen left ventricular myocardium of 7 normal (NL) dogs and 7 heart failure (HF) dogs. Left (bottom): Bar graphs (mean ± SEM) depicting differences of 4HNE-MITO in densitometric units (du) of 7 NL dogs and 7 HF dogs. $N = 7$.

B. Right (top): Western blot of 4-hydroxy-2-nonenal (4HNE) adducts to mitochondrial aldehyde dehydrogenase 2 (4HNE-mALDH2) from the frozen left ventricular myocardium of 5 normal (NL) dogs and 5 heart failure (HF) dogs. Right (bottom): Vertical point plots with mean ± SEM depicting differences of the ratio of 4HNE-mALDH2 to t-mALDH2 in 5 NL dogs and 5 HF dogs. *P*-values are based on a t-statistic for two means.

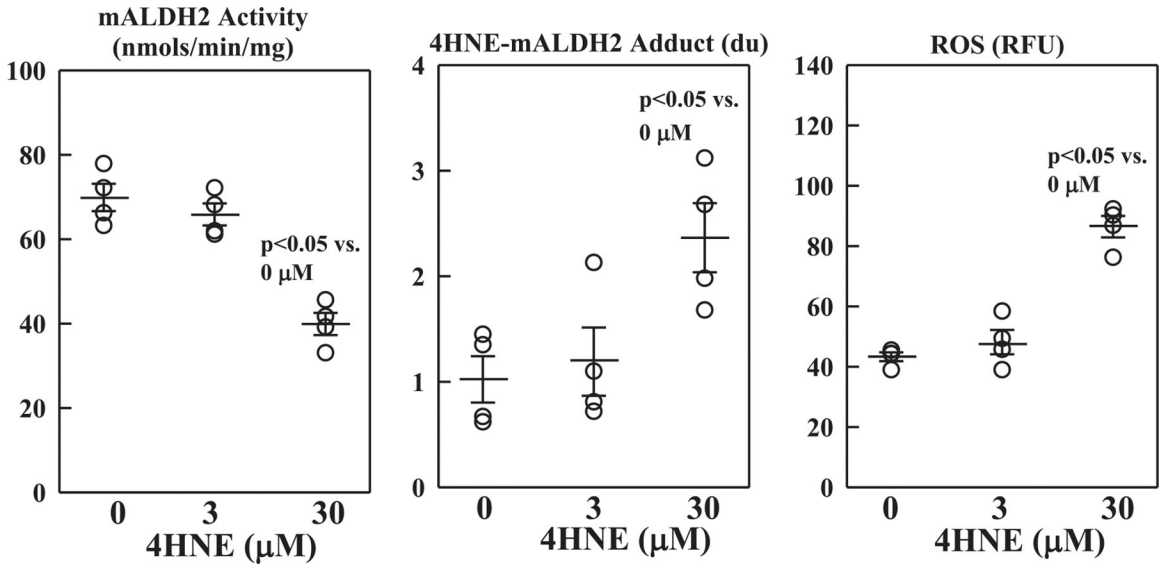

Fig. 4.

Vertical point plots with mean ± SEM depicting mitochondrial aldehyde dehydrogenase 2 (p-mALDH2) activity (left panel), 4-hydroxy-2-nonenal (4HNE) adducts to mitochondrial aldehyde dehydrogenase 2 (4HNE-mALDH2) (middle panel) and reactive oxygen species (right panel) after exposure of isolated mitochondria from fresh left ventricular myocardium of 4 dogs with heart failure to 4HNE at concentration of 0, 3, and 30 μM. P-values are based on Student-Neuman-Keuls test.

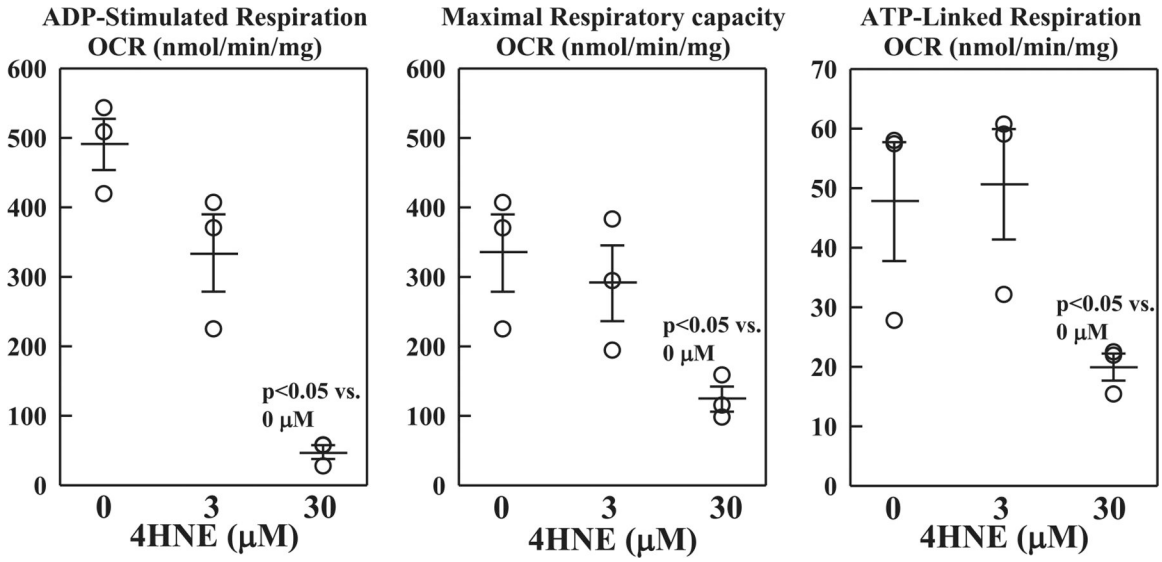

Fig. 5.

Vertical point plots with mean ± SEM depicting ADP-stimulated respiration (left panel), maximal respiratory capacity (middle panel) and ATP-linked respiration (right panel) after exposure of isolated mitochondria from fresh left ventricular myocardium of 3 dogs with heart failure to 4HNE at concentration of 0, 3, and 30 μM. OCR = oxygen consumption rate; ADP = adenosine diphosphate; ATP adenosine triphosphate. P-values are based on Student-Neuman-Keuls test.

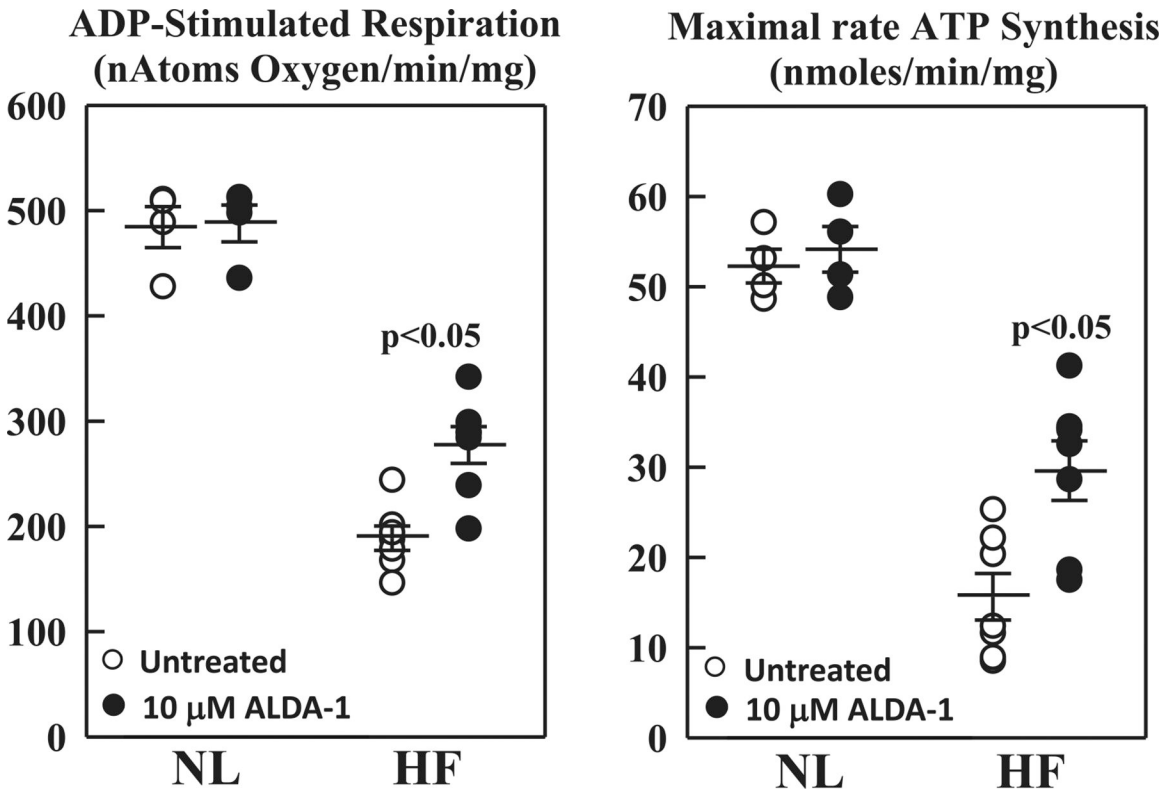

Fig. 6.

Vertical point plots with mean ± SEM depicting mitochondrial ADP-stimulated respiration (left panel) and mitochondrial maximal rate of ATP synthesis (right panel) after 1 h incubation of isolated cardiomyocyte in the absence of (black bars) and in the presence of 10 μM ALDA-1 (gray bars). Experiments were conducted in cardiomyocytes isolated from fresh left ventricular myocardium of normal dogs (NL, $n = 4$) and dogs with heart failure (HF, $n = 7$). ADP = adenosine diphosphate; ATP adenosine triphosphate. P-values are based on a t-statistic for two means.

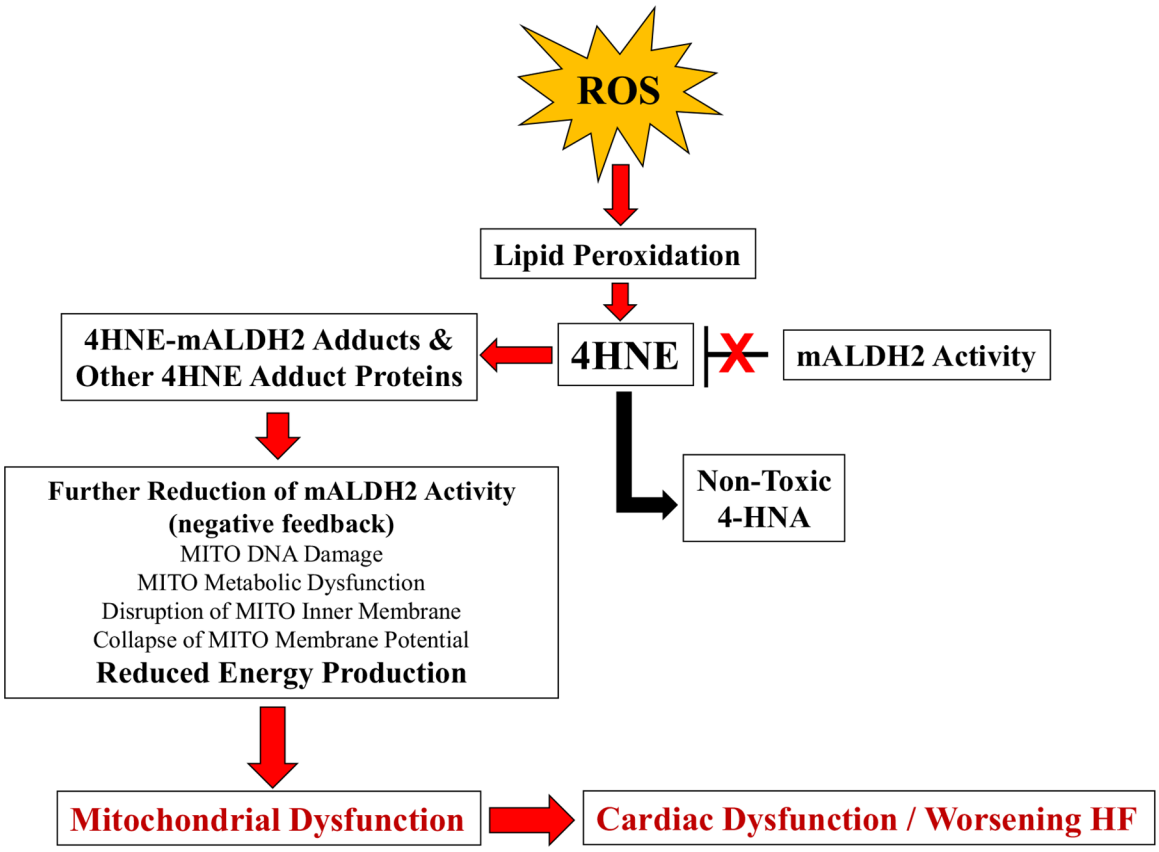

Fig. 7.

Schematic diagram illustrating the signaling pathway from formation of reactive oxygen species (ROS) to mitochondrial dysfunction and worsening heart failure (HF). When mitochondrial aldehyde dehydrogenase 2 (mALDH2) activity is normal, toxic 4-hydroxy-2-nonenal (4HNE) is converted to non-toxic 4-hydroxy-2-nonenoic acid (4-HNA) by mALDH2. When mALDH2 activity is reduced (red “X” and red arrow loop), toxic 4HNE increases, leading to formation of adduct protein, mitochondrial dysfunction and ultimately cardiac dysfunction and worsening heart failure (HF).