Molecular Mechanism of the Association Between Atrial Fibrillation and Heart Failure Includes Energy Metabolic Dysregulation Due to Mitochondrial Dysfunction

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ABSTRACT

Background: Atrial fibrillation (AF) and heart failure (HF) commonly coexist, yet the molecular mechanisms of this association have not been determined. We hypothesized that an energy deficit due to mitochondrial dysfunction plays a significant role in pathogenic link between AF and HF.

Methods and Results: Myocardial energy metabolism and mitochondria were examined in atrial tissue samples from patients and mice (cardiac-specific LKB1 knock-out) with HF and/or AF. There was significant atrial energy (ATP) deficit in patients with HF (11.5±1.3 nmol/mg, n=10; vs without HF 17±3.8 nmol/mg, n=5, P = .032). AF was associated with further energy depletion (ATP 5.4±1.2 nmol/mg, n=9) in HF (P = .001) and metabolic stress (AMP/ATP 1.6±0.1 vs 0.7±0.2 in HF alone; P = .043). The left atrium demonstrated lower ATP than the right (P = .004). Mitochondrial dysfunction and remodeling caused ATP depletion with impaired oxidative phosphorylation complexes (succinate dehydrogenase and cytochrome c oxidase), increased reactive oxygen species, and mtDNA damage in mice and human atria with AF and HF.

Conclusions: Molecular mechanisms of the association between HF and AF include an energy deficit due to mitochondrial dysfunction in atrial myocardium. Mitochondrial functional and structural remodeling in human and mouse atria is associated with energy metabolic dysregulation and oxidative stress that promote AF in HF and vice versa. (*J Cardiac Fail 2019;25:911−920*)

Key Words: Atrial fibrillation, heart failure, mitochondria, energy metabolism.

Atrial fibrillation (AF) and heart failure (HF) commonly coexist that, in combination, causes a major clinical problem with a higher risk of all-cause mortality and morbidity compared with either HF or AF alone.[1−9] HF begets AF and likewise AF begets HF. Presently, there is no effective management strategy for the prevention and treatment of the association between AF and HF. A lack of understanding of the molecular mechanisms of this pathogenic link between AF and HF has been the major obstacle to develop an effective management strategy.[5−11]

Arrhythmogenic atrial electroanatomical remodeling (AAEAR) is critical in development of AF and triggered by multiple clinical or molecular factors including stretch, neurohormonal activation, and oxidative stress.[2,8−13] Recently, we demonstrated progressive AAEAR in a mouse model of AF which developed HF, and also in patients with advanced HF who had high prevalence of AF.[11,12] Our studies in both mouse and human atria showed AAEAR as a shared pathogenic phenomenon in the coexistence of AF and HF.[5,11] Yet the molecular and cellular mechanisms of AAEAR have not been characterized. Myocardial energetics determine cardiac contractility and electrical properties.[14−16] Mitochondrial oxidative phosphorylation generates the vast majority of energy in cardiomyocytes.[14−19] Therefore, mitochondria can be the culprit for altered energetics and consequently AAEAR. Ventricular myocardial energetics is shown to be downregulated in patients with HF.[15−18] However, it is not known whether the changes in atrial energy metabolism can be the molecular and cellular interface between AF and HF disease processes with AAEAR and electrical instability.

In this study, we hypothesized that impaired atrial bioenergetics due to mitochondrial dysfunction associates AF and HF by causing AAEAR. To test this hypothesis, we studied atrial myocardial energy metabolism with nucleotide content, and mitochondrial function and structure in human and mice atrial tissue samples in sinus rhythm (SR) and AF with or without HF. Our study aimed to determine

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whether mitochondrial pathways were disrupted in the initiation and maintenance of AF in HF and vice versa.

Methods

Human Atrial Tissue Evaluation

The study protocol was approved by the Institutional Review Board at the University of Chicago Medical Center (UCMC). Atrial tissue samples were obtained from the UCMC Heart and Vascular Center Myocardial Tissue Biobank where the explanted human heart tissues were stored at the time of cardiac transplantation or surgery. All measurements were performed in human atrial tissue samples from failing and nonfailing heart with and without AF. The samples were harvested from the right and left atrial appendage. We studied a total of 29 atrial tissue samples from patients with advanced HF who underwent explantation of hearts during heart transplantation. Among those patients, 10 atrial tissue samples were from the patients with no known AF; 10 patients had history of paroxysmal AF (PAF) who were in SR at the time of explantation; and 9 patients were in AF at the time of explantation who had diagnosis of persistent AF (persAF). The patients were not on left ventricular assist devices. Control group included 5 atrial tissue samples from the patients with no documented HF who were in SR at the time of left atrial appendage tissue samples were obtained. We used multi-parametric approach to examine tissue samples as reported in next section. Clinical data regarding baseline characteristics of the patients were abstracted from a centralized electronic medical record at the UCMC.[11] Electrocardiograms, cardiac implantable electrical device interrogations and echocardiograms were reviewed to determine arrhythmia, atrial structure and left ventricular ejection fraction (LVEF) and others as reported previously.[11] AF was categorized as PAF and persAF based on the standard definition by the latest practice guideline.[1]

Mouse Model of AF and Mice Atrial Tissue Evaluation

The University of Chicago Animal Care and Use Committee reviewed and approved this research protocol and the experimental procedures. All experiments were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health. Cardiac-specific liver kinase B1 (LKB1) knock-out (KO) mice (*C57BL/6*) were generated by crossing LKB1*flox/flox* mice with transgenic mice expressing Cre-recombinase from the myosin heavy chain promoter.[12,20,21] LKB1 KO mice were born in SR and then developed spontaneous AF which followed with HF in persAF as we and others reported previously.[12,20,21] Wild-type (WT) mice were of the same strain as the corresponding transgenic mice (The Jackson Laboratory). During experimental procedures, isoflurane (0.5−4%) inhalation was used for deep anesthesia and termination.[12,22] Also, effective perioperative analgesia was provided with buprenorphine (0.1 mg/k^2) and carprofen (10 mg/kg^2). Electrocardiogram (ECG; PowerLab, ADI) and implanted telemetry system (TA11ETA-F10 transmitter, DSI) were used for monitoring heart rate (HR), rhythm, and conduction intervals as described previously.[12,22] Evaluation of *in vivo* cardiac structure and function including atrial volume quantification were performed by using cardiac magnetic resonance imaging (9.4-tesla scanner, Bruker, Germany) while animals were under light anesthesia with isoflurane.[12] Imaging and tissue harvesting were completed at the 3 months of age in each study and control group. After attaining optimal anesthesia, right and left atrial tissues were rapidly excised following anterior thoracotomy and freeze-clamped in liquid nitrogen.[12,22]

Nucleotide Profile, Mitochondrial Proteins, ROS, and MtDNA in Mice and Human Atrial Myocardium

Adenine nucleotide content including ATP, ADP, and AMP were quantified in atrial myocardial tissue from human and mice by using high performance liquid chromatography (HPLC) as described previously.[22,23] Atrial tissue extracts were prepared with K_2CO_3-MOPS−neutralized $HClO_4$-soluble before using in HPLC. Mitochondrial proteins were evaluated by using immunoblotting including succinate dehydrogenase (SDH), pyruvate dehydrogenase (PDH), cytochrome c oxidase (COX) IV, cytochrome c, voltage-dependent anion channel (VDAC), prohibitin 1, superoxide dismutase (SOD), caspase 3/9 and connexin 43 were studied.[12,22] Specific antibodies (Cell Signaling Technology) were visualized with enhanced chemiluminescence while an antibody against GAPDH was used as loading controls. Internal control for protein loading was performed by using cardiomyocyte-specific alpha cardiac actin monoclonal antibody (ACTC1) compared with GAPDH to confirm cardiomyocyte population in human and mouse atrial tissue samples with and without AF and HF (Supplementary Fig. 1). Bands were quantified with the Bio-Rad Chemi-Doc gel imager and Image Lab software. The results are presented as a ratio to total protein. In addition to the reactive oxygen species (ROS) scavenger, SOD, hydrogen peroxide (H_2O_2) in atrial tissue extracts was measured by using 2,7-dichlorofluorescein (DCF) diacetate fluorescence (Sigma-Aldrich) with a spectrofluorometer at 480 nm excitation and 530 nm emission as described previously.[12,22,23] Mitochondrial DNA (mtDNA) was studied by using mtDNA Extractor CT Kit (Wako, Richmond, VA), quantitative expand high fidelity PCR system (Roche) and NanoVue plus spectrophotometer (GE Healthcare).[24,25] A short 316 base pair (bp) mtDNA (5′-CGACAGCTAAGACCCAAACTGGG-3′ and 5′-CCCATTTCTTCCCATTTCATTGGC-3′ primers) and a long 8636-bp mtDNA (5′-TACTAGTCCGCGAGCCTTCAAAGC-3′ and 5′-GGGTGATCTTTGTTTGCGGGT-3′) fragment were coamplified with quantitative PCR.[24,25] PCR products (mtDNA fragment band intensity) were visualized and quantified by agarose gel electrophoresis using Bio-Rad Molecular Imager (ChemiDoc XRS).

Mitochondrial Ultrastructure in Atrial Cardiomyocyte

Mitochondria were visualized by using transmission electron microscopy after fixation of atrial tissue in EM-grade glutaraldehyde solution.[12,23,24] Each sample was stained, embedded and cut on an ultra-cut E ultra-microtome in thin sections. Mitochondria were micrographed with a JOEL FX1200 electron microscope. We examined mitochondria in 4 different mice atria in each group. Over 1000 mitochondria were quantified in each sample within 12−15 different cells. Mitochondrial density and ultrastructural analysis was performed on cardiomyocytes specifically. Intact mitochondria were defined as those with uninterrupted outer and inner membranes, thin intermembrane space, and regular cristae enfolding into a compact matrix. Swollen mitochondria were defined as distended mitochondria with increased intermembrane space and swollen cristae. Mitochondrial volume and density were quantified by the National Institutes of Health, ImageJ software. Mitochondria with damaged outer membranes were not included in measurement of volume or density.

Statistical Analysis

Comparisons between groups were performed by using parametric (Student *t* test; ANOVA) for paired samples and nonparametric (Mann−Whitney−Wilcoxon) tests if there was heterogeneity or non-normal data based upon the Shapiro−Wilk test. For ANOVA, Bonferroni correction test was conducted for post hoc analysis if there was a significant result among the group comparison. ANOVA was used in comparing all groups for any difference. *T* tests were used for pairwise comparisons. Data were analyzed by using a Microsoft Excel worksheet and Statistical Package for the Social Sciences software. A value of $P < .05$ was considered statistically significant for two-tailed tests. Data are presented as mean ± SD, and *n* represents the number of animals used for the experiments.

Results

Human Atria in HF and AF

We studied 29 atrial tissue samples from explanted hearts from the patients with advanced HF who underwent heart transplantation. Among those patients, 10 had no documented AF (median age 48 years, 80% male and 70% nonischemic cardiomyopathy); 10 had history of paroxysmal AF but were in SR at the time of explant (median age 55 years, 80% male and 70% nonischemic cardiomyopathy); and 9 had persAF who were in AF at the time of explant (median age 63 years, 77% male and 88% nonischemic cardiomyopathy). Control group included 5 patients with no HF (median age 68 years, 60% male) who were in SR at the time of atrial tissue sampling. As presented in Table 1, LVEF was significantly reduced ($P < .001$) and left atrial volume was increased ($P = .1$) in HF compared with the patients without HF. Left atrial dilation was worse in patients with HF and PersAF.

Table 1. Baseline Characteristics of the Patients With Advanced HF

Characteristic	HF (no AF) n=10	PAF n=10	persAF n=9	no HF n=5
Male/Female, n	8/2	8/2	7/2	3/2
Age, median years	48	55	63	68
LVEF, %	21±7	22±5	23±6	63±5
Left atrial volume, mL/m^2	49±22	46±10	71±15	36±16
Non/ischemic cardiomyopathy, n	7/3	7/3	1/8	NA

Energy Metabolic Dysregulation in Human Atria

Total ATP content was significantly low in atrial myocardium in patients with HF (11.5±1.3 nmol/mg) compared with the patients without HF (17±3.8 nmol/mg; $P = .032$; Fig. 1A). ATP level was similar in patients with HF and history PAF while with in SR (10.5±2.4 nmol/mg vs 11.5±1.3 nmol/mg, $P = .9$; Fig. 1A). However, the presence of AF was associated with severely reduced atrial ATP in HF as documented in patients with HF and persAF (5.4±1.2 nmol/mg) compared with all other study and control groups ($P = .001$). Also, there was significant metabolic stress in atrial myocardium with HF as revealed with increased AMP to ATP ratio (1.1±0.3 in HF alone; 1.2±0.2 in HF with PAF; 1.6±0.2 in HF with persAF; Fig. 1B). Metabolic stress was worse in the coexistence of persAF and HF (1.6±0.2) compared with patients without HF or AF (0.7±0.1; $P = .043$). Moreover, there was a significant difference between left and right atrial energetic in HF (Fig. 1C). Left atria contained lower ATP levels in HF compared with right atria (7.1±1.1 vs 12.9±1.7 nmol/mg protein, $P = .0041$) and HF with paroxysmal AF (7.5±1.4 vs 13.6±2.8 nmol/mg, $P = .002$) and persAF (3.6±0.6 vs 7.7±1.2 nmol/mg, $P = .01$). Thus, HF is associated with energy deficit and metabolic dysregulation with impaired nucleotide profile in atrial myocardium, which is worse in AF and in left atria.

Mitochondrial Electron Transport Chain Protein Complexes

SDH (complex II) and COX (complex IV) are unique mitochondrial electron transport chain complexes, which are essential for ATP production through oxidative phosphorylation. In atria, SDH was significantly reduced in HF with AF (0.15±0.07 AU) and without AF (0.22±0.03 AU) compared with atria with no HF (0.41±0.05 AU; $P = .01$ and $P = .02$, respectively; Fig. 1D). Similarly, COX was significantly diminished in HF (0.43±0.1 AU in HF alone and 0.54±0.4 AU in HF with AF) compared with no HF (0.87±0.08 AU; $P = .03$ and $P = .041$, respectively; Fig. 1E). This was associated with oxidative stress as reflected with increased SOD. To catabolize mitochondrial superoxide, ROS scavenger, SOD, was elevated in HF (0.61±0.09 AU) and HF with AF (1.58±0.3 AU) compared with atria without HF (0.31±0.02 AU; n=6−8; HF vs no HF, $P = .02$; HF and AF vs no HF, $P = .003$; Fig. 1F). This reflects increased

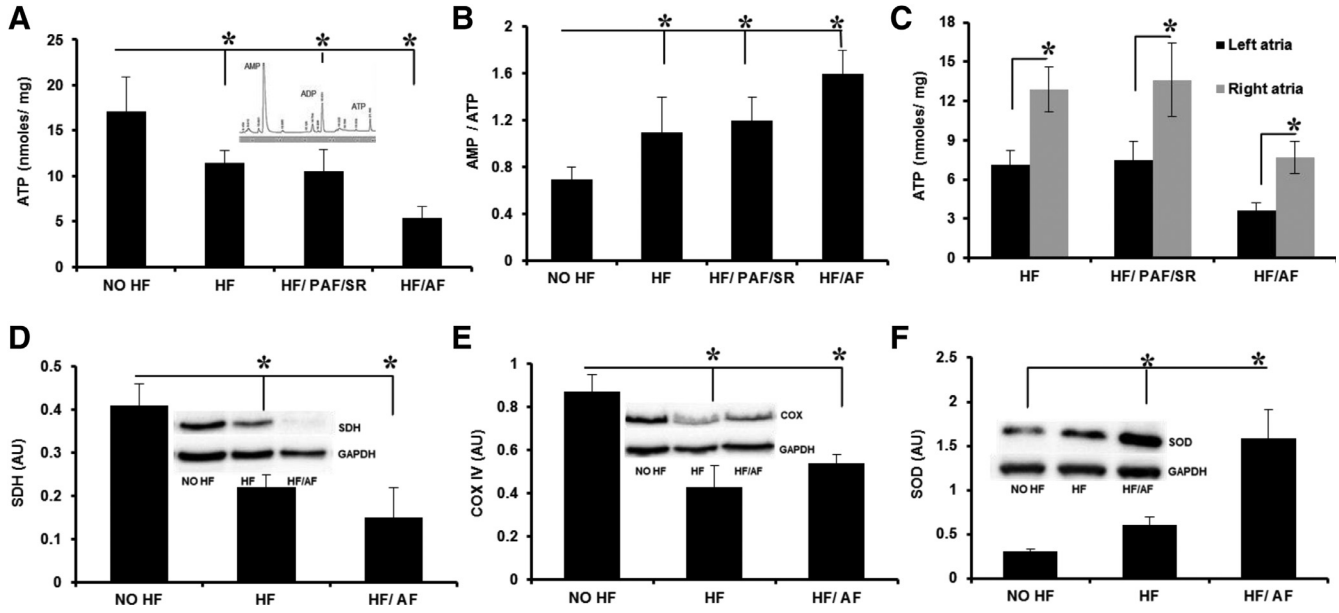

Fig. 1. Atrial energetics and mitochondrial dysfunction in human atrial tissue in patients with HF and AF. (**A**) Atrial myocardial energetic was significantly decreased in patients with HF and AF as shown with low ATP levels. Atrial ATP concentration was lowest in patients with persAF in HF compared with HF in SR. Thus, presence of AF severely reduces atrial ATP levels in HF. Inset shows HPLC measurement of nucleotide profile. (**B**) HF with or without AF was associated with significant metabolic stress in atrial myocardium as reflected with increased AMP−ATP ratio. Metabolic stress was more prominent in patients with coexisting persAF and HF. (**C**) Left atrium ATP content was significantly lower than right atrium in advanced HF with or without AF. However, left atrium has the worst energy deficit in AF. (**D**, **E**) Impaired energetic in atrial myocardium with HF was associated with reduced mitochondrial oxidative phosphorylation protein complexes: SDH (complex II) and COX (complex IV). These two essential mitochondrial electron transport complexes were significantly low in HF with AF. (**F**) Atrial myocardial energy deficit and metabolic stress in AF and HF were associated with oxidative stress as reflected with increased SOD generation as response to significantly higher reactive oxygen species generation. Insets show Western blotting for specific proteins in (**D**−**F**). The immunoblot analysis is corrected with loading control, antibody against GAPDH (*$P < .05$; ANOVA presents comparing all groups for any difference, *t* tests were used pairwise comparisons).

oxidative stress with significantly higher ROS generation in HF with and without AF.

Mice Atria in HF and AF

Similar to our previous publication, LKB1 KO mice developed spontaneous AF within 3−12 weeks of age in association with electrical and structural remodeling of atria (Fig. 2A−C). ECG analysis demonstrated that a mean HR in KO mice in SR (482±35 bpm, n=26) was similar to WT mice HR (503±65 bmp, n=13; $P = .1$; Fig. 2D). However, mean ventricular rate was slower in KO mice in AF (422±58 bpm, n=24) than WT mice ($P = .001$). PR interval (36.6±3 vs 34±6 ms) and P wave duration (13±2 vs 12±3 ms) were comparable in KO and WT mice in SR ($P = .8$). AF was associated with significant atrial structural changes with bi-atrial enlargement compared with WT mice (Fig. 2C,E; n=4). Mean atrial size was 21.7±3 AU in AF compared with 9.5±1 AU in SR in KO heart and 6.8±0.6 AU in WT heart ($P = .002$ and $P = .0007$, respectively; Fig. 2A,B,C,E). LVEF in KO heart in SR (70±4%) was similar to WT mice heart (73±5%; $P = 1$). However, LVEF was significantly reduced in mice with AF (52±7% vs 73±5%, $P = .002$). This was associated with HF phenotype with systemic edema and related weight gain (33.1±5.2 g in mice with AF and HF vs 25.7±3.2 g in WT mice at 12 weeks of age, $P = .001$). Whereas KO mice in SR demonstrated no clinical evidence of HF. Thus, LKB1 KO with AF developed HF with significant AAEAR and depressed LVEF.

Atrial Energy Metabolic Dysregulation in Mice

In addition to human study, we examined nucleotide profile including ATP, ADP, and AMP in mice atria. LKB1 KO mice with AF and HF demonstrated significant atrial energy deficit with severely reduced ATP content (0.7±0.28 nmol/mg protein, n=6) compared with KO mice in SR without HF (1.99±0.6 nmol/mg protein, n=7, $P = .003$) and WT mice with no HF (3.16±0.86 nmol/mg protein, n=4, $P = .002$; Fig. 2G). Similarly, KO heart ventricles in SR showed reduced ATP content (2.27±0.35 nmol/mg protein) compared with WT ventricles (4.12±0.62 to nmol/mg protein; $P = .01$). It was significantly lower in AF and HF (1.38±0.26 nmol/mg protein, n= 5; $P = .006$). Also, KO hearts with HF in AF or in SR had a lower atrial content of ADP (3.1±1.7 and 10.1±1.4 vs 13.1±2.1 nmol/mg protein, $P = .006$ and $P = .044$, respectively; Fig. 2H) and AMP (20.1±7 and 40.51±2 vs 48.7±6.3 nmol/mg protein, $P = .007$ and $P = .046$, respectively) compared with WT hearts. A marker of metabolic dysregulation, the AMP−ATP ratio was higher in KO hearts with AF and HF (31±7; $P = .006$) or in SR (25±6; $P = .02$) compared with

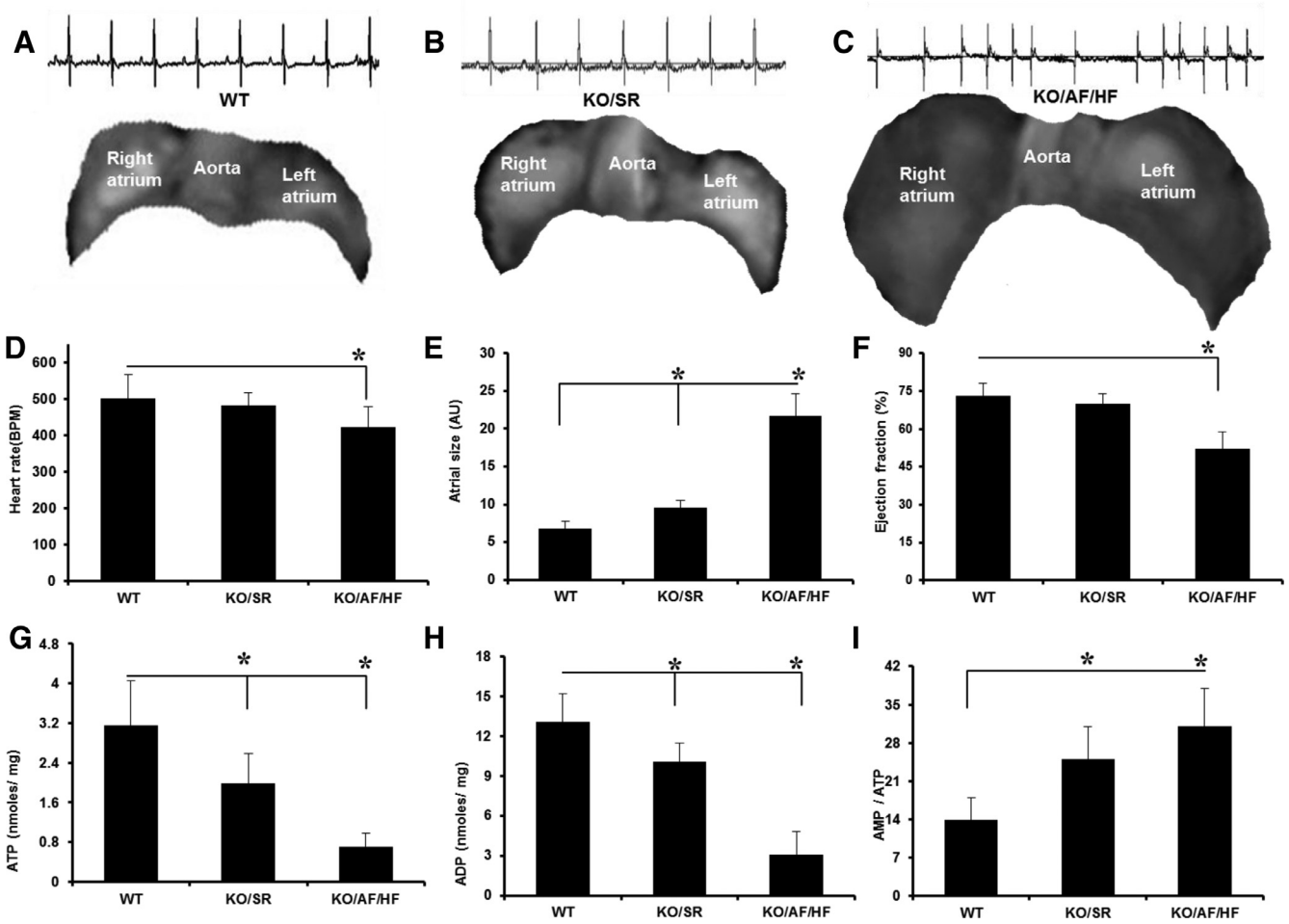

Fig. 2. Atrial energetics and mitochondrial dysfunction in mouse model of AF with HF. (**A**–**C**) As opposed to WT mice, a novel mouse model of AF shows significant electrical and structural remodeling. Electrocardiograms demonstrate SR in WT (**A**) and LKB1 KO mice (**B**). LKB1 KO mice develop spontaneous AF (**C**) and then HF. Cardiac magnetic resonance imaging showed significant right and left atrial enlargement in LKB1 KO mice in AF and HF (bottom). (**D**) ECG analysis demonstrated HR was similar in WT and KO mice in SR, whereas ventricular rate was slower in KO in AF and HF. (**E**) Atrial size significantly increased in AF and HF with progressive right and left atrial enlargement compared with WT mice. (**F**) LVEF was reduced in AF. (**G, H, I**) Disrupted atrial energetics and metabolism was associated with development of atrial remodeling, AF and HF. Atrial myocardial content of nucleotides including ATP (**G**) and ADP (**H**) was significantly lower in LKB1 KO mice in SR and AF compared with WT mice. As shown by HPLC measurement (**G**), ATP was significantly depleted in LKB1 KO atrial myocardium starting in SR and further decreased in AF. Metabolic stress, as reflected in AMP/ATP ratio (**I**), was more significant in LKB1 KO hearts with AF than in WT. Thus, AF is associated with impaired myocardial energetics and metabolism. The immunoblot analysis is corrected with loading control, antibody against GAPDH (*$P < .05$; comparisons between WT vs KO mice; Student *t* test, ANOVA).

WT (14±4) atrial myocardium (Fig. 2I). Thus, atrial myocardial energy metabolic disruption is critical in association of the AF and HF in mice as in human.

Mitochondrial Protein Complexes in Mice

Mitochondrial electron transport chain, matrix, and inner and outer membrane proteins were evaluated by specific antibodies in mice atrial tissue (Fig. 3). In LKB1 KO atria, SDH was significantly decreased in SR (2.77±0.19 AU, n=7) and in AF with HF (0.77±0.07 AU, n=8) compared with WT (3.54±0.16, n=6; $P = .031$ and $P = .015$, respectively; Fig. 3A). There was also significantly lower COX in AF with HF (0.23±0.05 AU, n=8), whereas it was preserved in WT atria (1.95±0.4 AU, n=6; $P = .001$; Fig. 3B). As a link between glycolysis and the citric acid cycle, PDH in mitochondrial matrix was significantly decreased in KO atrium in AF with HF (0.64±0.1 AU, n=8) compared with WT atria (2.7±0.47 AU, n=6; $P = .002$; Fig. 3C). An essential mitochondrial outer membrane protein, VDAC was also disrupted in mice with AF and HF (0.2±0.03 AU, n=7) compared with SR (1.07±0.12 AU, n=6; $P = .012$), whereas it was 1.37± 0.16 AU in WT atria (n=8; $P = .009$ and $P = .043$, respectively; Fig. 3D). Inner membrane proteins, cytochrome c (0.47±0.06 AU in AF and HF vs 2.54±0.15 AU in WT, n=6–8, $P = .002$) and prohibitin 1 (0.19±0.01 in AF with HF vs 0.47±0.03 AU in WT, n=6–8, $P = .034$), were lower in atria in AF and HF compared with WT (Fig. 3E,F).

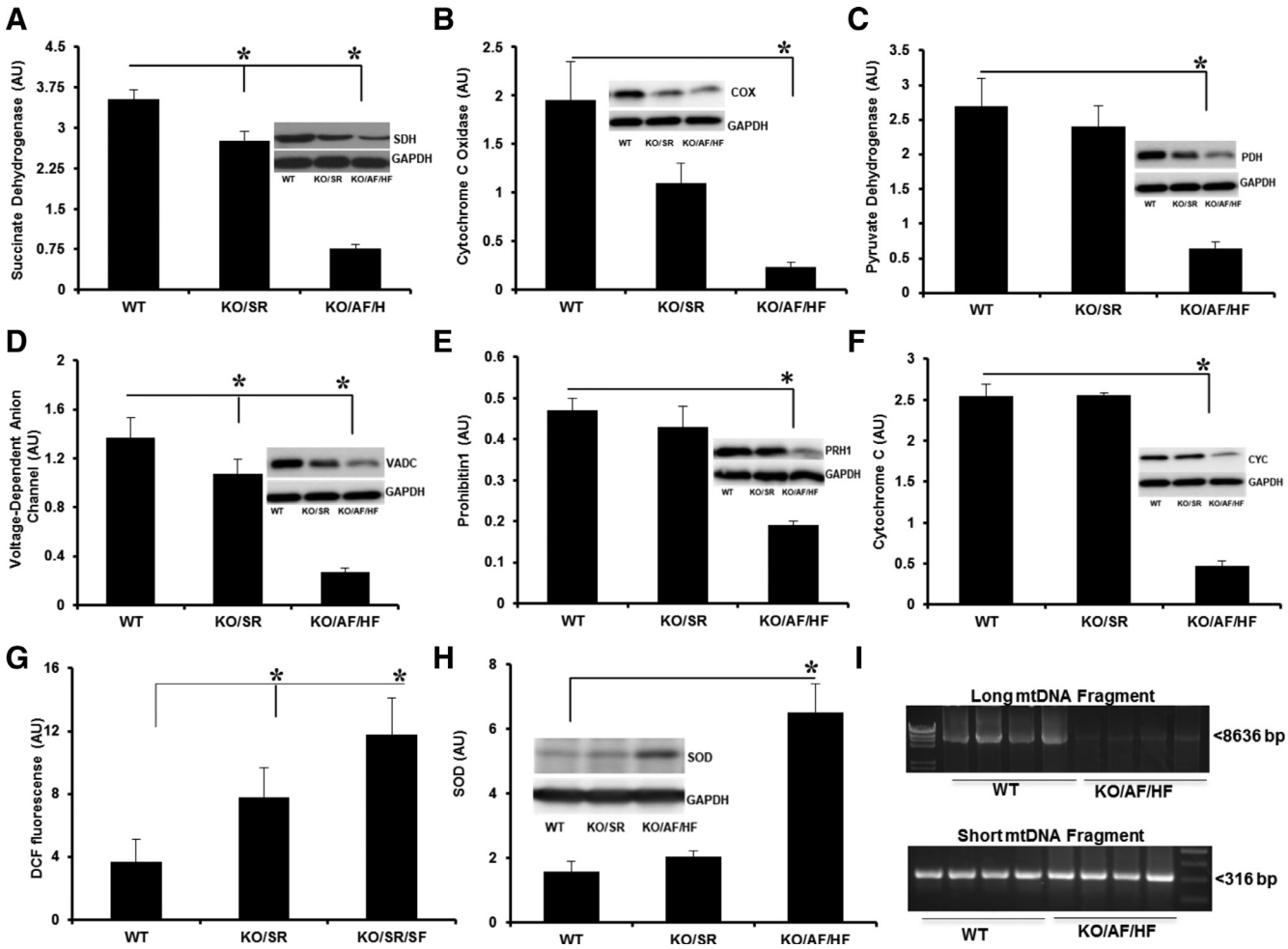

Fig. 3. Mitochondrial Dysfunction in mechanism of AF and HF in mice atria. (**A**−**F**) Mitochondrial electron transport chain, matrix, inner and outer membrane proteins were significantly impaired in LKB1 KO heart atria compared with WT atria, particularly in AF and HF coexistence. In KO atria with AF and HF, succinate dehydrogenase (complex II; **A**), cytochrome c oxidase (complex IV; **B**), pyruvate dehydrogenase (**C**), voltage-gated anion channel (**D**), prohibitin 1 (**E**), and cytochrome c (**F**) levels were significantly lower compared with WT atria and KO atria in SR without HF. Functional and structural impairment started in SR and then worsened in persAF and HF. (**G**−**I**) Mitochondrial dysfunction was associated with oxidative stress and mtDNA damage. There was significantly higher ROS generation in AF and HF compared with WT atrial tissue as shown in elevated level of hydrogen peroxide (H_2O_2) that was measured by using DCF diacetate fluorescence (**G**) and superoxide as shown elevated ROS scavenger, SOD (**H**). mtDNA (long fragment: 8636 bp) was significantly damaged in AF and HF (**I**, top). However, the short fragment of mtDNA was similarly amplified in both groups (**I**, bottom). Thus, impaired mitochondrial complexes and oxidative stress were associated with mtDNA damage in AF and HF. Insets show Western blotting for specific proteins in (**A**−**F**) and (**H**). The immunoblot analysis is corrected with loading control, antibody against GAPDH (*$P < .05$; comparisons between WT vs KO mice; The Student *t* test and the Mann−Whitney−Wilcoxon test for heterogeneous data).

However, cytochrome c (2.56±0.02 AU, $P = .7$) and prohibitin 1 (0.43±0.05 AU, n=6−8, $P = .4$) levels in KO atria in SR were comparable to WT atria.

Oxidative Stress and mtDNA Damage in AF and HF

Atrial myocardium in LKB1 KO mice demonstrates significant oxidative stress with increased ROS in AF and HF (Fig. 3G,H). DCF fluorescence showed high level of ROS (H_2O_2) in KO atria in SR (7.8±1.9 AU, n=7) and AF with HF (11.8±2.3 AU, n=6) compared with WT (3.7±1.4 AU, n=7; $P = .031$ and $P = .017$, respectively; Fig. 3G). Consequently, the ROS scavenger, SOD, was elevated in KO atria (2.04±0.19 AU in SR and 6.51±0.9 AU in AF with HF vs 1.58±0.33 AU in WT, n=6−8, $P = .041$ and $P = .013$, respectively; Fig. 3H). It was associated with significant damage in the long fragment (8636 bp) of mtDNA in AF and HF (4870±247 AU) compared with WT mice atria (9412±751 AU, n=8; $P = .007$; Fig. 3I, top). However, the short (316 bp) fragment of mtDNA was similarly amplified in KO atria with AF and HF (19,835±2030 AU) and WT atria (17,166±1052; n=8; $P = .3$; Fig. 3I, bottom). The long to short fragment ratio was 55±5% less in KO atria than in WT, indicating a high frequency of lesions in mtDNA in AF and HF. Thus, impaired mitochondrial complexes were associated with oxidative stress and mtDNA damage in AF and HF.

Mitochondrial Structural Remodeling in AF and HF

Electron microscopy demonstrated significant mitochondrial ultrastructural remodeling in LKB1 KO atria with AF and HF (Fig. 4A−F). The number of mitochondria in cardiomyocytes was higher in AF and HF (218±12, n=4 mice, n=56 cell) than in WT atria (178±13, n=4 mice, n=52 cells; P =.02; Fig. 4A). Mitochondrial volume was larger in AF and HF (149.6±9.7 AU, n=152) compared with WT in SR (134.5±11.7 AU, n=164; P = .0003; Fig. 4B). Mitochondrial matrix edema and disruption of the inner and outer membranes were evaluated by measuring crista density (Fig. 4C). In KO atria with AF and HF (n=4 mice), crista density was significantly lower (13.3± 2.06 AU, n=84) than WT atria in SR (19.9±1.8 AU, n=112; P = .03; Fig. 4C). Mitochondrial size and structure were within normal limits in WT atria (Fig. 4E,F, bottom) whereas ultrastructure was significantly impaired in AF and HF (Fig. 4D,F, top). This structural remodeling was parallel to the impairment of electron transport complexes and atrial dilation.

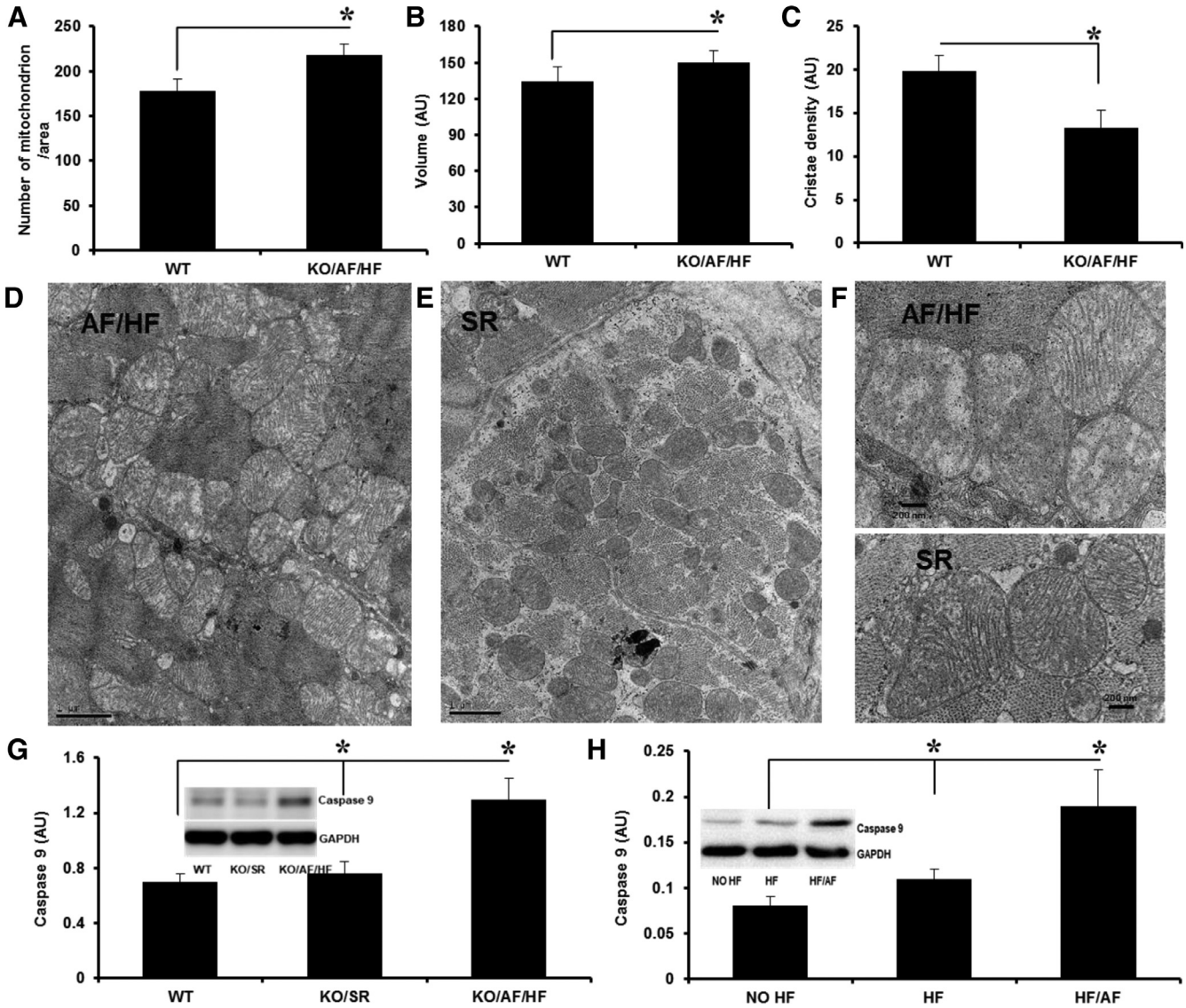

Fig. 4. Mitochondrial ultrastructural remodeling in mechanism of AF and HF. (**A**−**F**) Mitochondrial ultrastructure was significantly disrupted in AF and HF. Electron microscopy showed that atrial cardiomyocyte with AF and HF has higher number of mitochondria than in WT (**A**) and larger mitochondrion volume (**B**). Mitochondrial matrix edema and disruption of the inner and outer membranes were demonstrated by measurement of crista density that was significantly lower in AF and HF indicating mitochondrial damage (**C**). Electron microscopy images showed significantly impaired mitochondrial ultrastructure in cardiomyocytes with AF compared with WT in SR (**D**−**F**). Mitochondrial functional and structural damage were associated with activation of mitochondrial apoptotic cascade (caspase 9) in mice atria (**G**), and in human atria (**H**) with HF and AF. Mitochondrial apoptotic cascade was activated in KO atria in AF compared with WT atria. Insets show Western blotting for specific proteins in (**G**) and (**H**). The immunoblot analysis is corrected with loading control, antibody against GAPDH (*P < .05; comparisons between WT vs KO mice from (**A**) to (**G**); comparisons between patients with no HF vs patients with HF with/without AF, (**H**); Student t test and the Mann−Whitney−Wilcoxon test).

Mitochondrial Apoptotic Cascade in AF and HF

In association with impaired structural and functional integrity of mitochondria, mitochondrial apoptotic cascade (caspase 9) was activated in atrial myocardium with AF and HF in mice and human (Fig. 4G,H). Caspase 9 level was found higher in AF and HF (1.3±0.15 AU, n=13) compared with SR (0.76±0.09 AU, n=7; P = .02) in LKB1 KO atria and WT (0.7±0.06 AU, n=6; P = .018; Fig. 4H). Caspase 9 activated final common apoptotic cascade-protease, caspase 3, in KO atria (3.6±0.6 AU in SR and 9.2±1.3 AU in AF with HF, P = .011) compared with WT atria (1.6±0.5 AU, n=7−13; P = .025 and P = .007, respectively). Similarly, caspase 9 was activated in human atria with HF alone (0.11±0.01 AU, n=7) and HF with AF (0.19±0.04 AU, n=13; P = .028) compared with atria without HF (0.08± 0.01 AU, n=6; P = .039; Fig. 4H). Caspase 9 activated caspase 3 was high in HF with AF (9.2±1.3 AU) and without AF (3.6±0.6 AU in SR, P = .011) as opposed to human atria without HF (1.6±0.5 AU, n=7−13; P = .001).

Discussion

This study investigated the mechanisms by which myocardial energy metabolism provides a link between HF and AF in human and mice atria. We found significant energy metabolic dysregulation in atrial myocardium with decreased ATP content and increased AMP/ATP ratio in coexistence of HF and AF in both species, particularly in left atria. Energy depletion was associated with impaired mitochondrial oxidative phosphorylation complexes and pathological mitochondrial functional and ultrastructural remodeling. Mitochondrial dysfunction was associated with the development of AAEAR with reduced ATP production, metabolic dysregulation, increased ROS generation, and apoptotic cascade activation that contributed to the HF and AF association. Thus, our findings demonstrate that mitochondrial dysfunction is critical in the association between AF and HF and may represent an upstream mechanism of this disease process.

Cardiomyocyte function and survival depend on adequate ATP production and related energy metabolism that is governed by mitochondrial functional and structural integrity.[19,22−24] Our findings showed that impaired energetics in atrial myocardium plays significant role in cellular mechanism of the coexistence of AF and HF. Metabolic stress with ATP depletion was associated with mitochondrial dysfunction due to mitochondrial functional and ultrastructural remodeling. This was interconnected with AAEAR and susceptibility to the coexistence of AF and HF. The presence of mitochondrial dysfunction in LKB1 KO mice atria prior to the development of AF and HF confirmed the role of mitochondrial dysfunction between AF and HF. Mitochondria regulate myocyte energy metabolism, ROS, ionic homeostasis, calcium storage, membrane potentials, and cell survival.[14−18,19−22] In both HF and AF, we demonstrated disrupted mitochondrial electron transport chain complexes (II and IV), mtDNA damage, ATP depletion, profound ROS generation, and activation of mitochondrial apoptotic cascade. This promoted an oxidative and metabolic stress in atrial myocardium that likely initiated AAEAR. Oxidative stress developed with increased ROS generation at the onset of AF and persisted in AF with HF in mice. Metabolic stress occurred with significant energy depletion and disrupted nucleotide profile in human and mouse atria and ventricle. This ATP deficit was associated with impaired SDH and COX in the failing heart, which worsened with the development of AF. This helps understanding of the relationship between AF, HF, and atrial remodeling. In humans, all atrial tissue samples in both the study and control groups were from the right and left atrial appendage. This provided an objective comparison. However, there might be regional molecular variation in the different part of the atrial tissue that is not eliminated in this study. As known, the appendage is typically more trabeculated and serves different functions (reservoir, contractile, natriuretic peptide secretion, and others).

In mice atria, we also demonstrated that other inner and outer membrane proteins including cytochrome C, prohibitin 1, and VDAC, were significantly decreased before the initiation of AF and then during AF with HF. Mitochondrial matrix complex, PDH, which links glycolysis and the citric acid cycle, was also reduced in AF and HF. In parallel, pathologic mitochondrial ultrastructural remodeling developed with increased number and volume of mitochondria in the atrial cardiomyocyte with AF and HF. These were defective mitochondria with matrix edema and interruption of the inner and outer membrane structures and the likely cause of the energy deficit via reduced ATP production. Then again, disrupted energy metabolism can make the cell to churn out more mitochondria or change the shape of the organelle. The observed mitochondrial ultrastructural abnormality was associated with damage in the long fragment of mtDNA, ROS generation, and activation of the mitochondrial apoptotic cascade (caspase 9/3). These are interconnected processes with functional and structural remodeling of mitochondria in AF and HF. It triggered substantial disorganization of intracellular and extracellular structures as well. As we reported, LKB1 KO mice developed progressive atrial cardiomyopathy with bi-atrial enlargement in the presence of cardiomyocyte loss with apoptosis and necrosis, patchy fibrosis, and disrupted of cell-to-cell coupling with reduced gap junction proteins.[12] Now, we demonstrate mitochondrial dysfunction with remodeling is the part of the mechanisms of this pathogenic AAEAR. Impaired mitochondrial function, pathways, mtDNA, and ultrastructure can cause impaired ionic homeostasis, fibrosis, electrical instability with collapsed membrane potential, altered action potential morphology, and atrial effective refractory period.[12,9−13] Atrial chamber dilation with metabolic and oxidative stress in LKB1 KO heart and in human contributes to the vulnerable substrate

for AF and HF.[2,12,20,21] Thus, prevention of atrial remodeling may reduce incidence of AF in HF and vice versa.

The LKB1 KO mouse recapitulates the characteristics human AF, AAEAR, and HF.[12,20,21] LKB1 plays a major role in the regulation of cellular metabolism, proliferation and polarity as part of the AMPK superfamily.[26] Here, we demonstrated the role mitochondria/AMPK regulated cardiomyocyte energy metabolism in AF, HF, and AAEAR. In human, previous studies demonstrated mitochondrial structural damage, fatty acid metabolism, apoptosis, and downregulation of complex I and II in patients with postoperative AF.[27,28] However, those studies were not able to demonstrate mitochondrial function or structure before the development of AF and/or HF. Our study demonstrates that mitochondrial dysfunction occurs before the development of AF with HF in addition to worsening mitochondrial dysfunction in both disease processes with coexistence. This gives an opportunity to study whether preservation of mitochondrial functional and structural integrity may provide an innovative strategy for primary prevention of AF.

There are several limitations in our experimental study. Although we found energy deficit and metabolic stress in human atrial tissue with HF-reduced ejection fraction and then validated these findings in murine disease model, we did not study HF with preserved ejection fraction or different disease stages of HF or AF because of the difficulty of obtaining tissue samples from those patients. Therefore, a significant amount of experimental studies was performed in mice because we were able to use fresh atrial tissue samples in different stages of the disease process. Our model recapitulates human disease effectively. The spontaneous nature of AF initiation and development of HF provided an opportunity to study energy metabolism in molecular mechanisms of the association between AF and HF disease processes. Also LKB1 KO is cardiac-restricted and therefore there was no confounding effect from other organ systems. Even though we maintained standard methods for each experiment, we cannot completely eliminate plausible variations in human heart explantation, tissue sampling, and storage. The difference in ATP levels between left and right atria in the control patients was not studied because right atrial samples were not obtained from the patients. Similarly, no ventricular samples were available to study energetics in controls.

In conclusion, pathogenesis of the AF and HF coexistence includes energy deficit and metabolic dysregulation in human and mice atria due to mitochondrial dysfunction. Oxidative and metabolic stress as a result of mitochondrial dysfunction is an upstream mechanism of the initiation and maintenance of AF in HF and vice versa. AAEAR is critical substrate for the association between AF and HF that involves impaired mitochondrial function, pathways, mtDNA, and ultrastructure. Thus, preservation of mitochondrial integrity is a promising innovative therapeutic strategy for primary and secondary prevention of AF in HF and also HF in AF.

Disclosures

None.

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Supplementary materials

Supplementary data related to this article can be found at doi:10.1016/j.cardfail.2019.08.005.

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