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Effect of Hyperoxia on Cardiac Pathophysiology in Female Guinea Pig Hearts

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by

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ABSTRACT

Hyperoxia is widely implemented in critical care and ICU patients. The administration of a high concentration of inspired oxygen to the lung can unknowingly cause hyperoxia and thereby damaging the lungs and heart due to oxidative stress. Technically, hyperoxia occurs when the patient receives PaO2 > 200 mmHg. Major research focused on hyperoxia-induced lung injury, but nothing is known on its effect on heart. Our lab is the pioneer in understanding the effect of hyperoxia on cardiovascular remodeling using mice model. Previous results show that mice under 72 hours of hyperoxia present severe cardiac pathophysiology. This research uses Guinea pigs, which are better animals in terms of physiological similarities to humans. The study aims are to unveil the pathophysiology of the cardiovascular system concurrent with the age and sex that influence the outcome led to clinical use in the future. In this study, we exposed year old age, female guinea pig (n= 12) assessing the physiology, functional, and electrical data (ECG).

We found that hyperoxia groups show a severe reduction of body weight and severe lung edema. Comparing to male Guinea pigs, females show more severity considering lung edema, weight loss, and some ECG parameters including a decrease in QTc, PR interval, and increase in RR interval. In Guinea pigs even though some echocardiogram parameters are similar to the mice reports (LVIDs increase and CO, SV, %FS decrease), ECG data presents interestingly in a different trend. Since PR, QTc, JT, and QRS intervals in mice increase significantly but in Guinea pigs, QTc and PR intervals decrease significantly.

Even though the significant severity effect from hyperoxia to Guinea pigs are occur similar to mice. The distinct expression between species suggests that each species has a unique response when exposed to high oxygen level. This data is another step toward

expanding our boundaries in hyperoxia exposure in Guinea pigs in aspects of sex and species comparison and will pave the way for potential capabilities for precision medicine.

CHAPTER ONE: INTRODUCTION

1.1 Hyperoxia

One of the most essential treatments used to treat patients in Intensive Care Units (ICUs) around the world is oxygen therapy. Oxygen therapy assists patients in recovering from breathing obstructions and maintaining equilibrium. Since oxygen is required for all cells to function, administering additional oxygen as a treatment is always the primary curative therapy for many diseases, especially in respiratory disorders COVID-19 [1], or especially with patients in ICU conditions, for example, traumatic brain injuries [2], cardiac arrest [3], patient in the emergency department [4], Patients who present with respiratory distress are already weak due to their conditions; introducing the burden of hyperoxia will aggressively accelerate the progression of their pathology, increasing the severity of their conditions, and increasing their mortality rate [2-5].

Oxygen is the most common crucial gas for human, and one of the key reasons why oxygen treatment is so popular is because oxygen gas is inexpensive [5]. the other side of oxygen therapy that is casting a shadow on this treatment is hyperoxia, which should be highlighted. Many studies have shown the deleterious effect of hyperoxia and one recent evidence in 2021 recommends that PaO_2 level should not higher than $PaO_2 > 300$ mmHg (40 kPa) [6]. However, the specific optimal level of hyperoxia is remained a mystery. This might be because currently, the research for hyperoxia is mainly from heterogenous and observational study which gives an unsubstantial result [7]. To have more tangible information to support the definite terms, the hyperoxia study should be more conducted on a larger scale, randomized, or a controlled clinical trial [7].

Even though Hyperoxia can be simply explained as the partial arterial pressure (PaO_2) is greater than the normal level of the patient's age when breathing normal air [8]. It is simply characterized by the stage in which oxygen level in tissues and organs excess the normal range which should be between 92%-96% [9]. Various research over the past century, lacking the precision of today's advanced technology, provided contradictory data on the effects of oxygen therapy in normoxic cardiac patients. Although, supplemental oxygen is commonly used in cardiac patients. In these scenarios, errant hyperoxia normally appears because of fears to guarantee adequate oxygenation and also because hyperoxia is not considered to be detrimental [10].

Until recently, the data from acute hypoxemia patients treated with oxygen therapy are still limited [11]. This might be because the increases in blood oxygen saturation level are not detected when it approaches 100% and oxygen pressure is not monitored closely, particularly in the case of high-flow oxygen therapy [10]. Clinical data shows that oxygen toxicity will take at least 24 hours to develop effects on lung tissue [8]. According to a study in murine, after 72 hours of hyperoxia exposure, the gender differences cardiac-pathological is presented [12]. ICU stays for patients are typically 2.2 days [13].Expected observe a similar pathological progression, in this study we conducted 72 hours of hyperoxia exposure. The ARDS network which is a clinical research network of hospitals and clinical sites suggests the treatment of choice for hyperoxia that targeting the conservative oxygen therapy from arterial hemoglobin oxygen saturation at 88-95% to avoid hypoxemia [7]. Study shows that patients who are given 96% oxygen (hyperoxic) present an accumulation of lung fluid, which causes pulmonary dysfunction and oxidative stress in the heart [14-16]. One of the most effective strategies for avoiding hyperoxia is as follows: Only a physician should prescribe oxygen treatment, and whenever oxygen is administered, it should be titrated for target oxygen saturation and arterial PaO_2 level first. The pulse oximeter, which is now widely available, should be used to monitor

the patient while he or she is receiving oxygen treatment; this strategy will securely protect the patient from hyperoxia and its harmful outcomes [8].

Even though many patient guidelines support O_2 administration, The pathophysiology of hyperoxia is remains a hotly contested and poorly understood topic. We expect to discover full pathophysiology of heart exposed to hyperoxia, as well as the effects of age and gender on hyperoxia-induced cardiac remodeling and damage, particularly from functional and electrical profile, by utilizing an animal model. The results of this study will enhance clinical outcomes in intensive care units or even other cases that require oxygen treatment. Until recently, the majority of hyperoxia literature concentrated on lung injury, with little research into cardiovascular toxicity. As the heart and lung are known to work collaboratively, pulmonary function disruption will also affect cardiac performance. Our lab is the first to use a mouse model to study the effects of hyperoxia on cardiac pathophysiology, yielding major findings with therapeutic significance. The previous study has shown clearly that hyperoxia plays a major role in cardiac pathophysiology starting from the electrical, functional, and biochemical result that has altered during hyperoxia assaults. Although Mice are common use as an animal model in cardiovascular research, their fast heart rate, small size, short life span, and variances in cardiac ion channel expressions and contractile function make Guinea pigs become more ideally compatible to demonstrate the animal model in this research. Because some of their significant parameters are like humans, such as ventricular action potential, lipoprotein profile, and cholesterol metabolism [17], Guinea pigs are regarded as the ideal animal model for studying myocardial damage for hyperoxia attacks.

Understanding the specific mechanism of hyperoxia-induced cardiomyopathy is valuable not only for understanding disease development and progression but also for launching new opportunities for therapeutic strategies.

1.2 Pathophysiology of Hyperoxia to Lung

Hyperoxia can impact the body system through a plethora of mechanisms. Because when individuals inhale air into their lungs, the entire surface area of the lung epithelium is exposed directly to O_2 and other inhalation agents [18]. Hyperoxia has a consequence not only for both acute and chronic lung injuries but also on the Reactive Oxygen Species (ROS) that are produced after hyperoxia exposure which can attack the pulmonary cells and eventually lead to cell death [19].

Beginning with the early years of our lives, when newborns are exposed to hyperoxia, exposure to a high level of oxygen concentration release ROS, which then inhibits the development of lung vascular endothelial cells. which interrupt alveolar formation and block capillaries from developing, as well as poor vascularization and alveolarization this pathological characterization was diagnosed as Bronchopulmonary Dysplasia (BPD) [20, 21]. BPD is the most common newborn chronic lung disease, which can affect up to 15,000 or 43% of premature newborns case in the US, This histopathologic condition is caused by oxygen toxicity and ventilation-related lung development dysregulation [16, 18, 21]. There is no treatment available for BPD as of 2021 [21]. BPD has evolved into a lucid model for studying the chronic pulmonary effects after hyperoxia exposure. BPD effect both morphological and molecular changes. In terms of morphological changes, When the lungs are exposed to hyperoxia, the formation of free radicals induces an inflammatory response, which results in morphological abnormalities [22]. This abnormality includes a weakening of the alveolar architecture, resulting in fewer alveoli and a smaller surface area for gas exchange, followed by mild airway smooth muscle thickening as well as alveolar septal fibrosis that has developed [22, 23]. When considering the molecular alterations pathway, this is primarily focused on the plethora of pro-inflammatory that has been altered because of hyperoxia exposure. First, the Transforming Growth Factor β1 (TGFβ1), The study of expression levels

of Tgfb1 mRNA increased after hyperoxia in rats and proved to be related to alveolar interstitial fibrosis [24], Second, Interleukin-6 (IL-6), IL-6 is a protein that release in response to inflammation caused by significant DNA damages and cell death [25]. Since elevated IL-6 concentrations are related to the progression of BPD, IL-6 expression can serve as a reliable biomarker of BPD [26]. Third, the vascular endothelial growth factor (VEGF), VEGF is a growth factor that is abundantly expressed in the lung and appears to be necessary for the proper formation of alveolar tissue [27]. At 14 days old, rat pups treated with hyperoxia have significantly reduced VEGF mRNA levels in their lungs [28]. Another study shows that seven miRNAs that may have a major role on the development of BPD were shown to be deregulated in neonatal lungs exposed to hyperoxia (five are down-regulate and two are increasing) [20].

The majority of BPD animal model investigations are directly linked to hyperoxic exposure, It is crucial to design an animal model that demonstrates the same level of lung injury to fully understand the impact of hyperoxia in animal studies of BPD [21]. However, when selecting an animal model for BPD research, researchers must consider all these factors including strain, sex, and species. In human, gender differences have influenced the severity of BPD since males have a higher risk of disease progression when compared to the same age [29]. Additionally, there is the significant physiological differences between species. For example, in a healthy newborn baby, the saccular stage occurs entirely in utero of a mother. Prematurely born humans, whose lungs are in the saccular developmental stage are at an increased risk of problems from pulmonary immaturity and surfactant defect [30, 31]. Rodents, on the other hand, are born surfactant-sufficient, which reduces their risk of respiratory hardship during hyperoxic exposure [31]. Rabbits, unlike rodents, begin the alveolar stage of lung formation in utero, just like a complete human newborn [32, 33]. But it may not be as valuable to study this process in the complete rabbit, since the alveologenesis has already

started and rabbits are more expensive than rodents, thus there are strong rules and regulations in place to make sure they are handled, cared for, and treated properly [34]. Rodents eventually, serve as the only animal studies born during the saccular stage of lung development, which makes them suitable for imitating the characteristics of BPD in neonates [21].

Not only, long-term mechanical ventilation in associated with hyperoxia can cause lung injury. one study found that even though general anesthesia is typically used for a short period of time, it has a negative effect and damages the lungs [35]. Exposure for only 4 hours in rat's model. Hyperoxia can induced oxidative injury, SP-C and SP-D levels in the lung tissue homogenate and BALF were dramatically decreased [35]. This furthered the progression of pulmonary edema and respiratory dysfunction.

Despite their many advantages from a morphological and physiological standpoint, research using animal models alone cannot address all challenges; human-derived model systems are also necessary [21]. Furthermore, the majority of recent pre-clinical hyperoxia investigations concentrate on morphological characteristic to assess lung damage, which is rarely reflective of the changes that occur within the alveoli. Since hyperoxia-induced lung damage alters a plethora of intracellular signal transduction pathways that control various processes during the embryonic stage, which in turn leads to abnormal lung development. Analyzing gene expression will provide useful insights into when molecular damage occurs [21]. This will allow researchers and clinicians about when will pharmacological treatments be most effective by providing a point of correlation to genes affected in the neonate [21].

1.3 Oxidative Stress in the Lung.

Oxygen therapy has been used in all living things on the basis that our bodies must maintain a balance of oxidants and antioxidants. Normally, the body's cells maintain a balance between oxidants and antioxidants. When this balance is maintained, free radicals do not harm the body. However, this balance can be disrupted, which is known as oxidative stress. [16, 36].

Oxidative stress is the imbalance that results from too much ROS (Reactive oxygen species) or oxidants in comparison to the cell's ability to mount a successful antioxidant response [37]. ROS are formed by the cells when molecular oxygen (O_2) is reduced to water (H_2O), it primarily occurs under disease conditions or in macromolecular damage [37, 38]. ROS have been tied to hyperoxia since oxygen is a major factor in the production of ROS, Persistent hyperoxia exposure will results in an increase in the production of ROS, This increment is directly disrupting physiological homeostasis and, consumes all the enzymes and antioxidants that produced in the body causing cell and tissue damage [39, 40].

ROS that releases from prolonged oxygen exposure can lead to a variety of pathological alterations in the lungs, which eventually results in pulmonary toxicity. This is because the lungs are the first-line receptors that are directly exposed to all inhalation particles [41]. Retrieving the excessive oxygen intake can lead to lung injury, Hyperoxia-induced lung injury can be described by the induction of an extensive inflammatory response by increasing levels of pulmonary proinflammatory mediators such as NF-B and massive leukocyte infiltration [42].This will pose significant damage to the alveolar-capillary barrier, resulting in impaired gas exchange and pulmonary edema and eventually leads to pulmonary cell apoptotic [43, 44].

1.4 Pathophysiology of Hyperoxia to Heart.

Cardiac remodeling is a term to describe a collection of cellular, molecular, and interstitial changes that, occurs after cardiac injury. It alters the size, weight, structure, and function of the heart. According to the process' link to ventricular dysfunction and arrhythmias, the prognosis is poor [45]. The cause of cardiac injury is various starting from toxic, inflammatory to pressure or volume overload [45].

It is commonly known that ventricular arrhythmias, such as prolonged ventricular tachycardia and ventricular fibrillation, are related to cardiac remodeling [45]. There are three basic mechanisms related to this. The first mechanism is changed in gap junctional intercellular interaction, which is responsible for communication between cells and, thus, electrical linkage. Although it is usually located within an intercalated disc, remodeling causes a decrease in labeling density as well as a realignment of the protein along the lengthy ends of the cell. This process will further result in QT interval prolongation and cardiac arrhythmia [45-47]. The studies show that hyperoxia can induce an arrhythmia with a prolonged QTc as well as action potential length [48]. The second mechanism includes ion channel changes, such as sodium channel inhibition, calcium, and potassium channel changes, and sodium/calcium exchanger mechanism changes [46, 47]. The third mechanism is about cardiac remodeling and the increasing of collagen content or fibrosis that are proved to be related. Fibrosis may cause electrical conduction obstruction and relapse arrhythmia. There is supportive evidence that linked fibrosis to sudden death and heart arrhythmias [49].

1.4.1 Ventricular Remodeling

Hyperoxia, related to BPD, increased pulmonary vascular resistance (PVR), which causes an increase in pressure overload on the right ventricle. This is one of the cardiovascular pathophysiological effects of BPD. When Right Ventricle (RV) hypertrophy

and failure potentially result from this increased pressure load on the right ventricle, it will initially increase ventricular thickness as a compensatory response [50-53].

Practically Echocardiography is one of the most commonly used methods for clinical detecting morphological changes in cardiac remodeling [54]. Another laboratory-based diagnostic method is the detection of cell markers, this is because cardiac remodeling presented by the re-expression of fetal genes. Several markers, including changes in the expression of myosin heavy chain isoforms, with an increment in alpha- and a reduce in beta-myosin heavy chain (MHCα and MHCβ), may reveal a remodeling process [55-57]. We have previously shown that hyperoxia triggers MHC6 and MHC7, which then produces inflammatory cytokines that cause abnormalities to NFkB and metabolism [58].

MHC6 and MHC7**:** Studies of myosin filaments (proteins in cardiac muscles) have proven that, thru the changes in hemodynamics and cardiac output, Hyperoxia can generate cardiac structural remodeling, which in turn can lead to cardiac dysfunction and heart failure [15]. In the adult mice hyperoxia group, levels of MHCα/MHCβ mRNA, as well as proteins, were markedly increased, these results are further supported by the increased LV wall thickness and overall cross-sectional area by cryostat section with H&E staining [15]. Another study shows that adult mice adapted to hyperoxia circumstances differently from newborn mice, In newborn mice there are higher expressions of MHCα than MHCβ [15]. This research represents myosin heavy chain expressed differently between age.

The heart's myosin filaments are made up of α and β subunits. In the healthy cardiac tissue of rodent heart, MHCα levels are more prominent than MHCβ level because, in comparison to MHCβ subunits, MHCα subunits have higher ATPase efficiency and contractile strength. Cardiac enlargement can be indicated by a reduction in MHCα/MHCβ ratio. [59, 60] Similar to prior research and as expected given that structural remodeling will have an impact on cardiac output and other hemodynamic alterations, The adult heart subjected to

hyperoxia in our previous experiment showed a significant reduction in heart rate and cardiac output , and the newborns that had been exposed to hyperoxia also exhibited cardiac dysfunction, as evidenced by a marked increase in LV end systolic thickness and a decline in fractional shortening(FS) [58, 61-66].

1.4.2 Electrical Remodeling

Electric remodeling is a defining characteristic of several myocardiopathy, This results in left ventricular hypertrophy and heart failure. [15, 67].It is known that potassium channels control the pattern and length of the action potential [15].

Kv4.2: Kv4.2 and Kv1.5, two of the potassium channels, respond to alterations in oxygen [68, 69]. The Kv4.2 (Voltage gate potassium channel: Kv4.2), is the principal ion channel that supports the heart in retaining the repolarization capacity among the multiple potassium channel family (Kv1–12) members [15, 70]. We previously demonstrated that in mice in hyperoxia conditions, Kv4.2 expression critically declines just the same as protein articulation takes place [58].

Kv1.4: Heart failure frequently manifests in mechanical and electrical dysregulation with decreased Kv4.2 expressions and elevated Kv1.4 expressions [71, 72]. Previous research found that STZ-induced diabetic rats had lower Kv4.2 mRNA and protein levels while having higher Kv1.4 transcripts and protein levels [72].Our laboratory demonstrated for the first time for the adult mice heart under hyperoxia condition that Kv4.2 and Kv1.5 transcripts , as well as protein expression are reduced significantly in comparison to normoxia controls however, there is no significant change found in Kv1.4 [48]. Another study shows the relationship between this important Kv channel that after hyperoxia exposure there was an increase in Kv1.4 and decreasing in Kv 4.5 [58, 71]. Early literature has shown that both Kv4.2, as well as Kv1.5, are oxygen susceptible. Kv1.5, in addition to Kv4.2, the gradually inactivating potassium channel, is also an important indicator of action potential duration in ventricular

myocytes, and its reduced expression is intended to decrease cardiac repolarization reserve.[73-75]. It was found that Kv1.5, Kv4.5, and KChIP2 cause severe abnormalities in rodent Kv channels after hyperoxia exposure, causing morphological anomalies and changing the duration of action potentials [58]. We previously study the expression of the Kv1.5 gene in the heart of diabetic (db/db) and hyperoxia-treated mice, and we found that Kv1.5 is significantly decreased in both of these conditions. because of Kv4.2's inhibition [48].

Since transcriptional factors are important through gene regulation at the molecular level, particularly during disease states. We should further evaluate the expression levels of transcription factors that can regulate oxygen-sensitive genes (Kv4.2 and Kv1.5), such as 1. homeobox transcriptional factor Iroquois protein 5 (Irx5), 2. nuclear factor kappa B (NFB), 3. GATA, 4. Myocyte enhancer factor-2 (Mef2), 5. C-terminal binding protein (CtBP), and 6. SiRT1. In mice, the findings indicate that hyperoxia induce left ventricular remodeling as well as hypertrophy, and stimulates MHC6 and MHC7, which produce inflammatory cytokines that cause NFkB and metabolic disorder. It was discovered that Kv1.5, Kv4.5, and KChIP2 lead to severe anomalies in rodent Kv channels, causing morphological discrepancies and altering the length of action potentials [58].

1.5 Oxidative Stress in the Heart.

Since the heart and lungs are known to work synergistically, alterations in heart function will influence pulmonary function, and likewise. Exposure to hyperoxia results in vasoconstriction and an increase in microcirculatory heterogeneity, which compromises perfusion [76]. Vascular constriction of coronary vessels reduces the amount of oxygen delivered to the heart muscles, increasing the risk of ischemia, and also reducing the contractility of the heart muscles [77]. Through the agonistic effect of L-type Calcium channels and the closure of the ATP-dependent potassium channels, the increase in ROS negatively impacts the cells of smooth muscle in the vessels [76, 77] The heart may also be directly

inotropic ally affected by hyperoxia. Sarcoplasmic reticular and sarcolemma Calcium release pathways can be altered by ROS to stimulate intracellular Calcium discharge, which leads to cell damage [41]. This, in turn, generates inflammatory conditions such as cardiac arrest [78].

The effects of hyperoxia, known as oxygen toxicity, can be brought on by a variety of factors, such as atmospheric pressure, FiO_2, exposure time, and cumulative oxygen dose. One key indicator of oxidative stress following hyperoxia exposure is the percentage of inspired oxygen (FiO_2) in blood samples, which simply indicates that the quantity of antioxidants is out of balance. [79]. Since the body has various defense setting to reduce the damage of ROS by protecting, and healing the harmed tissue, other defense enzymes besides FiO_2 can also be an indicator of oxidative stress. These defenses consist of non-enzymatic substances like glutathione, vitamin A and E, and albumin as well as enzymes like glutathione peroxidase, superoxide dismutase, and catalase [80].

1.6 Cardiac Injury in Animal Subjects

The swine model shows that hyperoxia reduced myocardial oxygen level decreases regional wall motion, and eventually worsens cardiac ischemia [81]. Since mice may not be a good animal model because of their physiology and our lab which pioneering in investigating the effect of hyperoxia in mice for several years. We had a lot of experiments and data in mice, and We aimed to move from mice to another level of animal that shares more similar anatomy and physiology to humans than the mice. Guinea pigs, interestingly, are the currently suitable animal model the best fit for our lab because they share many characteristics with humans, such as, lipoprotein metabolic activity and hepatic enzymatic activity replicate the human enhances the possibility of utilizing them as model types to understand molecular mechanisms and metabolic pathway alterations [17]. A previous study in Hamster founds that hyperoxia-induced maldistribution of the perfusion in the vascular system [82]. There is strong evidence that coronary blood flow decreases in response to hyperoxia, but it is unclear whether the cause

comes from the form of biological autoregulation or a harmful pathological process that leads to tissue ischemia [83]. We aim to obtain more data to clarify this pathophysiology by using Guinea pigs as an animal model.

Not only because of its physiological resemblance to a human, the size of Guinea pigs are also big enough for the hyperoxia experiment in an airtight chamber available in our facility, unlike other larger mammals such as pigs and dogs, which may require larger chambers and more room. In this study, we choose a year-old age, according to Charles River Laboratory, currently, there is no exact mathematical formula to calculate the Guinea pug age compared to humans. Since Guinea pigs are mature much faster than human they were born and be able to walk and weaned at a young age whereas a human cannot, a rough estimate one-year-old, female guinea pig represent approximately 50-year-old age in human. Study reveals that the median age of patients admitted to cardiac intensive care units was 65 years (25th and 75th percentiles, 55-75 years) [13] .

In a molecular level, Kv channels yield a plethora of opportunities for the innovation of novel pharmaceuticals for cardiovascular disease [70]. It comprises of total 12 major families and 40 subgroups [70]. When expressed alone, several of these genes might not express functional channels because they remain electrically silent [84]. Besides that, every gene expression has manifested differently in each species, with some Kv are prominent in one species but not in another. These variations are important because they are related to arrhythmia susceptibility [85]. From a molecular basis, each type of K+ channel has unique kinetic and modulation, allowing control over specific action potential [86]. Key ion channels for Guinea pig heart are including Kv1.4, Kv4.2, Kv4.3, Kv2.1, Kv2.2, ERG, and Kv LQ (Kv1.4 and 4.2 are previously discussed in 1.4.1section),

Kv4.3: Kv4.3 is found in the heart, CNS (cortex and cerebellum), ventricular and atrial myocytes, and smooth muscle[70]. According to studies, For guinea pigs' Kv4.1, Kv4.2 and Kv4.3 mRNA levels were not detectable but they were present in rabbits [85].

Kv2.1, Kv2.2, and Kv4.2: According to the study, Kv2.1 channels have a crucial physiological role in controlling the excitability and contractility of guinea pig detrusor smooth muscles (DSM) [84]. DSM is the primary muscle of the urinary wall, its function as a contraction and relaxation during filling and urination [87]. Compared with the smooth muscle cells (SMCs) of the heart, Smooth muscle is characterized by short transverse striations that run through cardiac muscle cells as well as vascular at urogenital muscular layers [88]. Atherosclerosis, hypertension, and myocardial infarction all involved the function of vascular SMCs [88].

ERG and KvLQ: There are well-studied that KvLQT1 and MinK genes work collaboratively, KvLQT1 is known to generate weak, rapidly activating currents when MinK is not present, but when KvLQT1 and MinK are co-expressed, robust currents with I_{Ks} features are formed [89, 90]. There are three types of Kv channels: slow (I_{Ks}), rapid (I_{Kr}), and ultra-rapid (I_{Kur}) delayed rectifier [86]. The pore-forming subunits of I_{Kr} and the slow part of I_K (I_{Ks}), alternatively, are generated by alpha-subunits encoded by the ERG (ether-a-go-go-related gene) and KvLQT1 genes, which highlights the importance of ERG and KvLQ [89, 90]. Studies imply that I_K is probably of equal relevance in the human heart as it is in the heart of other species [91]. Guinea pigs had higher levels of both the MinK concentration and expression than either humans or rabbits reported [85]. Humans had the highest KvLQT1 concentration, and their protein expression was approximately 2- and 7-fold higher than that of rabbits and guinea pigs, respectively [85]. Because of variations in epitope sequence, ERG1 expression across species could not be compared, yet ERG1 concentration was higher in humans [85].

It can be deducted from the passage that significant interspecies differences in K channel expression exist and may be the cause of the guinea pigs heart's lack of transient outward current , due to Alpha-subunit transcription, as well as the guinea pig's large slow component of the delayed rectifier current (strong MinK expression) [85].

1.7 Aging and Sex-Related Hyperoxia.

Recent findings evaluating the effects of hyperoxia on male and female wild-type mouse heart revealed that female mice are more susceptible to hyperoxia-induced pathology than male mice, with 50% mortality after 72 hours of hyperoxia exposure [12] Another study found that aged females mice exposed to hyperoxia have more severe electrophysiological disturbances than males [92]. Furthermore, Gender differences in cardiac repolarization have been documented in a variety of species, for example, humans [93], murine [12], or mice [94] According to reports on mice, the female was more resistant to cardiac remodeling and rhythm disorder than males, the reason behind this is related to sex hormones [95]. Sex differences in Guinea pig is another further step of the animal model to represent the pathophysiology of heart affected by hyperoxia exposure. This study will further investigate females form previous data in our lab that conduct in Male last year, to thoroughly understand the differences between both sexes. Since sex differences show independently cardiovascular risk factors [12].

From Echocardiogram data in healthy Guinea pigs , female has smaller LV internal diameter at end-diastole compared to the male but fractional shortening are higher [96] and Age of Guinea pig has a positive correlated with LV wall thickness when measuring end-diastolic and the aortic diameter [96]. In this research, not only the echocardiogram were use, we also check electrocardiogram (ECG) under hyperoxia condition to thoroughly assess the pathophysiology during this condition.

Similar model of our study, the previous research of Dunkin Hartley Guinea pigs aged 5 -15 months has served as a model to study human musculoskeletal aging [97] , this study used 12

months (1 year) to represent the aged effect of cardiovascular disease. Since the mean age of the patients admitted to an ICU, and ventilated patients in the ICU has been estimated to be old age-related [98]. Understanding the etiology of hyperoxia-induced cardiac dysfunction is important, especially in a high-risk population.

Using Guinea pigs as an animal model at 1-year-old age as represent of age-related factors, we wanted to advance our understanding of hyperoxia-induced cardiac pathophysiology in high-risk age-related populations. This key information should be transferrable to clinical settings. Despite the potentially significant risk of cardiovascular problems in this cohort, there is currently no research on the impact of hyperoxia in an aging model of heart disease. Our method includes the use of Electrocardiogram (ECG), Echocardiography together with histological data which successfully allows us to monitor electrical and functional changes like those seen in ICU patients. This idea has the potential for strategic innovation, as this data will not only identify hyperoxia-induced pathophysiological changes, but it can also help to prevent them.

1.8 Current Objective

To study the effect of Hyperoxia on cardiovascular pathogenic mechanism in Female (years old age) guinea pig heart.

1.9 Long Term Objective

Using an animal model, we seek reveal the full etiology of the heart under hyperoxic settings, and also the differences between gender on hyperoxia-induced cardiovascular remodeling and injury.

CHAPTER TWO: MATERIALS AND METHODS

2.1 Materials

2.1.1 Animals

One year old-age female of Hartley Guinea pigs' weight between 900-1000g purchased from Charles River laboratory. The health status of Guinea pigs is Bright Active and Responsive (BAR). This protocol was approved by the Institutional Animal Care and Use Committee at the University of South Florida (Tampa, Fl) together with the US National Institutes of Health (NIH). We divided Guinea pigs into two groups, (n=6 per group)

2.2 Methods

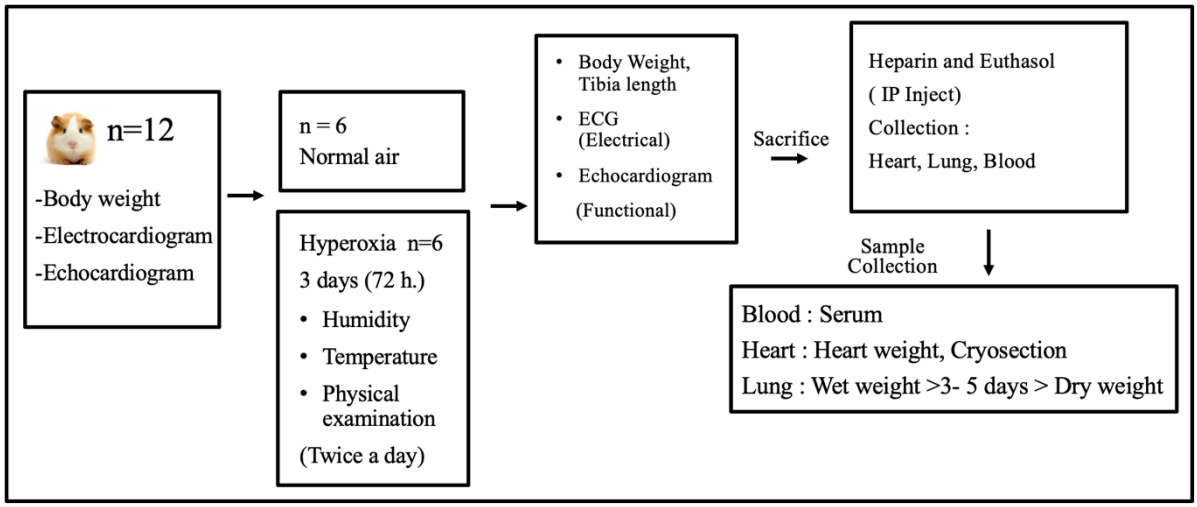

Figure 1. Schematic chart of materials and methods.

2.2.1 Hyperoxia Exposure

These female Guinea pigs were retired, female breeders. Separated randomly into two groups, The first group is hyperoxia (n=6), The second group is normoxia (n=6). Both groups were allowed to access food and water *ad libitum.* Guinea pigs in both groups were conserved on 12 hours day/night cycle. For the hyperoxia group, the Guinea pigs were treated with

>90% oxygen for 72 hours (3 days). They were paced in an airtight chamber size of 50cm × 50cm × 30cm with hermetically seal furthermore the level of oxygen in the chamber was monitored by an oxygen analyzer (Vascular Technology, Chelmsford, MA) to ensure the hyperoxia condition throughout experiment. Every 6 hours the animal was inspected for their health status including discomfort and humidity in the chamber. For the normoxia group, they were placed in the chamber with normal air for the same duration of 72hours (3 days).

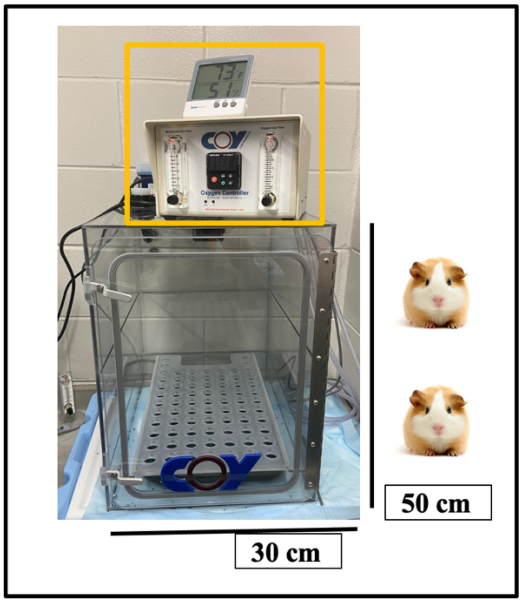

Figure 2. The oxygen level was produced continuously using an oxygen analyzer.

2.2.2 Physical Parameters

Physical parameters include body weight and the area of the heart collected. Guinea pigs in both groups were measured body weight before hyperoxia exposure and right after hyperoxia exposure to compare both groups. The tibia lengths were measured to normalize them corresponding to their weight.

2.2.3 Electrocardiogram (ECG)

Transthoracic electrocardiography was anesthetized with 1-2% isoflurane was used for the anesthetized. ECG was recorded a total of three intervals per stretch. Each stretch was collected for 30 seconds. ECG graphs was analyzed by LabChart 7.2 software (PowerLab system, AD instruments, UK). As the side effect of isoflurane which reduces the heart rate

after hyperoxia treatment, we tried to evaluate the heart rate during the time we collect the ECG data. All the ECG data were collected in a millisecond unit.

ECG is a non-invasive device to assess cardiac's electrical activity and others abnormality of the heart [99]. Every ECG wave begins with depolarization followed by repolarization (heart relax).There are several parameters assessing the ECG including's P-Wave (atrial depolarization).P-wave represents the atrial depolarization which start from SA node sensory input located in the right atrium. The signal in P wave sent from right to left atrium called atrial depolarization [100]. QRS complex (ventricular depolarization) describes the depolarization of the ventricles or during the ventricle's contraction furthermore, QRS complex also represented atrial repolarization, Because it occurs during ventricular depolarization, there is no clearly visible wave showing atrial repolarization in the ECG. T-wave represented the ventricular repolarization (ventricles are relaxing).PR intervals, PR intervals displays the period of the electrical activity moving from atria to ventricles (AV node), starting from the beginning of the P wave and ending at the beginning of the Q wave. RR interval is the time between two of the QRS complex, beginning from the peak of the first R wave and terminating at the second R wave. QT interval is the time taken of the depolarization of the ventricles to repolarization which starts at the QRS complex and ends by the finish line of the T-wave. In practice, the QT interval is denoted as a "corrected QT (QTc)" by dividing the QT interval by a square root of the R-R interval. JT interval was obtained by subtracting the QRS duration from the QT interval Similar to mice that place the A total of three leads including's Lead I in the axis of horizontal heart position (inserted between right and left forelegs), Lead II along the anatomical axis of the heart (the middle between right foreleg and left hind legs), and Lead III in the axis of the vertical heart (the middle between left foreleg and left hindleg). ECG lead are place in right arm (R), left arm (L) and left legs (L) [101].

2.2.4 Echocardiogram

Another procedure for assessing the cardiac function that has been widely used for screening and assessing the pathologist of the heart is Echocardiogram. The echocardiogram is a type of heart ultrasound. Utilizing the ultrasonic wave echocardiogram can give both morphological and physiological images of the cardiovascular system. Since it is a noninvasive technique that can provide a reliable result for evaluating the hemodynamic movement of the heart, Echocardiogram has become one of the main procedures in a critically ill patient [102, 103].

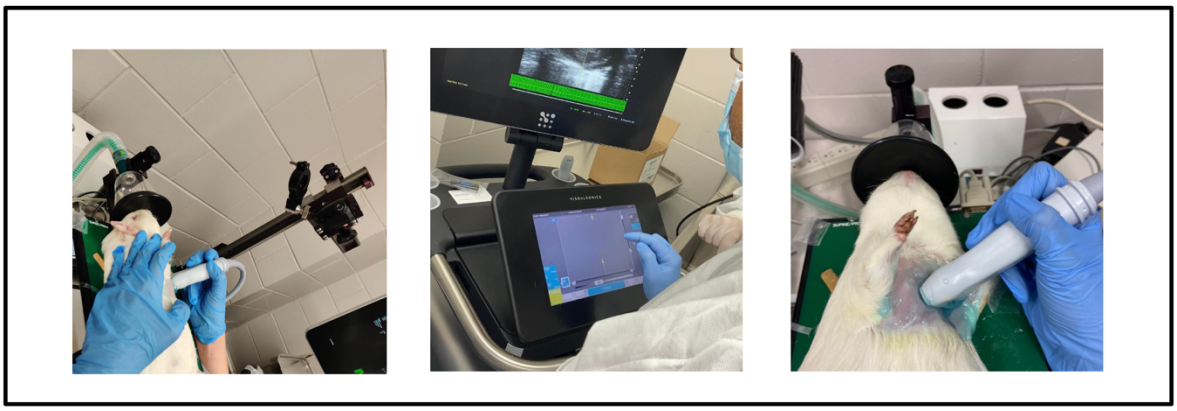

Figure 3. Transthoracic echocardiography (TTE): Vevo 3100.Ultrasonograph (VisualSonics) was used to analyze functional status both in normoxic and hyperoxic female Guinea pigs. After Guinea pigs were under anesthetized from ECG analysis, we maintained general anesthesia status concurrent with temperature 37°C monitoring. The parasternal long and short axis were imaged in 2D guided m-mode at the midpapillary muscle level.

Echocardiograms can evaluate various functions of the heart. In this study the criteria that we will mainly focus on are Cardiac output (CO), CO is the volume of the blood moved by the heart per minute [104]. CO can be calculated by Stroke Volume (SV) multiply Heart Rate (HR) or (CO = SV x HR). Stroke Volume (SV) is the volume of the blood that is pumped out from the left ventricle of each pump [105]. SV can be calculated from End-diastolic volume (EDV) and End systolic volume (ESV) as (SV = EDV – ESV) [105]. Left ventricular internal diameter-end diastolic (LVID d) and left ventricular internal diameter end systolic (LVIDs) can directly collect from the echocardiogram. % Ejection fraction (%EF) or volume of the blood per beat that eject from the left ventricle (EF%) was calculated using end-diastolic

volume (EDV) and end-systolic volume (ESV) as (EDV ESV) / (EDV ESV) * 100%. Fractional shortening (%FS) is the fraction of a cardiac cycle in which the left ventricle contracts (LVIDdLVIDs)/ LVIDd*100% was used to calculate fractional shortening (%FS). The percent change in left ventricular diameter throughout systole is calculated. Furthermore, we also collect LV mass and LV mass corrected to evaluate the mass weight of the left ventricle. Transthoracic echocardiography Vevo 3100.Ultrasonograph (Visual Sonics) was used to analyze functional status both in normoxic and hyperoxic female Guinea pigs. The previously published experimental protocols for echocardiographic measurements were used [58].

2.2.5 Collection of Organs

After ECG and Echocardiogram data were collected animals were euthanized by injecting euthasol dose of 50 mg/kg followed by heparin (Sigma-Aldrich, MA) dose of 500 IU/kg/10ml via IP injection (intraperitoneal). Thoracotomy was performed and blood collection and organs including heart and lungs were collected.

Blood: The blood sample was then centrifuged for 5 minutes at 5,500 rpm to collect plasma serum and then frozen at -80 °C in the refrigerator for future molecular investigation.

Heart: Both groups' heart were stored − 80 °C temperature for at least 24 hours to prepare for cryosection. After specimen preparation, cryosections were performed. The heart was then sectioned and stained with H&E (Hematoxylin and Eosin) to compare the heart area (heart size) between both groups.

Lung: The lungs' wet -to- dry ratio was calculated. To make a comparison of the total volume of lung edema between the hyperoxia and normoxia groups, the guinea pigs were abdominal dissected, and the lung was collected and measured as wet weight immediately. The lungs were then dried for 48 hours in a 60°C oven before being re-weighed as dry weight again.

2.2.6 Immunohistochemistry: Cryostat Section and Staining

The most frequently used technique of using immunostaining is immunohistochemistry (IHC). It involves a technique for selectively identifying antigens (proteins) in a tissue segment by reinforcing the idea that antibodies react to antigens in living tissues specifically.

Frozen section

1 Using a compatible tissue mold, the sample was frozen up to 2 cm in diameter in OCT (OCT is a liquid at ambient temperature but freezes at -200C).

2. On the metal grids that fit the cryostat, OCT was frozen with the sample.

3. In the cryostat at -20°C, mold was cut to attach to the slide of around 10 µm. /Sections

4. The first slide of each series was stained with toluidine blue, eosin, hematoxylin, or other stains to assess the preservation of the tissue. In the end, Slide has dipped into a fixative right away.

Hematoxylin and Eosin (H&E) Staining: H&E staining was used to determine the total cross-sectional area of each heart. H&E staining active substance include 100% alcohol, 95% alcohol, Rapid-Fixx, Gill 3 Hematoxylin, Bluing reagent, Eosin-Y, Xylene, and Shandon mounting medium. For 15 minutes, the cryostat slices on the slides were placed on hot plates at 45°C. To eliminate OCT from the slides, sections were washed three times with 1xTPBS. The following staining procedures were carried out:

1.After applying Rapid-Fixx for 5-7 seconds, distilled water was added, then The slide was left in Hematoxylin for 30 seconds before washing the slide again with distilled water.

2. Using a pipet, the slide was rinsed with a few drops of Bluing reagent until being rinsed with 95% alcohol.

3. Eosin-Y was added to the slide for 10 seconds.

4. A few drops of 95% alcohol, followed by100% alcohol two times was used to clean the slide. Finally, the slide was cleaned twice with Xylene and the frozen section was stained with H&E.

5.The slides were left to dry completely 24 hrs. before mounting them with coverslips and fluor mount medium (Fisher Scientific, USA)

Heart measurement: After cryosection was ,we use ImageJ software to measure and analyzed heart area. The mean (SE) results were presented in a bar diagram.

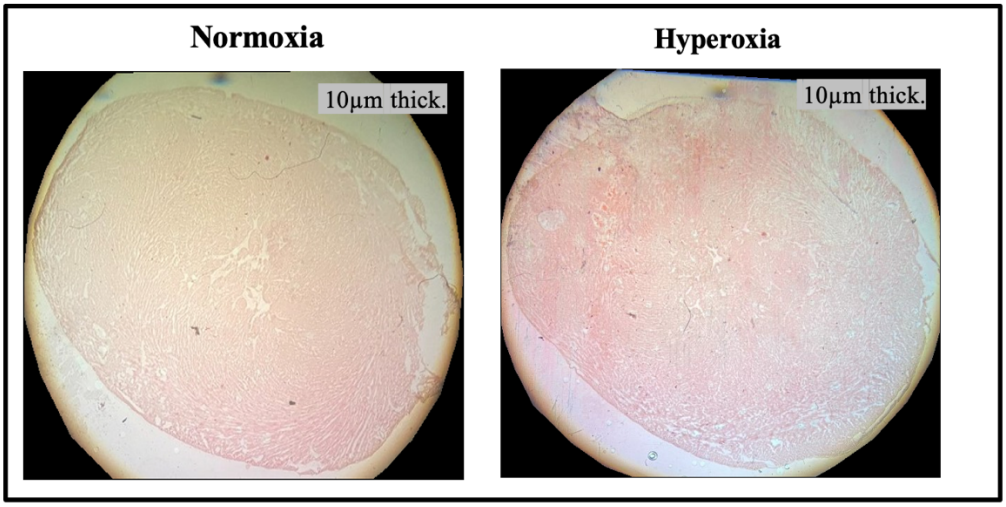

Figure 4. H&E-stained heart: Histological cross-sections in both age groups: normoxia and hyperoxia.

2.2.7 Statistical Analysis

For this study Female Guinea pigs with one year old age will be selected the breed that we expected to have is Hartley Guinea pigs. To calculate the sample size, we will use the sample size calculator website (http://biomath.info/power/prt.htm), To ensure that the analysis was done blindly, all the samples were labeled with unique numbers and codes. For example, hyperoxia and normoxia guinea pigs animal's heart and lungs samples were given to them at random by mentor and student who is analyzing the data was not informed of these numbering and coding so that he does not know the intervention of each random selection he

is analyzing to avoid biased interpretation of treatment. After collecting all the data, the mentor revealed his identity, and the analysis was completed with the proper grouping.

All mean (± SEM) values were depicted in a bar diagram, and student t-tests were used to determine significance (p-values). The information analysis was used to make comparisons the quantitative data populations of typical dispersion and equal variance, with a $p \leq 0.05$ value considered statistically significant. For all data sets, the error represent means(± SEM).

CHAPTER THREE: RESULTS

3.1 Physical Parameters

The hyperoxia caused the death of one Guinea pig. The remaining Guinea pigs in the experiment group had severe dyspnea, were depressed, were lethargic, and had significantly reduced responsiveness.

3.1.1 Body Weight and Heart Weight

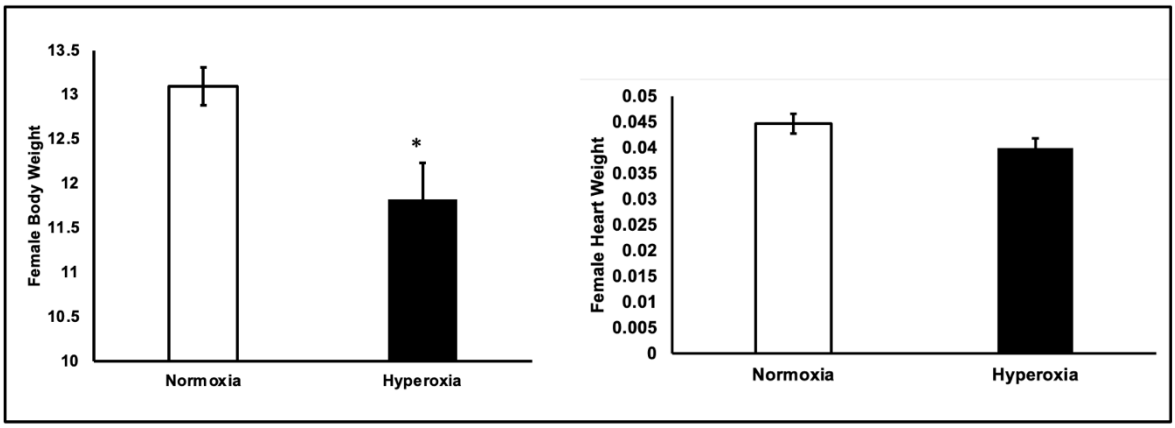

Figure 5. Physical parameters**:** body weight and heart weight: Hyperoxia exposure significantly reduced body weight (normalized with tibia length) compared to normal groups. Heart weights between the two groups are not different. For all data, error bars represent ± SEM. *$p < 0.05$, * Represents p-value between hyperoxia and normoxia. Body weight demonstrates that the hyperoxia groups' weight drastically decreased.

The decline in hyperoxia groups matched the findings from mice of both sexes and male Guinea pigs. [92]. We measured heart weight to see if there are any cardiac hypertrophy after hyperoxia treatment, although heart weight in the hyperoxia group was no different than in the normoxia group.

3.1.2 Heart Size

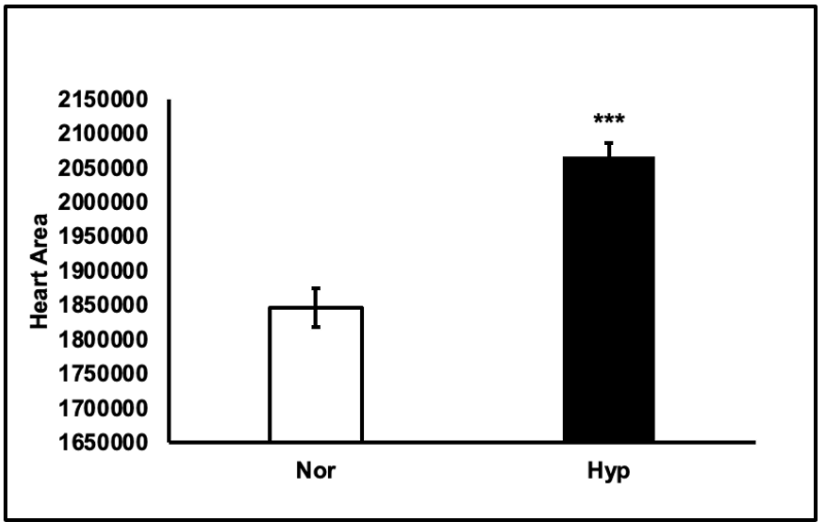

Figure 6. Heart size (ImageJ software): In female Guinea pigs, heart area in hyperoxia group increase significantly. error bars represent ± SEM. ***p < 0.0005 * Represents p-value between hyperoxia and normoxia.

We measured heart weight to see if there are any cardiac hypertrophy after hyperoxia treatment, although heart weight in the hyperoxia group was no different than in the normoxia group. The hyperoxia heart area measurement shows a significant increase when compared to the normoxia groups, This could imply that hyperoxia has changed and remodeled the female Guinea pig heart structure. The result is similar to that of male mice [92], but this change was not observed in female mice's heart areas because it was significantly lower than that of normoxia [12, 92]

3.1.3 Lung wet/dry Weight Ratio

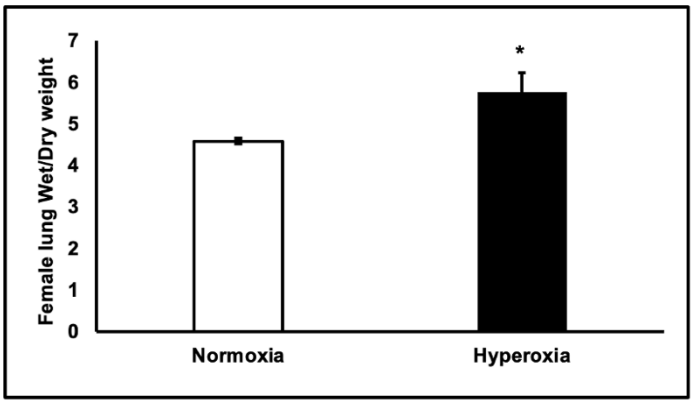

Figure 7. Lung wet/dry weight ratio: Hyperoxia group increase lung wet/dry weight ratio significantly. Error bars represent ± SEM. *p < 0.05, * Represents p-value between hyperoxia and normoxia.

From previous reports in mice both male and female present increasing the lung weight significantly after hyperoxia, lung edema has proved to be one of the significant complications from hyperoxia [92]. Pulmonary edema is an ailment caused by an excess of fluid inside the lungs. This fluid accumulates in the numerous air sacs in the lungs, impairing gas exchange and making breathing difficult. In most cases, cardiovascular system cause pulmonary edema, which can progress to respiratory failure, This is common in disorders caused by a failure of left ventricular systolic/diastolic activity to appropriately remove blood from the pulmonary circulation. [106].Female Guinea pig's hyperoxia groups show an increase in lung wet/dry weight ratio significantly similar to the result in Male Guinea pigs.

3.2 Electrophysiological Parameters - (Electrocardiogram, ECG)

As in previous studies[12], we analyze ECG using the LabChart Pro software protocol. Female Guinea pigs' RR interval increased significantly, and P wave decreased significantly after hyperoxia treatment, according to ECG data. They also have arrhythmia and a slower heart rate due to abnormal heart rhythms and missing beats in some beats. Bradycardia and an increase in the RR interval are also observed in male Guinea pigs and mice, both sexes[107].

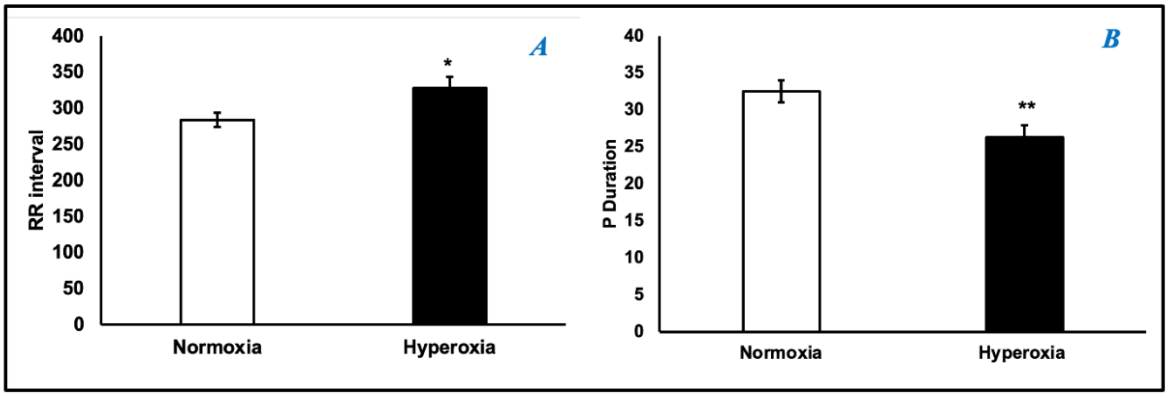

Figure 8. P-wave and RR interval: In hyperoxia group, RR interval (A) increased significantly, and P-wave duration (B) decreased significantly. For all data, error bars represent ± SEM. *p < 0.05, **p < 0.005.* Represents p-value between hyperoxia and normoxia.

Apart from the RR interval and P duration, which show changes, Female Guinea pigs show no significant changes in other parameters including's PR, QRS, QTc, and JT interval. Male Guinea pigs have similar results to females, except for QTc, which decreases significantly in hyperoxia groups (females only show decreasing trend but is not significant). Surprisingly, the PR, QRS, QTc, and JT intervals all increase significantly in mice both female and male [92].

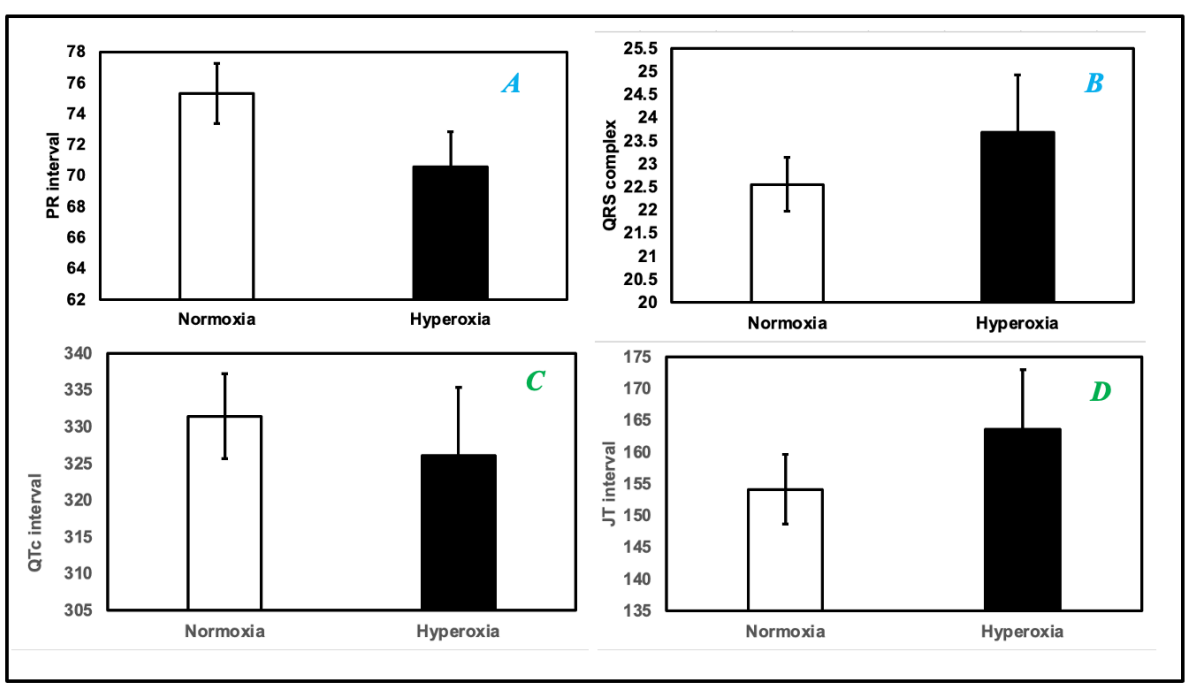

Figure 9. PR, QRS, QTc, JT interval: There are no significant differences according to PR (A), QRS (B), QTc (C), and JT (D) intervals from both hyperoxia and normoxia groups.

3.3 Functional Parameters – (Echocardiogram)

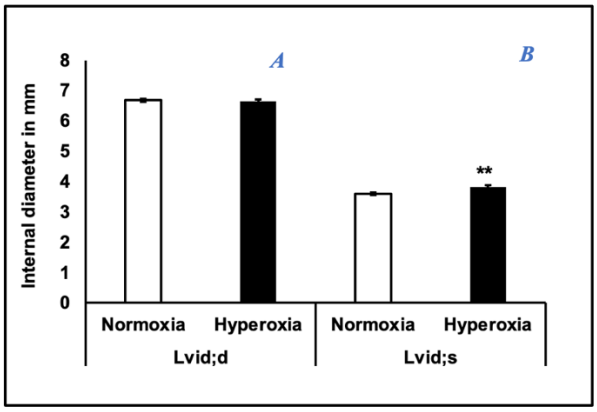

Figure10. Echocardiogram results (LVIDd (A) and LVIDs (B)): LVIDd shows no differences between the two groups. LVIDs increase in hyperoxia groups.**p < 0.005. * Represents the p-value between hyperoxia and normoxia.

According to 2D echocardiogram data, LVIDs in hyperoxia groups decrease significantly compared to the normal group. Since, the reduction of LVIDs represents the sign of the loss of ability to eject blood out of the ventricle. This abnormality will eventually increase the volume left over in the ventricle and increase the volume overload to the heart.

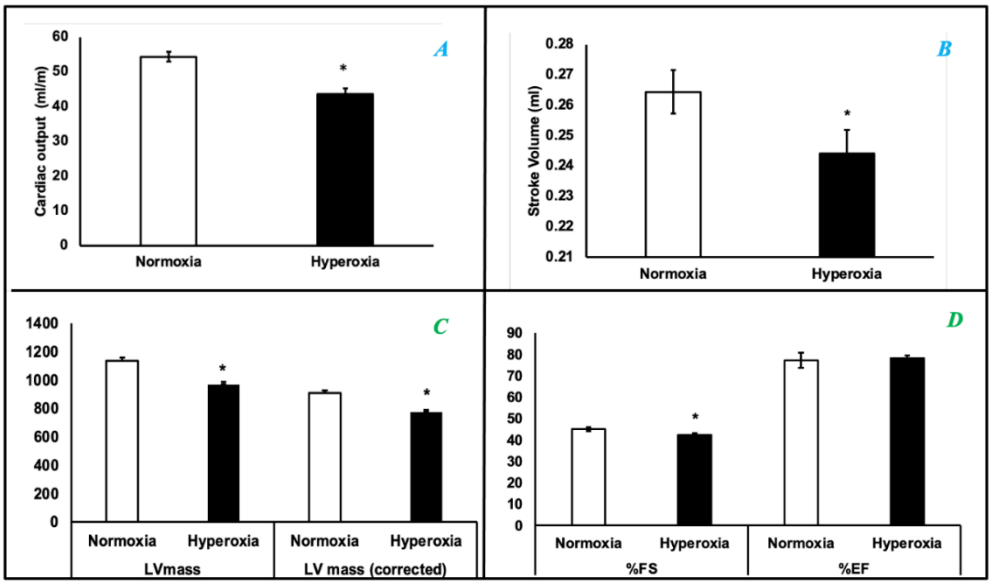

Figure 11. Echocardiogram result: According to the echocardiography Cardiac output (A), Stroke Volume (B), LV mass/LV mass corrected (C),and %FS (D) are decreasing significantly. Error bars represent ± SEM. *p < 0.05. * Represents the p-value between hyperoxia and normoxia

Furthermore, CO, SV, LV mass/LV mass corrected, and %FS that decrease significantly clearly represent the sign of eccentric left ventricular hypertrophy (Eccentric LVH). Eccentric hypertrophy is a type of cardiac remodeling that results from volume overload, eccentric hypertrophy is characterized by the dilation of the size of the ventricle which extended larger than the normal heart size [108].

Since, one of the most significant pathological features in heart failure is left ventricle remodeling [45]. With the %EF data that show no different between two groups we briefly categorized what happened with Guinea pigs heart as a HFpEF types of heart failure. In Heart failure with preserved ejection fraction (HFpEF) conditions, normally patient will %EF remains unchanged [109]. Comparing with mice, LVIDd, %FS, SV, CO show similar result in both Guinea pigs and mice (decrease significantly) and LVIDs (increase significantly) [92].

CHAPTER FOUR: DISCUSSION

This study clearly shows that female Guinea pigs have a severe cardiovascular abnormality, as evidenced by electrophysiological and functional changes that can lead to heart failure. When comparing female and male Guinea pigs based on ECG data, the female has more severe electrophysiological change (RR, PR, QTc interval) than the male. Regarding the age factor, because we are currently conducting in one age range (1-year- old), the data may not be conclusive for age comparison. In the future, we will further investigate in an extended experiment with a wide range of ages to compare between two age groups. However, according to the mice experiment, the elderly is more vulnerable due to physiological co-morbid factors such as body weight and lung edema, which have exacerbated when compared to the young [92]. When considering the age differences in Guinea pigs, Since the formula to calculate the exact age comparison between Guinea pigs and human are not available, The current formulation that we use in this study are an estimation of age compared to a human that we anticipate to be represented as an old age groups.

Using the Guinea pigs as a animal model has present that even though the ECG data from Guinea pigs female are similar to the male but, surprisingly it is different from the mice experiment. We have kept blood from both groups sample, in the future, serum will be further evaluate for the cardiac biomarker and mRNA expression profile to assess some key ion channel genes. Since report in mice shows that hyperoxia can down-regulate some key ion channel (Kv 1.5 and Kv2.1) and in mice hearts [92]. Comparing molecular expression between two animals will helps us clarify more and more clue of the signaling pathway and cell death mechanism which might help us to find a way to inhibit this process. However, the

expression of each species are unique. This distinct pathophysiology might be seen/not in human. In the long run, we may need to develop a unique proper model of the Kv ion channel to study the pathology of the heart. Even though animal models may not be a 100% exact match human physiology (not only small animal but also in large animals), using Guinea pigs as an animal models can clearly provide us the answer for our hypothesis and extend our boundaries from what we have from mice. This data is essential for another small steps of understanding the cardiovascular system response after hyperoxia conditions and will helps us to develop a therapeutic strategy in the future.

CHAPTER FIVE: CONCLUSION

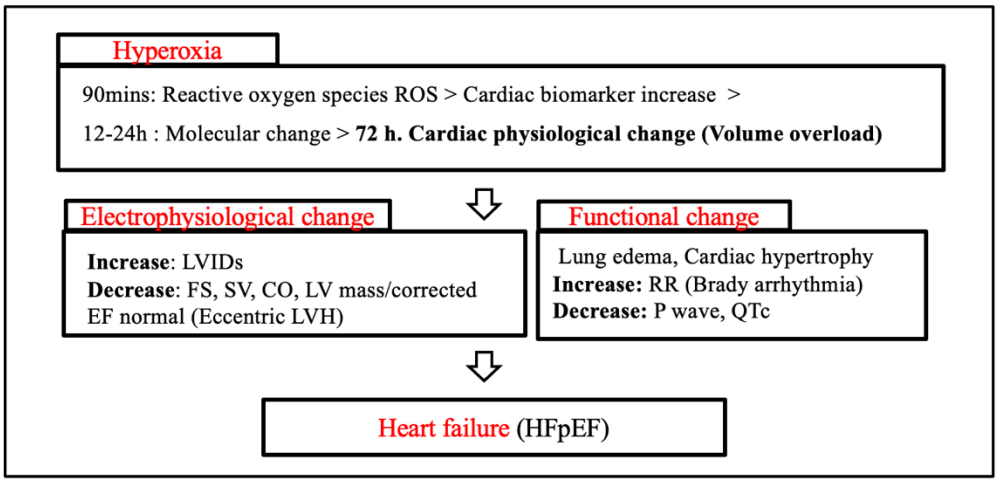

Figure 12. Conclusion of what happened in female Guinea pigs' heart.

According to the female Guinea pigs' experiment for this research we mainly focused on the cardiovascular system. Hyperoxia has the greatest impact on cardiac pathophysiology starting from physiological parameters (significant severe pulmonary edema) and severe weight loss. and one Guinea pig has died after hyperoxia was treated. Furthermore, electrical disturbances (cardiac arrhythmia and bradycardia) and functional abnormality including eccentric hypertrophy from ECG and echocardiogram data can imply the sign of heart failure (HFpEF type). Compare female and male Guinea pigs, female shows more severity according to electrical data. Considering two species mice and Guinea pigs show different electrical data. Using Guinea pigs as an animal provides us with a key difference between guinea pigs itself and mice, although animal models are not an exact match for human physiology, Guinea pigs are the models that can be used to test the hypotheses that we have set which helps us reveal some physiological response that animal and human shared.

As a conclusion, we propose that, even if oxygen supplementation in ICU is essential for many patients, thinking of oxygen as a medicine would be the best summary to help us understand the negative effects associated with this therapy. Utilizing excessive oxygen can be more damaging than we anticipated and have a significant impact on the heart's performance.

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APPENDIX A: IACUC APPROVAL

RESEARCH INTEGRITY & COMPLIANCE
INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

MEMORANDUM

TO:	Siva Panguluri,
FROM:	Farah Moulvi, MSPH, IACUC Coordinator Institutional Animal Care & Use Committee Research Integrity & Compliance
DATE:	3/23/2021
PROJECT TITLE:	Hyperoxia-induced Kv channel regulation in an aging mouse model Effect of diabetes, aging and hyperoxia on cardiac physiology Effect of hyperoxia stress on eye
FUNDING SOURCE:	Federal government or major agency that awards grants based on peer-reviewed proposals (NIH, NSF, DOD, AHA, ACS, etc.) USF department, institute, center, etc. National Institute on Aging
IACUC PROTOCOL #:	R IS00009004
PROTOCOL STATUS:	**APPROVED**

The Institutional Animal Care and Use Committee (IACUC) reviewed your application requesting the use of animals in research for the above-entitled study. The IACUC **APPROVED** your request to use the following animals in your **protocol for a one-year period beginning 3/23/2021:**

Mouse: B6.BKS-Leprdb/j (78wks/male and female)	2036
Mouse: C57BL/6 (8 to 10 wks/male and female)	2036
Mouse: B6.BKS-Leprdb/j (8 to 10 wks/male and female)	2036
Mouse: C57BL/6 (78 wks/male (Castrated))	2036
Guinea Pig: Hartley Guinea Pig/Strain code 051 (Retired breeders (12-18 months)/551g plus/male and female)	2036
Mouse: C57BL/6 (8 to 10 wks/male (Castrated))	2036
Guinea Pig: Hartley Guinea Pig/Strain code 051 (8-10 weeks/ 551g plus/male and female)	2036
Mouse: B6.BKS-Leprdb/j (78 wks/male (Castrated))	2036
Mouse: C57BL/6 (78 wks/male and female)	2036
Mouse: B6.BKS-Leprdb/j (8 to 10 wks/male (Castrated))	2036