Impact of age and sex on hyperoxia-induced cardiovascular pathophysiology

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ABSTRACT

Hyperoxia is characterized by pronounced inflammatory responses, pulmonary cell apoptosis, and adverse cardiac remodeling due to an excess supply of oxygen. Hyperoxic episodes are frequent in mechanically ventilated patients and are associated with in-hospital mortality. This study extends the analysis of prior published research by our group as it investigates the influence of age in male and female rodents exposed to hyperoxic conditions. Age is an independent cardiovascular risk factor, often compounded by variables like obesity, diabetes, and a decline in sex hormones and their receptors. This study simulates clinical hyperoxia by subjecting rodents to > 90 % of oxygen for 72 h and compares the changes in cardiac structural and functional parameters with those exposed to normal air. While in both sexes conduction abnormalities with ageing were discernible, aged females owing to their inherent higher baseline QTc, were at a higher risk of developing arrhythmias as compared to age-matched males. Quantitative real-time RT-PCR and western blot analysis reflected altered expression of cardiac potassium channels, resulting in conduction abnormalities in aged female rodents. Unaffected by age and sex, hyperoxia-treated mice had altered body composition, as evidenced by a considerable reduction in body weight. Interestingly, compensatory hypertrophy observed as a protective mechanism in young males was absent in aged males, whereas protection of hearts from hyperoxia-induced cardiac hypertrophy was absent in aged female mice, both of which may be at least in part due to a reduction in sex steroid receptors and the systemic steroid levels. Finally, statistical analysis revealed that hyperoxia had the greatest impact on most of the cardiac parameters, followed by age and then sex. This data established an imperative finding that can change the provision of care for aged individuals admitted to ICU by elucidating the impact of intrinsic aging on hyperoxia-induced cardiac remodeling.

1. Introduction

In the United States, the prevalence of cardiovascular disease (CVD) is around 40 % in men and women aged 40–59, but it exceeds 85 % in those over 80 years old (Yazdanyar and Newman, 2009). Consequently, there is a substantial increase (3.8 % per year) in the number of aged individuals admitted to intensive care units (ICUs) (Angus et al., 2004). However, retrospective cohort studies have reported a substantial decline in the long-term survival rate in the aged population following intensive care, stipulating hyperoxic exposure to present as a risk factor in aged individuals (Kristinsdottir et al., 2020; Ruggiu et al., 2018). In aged ICU patients, this life-saving maneuver can prevent the likelihood of the morbidity associated with hypoxia. However, emerging data suggest that the risk of developing hyperoxia with oxygen supplementation outweighs the benefits of this technique (Vincent et al., 2017; Young and Bellomo, 2019).

Hyperoxia occurs when the oxygen saturation (SpO2) is greater than 96 % or the partial pressure of oxygen (PaO2) is above 150 mm Hg (Ni et al., 2015). Unfortunately, patients in the emergency department had a median PaO2 of 189 mm Hg, particularly unfavorable in aged individuals (Page et al., 2018; Pawar, 2020). A recent study found that 19.7 % of patients experienced cardiac complications post ICU...
admission, yet oxygen supplementation remains a cornerstone of managing aged COVID-19 patients (Long et al., 2020). Despite the well-documented negative implications of hyperoxia such as causing alveolar and interstitial histopathological injury (Potter et al., 1999), altering lung metabolite profile (Magnusdottir et al., 2019), predisposing to hyperoxic acute lung injury (HALI) (Mizushina et al., 2015), causing neutrophil infiltration due to the activation of CXCR2, a potent neutrophil chemotactrant (Sue et al., 2004) and lung fibrosis due to the activation of RhoA, a GTPase-activating protein (Kondrikov et al., 2011), few studies have investigated the effects of hyperoxia on the cardiovascular system (Chapalamadugu et al., 2015; Panguluri et al., 2013; Rodgers et al., 2019b; Visser et al., 2010).

Liberal oxygen delivery causes hemodynamic changes like decreased stroke volume, heart rate, and cardiac output, subsequently increasing workload on the heart and triggering cardiac remodeling (Visser et al., 2013; Rodgers et al., 2019b; Visser et al., 2010). Additionally, prolonged hyperoxic exposure increased reductive workload on the heart and triggering cardiac remodeling (Visser et al., 2013; Rodgers et al., 2019b; Visser et al., 2010).

2. Material and method

2.1. Animals

The experiments were conducted on adult male and female mice (C57BL/6 strain), aged 8–10 weeks (n = 12) and 73 weeks old (n = 12) obtained from Jackson Laboratories (Chicago, IL). The mice were subjected to either normoxia (normal air) or hyperoxia (> 90 % oxygen) in an airtight chamber (Panguluri et al., 2013). During the experiment, mice were maintained on a 12-h light/dark cycle and were allowed to access food pellets and water ad libitum. The experimental protocols were strictly scrutinized and approved by the Institutional Animal Care and Use Committee (IACUC) at the University of South Florida, in compliance with the US National Institutes of Health.

2.2. Hyperoxia exposure

The experimental group was either subjected to hyperoxia (> 90 % O2) in an airtight chamber or normoxia (normal air) for the entire 72 h research period, according to previously established methods (Panguluri et al., 2013). During the span of the experiment, the oxygen levels were strictly maintained using an oxygen analyzer (Vascular Technology, Chelmsford, MA). The mice were sedated with isoflurane after 72 h to perform electrocardiography and echocardiography. The control and the treatment groups were euthanized for thoracotomy by intraperitoneal injection (IP) of 50 mg/kg euthasol. Immediately, blood was collected and spun at 5500 rpm for 5 min for the collection of plasma serum. Each heart was dissected for collection of atriums, septum, right and left ventricles, and the tissue was preserved at – 80 °C for later examination.

2.3. Physical parameters

Assessment of the body weights of male and female mice was performed before normoxia or immediately after hyperoxia exposure. The changes in the body weights were recorded in both young and aged groups of both sexes. The body weights were normalized with the tibia length. Lung wet to dry weight ratio was also recorded in all groups to assess hyperoxia-induced lung edema as described in our previous publications (Rodgers et al., 2021).

2.4. Histological analysis

Normoxia- and hyperoxia-treated hearts were sectioned and stained with hematoxylin and eosin (H&E) in both aged and young mice following the previously published technique (Panguluri et al., 2013). The stained cardiac sections were measured for overall heart area of cross section using a Keyence BZ-X800 microscope. The overall cardiomyocyte area was measured using fluorescent-labeled wheat germ agglutinin (WGA) and DAPI as described previously (Rodgers et al., 2021). The area of cross section for each group was measured using ImageJ software, and the mean (SE) results were displayed in the bar diagram.

2.5. Echocardiography

Transthoracic echocardiography was performed using a Vevo3100LT Ultrasoundograph (VisualSonics) equipped with a 30 MHz transducer. Cardiac function was measured in both normoxic and hyperoxic mice. We used previously published procedures for echocardiographic measurements (Rodgers et al., 2021). Briefly, the mice were anesthetized with 1–1.5 % isoflurane and maintained at body temperature (37 °C). A 2-D mode in parasternal long axis and the parasternal short axis at the mid-papillary muscle level were imaged. From this parasternal short axis view, the 2-D guided M-mode across the anterior wall and posterior wall were recorded. LVAW (left ventricular anterior wall), LVID (left ventricular interior diameter) and LVWP (left ventricular posterior wall thickness) at systole and diastole were measured. EF% (ejection fraction) was calculated as (EDV – ESV)/EDV * 100 %, and FS% (fractional shortening) was calculated by (LVID;d – LVID;s)/LVID;d * 100 %.

2.6. Electrocardiogram (ECG)

The experimental subjects in both groups were anesthetized using 1–2 % isoflurane, followed by the insertion of electrical probes in lead II configuration. The recordings were conducted in three consecutive intervals, each interval lasting 3–5 min as per the previously established protocol (Chapalamadugu et al., 2015). The ECG and HR recordings were processed and analyzed using the PowerLab system and LabChart Pro software (AD Instruments). As previously defined, RR, PR, QRS, and JT intervals were measured, with QT intervals measured at the start of Q peak up to T baseline. By using Bazett’s formula (QTc = QT/RR1/2), QTc (heart rate corrected) intervals were calculated.

2.7. RNA isolation and quantitative real-time PCR

The excised left ventricle tissue was weighed, and ~ 30 mg was used for RNA extraction using the step-by-step protocol mentioned in the miRCURY RNA Isolation kit (Exiqon). The total extracted RNA was quantified using NanoDrop spectrophotometer, and the quality was assessed by A260/280 ratio of ~ 2.0, as described in our previous publication (Rodgers et al., 2021). For cDNA synthesis, 1000 ng of RNA was utilized, and Real-time PCR was carried out in duplicates using iTaq SYBR green manufacturer protocol. Our lab has already published the list of potassium ion channel primers like Kv1.4, Kv1.5, Kv4.2, KChIP2 along with HPRT as a housekeeping gene (Panguluri et al., 2013; Rodgers et al., 2019b, 2021). The changes in the gene expressions were analyzed using the delta-delta CT method.
Fig. 1. Age and sex differences in physical parameters upon hyperoxia exposure. (a) Body weight for all experimental groups normalized to tibia length (g/cm), (b) Lung wet/dry ratio for all experimental groups, (c) H&E stained heart histological cross sections of male and female mice in both age groups under normoxia and hyperoxia, (d) Measured area for heart cross sections (au), (e) WGA-stained cardiac myocytes from young and aged mice in both sex under normoxia and hyperoxia. Cardiac myocyte area of male and female mice in both age groups under normoxia or hyperoxia from (f) LV cardiomyocyte area, (g) RV cardiomyocyte area, (h) Septum cardiomyocyte area, (i) Pooled cardiomyocyte area. For all data, error bars represent ± SEM. *p < 0.05, **p < 0.005, ***p < 0.0005. * Represents p-value between hyperoxia and normoxia of same sex and age; Y represents p-value between male and female mice of the same age and treatment; †† represents p-value between young and aged groups of same sex and treatment. H&E: hematoxylin & eosin; SEM: standard error of the mean.
2.8. Western blot

The experimental methods for protein quantification and analysis were all adapted from our previous publication (Panguluri et al., 2013; Rodgers et al., 2019b, 2021). Briefly, ~30 mg of left ventricle tissue was excised from both the groups and was homogenized in cell lysis buffer (Cell Signaling Technology, Danvers, MA) containing a protease cocktail (Sigma Life Science, Burlington, MA) and PMSF. The homogenized samples were centrifuged at 15,000 rpm for 30 min at 4°C and supernatant was quantified for 50 μg of equivalent proteins using Pierce™ BCA Protein Assay Kit (Thermo Scientific). Each sample was denatured and loaded to run in a SDS-PAGE precast gel (Bio-Rad Laboratories), blots were later blocked in 5% w/v nonfat dry milk for 1 h and probed in 1:1000 dilutions of primary antibodies for Kv1.4 and KChIP2 (Abcam, Cambridge, MA), Kv4.2 (Millipore, Billerica, MA), estrogen receptor beta (ER-β) (Thermo Fisher, CA), androgen receptor (Abcam, Cambridge, MA), and GAPDH (Millipore) antibodies; and 1:1000 dilutions of Kv1.5 antibody (Santa Cruz Biotechnology, Dallas, TX). The band intensities from GAPDH were used to normalize the target proteins using ImageJ software.

2.9. ELISA

Serum estradiol levels were analyzed using the Estradiol parameter assay kit (Cat #KGE014, R&D systems) as per the manufacturer’s protocol. The ELISA for testing serum estrogen was conducted with internal duplicates for young and old female mice under normoxic or hyperoxic conditions. Based on the standard curve, the final concentration of estradiol (pg/ml) was expressed. For analyzing serum testosterone levels, a mouse testosterone ELISA kit (Cat #80552, Crystal Chem) was used according to the manufacturer’s instructions. The data was obtained in (ng/ml) by assessing internal duplicates for young and old male mice under normoxic or hyperoxic conditions. For both the assays animal replicates (n = 6) were used from each group.

2.10. Statistical analysis

A multi-Way ANOVA was performed to estimate how the parameters studied were influenced by treatment, age, and sex. Pairwise multiple comparisons using Tukey Honest significant difference (Tukey HSD) post-hoc testing was performed after a multi-factor ANOVA. All these tests were run using R statistical program. The Pearson’s correlation matrix of outcomes (such as body weight, heart weight, and so on) was also generated using the corrplot package in R.

3. Results

3.1. Physical parameters

Previously, our group has reported alterations in body weights in young male and female mice due to 72 h of hyperoxic exposure (Rodgers et al., 2019b). The current study evaluated body weights and lung weights to further examine the effect of aging, sex, and hyperoxia on physical parameters. In accordance with our earlier reports (Panguluri et al., 2013; Rodgers et al., 2019b), exposure to hyperoxia significantly reduced the body weight (normalized to the tibia length) regardless of age and sex (Fig. 1a). Based on age differences, we observed that the older mice were heavier than the younger mice irrespective of exposure conditions and sex (Fig. 1a).

As we reported lung edema in hyperoxia-treated male and female young mice (Panguluri et al., 2013; Rodgers et al., 2019b), we also examined lung edema in both young and aged mice in this research by measuring the lung wet weight to dry weight ratio. Our data showed that aged mice groups in both the sex showed significant lung edema after hyperoxia treatment (Fig. 1b), similar to our previous reports (Rodgers et al., 2019b, 2021). On comparison between male and female, young female lungs are more susceptible to the development of lung edema after hyperoxia as compared to age-matched male, with no significant differences between other groups. When compared between the age groups, aged male lungs showed significantly higher edema compared to the younger group after hyperoxia treatment (Fig. 1b).

3.2. Histological analysis

We further investigated the histology of the heart in hyperoxia-treated young and aged mice to study the changes in cardiomyocyte
Fig. 2. Functional abnormalities between different age groups and sex under normoxia and hyperoxia. (a) M-mode parasternal short axis view in two-dimensional echocardiography for normal air and hyperoxia groups (young and aged; male and female), (b) left ventricular internal diameter at diastolic (LVID;d), (c) left ventricular internal diameter at systolic (LVID;s), (d) percent fractional shortening (%FS), (e) percent ejection fraction (%EF), (f) stroke volume (SV), and (g) cardiac output (CO) in hyperoxia/normoxia treated mice. For all data, error bars represent ± SEM. *p < 0.05, **p < 0.005, ***p < 0.0005. * Represents p-value between hyperoxia and normoxia of same sex and age; Y represents p-value between male and female mice of the same age and treatment; Ž represents p-value between young and aged groups of same sex and treatment.
Similar to our previous reports (Rodgers et al., 2019b), H&E staining showed that male heart size is significantly larger after hyperoxia exposure in the young group compared to normal air, whereas female hearts showed significantly reduced size after hyperoxia compared to their normal air controls (Fig. 1c and d). In contrast, hyperoxia treatment significantly reduced male heart size in aged group compared to their normal air controls, whereas aged female hearts showed a significant increase in size after hyperoxia treatment compared to their normal air controls (Fig. 1c and d). When compared between male and female, female hearts showed a significantly smaller size compared to male at both normal air and hyperoxia, except in aged female, which displayed significantly larger hearts than age-matched male after hyperoxia treatment (Fig. 1c and d). Further evaluation of cardiomyocyte size using WGA staining revealed a significant increase (p < 0.0005) in cardiomyocyte size upon exposure to hyperoxia irrespective of age and sex (Fig. 1e-i). Comparison of cardiomyocyte size between aged and young hearts revealed significantly smaller cardiomyocyte size in LV, RV, and spectrum at both normal air as well as after hyperoxia in mice group (Fig. 1f-i). A similar pattern of diminished cardiomyocyte size in aged females was observed under normal air and hyperoxic conditions when compared to young hearts, indicating age itself reduces cardiomyocyte area dramatically (Fig. 1i). In terms of sex differences, females inherently have smaller cardiomyocytes as compared to males regardless of age and hyperoxic stress (Fig. 1i).

3.3. Echocardiogram parameters

As we observed significant changes in physical parameters, we also investigated if these changes had any significant effect on cardiac functioning using 2D echocardiography (Fig. 2a). Our data showed that hyperoxia significantly increased %FS and %EF in all groups except aged
females (Fig. 2d and e). This is due to increased LVID;d in aged females under hyperoxia with no significant difference in LVID;d, which significantly reduced fractional shortening and ejection fraction in this group (Fig. 2b and c). Although there was no significant difference observed in LVID;d and LVID;s between young and aged groups at normal air in both male and female, significant increase in LVID;d in aged group (both male and female) after hyperoxia treatment was observed compared to young ones (Fig. 2b and c). Additionally, we also observed significant decrease in stroke volume (SV) and cardiac output (CO) in all the groups after hyperoxia treatment irrespective of age and sex (Fig. 2f and g). When compared between male and female groups, the young female group showed significantly reduced SV and CO under hyperoxia than the young male, whereas the aged male showed significantly reduced SV and CO under normal air compared to the age-matched female. Similarly, when compared between young and aged groups, aged female showed improved SV and CO under hyperoxia than their young female counterparts, whereas aged males showed reduced CO compared to young males under normal air.

3.4. Electrophysiological parameters

Under mild anesthesia, the effect of hyperoxia on mice is evident based on the electrocardiogram (ECG) records. The original traces and representative waveforms in young male and female mice are reported in our previous studies (Rodgers et al., 2019b). Here we report the ECG records of aged mice under normal air versus under hyperoxic stress for both sexes, with a clear indication of arrhythmias in the hyperoxia group (Fig. 3a). This study corroborates with our previous findings in young mice as a general trend of increasing RR, PR, QRS, QTc, and JT intervals in both sex of aged mice under hyperoxic conditions is noticed (Fig. 3b–f). Furthermore, pertaining to age under normoxic conditions males had a distinguishable increase in PR, QTC, and JT intervals as compared to young males, suggesting aged males to be more susceptible to alterations in cardiac physiology. Also, females did demonstrate a significant increase in QRS interval as compared to young females with age (Fig. 3d). In terms of sex differences, hyperoxia exposed females demonstrated considerably longer RR intervals than males in both young and aged groups (Fig. 3b).

3.5. Ion channel expression and transcription changes

In order to understand how aging alone affects the expression of the Kv ion channel in the absence of any other confounding factor (hyperoxia), we examine protein profiles of Kv channel genes and their interacting proteins (Fig. 4a–d). Although no significant change was observed in protein levels of these genes in male mice, aging alone could significantly downregulated Kv1.4 and upregulated KChIP2 (Fig. 4a–d).

We also evaluated expression profiles of some of these key cardiac Kv channels along with myosin heavy chain (MHC) 6 and 7 in all mice groups after normoxia or hyperoxia treatment using real-time quantitative RT-PCR (qRT-PCR). When compared to normal air controls, qRT-PCR data from our current investigation revealed a significant reduction
of Kv4.2, Kv1.5, Kv2.1, MHC 6 and KChIP2 expression in the LVs of aged males after hyperoxia treatment (Fig. 5a–f). Similarly, transcript levels of Kv2.1, Kv1.5, and MHC 6 were significantly lowered in aged female rodents, whereas an increase in KChIP2 with no significant change in Kv4.2 expressions was observed after hyperoxia (Fig. 5a–f). On the other hand, hyperoxic exposure resulted in upregulation in the expression levels of Kv1.4 and MHC7 in both sexes (Fig. 5e and g). The investigated data highlighted gender-differences with aging as female rodents had lower expression of Kv channels (Kv 4.2, Kv 2.1, Kv 1.5, and KChIP2) compared to age matched males even at normal air (Fig. 5a–e).

We additionally evaluated if these transcript changes were reflected in the protein profiles of these aged rodents. Hyperoxic insult alters protein profile of Kv ion channel including Kv1.5, Kv4.2, KChIP2, and interestingly Kv1.4 as a significant downregulation was observed in aged male mice. This trend was also observed in aged female rodents (Fig. 6a–e).

### 3.6. Serum hormone profile

We performed ELISA to analyze if aging has any effect on serum testosterone and estrogen levels. Our data showed that serum testosterone levels were significantly lower in the aged group compared to young males at both normal air and hyperoxia (Fig. 7a). While female mice showed a significant reduction of serum estradiol levels in the aged group under normal air, no significant difference was observed in the hyperoxia treated group between aged and young female mice (Fig. 7b). Additionally, we also investigated the expression profiles of androgen receptor (AR) and estrogen receptor beta (ER) in aged as well as young mice hearts using western blotting. As the studies reported previously suggests role of ER-β on cardiac hypertrophy (Skavdahl et al., 2005) and expressed in cardiac myocytes without any sexual dimorphism (Groh et al., 1998), we examine the expressions of ER-β, specifically in this study. Our data further confirms that aged mice not only reduced serum testosterone and estrogen levels in their hearts but also reduced their receptor levels (Fig. 8a–c).

### 4. Discussion

This study determined the negative implications of hyperoxia on aged individuals and the variability in the hyperoxic stress responses in males and females. As hyperoxia increases myocardial oxygen stress, incidence of arrhythmias, disrupts coronary blood flow, fluctuates redox status of intracellular milieu, and generates free radicles that damage the linings of cardiac tissue (Farquhar et al., 2009; Lodato, 1989), it is imperative to study negative implications of hyperoxia in aged individuals at higher risk. We previously reported biochemical alterations in oxygen-sensitive voltage-gated potassium channels (Kv) leading to conduction abnormalities upon 72 h of hyperoxia exposure to young
male mice (Chapalamadugu et al., 2015; Panguluri et al., 2013; Rodgers et al., 2019b). In a comparative study, we have also determined that the impact of hyperoxia differs based on sex, as severe bradycardia and high mortality were observed in females rodents as opposed to males (Rodgers et al., 2019b). This study aims to compares multiple variables (sex, age, and treatment) and determines the risk factor having highest bearing to modify cardio physiology.

4.1. Advanced age as an independent risk factor affecting cardiac physiology

4.1.1. Aging affects physical changes irrespective of sex

Age-related body composition findings in our study showed a substantial weight gain for aged rodents as compared to young rodents (Fig. 1a). This association could be driven by the decrease in the basal metabolic rate and physical activity, which causes an increase in the
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total body fat (adiposity) and decreases the lean body mass (Kuk et al., 2009). Unlike aged male mice, aged female mice gained a significantly higher percentage of body weight (Fig. 1a). This peculiar weight gain in aged female sex over male was also reported by other studies (Misso et al., 2003; Rogers et al., 2009; Stubbins et al., 2012). The increase in body weight in aged females is, at least in part, attributed to the resulting estrogen deficiency with age as compared to estrogen availability in healthy-young females (Sullivan et al., 2016). Although the serum estrogen levels in aged female rodents had a minor yet significant fall as compared to young female mice (Fig. 7a), this could be a contributing element to weight gain. Consequently, downregulation of cardiac estrogen receptor beta (ER-β), due to estrogen deficiency in aged females was observed in this study (Fig. 8b). Similarly, the weight gain in the older male rodents (Fig. 7b), can be associated, at least in part, to the decreased testosterone levels, as some clinical studies do delineate a link between reduced levels of serum testosterone and increased obesity with age (Feldman et al., 2002; Kelly and Jones, 2015; Snyder et al., 2016). Subsequently, a significant reduction of cardiac androgen receptors in aged male rodents was observed as compared to the young male mice group (Fig. 8c).

As compared to the younger females, our analysis revealed a significantly smaller cardiomyocyte (CM) size for aged females (Fig. 1i), this decrease was also reflected in the overall heart cross-section area for aging females (Fig. 1d). A similar decrease in CM size was observed for aged males as compared to young males (Fig. 1h). Potentially, this cardiomyocyte shrinkage could be a result of the progressive apoptosis altering the morphology of the differentiated adult cardiomyocytes (Sheydina et al., 2011). With advancing age, there is an increase in cardiomyocyte apoptosis mainly induced by caspase-dependent pathways (Nitaхara et al., 1998; Olivetti et al., 1997). Taken together, aging not only affects physical parameters but also alters cardiac histopathology equally in males and females, increasing their susceptibility to cardiac diseases.

### 4.1.2. Aged males are more susceptible to age-induced cardiac dysfunction

Echocardiography was used to evaluate if changes observed in physical parameters are also reflected in cardiac functioning. Our data indicated that aging significantly increased %FS and %EF in aged female mice compared to their young counterparts, and this increase was also significant when compared with age-matched males (Fig. 2d and e). The increase in %FS and %EF in aged females compared to age-matched males was due to significantly lower LVID;s, with no significant change in LVID;d (Fig. 2b and c). This phenomenon observed in aged female mice is very similar to the condition called heart failure with preserved ejection fraction (HFpEF), which is prevalent in the majority of elderly patients, particularly women (Upadhya and Kitzman, 2017). In contrast, aged male mice showed reduced cardiac output (CO) compared to their young counterparts (Fig. 2g), which was also significantly lower than age-matched female. This reduced CO in aged male is due to reduced heart rates compared to the younger male group. Preserved CO in aged females could be due to increased %EF (Fig. 2e), which efficiently compensated for the reduced heart rates in the aged group compared to their young counterparts, which was completely missing in aged male mice. From these observations, it is evident that the male is at a higher risk for age-related cardiac functional abnormalities than the female.

### 4.1.3. Aged females are more susceptible for developing cardiac arrhythmias

We observed significant changes in the ECG parameters, predictors of cardiac physiology, due to aging alone (Fig. 3a–f). The PR interval reflects the time duration for an electric pulse to advance from the sinus...
increase in PR interval as compared to aged males (AF) (Bidstrup et al., 2013). In our data, aged females had a discernible transition (Hung et al., 2015), and most importantly, to atrial fibrillation—resulting from increased epicardial adipose tissue deposition (Atwater et al., 2019), underfeeding, reduced water intake, muscle wasting, and a slower recovery rate (Heyland et al., 2003; Reid et al., 2008). A significant weight loss post hyperoxia irrespective of sex was observed in aged rodents (Fig. 1a), consistent with our prior findings in young mice (Panguluri et al., 2013). Previous reports in rats, suggested short-term (2–4 h) as well as long-term exposure (60 h) to hyperoxia resulted in pulmonary edema characterized by an increase in the W/D ratio of lung tissue (Kawamura et al., 2013; Wang et al., 2020). Clinically, poor pulmonary outcomes are routinely observed in mechanical ventilated patients (Damiani et al., 2014). Similar to our previous reports in young rodents (Rodgers et al., 2021), lung edema was evident as a detrimental consequence of hyperoxemia in aged mice irrespective of sex (Fig. 1b). Furthermore, studies have investigated increase in lung W/D ratio to be an effect of decreased concentrations of pulmonary surfactants required to maintain alveolar surface tension (Jin et al., 2018; Wang et al., 2020). In males, we also observed age-based differences, as aged rodents were at higher risk of developing pulmonary edema compared to young rodents on hyperoxic exposure (Fig. 1b).

For young males, the cardiomyocyte area increases significantly, which is also reflected by the overall increase in heart size (Fig. 1d). This is consistent with our previously reported data for signs of protective and adaptive cardiac hypertrophy in young males (Rodgers et al., 2019b). However, this compensatory mechanism diminishes with age. A potential reason could be the decreased androgens with age, which play a pro-hypertrophic role by modulating intracellular calcium homeostasis (Kraemer et al., 2020). In preclinical studies on mice, treatment with finasteride, an anti-androgen, attenuated cardiac hypertrophy underpinning the critical role of testosterone (Zwadlo et al., 2015). For aged males, even though the magnitude of cardiomyocyte area tends to increase, it may not be significant enough to increase the overall heart area (Fig. 1d). Thus, for the first time, we report the absence of compensatory hypertrophy in aged males exposed to hyperoxia. In females, hyperoxia results in an overall increase in cardiomyocyte area for both young and aged females (Fig. 1f). This can be a consequence of transient homotypic fusion, which results in large cardiomyocytes as a response to counter hyperoxia-induced stress. Cardiomyocyte multinucleation and polyploidization have been documented as a self-protective response to hypoxia (Ali et al., 2020; Bergmann et al., 2009; Jiang et al., 2020). However, the occurrence of this phenomenon as a mechanism to regenerate cardiomyocytes from hyperoxia-induced damage requires additional exploration. Nevertheless, the total heart size only increases for aged females as the young female hearts to some extent may be protected from cardiac hypertrophy due to the presence of estrogen (Van Eickels et al., 2001; Wu et al., 2020). The relationship between increased cardiovascular risks and estrogen deficiency is well reported in epidemiologic studies (Eaker et al., 1993; Hayward et al., 2000; Rodgers et al., 2019a).

4.1. Alteration in Kv channels is pronounced in aged females

Voltage-gated potassium K+ channels (Kv) are responsible for cardiac repolarization, action potential signaling across the cell membranes, and maintenance of vascular tone. These channels are dramatically diminished in aged coronary arteries (Marijic et al., 2001; Sadan and Grably). This study only examines whether the conduction abnormalities could be correlated to the imbalances in Kv channels. A study investigated a causal link between prolonged QT interval associated with dysregulation of Kv4.2, in a genetically engineered transgenic mice carrying point mutation in Kv4.2 (Barry et al., 1998). Despite no significant decrease in gene expression of Kv4.2 with aging (Fig. 4d), aged females did demonstrate a considerable increase in KCNH2 (Fig. 4c), which modulates the surface expression of Kv4 channel and tends to increase the arrhythmogenic potential. The data also showed aged females have a significantly lower level of Kv1.4 as compared to young females (Fig. 4b), resulting in an increased cardiac action potential that was also reflected by prolonged QRS interval (Fig. 3d). We did not see significant changes in Kv channels for males, implying that the resulting conduction abnormalities were not predominantly contributed by potassium channels but may be due to other calcium or sodium channels or lipid peroxidation due to elevated ROS activity with age (Rizvi et al., 2021; Sovari, 2016), which remains a subject of intense investigations.

4.2. Effect of hyperoxia and aging on cardiac remodeling

4.2.1. Hyperoxia worsens physiological parameters in aged individuals

In ICU patients, body weight perturbation due to mechanical ventilation is a commonly reported physical change, as approximately 30% of the admitted patients lose more than 10 kg of total body weight or remained underweight post 12 months of discharge (Helliwell et al., 2006). A few plausible explanations for the rapid weight loss could be underfeeding, reduced water intake, muscle wasting, and a slower weight loss post hyperoxia irrespective of sex was observed in aged rodents (Fig. 1a), consistent with our prior findings in young mice (Panguluri et al., 2013). Previous reports in rats, suggested short-term (2–4 h) as well as long-term exposure (60 h) to hyperoxia resulted in pulmonary edema characterized by an increase in the W/D ratio of lung tissue (Kawamura et al., 2013; Wang et al., 2020). Clinically, poor pulmonary outcomes are routinely observed in mechanical ventilated patients (Damiani et al., 2014). Similar to our previous reports in young rodents (Rodgers et al., 2021), lung edema was evident as a detrimental consequence of hyperoxemia in aged mice irrespective of sex (Fig. 1b). Furthermore, studies have investigated increase in lung W/D ratio to be an effect of decreased concentrations of pulmonary surfactants required to maintain alveolar surface tension (Jin et al., 2018; Wang et al., 2020). In males, we also observed age-based differences, as aged rodents were at higher risk of developing pulmonary edema compared to young rodents on hyperoxic exposure (Fig. 1b).

For young males, the cardiomyocyte area increases significantly, which is also reflected by the overall increase in heart size (Fig. 1d). This is consistent with our previously reported data for signs of protective and adaptive cardiac hypertrophy in young males (Rodgers et al., 2019b). However, this compensatory mechanism diminishes with age. A potential reason could be the decreased androgens with age, which play a pro-hypertrophic role by modulating intracellular calcium homeostasis (Kraemer et al., 2020). In preclinical studies on mice, treatment with finasteride, an anti-androgen, attenuated cardiac hypertrophy underpinning the critical role of testosterone (Zwadlo et al., 2015). For aged males, even though the magnitude of cardiomyocyte area tends to increase, it may not be significant enough to increase the overall heart area (Fig. 1d). Thus, for the first time, we report the absence of compensatory hypertrophy in aged males exposed to hyperoxia. In females, hyperoxia results in an overall increase in cardiomyocyte area for both young and aged females (Fig. 1f). This can be a consequence of transient homotypic fusion, which results in large cardiomyocytes as a response to counter hyperoxia-induced stress. Cardiomyocyte multinucleation and polyploidization have been documented as a self-protective response to hypoxia (Ali et al., 2020; Bergmann et al., 2009; Jiang et al., 2020). However, the occurrence of this phenomenon as a mechanism to regenerate cardiomyocytes from hyperoxia-induced damage requires additional exploration. Nevertheless, the total heart size only increases for aged females as the young female hearts to some extent may be protected from cardiac hypertrophy due to the presence of estrogen (Van Eickels et al., 2001; Wu et al., 2020). The relationship between increased cardiovascular risks and estrogen deficiency is well reported in epidemiologic studies (Eaker et al., 1993; Hayward et al., 2000; Rodgers et al., 2019a).

4.2.2. Distinct functional abnormalities between males and females after hyperoxia

Although aged female mice showed conditions similar to heart failure with preserved ejection fraction (HFpEF) due to preserved systolic function under normal air compared to age-matched males as well as their young counterparts (Fig. 2), hyperoxia significantly reduced %FS and %EF in aged females compared to age-matched males as well as their young counterparts (Fig. 2d and e), which is due to systolic dysfunction in hyperoxia treated aged female mice (Fig. 2c). Although hyperoxia significantly reduced SV and CO in all groups irrespective of age and sex when compared to their normoxia controls, aged mice (both male and female) after hyperoxia treatment showed improved SV and CO than their young counterparts due to a significant increase in LVIDd (Fig. 2b). A clinical study also reported significantly higher stroke volume in older participants than younger participants, but showed a decrease in cardiac output due to reduced heart rates (Houghton et al., 2016). As we know that hyperoxia causes bradycardia (Fig. 3b) in both young and aged mice (Panguluri et al., 2013; Rodgers et al., 2019b, 2021), the overall increase in the SV in aged group significantly
increased CO as well in these aged mice compared to young ones in our study.

4.2.3. Hyperoxia induces transcript changes in the molecular markers of hypertrophy

Early development of cardiac hypertrophy has been reported due to hyperoxia (Greco et al., 2019). The qRT-PCR data showed a significant increase in the mRNA expression of hypertrophic markers (MHC-6 and MHC-7) in the left ventricle of the young mice (Panguluri et al., 2013). Here we report downregulation of MHC-6 and elevated expression of MHC-7 (Fig. 5f and g) in aged mice, suggesting young mice behave differently to oxygen exposure as compared to aged mice. The relative expression of the two isoforms of MHC can be altered during cardiac hypertrophy as a shift from MHC-α to MHC-β was observed in rodents and human hearts (Jones et al., 1996; Miyata et al., 2000). This shift also occurs as an adaptive response to hyperoxia in order to conserve energy, as MHC-β has lower adenosine triphosphatase (ATP) activity due to low filament sliding velocity (Holubarsch et al., 1985). However, this event decreases the contractile function, predisposing individuals especially those with advanced age, to disease conditions like left ventricular (LV) outflow tract (LVOT) obstruction associated with cardiac hypertrophy (Krenz and Robbins, 2004; Slama et al., 2016).

4.2.4. Hyperoxia worsens electrical remodeling and gene expression profiles in females

The activity of the Kv channels is modulated by the pyridine nucleotides NAD(P)/NAD(P), major intracellular redox modulators, and hyperoxia alters the cellular redox balance triggering cardiac ion channel disturbances leading to arrhythmias (Chapalamadugu et al., 2015; Panguluri et al., 2013). The development of arrhythmias in young mice due to hyperoxia has been previously investigated (Rodgers et al., 2021), and here we extended these findings by studying and reporting electrical remodeling in aged mice. Under hyperoxia, we observed prolonged PR intervals regardless of sexes in aged rodents (Fig. 3c). This corresponds to a higher risk of developing atrial fibrillation, which manifests molecularly as decreased expression of the Kv1.5 channel at transcript and protein level with hyperoxia (Figs. 5c and 6d). We observed gender disparity as aged females had longer PR intervals as compared to age-matched males under hyperoxia, this was not observed in young female rodents with similar PR prolongations as compared to males. Another group examining sex-based mRNA expression changes in human cardiac tissue reported compromised function of Kv1.5 in aged females as compared to males, suggesting women have higher odds of developing atrial fibrillation with age (Ambrosi et al., 2013; Olson et al., 2006). The observed increase in RR interval in aged mice (Fig. 3b), clinically translates to changes in the vagal tone associated with heart rate variability (HRV), allowing for the prediction of poor outcomes associated with hyperoxia (Gonder et al., 1979). Another observation on hyperoxic insult was the presence of longer QTc interval in aged female rodents than aged male rodents (Fig. 3e). This complication adds to an increased risk of developing Torsade de points (TdP), a polymorphic ventricular arrhythmia in aged females (Baracaldo-Santamaría et al., 2021). The prolonged QRS-interval and JT intervals, predictors of intraventricular conduction delay associated with intraventricular arrhythmias (Alfraidi et al., 2019), at least in part, linked to the down-regulation of Kv4.2, Kv1.4, and its auxiliary subunit KChIP2 at protein and transcript levels with hyperoxia (Fig. 6). Results given from our present data indicate that cardiac repolarization and progression of cardiac arrhythmias is linked with hyperoxia exposure and advanced age, especially in women, increases the probability to develop cardiac abnormalities.

4.2.5. Statistical correlation

To determine the mathematical impact of the risk factors studied (hyperoxia, sex, and age), a Multi-Way Analysis of Variance (ANOVA) was performed. From our analysis, we determined physical parameters including body weight and cardiomyocyte size; RR, QRS, and JT from electrophysiological parameters were influenced by all three risk factors (Table 1). Among the risk factors, treatment (hyperoxia) had the highest statistical significance, followed by age, and sex. Moreover, for almost all groups, correlation analysis indicated a positive correlation between electrophysiological parameters and lung wet/dry weight ratio, whereas a negative correlation was observed between body weights and electrophysiological parameters (see Supplemental material), indicating a possible influence of lung edema on cardiac electrophysiology. Taken together, this data suggests that hyperoxic treatment has direct implications on cardiovascular remodeling and function, which may augment inherent cardiac challenges with advanced age and sex differences.

5. Conclusion

Although oxygen supplementation is a standard treatment for treating hypoxia in the ICU, it is associated with physiological and cardiovascular risks. We previously established that hyperoxia associated pathophysiological events occur in a time-dependent manner in young mice. In this study, we provide a new insight by comparing pathophysiological changes between aged and young mice of both sexes under normoxia and hyperoxia exposure. This research found that age-influenced cardiac physiology is distinct between males and females. Although physical parameters are affected equally by aging in both sex, functional abnormalities are more severe in males than females. Whereas electrophysiology revealed that vulnerability of aged female compared to the age-matched male for the early development of cardiac arrhythmias due to atrioventricular and intraventricular abnormalities.

When we examine the impact of hyperoxia on cardiac pathophysiology, it is evident that hyperoxia-induced pulmonary complications are significantly higher in aged male compared to their young counterparts. Hyperoxia-induced cardiac hypertrophy that was observed in young male mice was completely absent in aged mice, while estrogen conferred protection against hyperoxia-induced cardiac hypertrophy was absent in aged females. As reported in human studies, our mice data also showed that hyperoxia significantly improved stroke volume in the aged group compared to their young counterparts. Hyperoxia-induced cardiac repolarization defects and arrhythmias are found to be significant in both sexes, which gets worse with advanced age especially in females with high frailty to develop cardiac defects. Statistical analysis further confirms our hypothesis that hyperoxia has the maximum impact on observed cardiac pathophysiology, followed by age and sex. Taken together, this data suggests that hyperoxic treatment has direct implications on cardiovascular remodeling and function, which may augment inherent cardiac challenges with advanced age and sex differences.

Data Availability

Data will be made available on request.
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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mad.2022.111727.

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