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Original Article

Mouse strain-specific responses of mitochondrial respiratory function and cardiac hypertrophy to isoproterenol treatment

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Abstract: Cardiac hypertrophy is a common pathological process of various cardiovascular diseases and eventually develops into heart failure. This paper was aimed to study the different pathological characteristics exhibited by different mouse strains after hypertrophy stimulation. Two mouse strains, A/J and FVB/nJ, were treated with isoproterenol (ISO) by osmotic pump to induce cardiac hypertrophy. Echocardiography was performed to monitor heart morphology and function. Mitochondria were isolated from hearts in each group, and oxidative phosphorylation function was assayed *in vitro*. The results showed that both strains showed a compensatory enhancement of heart contractile function after 1-week ISO treatment. The A/J mice, but not the FVB/nJ mice, developed significant cardiac hypertrophy after 3-week ISO treatment as evidenced by increases in left ventricular posterior wall thickness, heart weight/body weight ratio, cross sectional area of cardiomyocytes and cardiac hypertrophic markers. Interestingly, the heart from A/J mice contained higher mitochondrial DNA copy number compared with that from FVB/nJ mice. Functionally, the mitochondria from A/J mice displayed faster O_2 consumption at state III with either complex I substrates or complex II substrate, compared with those from FVB/nJ mice. ISO treatment did not affect mitochondrial respiratory control rate (RCR), but significantly suppressed the ADP/O ratio generated from the complex II substrate in both strains. The ADP/O ratio generated from the complex I substrates in A/J mice declined by 50% after ISO treatment, whereas FVB/nJ mice were not affected. These results suggest that, compared with FVB/nJ mice, A/J mice possesses a poor integrity of mitochondrial respiratory chain that might contribute to its vulnerability to ISO-induced cardiac hypertrophy.

Key words: mouse strain; cardiac hypertrophy; isoproterenol; mitochondria; oxidative phosphorylation; ADP/O ratio

小鼠对异丙肾上腺素处理的品系特异性反应

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摘 要：心肌肥厚是各种心血管疾病共同的病理过程，最终发展为心力衰竭。本文旨在研究不同品系小鼠在肥大刺激后表现出的不同病理学特征。通过动物微量注射泵泵入异丙肾上腺素(isoproterenol, ISO)诱导A/J和FVB/nJ小鼠心肌肥厚，用超声心动图监测心脏形态和功能。从各组小鼠心脏中分离线粒体，检测其氧化磷酸化功能。结果显示，两种品系小鼠在1周ISO处理后均表现出心脏收缩功能的代偿性增强。A/J小鼠(而非FVB/nJ小鼠)在3周ISO处理后出现明显的心肌肥厚，主要表现为左室后壁厚度、心重/体重比、心肌细胞横截面积和心肌肥大标志物表达均显著增加。与FVB/nJ小鼠相比，A/J小鼠心脏含有更高的线粒体DNA拷贝数；且无论是在复合物I底物还是复合物II底物中，A/J小鼠线粒体在state III时的耗氧速率都更高。ISO

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处理对两种品系小鼠心脏线粒体呼吸控制率没有显著影响，但显著抑制两种品系小鼠自复合物II底物产生的ADP/O比值。在ISO处理后，A/J小鼠线粒体自复合物I底物产生的ADP/O比值下降了约50%，而FVB/nJ小鼠不受影响。以上结果提示，相对于FVB/nJ小鼠，A/J小鼠线粒体呼吸链的完整性较差，这可能是其易受ISO诱导产生心肌肥厚的原因之一。

关键词：小鼠品系；心肌肥厚；异丙肾上腺素；线粒体；氧化磷酸化；ADP/O比值
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Cardiac hypertrophy occurs in response to a chronic or recurrent pressure-overload stimulus, and it is mainly characterized by enlargement of individual cardiomyocyte and accompanied by interstitial and perivascular fibrosis and cardiomyocyte death [1]. Mitochondrion is the major energy plant for cardiac output, and mitochondrial disorder contributes to the pathogenesis of cardiac hypertrophy [1]. However, it remains unclear how mitochondrial function affects the pathological progress of cardiac hypertrophy.

Mouse cardiac hypertrophy models provide extensive mechanistic insights into the pathogenesis of cardiac hypertrophy. There are several ways to establish pathological cardiac hypertrophy in mice, mainly including pressure overload, volume overload and pharmaceutical treatment [2–4]. Isoproterenol (ISO), a beta-1 and beta-2 adrenergic receptor agonist, is a commonly used drug to induce cardiac hypertrophy [4]. ISO could activate adenylyl cyclase (AC), yield cyclic AMP (cAMP) and activate protein kinase A (PKA) through β-adrenergic receptor coupled with Gs protein (Gαs). It then results in increased intracellular calcium through phosphorylating L-type calcium channels. Increased intracellular calcium enhances cardiomyocyte contractility and accelerates heart rate by increasing the slope of phase 4 [1]. Chronically increased workload causes the heart to develop hypertrophy to compensate the ventricular stress and maintain normal contractile function.

Clinical manifestation in human heart diseases shows highly heterogeneous because of different genetic background [5]. Previous studies have evaluated structural and functional outcomes of the heart from 104 inbred mouse strains in response to chronic ISO infusion [6–8]. The findings suggest that genetic background has a high impact on the cardiac response to ISO treatment, and the heritability of the left ventricular mass was estimated to be between 61% and 81% [6–8]. By analyzing the changes in gene expression of these mouse strains during cardiac hypertrophy and heart failure, Santolini *et al*. [9] found that genes related to the severity of the disease were not consistently expressed among different strains. These studies support that mice have phenotypic heterogeneity in ISO-induced cardiac hypertrophy due to strain-specific genetic background. However, the underlying mechanism for different mouse strains to adapt to hypertrophic stress has not been clarified yet.

Here we examined the responses of two mouse strains, A/J and FVB/nJ, to ISO-induced cardiac hypertrophy. The A/J inbred strain is highly susceptible to cortisone-induced congenital cleft palate and widely used in cancer and immunology research [10]. The FVB/nJ strain is inbred for the *Fv1*^{b} allele which confers sensitivity to the Friend leukemia virus B strain. Due to the prominent pronuclei in their fertilized eggs and the large litter size, FVB/nJ mice are commonly used for transgenic injection [11]. In the present study, we compared the differences between A/J mice and FVB/nJ mice in response to ISO-induced cardiac hypertrophy, and explored the role of mitochondrial function in it.

1 MATERIALS AND METHODS

1.1 *Animal care*

All mice used in the present study were male mice aged 10–12 weeks. All mice were housed in the Animal Experiment Center of Renmin Hospital of Wuhan University under specific-pathogen-free (SPF) conditions with controlled temperature, humidity, and light and free access to food and water. All animal experiments in this work were approved by the Animal Care and Use Committee of Renmin Hospital at Wuhan University and performed conforming to the 8th Edition of the Guide for the Care and Use of Laboratory Animals (Guide NRC, 2011) published by the US National Institutes of Health.

1.2 *ISO administration*

Cardiac hypertrophy was induced by subcutaneous osmotic minipump infusion of ISO (30 mg/kg per day) for 3 weeks. An osmotic minipump (Alzet, Cupertino, CA, USA) was implanted subcutaneously to deliver ISO in the interscapular region under pentobarbital (50 mg/kg,

i.p.) anesthesia [12]. In this experiment, mice treated in parallel with same amount of saline served as controls.

1.3 *Echocardiography*

Echocardiography was performed 3 days after the end of ISO. Mice were first anesthetized with 1.5%–2% inhaled isoflurane. Then, transthoracic echocardiography was conducted utilizing a VINNO6 ultrasound system (VINNO Technology Co., Suzhou, Jiangsu, China) as previously described [13]. M-mode echocardiograms were recorded by a skilled ultrasound technician at the mitral papillary muscle level under the left ventricular short axis views. According to the American Society of Echocardiography guidelines, relevant echocardiographic parameters, including systolic interventricular septum (IVS;s), diastolic interventricular septum (IVS;d), systolic left ventricular posterior wall thickness (LVPW;s), diastolic left ventricular posterior wall thickness (LVPW;d), left ventricular end systolic inner dimension (LVID;s), left ventricular end diastolic inner dimension (LVID;d), ejection fraction (EF) and fractional shortening (FS) were measured or calculated in at least three consecutive heartbeats.

1.4 *Tissue preparation*

At the end of the experiment, mice were weighed and then sacrificed for harvesting hearts under the condition of pentobarbital (50 mg/kg, i.p.) anesthesia. The weight of hearts was measured after being rinsed in iced phosphate buffered saline (PBS) and dried with absorbent paper. Subsequently, the heart was divided into several pieces, one part of which was placed on ice for the following experiments, another part of which was fixed with 4% paraformaldehyde for the histopathological analysis, and the third part of which was stored at −80 °C after quick freezing with liquid nitrogen.

1.5 *Hematoxylin-eosin (H&E) and wheat germ agglutinin (WGA) staining*

The heart tissues were fixed in 4% paraformaldehyde at room temperature for 24 h and then embedded in paraffin. The heart tissues were cut into 5 μm sections, then stained with H&E and WGA respectively, and were imaged under an Olympus microscope. Image Pro Plus 6.0 (Media Cybernetics Inc.) was used to analyze WGA staining to measure the cross sectional area of cardiomyocytes. No less than 100 cardiomyocytes were taken from each group to calculate the cross sectional area of cardiomyocytes.

1.6 *Quantitative real-time PCR (qRT-PCR)*

According to the manufacturer's instructions, TRIzol (Invitrogen, Waltham, MA, USA) was used to extract total RNA from frozen mouse heart tissues. Then, cDNA was synthesized using a cDNA synthesis kit (Roche, Mannheim, Germany). Then, SYBR Green PCR Master Mix (Roche, Mannheim, Germany) was used to perform qRT-PCR, and GAPDH was used to quantify the relative expression level of the target gene. The primer sequences used for amplification were as follows: Atrial natriuretic peptide (ANP): 5'-TTTCAAGAACCTGCTAGACCACC-3' (forward) and 5'-GATCTATCGGAGGGGTCCCA-3' (reverse), product length: 207 bp; Brain natriuretic peptide (BNP): 5'-CGCTGGGAGGTCACTCCTAT-3' (forward) and 5'-CTTCAGTGCGTTACAGCCCAA-3' (reverse), product length: 293 bp; Myosin heavy chain 7 (Myh7): 5'-GGCCTGGGCTTACCTCTCTA-3' (forward) and 5'-ACAGTCACCGTCTTGCCATT-3' (reverse), product length: 266 bp; GAPDH: 5'-TCCTGCACCACCAACTGCTTAG-3' (forward) and 5'-GATGACCTTGCCCACAGCCTTG-3' (reverse), product length: 213 bp. The primers were synthesized in Sangon Biotech (Shanghai) Co., Ltd.

1.7 *Mitochondrial DNA content*

qRT-PCR was utilized to determine mitochondrial DNA content as described previously [13]. First, total heart tissue DNA was extracted using the Animal Tissues/Cells Genomic DNA Extraction Kit (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) according to the manufacturer's instructions. Mitochondrial gene (cytochrome b, mCytb, forward primer: 5'-ATGGAGCGATGGTTGTCGG-3', reverse primer: 5'-CACCCTCACTCGGCTTCTTT-3', product length: 235 bp) and a reference nuclear gene (H19, forward primer: 5'-CAACATCCCACCCACCGTAA-3', reverse primer: 5'-CAGTGCCTCATGGGAATGGT-3', product length: 225 bp) were amplified separately with the Lightcycler 480 system (Roche, Mannheim, Germany) to quantify mitochondrial DNA relative level. The primers were synthesized in Sangon Biotech (Shanghai) Co., Ltd.

1.8 *Mitochondria isolation*

Mitochondria from the heart tissues were isolated utilizing a Mitochondrial Extraction Kit (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) following the manufacturer's instructions. Briefly, the heart tissues were homogenized in Lysis Buffer and then subjected to centrifugation several times at different rotational speed. Finally, the mitochondria were resus-

pended in the Store Buffer and placed on ice. All above steps were performed under conditions of ice or 4 °C. Mitochondrial protein was quantified using a BCA Protein Quantitative Kit (Applygen Technologies Inc., Beijing, China).

1.9 *Mitochondrial respiratory function*

Mitochondrial respiratory function was analyzed utilizing a Clark oxygen electrode (Rank Brothers Ltd., Cambridge, UK) under conditions of sealed chamber and 25 °C room temperature as described previously [14, 15]. Briefly, the above mitochondrial suspension was diluted by mitochondrial assay buffer (220 mmol/L mannitol, 70 mmol/L sucrose, 10 mmol/L KH_2PO_4, 5 mmol/L $MgCl_2$, 2 mmol/L HEPES, 1 mmol/L EGTA, 0.2% fatty acid-free bovine serum albumin, pH 7.4) to yield a protein concentration of 4 mg/mL. Then, the complex I substrates (5 mmol/L glutamate, 5 mmol/L malate and 5 mmol/L pyruvate) and the complex II substrate (5 mmol/L succinate) were used to measure mitochondrial state II respiration separately. Mitochondrial state III respiration was occurred after the injection of ADP (250 mmol/L). The mitochondrial respiratory control ratio (RCR) was calculated by state III respiratory rate/state II respiratory rate. Meanwhile, the mitochondrial ADP/O ratio was calculated by ADP added/oxygen consumption.

1.10 *Statistical analyses*

All data were presented as mean ± SEM. Students' *t*-test and one-way ANOVA, analyzed by IBM SPSS statistics version 20 (Chicago, IL, USA), were used to determine the statistical significance between two groups and among three or more groups, respectively. Statistically significant difference was defined as $P < 0.05$.

2 RESULTS

2.1 ISO induced cardiac hypertrophy in A/J mice but not in FVB/nJ mice

During ISO treatment, the body weight was monitored weekly. A/J mice showed a generally lower body weight than FVB/nJ mice at the same age (Fig. 1*A*). ISO treatment (30 mg/kg per day) did not significantly alter the body weight in both strains except for a slight decrease (no statistical significance) in A/J mice after the first week of ISO treatment (Fig. 1*A*). Compared with control groups treated with saline, 3-week ISO treatment significantly increased the heart weight/body weight (HW/BW) ratio in A/J mice, but not in FVB/nJ mice (Fig. 1*B*). Moreover, the HW/BW ratio in A/J mice was significantly higher than FVB/nJ mice after 3-week ISO treatment (Fig. 1*B*).

In both A/J and FVB/nJ mice, the heart responded to ISO treatment by a compensatory increase of EF and FS after the first week and then recovered to baseline (Fig. 2*A*–*C*), suggesting an equivalent stimulation on contractile function by ISO and an adaption of the heart to the stress as expected. Both EF and FS did not show a remarkable decline after three weeks of ISO treatment (Fig. 2*A*–*C*), indicating that the heart did not progress into heart failure. However, LVPW;s and

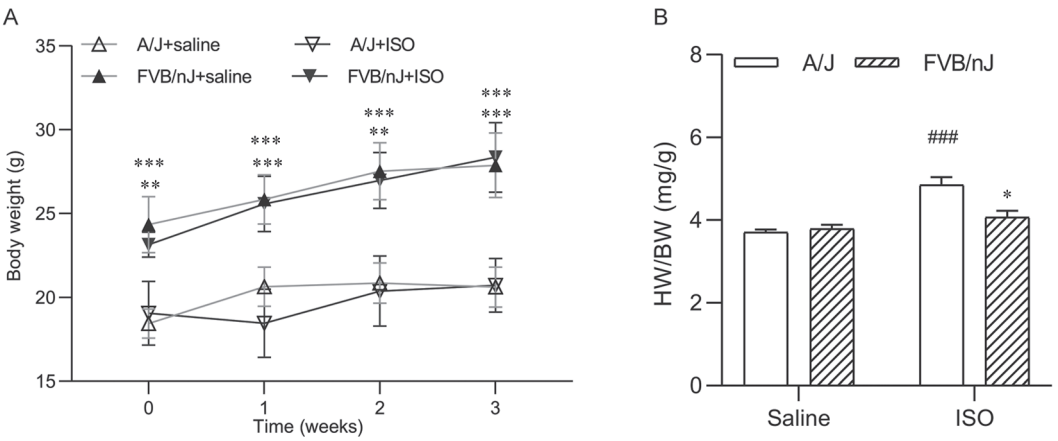

Fig. 1. A/J mice developed more severe cardiac hypertrophy than FVB/nJ mice in response to ISO treatment. *A*: Body weight over 3 weeks in A/J mice and FVB/nJ mice treated with saline or ISO (30 mg/kg per day). *B*: Heart weight/body weight (HW/BW) ratio in A/J mice and FVB/nJ mice after 3-week treatment with saline or ISO. Mean ± SEM, $n = 5$. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* A/J mice (In inset *A*, upper: FVB/nJ+saline mice *vs* A/J+saline mice; below: FVB/nJ+ISO mice *vs* A/J+ISO mice). $^{\#\#\#}P < 0.001$ *vs* A/J+saline; $^{*}P < 0.05$ *vs* A/J+ISO (In inset *B*).

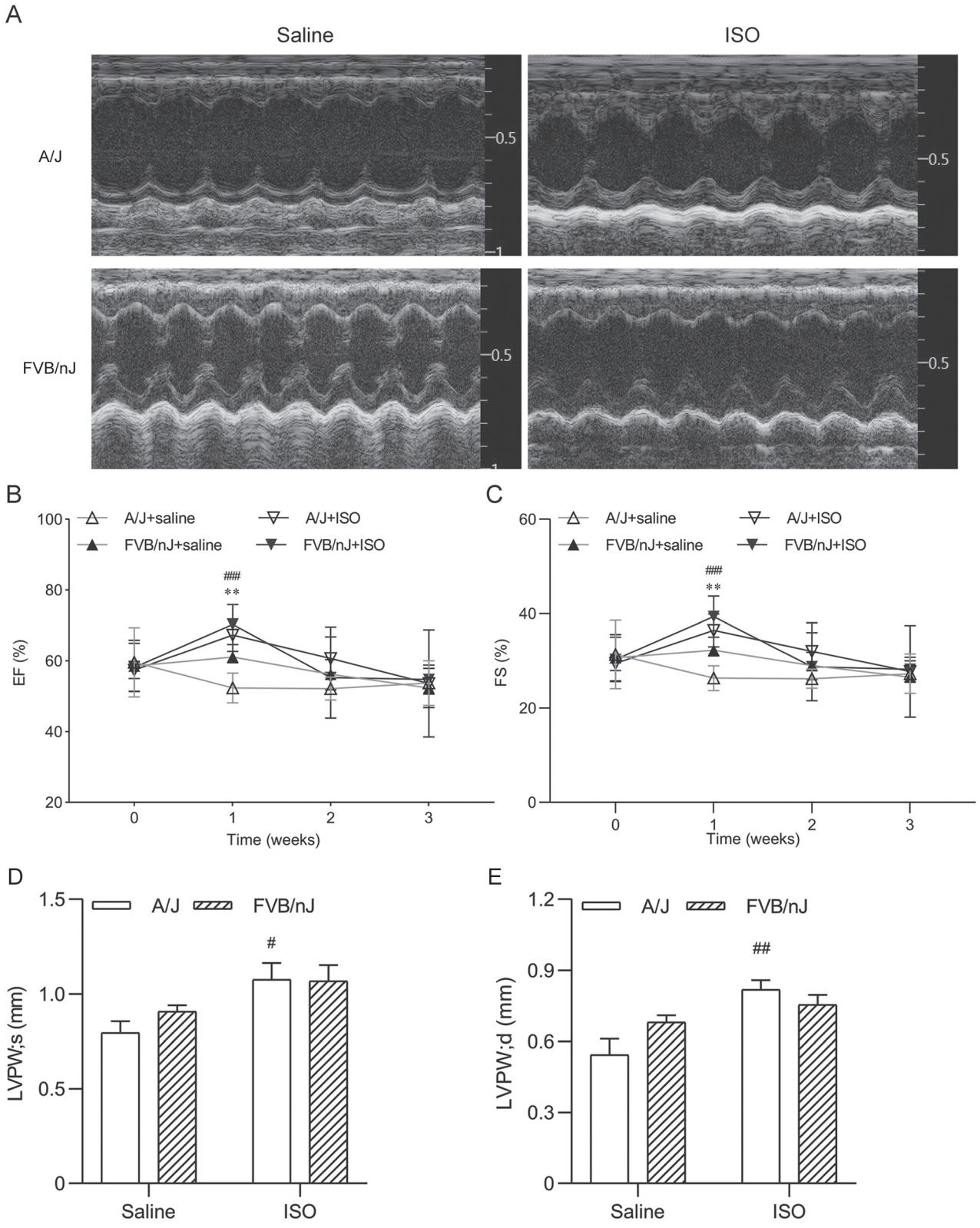

Fig. 2. Echocardiogram of A/J mice and FVB/nJ mice in response to ISO treatment. *A*: Representative echocardiogram of A/J mice and FVB/nJ mice after ISO treatment for 3 weeks. *B*, *C*: EF (*B*) and FS (*C*) over 3 weeks in A/J mice and FVB/nJ mice treated with saline or ISO. *D*, *E*: LVPW;s (*D*) and LVPW;d (*E*) in A/J mice and FVB/nJ mice treated with saline or ISO for 3 weeks. Mean ± SEM, $n = 5$. $^{**}P < 0.01$, FVB/nJ+ISO *vs* FVB/nJ+saline; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, A/J+ISO *vs* A/J+saline.

LVPW; d significantly increased in A/J mice in response to ISO infusion for 3 weeks, but the changes were comparably mild in FVB/nJ mice (Fig. 2*D* and 2*E*). The cross sectional area of cardiomyocytes (Fig. 3*A*, *B*) and cardiac hypertrophic makers (Fig. 3*C*–*E*) showed a similar trend. These data suggest that FVB/nJ mice are more resistant to ISO-induced cardiac hypertrophy than A/J mice.

2.2 The heart from A/J mice contains a higher mitochondrial DNA copy number than that from FVB/nJ mice

Since mitochondrial energy metabolism plays a key

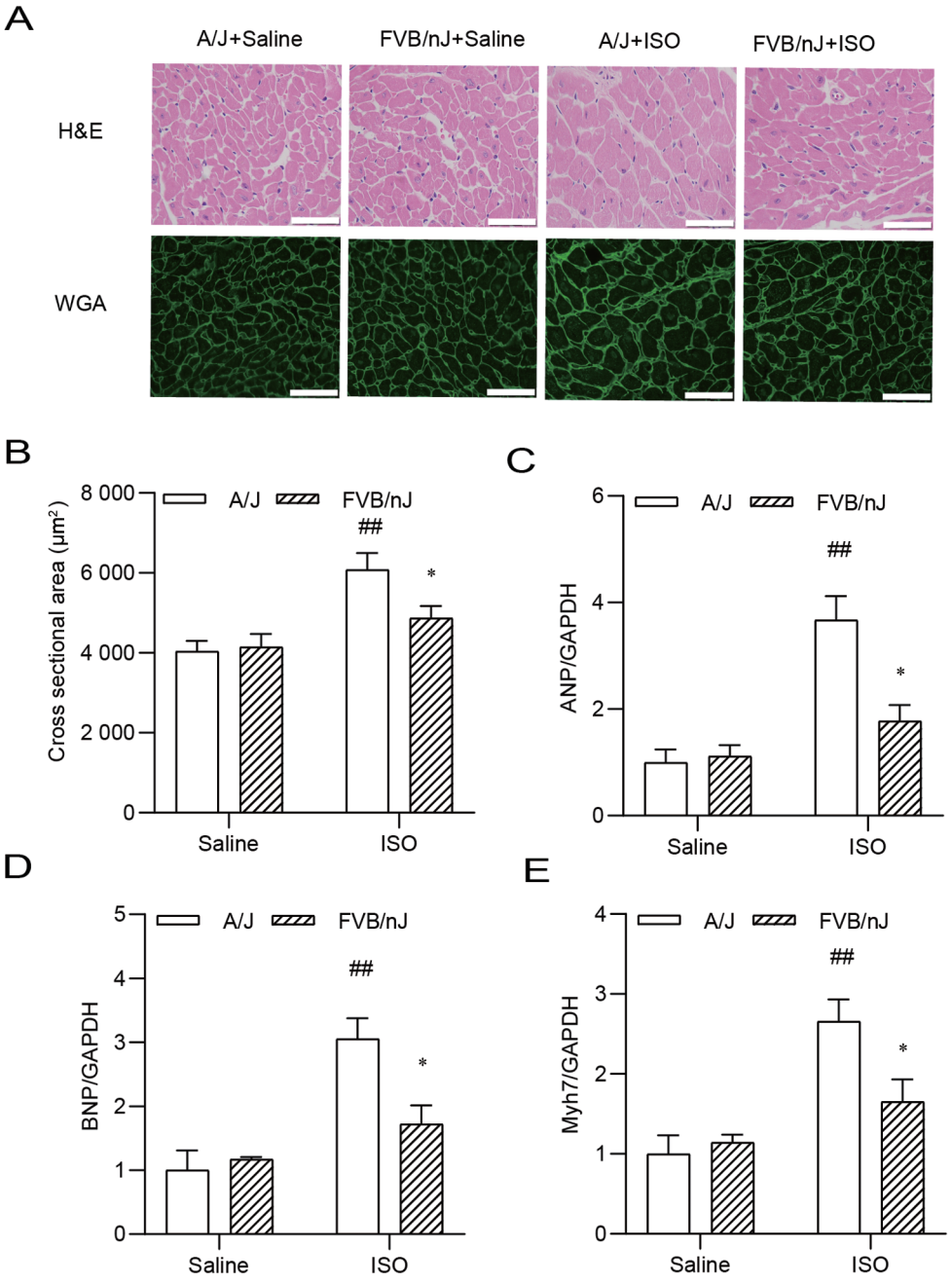

Fig. 3. Histopathology and cardiac hypertrophic markers of A/J mice and FVB/nJ mice in response to ISO treatment. *A*: Representative H&E and WGA staining images of A/J mice and FVB/nJ mice after saline or ISO treatment for 3 weeks. Scale bar, 50 μm. *B*: Quantitative statistical results of the cross sectional area of cardiomyocytes in A/J mice and FVB/nJ mice after treated with saline or ISO for 3 weeks; *C*–*E*: ANP (*C*), BNP (*D*) and Myh7 (*E*) mRNA expression levels in A/J mice and FVB/nJ mice after treated with saline or ISO for 3 weeks. Mean ± SEM, $n = 5$. $^{*}P < 0.05$ *vs* A/J+ISO; $^{\#\#}P < 0.01$ *vs* A/J+saline.

role in maintaining normal heart function, we then evaluated the status of isolated mitochondria from heart tissues of all four groups. To our surprise, the heart from A/J mice contained significantly higher mitochondrial DNA copy number than that from FVB/nJ mice, as evidenced by measuring mitochondrially encoded cytochrome b (mCytb) in contrast to nuclear H19 gene (Fig. 4*A*). However, the mitochondria yield in terms of protein content was comparable in both A/J and FVB/nJ mice (Fig. 4*B*). Neither the mitochondrial DNA copy number nor the mitochondria protein content yield from the two strains was affected by ISO treatment (Fig. 4).

2.3 ISO differentially affects mitochondrial respiratory chain integrity in the hearts from A/J and FVB/nJ mice

Mitochondrial oxygen consumption was measured under conditions of complex I substrates (glutamate, malate and pyruvate) or complex II substrate (succinate), respectively (Fig. 5*A* and 5*B*). No matter with or without ISO treatment, compared with the mitochondria from A/J mice, the mitochondria from the FVB/nJ mice displayed slightly lower (no statistical significance) oxygen consumption rates at state II under complex I substrates and complex II substrate, except for that at state II under complex II substrate (statistical significance) with saline treatment (Fig. 5*C–D*). However, compared with the mitochondria from A/J mice, the mitochondria from the FVB/nJ mice displayed significantly lower oxygen consumption rates at state III under complex I substrates and complex II substrate, which were not affected by ISO treatment (Fig. 5*E–F*). The calculated RCR showed no difference among all groups with complex I substrates (Fig. 5*G*); however, the RCR with complex II substrate in FVB/nJ mice was significantly lower than A/J mice at baseline but not after ISO treatment (Fig. 5*H*).

ADP/O ratio reflects the integrity and efficiency of the mitochondrial respiratory chain to generate ATP by utilizing oxygen. Interestingly, we observed a significant decrease of ADP/O ratio through both complex I and II after ISO treatment in A/J mice (Fig. 6). However, the ADP/O ratio in FVB/nJ mice showed no difference with complex I substrates but a decrease with the complex II substrate (Fig. 6). Compared with A/J mice, the post-ISO decrease of ADP/O ratio through complex I was largely preserved in FVB/nJ mice (Fig. 6*A*), reflecting a major difference in mitochondrial oxidative phosphorylation function between the two strains. These data suggest that the integrity of mitochondrial respiratory chain in A/J mice is more vulnerable to ISO treatment than that in FVB/nJ mice.

3 DISCUSSION

Cardiac hypertrophy and subsequent heart failure are the core pathological process of multiple cardiovascular diseases [16]. However, the underlying mechanisms about the occurrence and development of cardiac hypertrophy remain to be elucidated. Currently, mouse

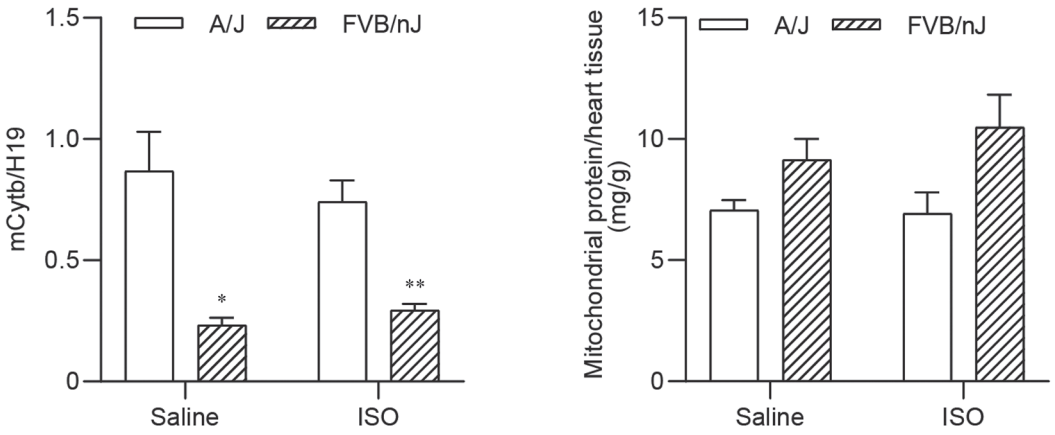

Fig. 4. A/J mice showed higher mitochondrial DNA content than FVB/nJ mice with or without ISO treatment. *A*: Mitochondrial DNA copy number was determined by mCytb DNA levels normalized with nuclear H19 measured by qRT-PCR. *B*: Mitochondrial protein content presented as mitochondrial protein/heart tissue (mg/g) ratio after homogenization. Mean ± SEM, $n = 5$. $^{*}P < 0.05$ *vs* A/J+saline; $^{**}P < 0.01$ *vs* A/J+ISO.

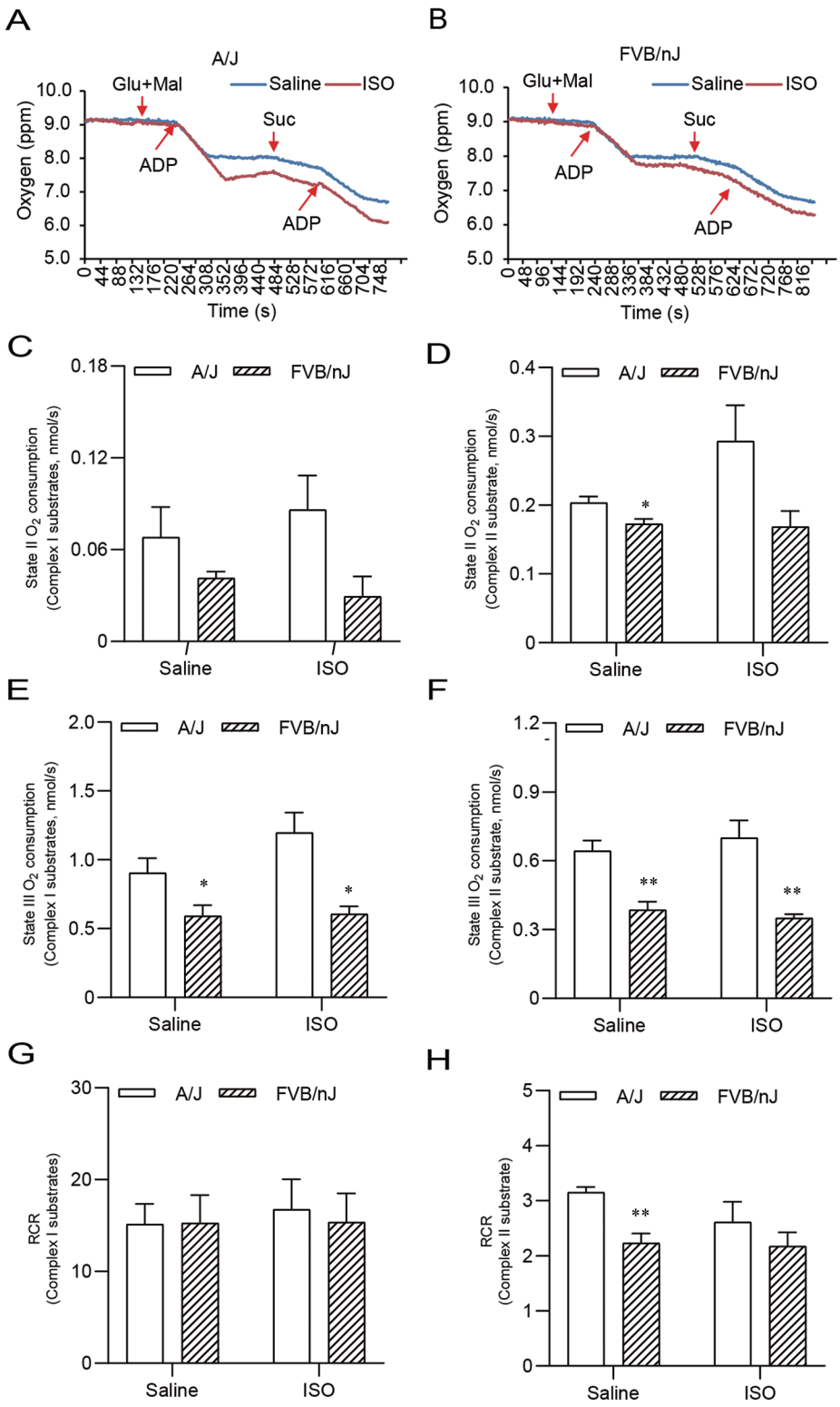

Fig. 5. Mitochondrial respiratory function in A/J and FVB/nJ mouse hearts with or without ISO treatment. *A*, *B*: Representative record chart of mitochondrial respiratory chain oxidative phosphorylation function detection in A/J mice (*A*) and FVB/nJ mice (*B*). *C*, *D*: Oxygen consumption rate of mitochondrial state II respiration with the substrates for complex I (*C*; 5 mmol/L glutamate, 5 mmol/L malate and 5 mmol/L pyruvate) or complex II (*D*; 5 mmol/L succinate) among these 4 groups. *E*, *F:* Oxygen consumption rate of mitochondrial state III respiration with the substrates for complex I (*E*) or complex II (*F*) in different groups. *G*, *H*: The mitochondrial RCR with the substrates for complex I (*G*) or complex II (*H*) in different groups. Mean ± SEM, $n = 5$. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* corresponding A/J mice. Glu, glutamate; Mal: malate; Suc, succinate.

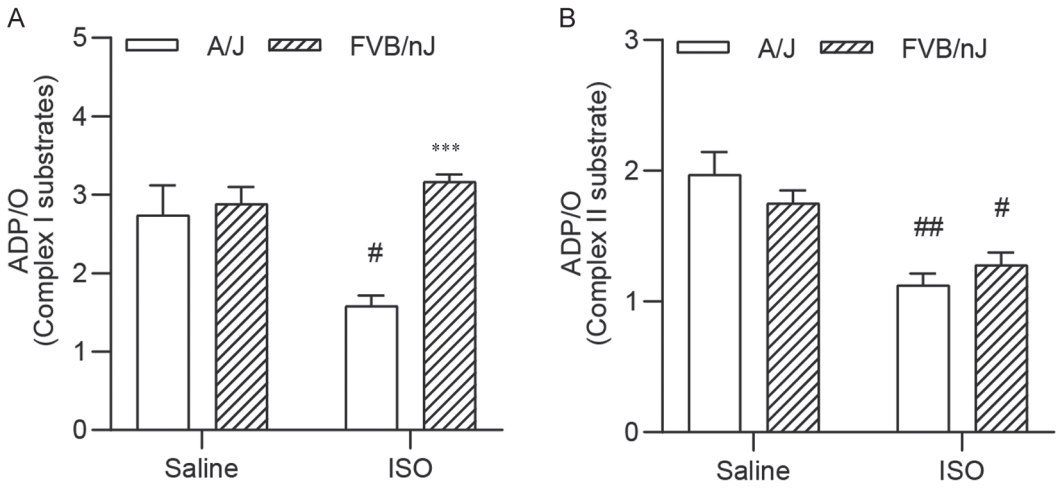

Fig. 6. Mitochondrial ADP/O ratio in A/J and FVB/nJ mouse hearts with or without ISO treatment. *A*, *B*: The mitochondrial ADP/O ratio with the substrates for complex I (*A*) or complex II (*B*) among these 4 groups. Mean ± SEM, $n = 5$. $^{***}P < 0.001$ *vs* A/J+ISO; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs* corresponding control (saline).

is the most commonly used animal to establish cardiac hypertrophy model and to investigate the molecular mechanisms, but different mouse strains display highly heterogeneous outcomes [12, 17]. In the present work, we treated A/J mice and FVB/nJ mice with ISO to induce cardiac hypertrophy and observed that the mitochondrial respiratory chain integrity in the two strains differentially responded to ISO treatment. Our findings support that strain-specific mitochondrial function accounts for different cardiac hypertrophy outcomes in mouse models.

Commonly used mouse strains for heart diseases include A/J, FVB/nJ and C57L/B6 mice [18–20]. Here we found that the A/J strain was more sensitive to ISO-induced cardiac hypertrophy than FVB/nJ. This is consistent with a previous report that A/J mice developed more severe cardiac hypertrophy than C57L/B6 mice under ISO treatment [21]. In addition, our results showed that FVB/nJ mice were resistant to ISO treatment, suggesting that FVB/nJ is not an optimistic mouse strain for cardiac hypertrophy studies.

Mitochondrion is the power plant of cardiomyocytes, and occupies approximately 30% of cardiomyocyte volume and supplies >95% of the ATP consumed by the heart [22, 23]. Pathological cardiac hypertrophy is commonly accompanied by the impairment of energy metabolism, whereby mitochondrial dysfunction has been regarded as one of major risk factors in the pathology of cardiac hypertrophy. Although the number of mitochondria has been proposed to be increased in cardiac hypertrophy due to enhanced mitochondrial biogenesis and protein synthesis [24], we failed to observe any significant increase of mitochondrial DNA copy number or protein content after 3-week ISO treatment in both A/J and FVB/nJ mice. In contrast, hypertrophied cardiomyocytes usually have reduced maximal mitochondrial oxidative phosphorylation capacity, which is partly attributable to activity defects of the respiratory chain complexes and the ATP synthase [25–28]. In the process of cardiac hypertrophy, the main cardiomyocytes energy source switches from fatty acid oxidation to a less efficient glucose oxidation, and this results in insufficient ATP supply [29, 30]. Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) regulates mitochondrial biogenesis and fatty acid oxidative capacity, and its expression decreases in the hypertrophied and failing heart [31, 32]. Adenine nucleotide translocator (ANT) is a carrier responsible for the transport of ATP from mitochondria to the cytosol, and reduction of ANT activity and alteration of ANT isoform expression occur in human heart failure [33, 34]. These evidence implicates that the regulations of mitochondria dynamics and respiratory function in cardiac hypertrophy are a complicated process.

Although the hearts from A/J mice possessed more mitochondrial DNA copy number, they did not yield more mitochondrial mass, implicating a potential increase of mitochondria fission. It has been shown that increased mitochondria fission would lower the mitochondrial oxidative phosphorylation activity compared with mitochondria fusion [35]. Moreover, the

mitochondria from A/J mouse hearts displayed faster oxygen consumption but lower ADP/O ratio, suggesting a defect in mitochondrial respiratory chain integrity. Consistently, Faulx *et al*. [21] found that A/J mice exhibited more severe cardiac hypertrophy than C57L/B6 mice under ISO administration because of poorer activity of mitochondrial enzymes regulating fatty acids and carbohydrate oxidation. Whether this is resultant from variations in mitochondria genome or nuclear-coded mito- chondrial genes needs further investigation.

Mitochondrial energy metabolism has a direct impact on cardiac hypertrophy. Lacking PGC-1α can accelerate transthoracic aortic constriction (TAC) induced cardiac dysfunction in mice, which is accompanied by a dramatic decline in cellular ATP content [36]. PGC-1α overexpression can protect cardiomyocytes from hypertrophy, although moderate overexpression of PGC-1α is unable to improve cardiac function in response to chronic pressure overload [37, 38]. Mice with *ANT1* knockout develop enlarged hearts and mitochondrial proliferation [39]. Meanwhile, cardiac-specific ANT1 overexpression protects against hypertension-induced cardiac hypertrophy in the rats owing to improvement in mitochondrial function [40]. Together with our findings, these evidence suggests that the disorder of mitochondrial energy metabolism plays a crucial role in the pathogenesis of cardiac hypertrophy.

In conclusion, A/J mice develop more severe cardiac hypertrophy than FVB/NJ mice in response to ISO administration, which might be attributable to the mitochondria dysfunction caused by defected mitochondrial respiratory chain integrity. On the other hand, strategies to improve mitochondrial function may be the key to treat cardiac hypertrophy and heart failure.

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