**Time course of hypoxic-induced changes in pulmonary arterial pressures in
anesthetized dogs exposed to FiO₂s of 12% and 10%--a model of vascular
pulmonary hypertension.**

THESIS

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in
the Graduate School of The Ohio State University

By

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2010

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Abstract

Goals: This study was conducted to characterize, more completely, the hypoxic canine model of pulmonary hypertension, with the intent of using the model for evaluating pathophysiology, diagnosis, and treatment.

Methods: Nine healthy dogs were anesthetized with acepromazine/butorphanol/propofol, and instrumented to record electrocardiograms, pressures from the right-side of the heart, and systemic arterial blood gasses. Respiratory rate was counted, and effort of ventilation was approximated as the difference in right ventricular end-diastolic pressure between the respiratory pause and during peak inspiration. In addition to systolic and diastolic pulmonary arterial pressures, pulse pressure was calculated. Energetics of ventilation was approximated as the “double-product” of respiratory rate and effort. Ventilation was spontaneous. Dogs were then exposed to FiO_2 's of 0.21, 0.12, and 0.10 and physiological measurements were made for 5 minutes because all values appeared to plateau by that time after exposure.

Results: Within 2 minutes of substituting an FiO_2 of 0.21 to either 0.12 or 0.10, steady states of most parameters occurred. Because the dogs breathed gasses with 5% CO_2 , their

PaCO₂ remained unchanged, however PaO₂ decreased from a baseline of 66 mmHg to 39 mmHg at 0.12 FiO₂, and to 29 mmHg at 0.10 FiO₂. The differences between baseline and during reduced FiO₂ were significant (p=0.005), and the differences between 0.12 FiO₂ and 0.10 FiO₂ were also significant (p=0.03). Respiratory rate increased from a baseline of ~13/minute to ~28/minute. The difference between right ventricular end-diastolic pressure during the respiratory pause and during peak inspiration increased from ~6 mmHg to ~16 mmHg for 0.12 FiO₂ and ~14 mmHg for 0.10 FiO₂. The difference between 0.12 FiO₂ and 0.10 FiO₂ was not significant. However the “double-product” during the final minutes of recording either plateaued for 0.12 FiO₂ or decreased for 0.10 FiO₂. Pulmonary arterial pulse pressure—determined by stroke volume and the elasticity modulus of the pulmonary trunk—increased from a baseline value of ~11.5 mmHg to ~12.5 mmHg for 0.12 FiO₂ and ~14 mmHg for 0.10 FiO₂. The differences were not significant.

Discussion/Importance: Hypoxia-induced, acute pulmonary hypertension has been used for years to investigate pathophysiology and therapy. However subtle changes in pulse pressure have never been reported and the increases in oxygen demand due to labored respirations have not been addressed. This study confirmed the effects of hypoxia on PaO₂, respiratory rate and ventilatory effort. A tendency for stiffening of the pulmonary

trunk for both 0.12 FiO₂ and 0.10 FiO₂, but greater for 0.10 FiO₂ was seen. Moreover the “double-product” increased for both levels of hypoxia, but tended to either plateau or actually decrease after 5 minutes of hypoxia, implying fatigue of muscles of respiration due to an energetic imbalance.

Limitations: The number of dogs studied precluded obtaining statistical significance for the differences between 0.12 FiO₂ and 0.10 FiO₂ in some parameters. It would have been desirable to measure cardiac output and calculate both stroke volume and pulmonary vascular resistance. It would be desirable, also, to conduct a study on more dogs using chloralose-urethane anesthesia, a regimen known to permit nearly intact control of respiration.

Dedicated to my Family and friends from Colombia and Columbus, Ohio.

Acknowledgments

The author would like to thank the economical support received from Qtest laboratories.

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Chapter 1: Introduction

Pulmonary hypertension is a serious disease that occurs in humans and infrahuman animals, and may be of particular issue for animals living at altitude. For example, in Bogota, Colombia (altitude 2,650 M), the usual PaO_2 for humans is 69 mmHg. To improve both the understanding of the disease and therapy, Animal models have proven helpful in improving both the understanding of the disease and its therapy, but humans with pulmonary hypertension have a 2.8 year life-expectancy after diagnosis even when enormously expensive treatments are applied. This progressive disease occurs most commonly in young and middle-aged women (Gaine and Rubin, 1998). Pulmonary hypertension has been reported with increasing frequency in dogs, possibly because of the ability to identify increased right ventricular systolic pressure by Doppler interrogation of tricuspid regurgitation. In dogs a survival rate of 3 to 3.5 days after diagnosis has been reported (Johnson L et al 1999). This study was initiated as part of a broader program to characterize known models of pulmonary hypertension that might be useful to identify therapeutic approaches.

Chapter 2: Literature Review

Pulmonary hypertension (PH or PHT) is defined in dogs and in humans as an increase in peak, systolic pulmonary arterial pressure to greater than 25 mmHg at rest, or to 30 mmHg with exercise (Schierer and Bansal, 2008; Gaine and Rubin, 1998). In larger animals (e.g., cattle, horses) normal peak systolic pressures may exceed 30 mmHg at rest and elevate to 70 mmHg during exercise (Manohar M and Goetz TE, 1996). In cows living at high altitude normal values are between 34 and 44 mmHg (Holt TN, Callan RJ, 2007).

Originally, PH was divided into two classifications: primary PH and secondary PH, according to its origin. Primary pulmonary hypertension referred to cases without a known cause and is rare in dogs. Secondary PH referred to either pulmonary disease (fibrosis, heartworms) or left-sided heart failure. The origin of the disease is frequently multifactorial, that is it could be related to one predisposing factor and 1 or more inciting stimuli which is called the “multiple hit hypotheses”, (circulation, pulmonary arterial pressure) as is seen when the left atrial pressure increases or the pulmonary vascular resistance rises. Common specific causes include: mitral valve disease, pulmonary

thromboembolism, and dilated cardiomyopathy. Currently, the accepted classification for pulmonary hypertension is the one developed in 2003 in the 3rd World Symposium on Pulmonary Arterial Hypertension (Simonneau et al 2004).

The classification is as follows:

- WHO Group I - Pulmonary arterial hypertension (PAH)
 - Idiopathic (IPAH)
 - Familial (FPAH)
 - Associated with other diseases (APAH): Collagen vascular diseases, congenital shunts between the systemic and pulmonary circulation, infection, drugs, toxins, or other diseases or disorders
 - Associated with venous or capillary disease.
- WHO Group II - Pulmonary hypertension associated with left heart disease, pulmonary venous obstruction and left-sided heart disease.
- WHO Group III - Pulmonary hypertension associated with lung diseases and/or hypoxemia, Interstitial lung disease, chronic exposure to high altitude, developmental lung
- WHO Group IV. Associated with thromboembolic disease.

- WHO Group V - Miscellaneous

Although in dogs, one of the most common causes of increase in the resistance to pulmonary flow is heartworms located in the pulmonary artery, impairing the normal outflow of blood from the right ventricle, some reports have found that indeed the most common predisposing factor is mitral valve disease (Johnson L. et al 1999). Some of the most common clinical signs found in dogs with PH are cough, dyspnea, exercise intolerance, syncope and cyanosis (Steele and Henik, 2004). This condition has a very bad prognosis both in human as in dogs.

2.1 Pulmonary Arterial Pressure and Its Determinants.

The pulmonary flow carries blood from the right ventricle to the left atrium. This blood, contrary to that which leaves the left ventricle, is deoxygenated. Pulmonary arterial blood reaches the alveolus with a low concentration of oxygen (16 ml oxygen/100 ml blood) and a high concentration of CO₂ (50 ml carbon dioxide/100 ml blood) that is primarily determined by the production CO₂ by the tissues (Looney JM and Jellinek EM, 1937). When the red cell passes along the alveolus through alveolar capillaries, the pressures of gasses equilibrate according to the Fick's law of diffusion ($Qd = (\Delta P \bullet A_c) / (MW^{1/3} \bullet X)$). The gradient for the diffusion of oxygen is from the

alveolus into the blood whereas the gradient for diffusion of CO₂ is from the blood into the alveolus (West JB, 2008).

Pulmonary circulation is a low pressure system. Pressures reach to levels one fifth to one sixth of the pressure reached by the systemic circulation during rest with pressure gradients between the RV and LA of only 10 mmHg compared to the 80 to 100 mmHg in the systemic circulation (Stone and Klinger, 2008; McLaughlin VV and McGoon MD, 2006).

The RV is a relatively thin muscle that forms a crescent covering the interventricular septum. Its shape and function are very different from those of LV. The pressures generated by the right ventricle are only those necessary to deliver all the cardiac output into and through the lungs. Pulmonary arterial pressure (PAP) remains relatively unchanged despite the 5-fold increase in cardiac output that occurs during exercise, due to the ability of the pulmonary capillaries to dilate and to open-up previously collapsed capillaries (Stone and Klinger, 2008).

Three mechanisms have been described as generators of increased right ventricular afterload: 1) impedance of pulmonary venous drainage like that occurring in left heart failure (Rich S et al, 1997); 2) increase in the pulmonary blood flow, associated

with congenital cardiac anomalies like atrial and ventricular septal defects and patent ductus arteriosus with left to right shunts (Johnson L, 1999) and 3) increased pulmonary vascular resistance, resulting from entities that obstruct or obliterate the pulmonary vessels (Saldana MJ, Arias-Stella J, 1994; Virmani R et al, 1994).

Pulmonary arterial pressure (PAP) is determined by flow (Q) times pulmonary vascular resistance (PVR). PVR in turn is the summation of the resistances imposed by arteries, arterioles, capillaries, and veins (Opie LH, 2004). Whereas systemic vascular resistance is imposed predominantly by arterioles and little by capillaries, pulmonary vascular resistance is imposed equally by arteries/arterioles and capillaries.

Poiseuille's law [$Q = (\Delta P \pi r^4) / 8 \mu l$] is used to describe the determinants of cardiac output (Q) under steady-state conditions. Its application is imperfect because blood flow is pulsatile and the blood vessels are compliant (the law is only applicable to steady-state flow of a Newtonian fluid through a rigid tube.). Peripheral resistance is inversely proportional to the fourth power of the radius. Thus, a reduction in the radius of the vessel by 50%, increases PVR 16-fold. Length is not altered and viscosity is altered only in chronic processes like hypoxia by means of an increase in the production of erythrocytes (Opie LH, 2004; Steele JL and Henik R, 2004).

1. $Q=(\Delta P \pi r^4)/8\mu l$; or, if the constants (π , l , and 8) and viscosity are

neglected:

$$2. Q= \Delta P r^4$$

PVR is related to $(1/\text{radius})^4$ power, therefore equation 2 can be rewritten as:

$$3. Q=\Delta P \times (1/PVR)$$

This equation can be rewritten as:

$$4. Q(PVR)= \Delta P$$

Solving for PVR generates the following equation:

$$5. PVR=(\Delta P)/Q, \text{ comparable to } R = E/I \text{ (Ohm's law).}$$

2.2 Pulmonary Artery Pulse pressure.

Figure 1. Pulmonary artery pulse pressure wave and its determinants. (Castelain et al, 2001).

The ascending (anacrotic) limb of the pulmonary artery pulse pressure is generated during systole when the RV ejects a stroke volume (SV) into the relatively compliant pulmonary trunk, thus the rise of pressure from diastole to systole depends upon SV and the relative incompliance [i.e., elasticity modulus (E) or stiffness] of the pulmonary trunk. The final part of the ascending limb is contributed by late systolic

augmentation, and is related to a reflection from the periphery. The peak of the curve is the systolic pressure, which is determined by SV and E. The greater E and the SV, the higher the pulse pressure. The descending (catacrotic) limb has two or more components: an incisura originated by the closure of the pulmonary valve, a second reflection possibly related to an impedance mismatch. The absolute value of the pulmonary arterial pressure pulse is of importance for 2 reasons. First it hinders the ejection of blood into and through the pulmonary vasculature; second it is the force from the rear (*vis a tergo*) generating pulmonary venous pressure that drives blood into the left atrium. Pulmonary hypertension results from an increase of the opposition to the mean component of flow (pulmonary vascular resistance). However the study of the pulse wave is also important (Bergel DH, Milnor WR, 1965, Castelain et al, 2001), since the opposition to flow occurs due to the stiffness of the pulmonary trunk (impedance) and the stricture of the pulmonary arterioles and capillaries (resistance). In pulmonary hypertension, some studies have found that both decreased PA compliance and increased resistance are equally important in the elevation of right ventricular afterload (Milnor et al, 1969).

2.3 Etiology.

As mentioned before, PH has been classified into different groups according to its origin.

2.3.1. The first group—It is called “pulmonary arterial hypertension”, includes PH idiopathic or sporadic, familial hypertension, diseases related to connective tissue, congenital heart disease, portal hypertension, human deficiency virus, drugs and toxins, venule/capillary involvement, persistent fetal circulation, and others (Roy and Couriel 2006; Simonneau *et al* 2004). Some drugs and toxins will be discussed further in this work.

2.3.2 The second group-- It refers to pulmonary venous hypertension due to pulmonary venous obstruction and left sided heart failure. PH secondary to LV systolic dysfunction is present in 68% to 78% of human patients with advanced heart failure. PH in heart failure results from congestion and passive increases in pulmonary venous pressure and from displacement of the interventricular septum into the right ventricular cavity. The reduced capacity of the LV to remove blood from the pulmonary venous system is also believed to be responsible. The increase in end-diastolic pressure (EDP) produces increases of local cytokines that lead to pulmonary artery dysfunction with reduction of nitric oxide (NO) and increases in the vasoconstrictor, endothelin-I (ET-I). This process induces pulmonary vasoconstriction and vascular remodeling. The increase in the pulmonary vascular resistance is then transmitted to the pulmonary arterial circulation, increasing RV afterload.

That in turn could lead to RV hypertrophy (Moraes et al, 2000, Roy and Couriel 2006; Simonneau *et al* 2004; Shah RV, Semigran MJ, 2008; Shin and Semigran, 2009).

2.3.3 The third group –It includes PH associated with disorders of the respiratory system or hypoxia. PH occurs most commonly due to parenchymal lung disease (e. g., chronic obstructive lung disease), extrinsic factors (e.g., upper airways obstruction, neuromuscular disorders or deformities of the thoracic cage), and others like high altitude and central hypoventilation disorders.

2.3.3.1 Acute and chronic hypoxia -- A common cause of PH is the exposure to low FiO_2 . It is known that the partial pressure of oxygen (O_2) in high altitude is reduced therefore the P_{AO_2} and PaO_2 are reduced. That reduction in P_{AO_2} below certain levels induces constriction of the pulmonary arteries as a means to adjust capillary perfusion to alveolar ventilation (Biernacki, 1988). This effect seems to be attributable to endothelial substances like endothelin-1 and serotonin, and to inhibition of voltage-gated potassium channels resulting in increased calcium ion concentrations in the cytoplasm inducing smooth muscle contraction. (Ghofrani et al. 2004; Huez et al. 2007; Ricart et al. 2005; Richalet et al. 2005; Pyle et al, 2004; Roy and Couriel, 2006; Farber HW, 2008; West JB, 2008).

Movement of gas across the alveolar-capillary membrane depends on the pressure difference between the blood and alveolus, the molecular weight of the gas, the thickness of the diffusing membrane, and the solubility of the gas in the membrane wall (Fick's law of diffusion). At sea level the pressure of the mixed gases comprising air is 760 mmHg (1 atmosphere). Of that, ~21% is oxygen, therefore partial pressure of oxygen is only 160 mmHg (Guyton and Hall, 2006). The pressure exerted by water vapor in the airways at body temperature is 47 mmHg. Thus, the actual partial pressure of oxygen reaching the alveoli at sea level is $(20.93/100) \times (760-47) = 149$ mmHg. The partial pressure of CO₂ must be subtracted to establish the actual PAO₂ (alveolar gas equation).

$$p_{AO_2} = F_{IO_2}(P_{ATM} - p_{H_2O}) - \frac{p_aCO_2(1 - F_{IO_2}[1 - RQ])}{RQ}$$

The PaO₂, on the other hand, is reduced by the presence of physiological dead space and right to left pulmonary shunts, with typical values of approximately 100 mmHg. The diffusion gradient for oxygen across the alveolar-erythrocyte barrier is dependent on that pressure and on the pressure within the erythrocyte in the capillary (~60 mmHg). The oxygen pressure in the alveoli is higher than in the venous blood, which drives oxygen from the alveoli to the blood. The opposite happens with CO₂. At high altitude or under

certain laboratory circumstances, the partial pressure of oxygen can be reduced significantly, triggering the phenomena previously discussed.

Acutely, the medullary respiratory centers and peripheral chemoreceptors induce an increase in minute ventilation (first by increasing tidal volume, then by increasing respiratory rate) when either PaCO_2 rises or PaO_2 falls. This, of course, tends to modulate the blood gas derangement. Nevertheless, when the PO_2 continues descending, the delivery of oxygen to the tissues also decreases (West, 2007; West 2008). Ultimately, low arterial oxygen content is associated with decreases in cardiac output (CO) probably due to the hypoxia induced vasoconstriction and possibly to decrease in stroke volume arising from decreases in contractility due to depletion of ATP.

The acute increase in pulmonary arterial pressure induces a sudden increase in the right ventricular afterload with lengthening of the preejection period of the right ventricle and a decrease in stroke volume (Alexander JK and Grover RF, 1983). In susceptible individuals, ascending rapidly to high altitude can also induce pulmonary edema due to an excessive rise in pulmonary arterial pressure (Bartsch, P et al, 2005). During chronic exposure to high altitude/low O_2 the ventricle is capable of developing hypertrophy, but when the pressure rises faster than the ability of the ventricle to adapt, it becomes dilated, altering the diastolic properties of the left ventricular function due to the leftward

displacement of the interventricular septum. Furthermore, RV dilation alters the functional anatomy of the tricuspid valve leaflets altering their ability to close after the ventricular filling. Thus, even normal leaflets cannot coapt causing some degree of tricuspid regurgitation or increasing preexistent regurgitation from the RV to the right atrium (Alexander JK and Grover RF, 1983; Huez S et al, 2005; Kjaergaard et al, 2007; Stone A and Klinger J, 2008).

2.3.4 Fourth group--Chronic thrombotic and/or embolic disease. Pulmonary embolism, originating mostly from thrombi formed in the deep veins of the legs, can be asymptomatic, incidentally discovered, or may cause immediate death. Different from other forms of pulmonary hypertension, chronic thromboembolic pulmonary hypertension has the possibility of being potentially curable by surgical extraction of the offending thrombi. Although the mechanisms of the origin of this entity are not completely understood it is known that in patients without preexistent cardiac disease a 30% obstruction of the pulmonary vascular bed is necessary to induce pulmonary hypertension. The main consequence is the anatomical obstruction of the pulmonary vascular bed. However, the release of vasoactive and bronchoactive substances like serotonin may alter ventilation/ perfusion matching. The results are a fall in PaO₂, and

an increase in the RV afterload which leads to increase in oxygen consumption and RV failure (Elliott CG., 1992, Tapson, 2008, Pengo, 2004, Test, 2008).

2.4 Models.

Several models have been used to study the physiological responses to PH. Due to the multifacted origin of this entity; several models have been developed to mimic one or several of those origins.

2.4.1 Hypoxia (acute and chronic)--Hypoxia can be induced in anesthetized or conscious animals. This is probably the most used model to induce acute but also sustained pulmonary hypertension and to evaluate pulmonary vasoreactivity. A reduction of atmospheric pressure (hypobaric hypoxia like in high altitude) or a reduction of PaO₂ at atmospheric pressure (normobaric pressure) can be achieved in the laboratory setting with the use of especially mixed gases (Marsboom GR and Janssens, 2004). Gases are usually mixed with different concentrations of CO₂ and nitrogen to produce normocarbic or hypercarbic conditions. The reduction in the inspired oxygen leads to a reduction in the alveolar O₂ with subsequent reduction in the end capillary O₂. The mismatch between blood flow and oxygenation induces changes in the pulmonary circulation that redirect flow to the more oxygenated areas, thus reestablishing normal V/Q. This is achieved by

means of the vasoconstriction of small arterioles (Allison et al. Invest radiol, 1980). The main consequence of this phenomenon is an increase in the pressure of the pulmonary artery. The mechanism was previously described in this work.

2.4.2 Monocrotaline--Monocrotaline is a phytotoxin derived from the seeds of *Crotalaria spectabilis*. After being injected subcutaneously it induces damage to the lungs such as interstitial edema, hemorrhage, and fibrosis. It also induces endothelial cell injury, medial hypertrophy of the pulmonary arteries, and severe pressure overload-induced right ventricular hypertrophy. (Meyrick B. et al, 1980; Schermuly RT, 2004). Other models include the use of toxic and chemical stimuli (besides monocrotaline) like alpha-naphthylthiourea, bleomycin, group B streptococcus; molecular stimuli like VEGFR-2 inhibition + hypoxia and angiopoietin-I overexpression; or genetic stimuli (Marsboom GR and Janssens, 2004).

2.4.3 Microspheres, air and clot embolism--Injection of polydextrane microspheres, fresh clots, or air mimics the effect of clots or parasites (e.g., *Dirofilaria immitis*) that can naturally occlude pulmonary arterioles and induce acute and/or chronic pulmonary hypertension (Marsboom GR and Janssens, 2004).

2.4.4 Increased flow--Increasing pulmonary blood flow may also increase pulmonary vascular pressures, however it must be remembered that when pulmonary blood flow increases acutely (up to 5 times during vigorous exercise) pulmonary vascular pressures do not increase significantly. However if that flow is increased chronically, as with left to right intra- or extra-cardiac shunts PH and vascular remodeling may occur (Rondelet, B. et al. 2003).

2.5 Diagnosis.

Diagnosis of PH as well as the identification of the underlying pathology is extremely important. Diagnose PH due to chronic embolism is quite different from diagnosis of PH due to left heart failure. Different therapeutic approaches are required dependent on the inciting cause (Oudiz R, 2008). Diagnosis starts with a complete physical examination. History of acute respiratory signs should persuade the clinician to start a more definitive and specific evaluation. Dyspnea on exertion and shortness of breath (SOB), although is not a specific for PH, are important signs. Auscultation can reveal abnormalities such as increased intensity of the pulmonic component of the second heart sound or splitting of the second sound due to a delayed pulmonary valve closure. Additionally, clinical signs of right heart failure (ascites, jugular pulse, pulmonic regurgitation murmur, tricuspid insufficiency murmur and peripheral edema) can be

found (Johnson L, 1999; Johnson LR, Hamlin RL 1995; Rich S et al, 1987, Steele and Henik, 2004).

New technologies have made the diagnosis of pulmonary hypertension easier using noninvasive modalities. Pulmonary hypertension can be diagnosed with the use of echo-Doppler. The measurement of the velocity of the tricuspid and pulmonic regurgitation jets and the use of the Bernoulli's modified equation ($P = 4 \times v^2$) are used to establish the value of systolic and diastolic pressures respectively (Johnson et al, 1999; Schober and Baade, 2006). This assumes that the right atrial pressure is either normal or is known, since the P is actually the gradient in pressure between the right ventricle and right atrium. Chest radiography could be normal in some cases of pulmonary hypertension, but more commonly, right ventricular enlargement or dilated pulmonary arteries can be found (Hawkins E C, 1995). Right ventricular enlargement is not often detectable by electrocardiography. When found, it is usually expressed as deep sharp S wave (Tilley, 1992).

Determination of PH by means of catheterization of the right ventricle is still the "gold standard." Invasively pulmonary arterial pressure can be determined by using of a flow-directed (Swan-Ganz) catheter placed in the right ventricle or pulmonary artery via an external vein. The aim of right heart and pulmonary artery catheterization is to

determine the pressure at the pulmonary artery and right ventricle, “wedge” (pulmonary capillary) pressure, pulmonary vascular resistance and cardiac output. This technique also provides very useful and necessary information when there is not tricuspid or pulmonic regurgitation to utilize echo-Doppler options (Oudiz R, 2008). Arterial blood gases are useful to establish the presence of hypoxia. Elevated troponin levels help in the identification of patients with acute pulmonary embolism at high risk for acute death (Becattini, 2007). In humans, part of the diagnosis of pulmonary hypertension is based on genetic screening for mutations. (McGoan M et al, 2004)

2.6 Therapy

To start the discussion of therapy for PH it is important to describe goals to be achieved. Besides controlling dyspnea, edema and fatigue, potential therapies should reverse vascular remodeling, thrombosis, and vascular damage, as well as prevent further myocardial damage and permit repair of already existing pathology (Hill et al, 2008). Currently, therapies are suboptimal; they have demonstrated only modest improvement in pulmonary hemodynamics with pulmonary arterial pressures remaining elevated (Chin and Rubin, 2008; Hirschrift, 2008). In addition some of the therapies have potential adverse effects, their efficacy can be brief, and they may be extraordinarily expensive and difficult to deliver.

2.6.1 Prostacyclins -- Based on the concept that an imbalance between vasoconstriction/vasodilation is found in idiopathic PH, prostacyclins were proposed for use in the treatment of PH. Prostacyclin is a metabolite of arachidonic acid produced primarily in vascular endothelium that has vasodilator effects in the pulmonary and systemic circulations. Prostacyclins bind to cell receptors and increase the production of cGMP due to stimulatory effects on the adenylate cyclase (Hill et al, 2008; Badesch, 2004; Rubin LJ et al 1990; Barst et al, 1996). The first one studied was epoprostenol. A report from 1990 showed that epoprostenol improved pulmonary hemodynamics and exercise tolerance. Survival percentage times at 1, 2 and 3 years also increased compared to placebo (Rubin et al, 1990). Another study in 81 severely ill patients using epoprostenol IV plus conventional therapy found improvements in the 6 minute walk test (6MWT) compared to conventional therapy alone ($p < 0.002$). Also, quality of life and hemodynamics (mean PAP and PVR) improved in the epoprostenol group. The study concluded that infusion of epoprostenol produced symptomatic and hemodynamic improvement in severely ill patients. Never-the-less, another study concluded that epoprostenol is more useful depending on the severity at baseline and the 3-months response to therapy. Side effects include headache, nausea, and diarrhea. The use of epoprostenol is labor-intensive and complicated because it has to be administrated as a continuous IV infusion and requires a

central catheter for its administration, thus increasing the risk of infections. Epoprostenol is contraindicated when ejection fraction is <30% and in patients with coronary disease. Other prostanoids used in the treatment of PH are treprostinil, iloprost and beraprost (Hill et al, 2008; Barst et al, 1996).

2.6.2 PDE 5 inhibitors -- Cyclic guanosine monophosphate (GMP) is a mediator for the relaxation of pulmonary vessels. It also inhibits cell proliferation. Cyclic GMP is hydrolyzed by phosphodiesterases (PDE). Among them, PDE5 is the most prevalent in the lung. Cyclic GMP is produced by the interaction of nitric oxide (NO) and natriuretic peptides, however the use of these substances in the long term clinical setting is complicated. PDE5 inhibitors reduce the metabolism of c GMP thus increasing its levels in the pulmonary vascular bed. There is much information regarding the effect of PDE5 inhibitors on PH. Among them, sildenafil has been studied most. It has been associated with reduction in PAP and improvement in physical symptoms in patients with PH. Sildenafil has demonstrated improvements in pulmonary hemodynamics in healthy subjects with hypoxia at rest and with exercise (Kojonazarov et al 2007, Fesler et al, 2006; Preston, 2008)

2.6.3 Endothelin 1 (ET1) inhibitors -- Endothelin-1 is a potent vasoconstrictor produced by endothelial cells, and it is considered a provocateur of PH. The effect of endothelin-1 is

mediated by a second messenger and is related to the suppression of K channels leading to opening of calcium channels and activation of vascular smooth muscle. ET1 has effects on 2 receptors, ET_A and ET_B. They affect vascular cell proliferation. Also ET_A mediates vasoconstriction. The most studied ET1 blocker is bosentan. Two studies have demonstrated improvements in 6 minute walk test, mean PAP, PVR and most importantly. it also improved survival time. However, side effects include dramatic increases in hepatic enzymes as well as teratogenesis (Yanagisawa M, 1988; McLaughlin VV, 2006; Channick RN, 2001; Rubin, 2002).

Other medications such as calcium channel blockers and statins are reported to be beneficial for the treatment of PH. Of course oxygen, diuretics, anticoagulants, and phlebotomy in cases of high hematocrit (Hill and Kling, 2008; McLaughlin VV and McGoon MD, 2006) are more conventional agents used to treat PH. In patients with poor response to treatment, lung transplantation and atrial septostomy are options and have been shown to increase survival (Trulock, 2008).

Chapter 3: Materials and Methods

Summary of Entire Experiment

Figure 2. Summary of the entire experiment.

This study was approved by the IACUC of QTest Labs. At the end of the study, dogs were euthanatized with sodium pentobarbital (Somnasol) given IV.

Nine healthy, mature, male Beagle dogs were randomly selected from a colony. They received 0.02 mg/kg of acepromazine IV and 0.25 mg/kg of butorphanol IV for sedation. Immediately thereafter, 6 mg/kg of propofol was given IV and an endotracheal tube was inserted. Electrode pads were placed on the four limbs, left thorax and over the dorsal spinous process of the 7th thoracic vertebra to obtain orthogonal lead

electrocardiograms. Leads I, II, avF V3 and V10 were continuously recorded during the experiment. An infusion of propofol, 0.5 mg/kg/min, was given using a constant rate infusion pump. Dogs were kept in right lateral recumbency. The left jugular vein was exposed to place a catheter introducer. A 4 French Millar catheter with two sensors was advanced via the catheter introducer through the right ventricle and into the pulmonary artery to obtain right ventricular and pulmonary arterial pressures. The left femoral artery was exposed and a catheter introducer was placed. A 6 French Millar catheter was advanced into the left ventricle to obtain measurements of the left ventricular and aortic pressures. A pulse oximeter was placed on the tongue to measure SPO₂ and heart rate. Catheters and electrode leads were connected to an EMKA system. A baseline recording was obtained with dogs breathing room air (~21% oxygen). A sample of arterial blood was obtained and pH and blood gases were measured. After 10 minutes of baseline recording, dogs breathed a mixture of 12 % oxygen, 5% CO₂ and 83% N₂ for 5 to 10 minutes¹. Dogs were allowed to breathe room air for a 20 minute washout period before breathing a mixture of 10 % oxygen and 5 % CO₂ for 5 minutes followed by a 10 minutes recovery. The methods are summarized (Figure 2).

¹ Gasses came from DeLille Oxygen Company, Columbus, Ohio 43207, and were accurate to <0.05%.

Each parameter was measured during baseline and during minutes 1, 2, 3, 4, and 5 while dogs breathed room air (baseline), 12% O₂, and 10% O₂. Pulmonary arterial pressures were also measured during minutes 6, 7, 8, 9, and 10 for 3 dog only. Normality of distribution for each parameter was determined by a Kurtosis test. Means of each parameter at all times and for all FiO₂'s were compared by ANOVA. When a significant F-statistic was obtained, specific means were compared using Bonferroni correction. The differences between FIO₂ means at same time points were compared using T test. The software used was Grad Pad Prism 4.

Chapter 4: Results/Discussion

The goal of this study was to characterize pulmonary arterial and right ventricular pressures in response to reductions in FiO_2 under isocapneic conditions; i.e., the effects were attributable only to reduction in PaO_2 and were not obfuscated by alterations in PaCO_2 since both a reduction in PaO_2 and an increase in PaCO_2 produce similar—but not identical—physiological consequences.

Recordings of ECG and right ventricular and pulmonary arterial pressures typical of those obtained are shown, autoscaled*, for baseline and while breathing 12% and 10% O_2 (Figure 3). Mean values for each parameter either measured (e.g., PAP, , PaO_2 , pH) or calculated (e.g., PAP pulse pressure, RV EDP difference between inspiration and expiration, product of respiratory rate and difference in RV EDP) are shown (Tables I II, III and figure 3).

*Autoscaled means that amplitudes of all curves are adjusted to occupy the same space on the paper, so values of amplitude must be obtained from reading the coordinates of the Y-axis for each tracing. Time is unaffected by autoscaling.

12%

	Room air	Min1	Min2	Min3	Min4	Min5
PA Syst PAP	24.1	28.2	33.3	32.0	31.8	31.9
PA Mean PAP	18.9	22.9	28.8	27.7	27.5	27.3
PA Diast PAP	13.7	17.6	19.9	19.8	19.9	19.9
PA Pulse pressure	10.7	10.0	9.9	10.3	9.5	10.2
RV Syst Pressure	26.6	31.1	34.7	34.0	34.0	34.0
HR	111.2	122.9	138.1	138.9	140.7	142.4

Table 1. Summary of the variables measured during baseline and exposure to 12% oxygen.

10%

	Room air	min 1	min 2	min3	min 4	Min 5
PA Syst PAP	24.4	28.7	35.6	34.3	34.6	31.5
PA Mean PAP	17.8	22.8	27.6	26.8	26.1	22.4
PA Diast PAP	14.7	18.7	23.0	22.7	22.7	21.6
PA Pulse pressure	9.5	10.1	12.0	11.5	11.1	8.9
RV Syst Pressure	27.4	30.4	36.5	36.2	35.8	36.0
HR	119.5	130.0	145.3	153.2	155.2	156.6

Table 2. Summary of the variables measured during baseline and exposure to 10% oxygen.

	BL Room air	O ₂ 12%	O ₂ 10%	P value comparing BL and 12%	P value comparing 12% and 11%
Delta EDP (parallels effort of breathing)	5.89	15.56	14.00	< 0.01	>0.05
Respiratory Rate	12.9	28.2	27.1	< 0.01	>0.05
PO ₂ mmHg	66.2a	38.75b	29.2c	0.005	0.03
PCO ₂ mmHg	55.06a	57.65a	55.1a	>0.05	>0.05
pHa	7.3142a	7.28675a	7.3152a	>0.05	>0.05
SO ₂ %	88.6a	63.75b	48.2c	<0.05	<0.05
HCO ₃ mm/L	27.92a	27.575a	27.24a	>0.05	>0.05

Table 3. dEDP and independent variables.

Figure 3. Recordings of ECG and pressures during different time points.

Independent Variables: As expected the changing FiO_2 produced changes in PaO_2 and SO_2 that served as the independent variables producing changes in the parameters of the system that depended upon the independent variable (Figure 4).

Figure 4. Effects induced by the independent variables over the dependent variables.

A decrease in arterial pressure of oxygen (PaO_2), and saturation of oxygen (SaO_2) was found as expected during the experiment. The baseline value of PaO_2 was ~65 mmHg and decreased to ~30 mmHg at 12% and 20 mmHg at 10 % oxygen. For SaO_2 , baseline values of ~90%, decreased to ~63% with 12 % oxygen, and to ~48% with 10% oxygen. The difference in PaO_2 between baseline and during reduced FiO_2 was significant

($p=0.005$), and the difference between 0.12 and 0.10 was also significant ($p=0.03$). The low value of PaO_2 (66 mmHg) at baseline was due, no doubt, to hypoventilation related to anesthesia (propofol) without positive pressure ventilation. Of course changes in SaO_2 should be similar to changes in PaO_2 since the percent saturation depends upon the partial pressure of oxygen unless, of course, affinity to hemoglobin is changed.

Since the dogs ventilated continuously with variable FiO_2 but with a constant FiCO_2 of 5% PaCO_2 , HCO_3 , and pH_a remained unchanged from baseline breathing with an FiO_2 of 21% with an inspired tension of CO_2 of 0. This constitutes isocapnea, therefore none of the observation could be attributable to alterations in CO_2 . PaCO_2 's for baseline and during all FiO_2 's varied trivially between 55.06 mmHg (baseline) and 57.65 mmHg during exposure to an FiO_2 of 0.12. Of course the PaCO_2 will possess an equilibrium with the P_ACO_2 , and both depend upon the FiCO_2 , the rate of metabolic production of CO_2 and the rate of alveolar ventilation. As will be shown, production of CO_2 increased due to the increased work of breathing, alveolar ventilation (not measured) also increased since respiratory rate tripled, while FiCO_2 was held constant. That PaCO_2 varied so minutely may be explained by a balance between the CO_2 production and increased alveolar ventilation. It would be anticipated that PaCO_2 would be fairly constant, since PaCO_2 is a prime determinant of alveolar ventilation via the biological control mechanism. That

PaCO₂ elevated slightly over baseline despite the biological control system, is consistent with reduced gain of this system due to the anesthetic regimen.

4.1 Respiratory rate.

Respiratory rate, compared to baseline values, increased as expected during the administration of FiO₂'s of 12% and 10%. Respiratory rate is determined by rate of oscillations of medullary respiratory centers that are affected principally by central (e.g., medulla, ventral respiratory group, retroambiguus, nucleus ambiguus, nucleus parambigualis, the pre-Botzinger complex, the dorsal respiratory group, pneumotaxic center, apneustic center, further integration in the anterior horn cells of the spinal cord) and peripheral chemoreceptors (aortic and carotid bodies), and by pulmonary pressures (e.g., Hering-Breuer). Minute ventilation is the product of tidal volume and respiratory rate. In general, rate of respiration is influenced most by pulmonary injury (e.g., edema, pneumonia), whereas depth of respiration is influenced most by blood gas derangement (i.e., decreased PaO₂, increased PaCO₂, decrease in pHa). With profound blood gas derangement, however, as produced by FiO₂'s of 12% and 10%, increases in both rate and depth of respiration occur and were observed in this study. These results are consistent with the biological control system "keeping" PaCO₂ in the normal range and PaO₂ above 50mmHg (West, 2008).

Most deaths from cardiac or pulmonary disease that are not sudden result from asphyxia due to fatigue of muscles of ventilation. Fatigue results most often from an energetic imbalance in which oxygen consumption by working muscles exceeds oxygen delivery, resulting in depletion of ATP, the source of fuel for ventilation. The FiO_2 decrease initiates tachypnea (increased rate of respiration) and hyperpnea (increased depth/effort of respiration), thus the increased rate and effort of ventilation produces a dramatic increase in oxygen consumption. At the same time the increased tension developed by the muscle (to allow for hyperpnea) causes compression of capillaries and veins of the muscles of respiration, decreasing the blood flow and oxygen delivery. These 2 phenomena result in an energetic imbalance, oxygen “starvation”, failure to ventilate adequately, and in asphyxia (decrease in PaO_2 and SaO_2) (Grassino and Macklem, 1984; Aubier M, 1989).

In this study, muscle blood flow was not measured; however oxygen demand (consumption) was approximated as the product of respiratory rate and effort (difference in end-diastolic pressures between inspiration and expiration). It can be observed that this “double product” increased from a baseline value of $<1400 \text{ mmHg/min}$ to $>4000 \text{ mmHg/min}$ when dogs breathed FiO_2 's of 12%. There was no additional increase in double product when dogs breathed FiO_2 's of 10%. Thus the asphyxia no doubt lead also, to

depression of respiratory function as manifested by the fact that respiratory rate was slower with a FiO_2 of 10% than with an FiO_2 of 12%.

4.2 Pulmonary and right ventricular pressures.

Pulmonary arterial systolic (Figure 5A, 5B, 5C), mean (Figure 6), diastolic (Figure 7) and pulse (Figure 9) pressures are graphed versus exposure to 12% and 10% FiO_2 's, for the 1st 5 minutes of exposure, to evaluate both values and the time-course of change with exposure. Similar displays are shown for right ventricular pressure (Figure 10). An example of contours of pulmonary arterial pressure pulses for a dog breathing 21%, 12%, and 10% O_2 is shown (Figure 11).

All pressures (except pulse pressure) from both pulmonary artery and right ventricle, increased with exposures to reduced FiO_2 and reached a maximum by 2 minutes after exposure, however the rates of rise of right ventricular pressure remained constant, for the 5 minute period of surveillance, between FiO_2 's of 21% and 12%, but decreased after 3 minutes for dogs breathing FiO_2 of 10% (Figure 12).

Of course the time changing in FiO_2 and changes in ventilation or pressures are influenced by both rate of response of neural pathways and autonomic centers and by rate of "washout" of the fractional residual capacity (FRC) by "new" concentrations of gas. The latter, in turn, depends upon the volume of the anesthetic apparatus.

dRVP/dtmax (the maximal rate of rise of right ventricular pressure) is determined by both myocardial contractility (inotrope) and preload (heterometric autoregulation), and in addition, contractility changes with changes in heart rate chronotropic-inotropism (or treppe) (figures 13 and 14) (Opie, 2004).

An increase in dRVP/dtmax could be attributed to either an increase in RVEDP (heterometric autoregulation or the Cyon-Frank-Starling law-of-the-heart) or to an increase in myocardial contractility. However as shown in Figure 15, it can be observed that RVEDP actually decreases, therefore the increase in dRVP/dtmax must be attributable to positive inotropy. Since it will be argued subsequently that the hypoxic hearts were in fact energy starved and should therefore have been under a negative inotropic influence, it follows that the positive inotrope must have been a consequence of the positive inotrope subsequent to the increase in heart rate—chronotropic-inotropism. There were no differences in RVEDP of statistical significance between a FiO₂ of 12% and 10% in either magnitude or time-course, although it appeared that the pressures were greater for 12% than for 10%, but the differences were <3 mmHg. No doubt had a larger number of dogs been studied, these differences would have achieved statistical significance although clearly neither clinical nor toxicological significance. These increases in right-sided pressures are consistent with knowledge that decreases in PaO₂

result in constriction of smooth muscle in the pulmonary vasculature that results in elevation of pulmonary vascular resistance and pulmonary arterial pressure. On the other hand the decrease in RVEDP observed in response to hypoxia reflects systemic venous dilatation and reduction in right ventricular filling that invokes the Cyon-Frank-Starling law-of-the-heart.

A

B

C

Figure 5. The time course of hypoxia induced pulmonary hypertension after the administration of 12% and 10% oxygen. Notice the difference with baseline, the peak value at minute 2 in both cases and the steady state reached with 12 % oxygen

Figure 6. Mean PAP during different time points

Figure 7. Diastolic PAP during different time points

4.3 Pulse pressure.

Pulmonary arterial pulse pressure (systolic minus diastolic) is a special case depending upon possible differences in changes between systolic and diastolic pressures. Pulse pressures are shown (Figure 8), and although there are no differences of statistical significance between dogs breathing 12% and 10% FiO₂'s, it appears that pulse pressure rose more from 2 to 4 minutes for dogs breathing 10% than for dogs breathing 12%. Pulse pressure may elevate because of either an increase in stroke volume or an increase in elasticity modulus (i.e., stiffness) of the pulmonary artery. The increase in dRVP/dtmax may have been translated to an increase in stroke volume (not measured in this study), but the increase in pulse pressure may be attributable to increased stiffness of the pulmonary artery caused by activation of smooth muscle in its wall. This is consistent with greater activation at 10% than at 12% FiO₂.

Pulse pressure in the pulmonary trunk (main pulmonary artery), into which the right ventricle ejects its stroke volume, is determined by the right ventricular stroke volume and the stiffness (elasticity modulus) of the pulmonary trunk. This is in contradistinction to mean or diastolic pulmonary arterial pressures that are determined by the product of cardiac output and pulmonary vascular resistance. Pulmonary vascular resistance is determined by the degree of constriction or relaxation of smooth muscle in

the smaller pulmonary arteries, pulmonary capillaries, and pulmonary veins. Both components (impedance and resistance) constitute the hindrance to ejection. The impedance is thought to contribute <25% of the effort of the ventricle; the resistance is thought to constitute the majority of the effort of the right ventricle. If pulmonary vascular resistance increases and pulmonary arterial pressure elevates, the entire pulmonary vascular tree will become stiffer (lowering the compliance) (Castelain V et al, 2001) so an increase in resistance might be expected to also increase impedance. However in the systemic circulation, it is known that some drugs and diseases may decrease systemic vascular resistance but actually increase impedance; that is peripheral systemic arterial pressure falls but central aortic pressure (“what the ventricle sees”) increases or at least falls much less. The CAFE study, prospectively evaluated the effects of cardiovascular drugs on derived central aortic pressures and hemodynamics. It showed that brachial blood pressure is not always a good surrogate for the effect of blood pressure-lowering drugs on arterial hemodynamics. For example (Figure 8), atenolol±thiazide-based treatment was much less effective than amlodipine±perindopril-based treatment at lowering central aortic pressures despite equivalent lowering of peripheral pressures. That is, whereas equivalent peripheral arterial pressure pulse were obtained for both drugs, the central aortic pressure pulses differed significantly (Williams

Figure 8. Comparison between Amlodipine and Atenolol and brachial and systemic pressure. (Taken from Williams B, Lacy, 2006.)

In this model an FiO₂ of 10% oxygen induced an approximate 25% (9.5 mmHg to 12 mmHg) increase in pulse pressure by the 2nd minute of exposure. An FiO₂ of 12% produced only a 9% increase in pulse pressure P<0.05).

Figure 9. Pulse PAP

Figure 10. RV systolic pressure.

Figure 11. Example of contours of pulmonary arterial pressure pulses for a dog breathing 21%, 12%, and 10% O₂.

Figure 12. dp/dt

Figure 13. dP/dT max vs HR at 12% Oxygen

Figure 14. dP/dT max vs HR at 10% Oxygen

Figure 15. EDP vs Time

Figure 16. Energetics of respiration

4.4 Energetics of Respiration.

Energetics refers to the balance between useful work performed by a machine (in this case ventilation of the lung) and the oxygen consumed to perform that work. If either the useful work decreases or the oxygen demand increases, the organ is said to be in a negative energetic state. This might decrease oxidative production of ATP, resulting in “fuel” starvation and depressed function. In contracting respiratory muscles, the oxygen they consume would be estimated as the product of respiratory rate and difference in intrapleural pressure. In this study pleural pressure was not measured, but the difference in right ventricular end-diastolic pressure served as a surrogate. When the dogs in this

study breathed with reduced FiO_2 , the hypoxia resulted in a dramatic increase in both respiratory rate (RR) and fluctuations in right ventricular end-diastolic pressure (Figure 16), thus the double product ($\text{RR} \times \Delta \text{EDP}$) increased still more. Thus demand for oxygen must have increased dramatically. However during times when the respiratory muscles tense as they perform work, the increased muscle tension compresses blood vessels within and obstructs blood flow and oxygen delivery. Furthermore since rate also increased and the muscles spent a lesser time in relaxation, the increase in rate also results in decreased oxygen delivery. Thus on both accounts (i.e., increased oxygen demand, decreased oxygen delivery), muscles of respiration and probably the myocardium as well become oxygen starved. This was demonstrated when dogs breathing 12% O_2 for 5 minutes, did not increase $\text{dRVP}/\text{dt}_{\text{max}}$ further, and dogs breathing 10% actually decreased $\text{dRVP}/\text{dt}_{\text{max}}$.

Chapter 5: Limitations

Despite of the fact that the n used in this study (9) was statistically adequate for many of the parameters evaluated (e.g., PAP, RVP, PaO₂ between baseline and reduced FiO₂s), the number of dogs studied precluded obtaining statistical significance for differences between 0.12 and 0.10 in others (e.g., PaO₂ between 0.12 and 0.10, dRVP/dt_{max} between 0.12 and 0.10). On the other hand, it would have been desirable to measure cardiac output and calculate both stroke volume and pulmonary vascular resistance. That information, in conjunction with the change in pressures, would have given us a most accurate understanding of the changes in energetics and, directly, of activity of vascular smooth muscle during hypoxia and the adaptative mechanisms that are generated in acutely induced pulmonary hypoxia.

The baseline values of arterial blood gases obtained with this anesthesia protocol are considered borderline for hypoxia at sea level, however in Bogota, Colombia (3,650 M), a PaO₂ of 66 mmHg is considered normal. No doubt the hypoxia at baseline arose from well-known depression of ventilation by the preanesthetic (butorphanol) and anesthetic (propofol). In fact, many of the dogs became transiently (for < 1 minute) apneic

immediately after induction, and were ventilated artificially before baseline measurements were obtained. It would be desirable to conduct a study on more dogs using chloralose-urethane anesthesia, a regimen known to permit nearly intact control of respiration, however the effect of volume expansion and hemodilution produced by infusion of large volumes of the anesthetic may also confound the results. The anesthetic use in this study conforms loosely to that used in clinical practice.

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