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The Effects of Pulmonary Hypertension in Diabetic Zucker Rats on Pulmonary Vascular Contraction and Right Ventricular Size

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Literature Review

Introduction

Pulmonary hypertension is a life-threatening disease that is identified by a resting

mean pulmonary arterial pressure over 25 mmHg and established by right heart

catheterization (Saglam et al., 2015). The symptoms commonly associated with

pulmonary hypertension include fatigue, dyspnea, syncope, and chest pain, which

severely limit quality of life in diagnosed patients (Saglam et al., 2015). According to

recent studies in patients with pulmonary hypertension, the one, three and five year

mortality rates are 8%, 25%, and 34%, respectively (Burudpakdee, Shah, Joish, Divers, &

Yaldo, 2014). Although some advances have been made in therapies for pulmonary

hypertension, the prognosis is still poor, and there is a lack of understanding of its

mechanisms (Voelkel, Gomez-Arroyo, Abbate, Bogaard, & Nicolls, 2012). This study

aims to examine and understand some of those mechanisms in the Zucker Rat.

Additionally, previous analyses have shown that individuals with diabetes

mellitus have an increased incidence of pulmonary hypertension (Gurney, & Howarth,

2009). The number of individuals with diabetes has skyrocketed in previous years, and

the International Diabetes Federation shows that the worldwide prevalence of diabetes as

of 2013 is 382 million (Shi, & Hu, 2014). Additionally, this number is expected to rise

and could be as high as 592 million by 2035 (Shi, & Hu, 2014). Dynamics that could

bring about pulmonary hypertension associated with diabetes include microvascular

disease, autonomic neuropathy, and metabolic derangements such as hyperglycemia and dyslipidemia (Blendea, McFarlane, Isenovic, Gick, & Sowers, 2003). The mechanisms of these dynamics are poorly understood, and the relationship between pulmonary hypertension and diabetes should be examined. The purpose of this study was to examine the difference in effect of diabetes on pulmonary hypertension using an animal model.

Pulmonary Hypertension

Pulmonary hypertension (PH) is a dynamic disease distinguished by the uncontrolled growth of smooth muscle and pulmonary vascular endothelial cells causing constriction of the pulmonary arteries which results in abnormally high vascular resistance and failure of the right heart characterized by right ventricular hypertrophy (Moral-Sanz et al., 2011). Right ventricular hypertrophy eventually causes heart cells to die, which has been demonstrated to be a crucial event during the end stages of heart failure (Doggrell, & Brown, 1998). A number of possible causes of PH have been identified, but the mechanisms of the disease are complicated and poorly understood (Brunner et al., 2014). Although progress has been made in the management of PH, the prognosis is still poor, and there is currently no curative treatment for this destructive disease (West et al., 2014; Farkas, & Kolb, 2013). Current vasodilator treatments act to increase circulation in the lungs and help to stave off increased pulmonary vascular pressures resulting in right ventricular hypertrophy. However, this does not target the underlying problem of the disease, which is pulmonary vascular remodeling (Farkas, & Kolb, 2013).

Diabetes Mellitus Type II

Diabetes is a disease chiefly distinguished by high blood sugar levels that increase the risk of developing microvascular damage such as retinopathy, neuropathy, and nephropathy. According to the World Health Organization, it decreases life expectancy and shows appreciable morbidity due to diabetes related microvascular problems, a lesser quality of life, and serious macrovascular problems such as heart disease, peripheral vascular disease, and stroke. Diagnosis of diabetes has seen a startling increase in the past twenty years, and current statistics show approximately 6.4% of the world's population has some form of diabetes (Carter, Gray, Troughton, Khunti, & Davies, 2010).

According to Movahed and colleagues (2005), the prevalence of PH in diabetic patients is significantly higher independent of other comorbidities such as coronary artery disease, hypertension, heart failure, or smoking. One of the complications of uncontrolled glucose levels in diabetes is the development of pulmonary hypertension (Gurney, & Howarth, 2009). Recent studies have also linked endothelial dysfunction as an important factor in the progression of diabetic retinopathy, nephropathy, and atherosclerosis in both types of diabetes (Lopez-Lopez et al., 2008). Despite the close relationship with systemic and pulmonary cardiovascular disease, it has only been shown recently that type 1 and type 2 diabetes are risk factors for pulmonary hypertension. Limited research has been conducted to examine this relationship (Lopez-Lopez et al., 2008).

Pharmacology

Potassium Chloride (KCl). Potassium chloride (KCl) has been used in a number of studies to produce substantial contractions in mammalian cardiac muscle. It has also

been used to avoid G-protein coupled receptor (GPCR) activation and mobilize smooth muscle by altering the K^+ equilibrium potential and fastening membrane potential above the resting level. It has been used in other studies to induce maximum contractions in vascular smooth muscle that can then be used as a reference for further experimentation (Schuh et al., 2003). Because of these substantial contractions, KCl was used in the study to test pulmonary arterial vascular rings for contractility (Bassett, & Wiggins, 1976).

Phenylephrine. Phenylephrine is a sympathomimetic vasoconstrictor similar in structure to epinephrine. Its chemical structure deviates from epinephrine only in the absence of one hydroxyl group on the benzene ring (Eccles, 2007). Phenylephrine is a somewhat discriminatory α_1 agonist, with poor α_2 agonist activity (Eccles, 2007). It induces contraction of muscle through various mechanisms and calcium channels such as L-type voltage-operated calcium channels and receptor-operated calcium channels (Kim, et al., 2014). Phenylephrine was used in this study to initiate pulmonary arterial vascular contraction.

Acetylcholine Acetylcholine is a neurotransmitter that plays an important role in many biological processes of both the central and peripheral nervous system (Pinheiro et al., 2015). The synthesis of acetylcholine occurs in an uncomplicated biochemical process. It involves the transfer of the acetyl group from acetyl-coenzyme A to choline by an enzyme called choline acetyltransferase (Schwarz et al., 2013). Once synthesized, acetylcholine is the excitatory neurotransmitter used at the synapse between the neuromuscular junctions in mammals (Bagnall, du Lac, & Mauk, 2013). It has also been documented to induce smooth muscle dilation in vivo in mammalian pulmonary arteries

and systemic arteries and was used in this study to induce vasodilation of pulmonary

arterial vasculature (Vanhoutte, Rubanyi, Miller, & Houston, 1986)

Monocrotaline ($C_{16}H_{23}NO_6$). Monocrotaline (MCT) is a toxic pyrrolizidine

alkaloid derived from the seeds of the *Crotalaria spectabillis* plant. When administered

in small doses caused delayed and progressive lung injury resulting in pulmonary

vascular remodeling, pulmonary hypertension and resultant right heart hypertrophy

(Schultze & Roth, 1998; Broderick, Wang, Gutkowska, Wang, & Jankowski, 2010).

Pulmonary hypertension produced by MCT injection is well documented in numerous

rodent studies of pulmonary hypertension (Broderick, T. L., & King, T. M. 2008).

Animal Models

The utilization of the rat animal model is economically reasonable and allows for

measurement of important hemodynamic and cardiac parameters in vitro (Doggrell, &

Brown, 1998). The Obese Zucker rat has been used in many studies and has become a

popular model for type II diabetes and its links to systemic vascular dysfunction (Moral-

Sanz, Moreno, Cogolludo, & Perez-Vizcaino, 2014). These Zucker rats typically develop

obesity and insulin resistance around seven weeks of age (Schmidt, Dorsey, Beaudet, &

Peterson, 2003; Shiota, & Printz, 2012).

This study examined a variety of factors including right ventricular pressures and

heart weights, right ventricular ratios, and contractility properties of the pulmonary

vascular ring using a Schuler tissue bath. Elevation of right ventricular pressures and the

development of right ventricular hypertrophy are clinical signs of pulmonary

hypertension resulting in pulmonary arterial vascular remodeling with thickening of the vascular wall which may impact contractile and/or relaxant ability of the vessel.

Specific Aims

The aims of this study are to compare the effects of pulmonary hypertension between non-diabetic control Zucker rats and Zucker rats with PH and PH plus Type II diabetes in terms of pulmonary artery relaxation function and right ventricular hypertrophy.

Hypothesis

The hypotheses of this study are

1. The pulmonary arterial vascular contractile responses will be decreased in the PH and PH plus type II diabetic rats as compared to the control rats; and

2. The pulmonary arterial vascular relaxant responses will be decreased in the PH and PH plus type II diabetic rats as compared to the control rats.

Methodology

This study was performed following approval of University of Arkansas-Fayetteville Institutional Animal Care and Use Committee (IACUC). All University and Laboratory Animal Training Association (LATA) protocols and guidelines for animal handling were followed.

Sample

An a priori power analysis was conducted to establish group sizes of 8 were sufficient to achieve a power value of $>.80$. A three-group experimental design was used.

The groups consisted of age-matched Zucker rats including 9 control Zucker rats, 6 lean PH Zucker (Fa/fa) rats and 4 type II diabetic PH Zucker (fa/fa) rats(Harlan Laboratories, Indianapolis, IN). The study experienced a 50% death rate (n=4) in the lean Zucker (Fa/fa) rats and 1 death in the type II diabetic Zucker (fa/fa) group prior to the experiment. Additionally the study experienced 1 type II diabetic Zucker (fa/fa) pulmonary artery tissue death during the experiment accounting for the variable number of rats in each group.

Animal Care

Animals were received into the Central Live Animal Facility (CLAF) facility and allowed to acclimate to their surroundings for two weeks. Animals were housed two rats per cage (10.25"W X 19"D X 8"H) with micro-filter tops and autoclaved pine litter. The animals were allowed standard rat chow food and water ad libitum. A reversed 12:12 light-dark cycle was used and the rats were allowed unrestricted cage activity. All procedures were performed under a laminar hood in the CLAF facility until the terminal phase of the experiment. At that time, animals were transported to Dr. Smith-Blair's laboratory in HHPR 321 and allowed to acclimate for a minimum of 1 hour prior to the beginning of the experiment.

Pre-Experiment Protocol

Monocrotaline (MCT) Injection Procedure Following the arrival and a two week stabilization period in the CLAF, MCT 40 mg/kg was injected subcutaneously into the experimental groups of rats. This dose of MCT has been demonstrated by Handoko and colleagues (2009) to produce stable pulmonary hypertension with a preserved cardiac

output. The animals were observed daily for four weeks for clinical signs of complicating right heart failure and compromised cardiac output (respiratory distress, cyanosis, lethargy, weight loss, and decrease in responsiveness to stimulation). Five animals were euthanized according to the approved IACUC protocol due to progressive pulmonary decompensation with persistent weight loss and lethargy.

Body Weight/Blood Glucose Body weight was measured daily and blood glucose levels measured weekly on all animals. Blood glucose levels were obtained using a micro-lancet to obtain blood from the rat tail and measured using the Accu-Check Active™ meter.

General Experimental Procedures

On the day of the terminal phase of the experiment, the animals were transported to the laboratory and allowed to acclimate for a minimum of 1 hour. Following obtaining a surgical plan of anesthesia using pentobarbital sodium (50 mg/kg body weight), a tracheotomy was performed and a stainless steel tracheotomy cannula with Luer adapter, 1.8 mm OD, 25 mm in length (Harvard Apparatus, Holliston, MA) was inserted and sutured in place. The tracheal cannula was connected to pressure-controlled rat ventilator. The settings of the rodent ventilator were 10 mL/kg (tidal volume) and 60 cycles/min.

The diaphragm was exposed via a midline abdominal incision. A 20G needle attached to a low pressure transducer was inserted through the diaphragm into the right ventricle. Right ventricular pressure was measured via the Digi-Med™ (Model 200) low-pressure transducer. This model of transducer measures pressures within an error range of + 0.1 mmHg.

Following obtaining the right ventricular pressure, the animal was given an overdose of pentobarbital (100 mg/kg body weight). The thorax was quickly opened surgically and the heart and lungs removed. The heart and lungs were submerged in a dissection dish containing Krebs solution bubbled with low flow 95% oxygen/5% CO_2.

Vascular Reactivity Studies The pulmonary artery rings (~2 mm long and an internal diameter ~ 0.3-0.5 mm) were dissected and mounted on a tissue hook supports in a Schuler tissue bath™ (Harvard Apparatus, Holliston, MA). After stretching to give an appropriate resting tension (equivalent to 0.75 g), tissues were continuously bathed in the chamber with Krebs solution at 37^0C with a mixture of 95% O_2-5% CO_2 as previously described by Cogollundo et al (2001).

KCL contraction challenge. Following equilibration, the vascular muscle contractile responses were evaluated using a KCL 96mM solution infused into the tissue bath chamber. This procedure was performed to obtain a baseline measurement of the viability of each pulmonary artery vessel to ensure the artery was not damaged during isolation or mounting of the vessel. The KCL was allowed to saturate the vascular ring for 2 minutes after which the peak contraction was recorded. Following measurement, the vessel was thoroughly rinsed with Krebs' solution and the vascular ring allowed for equilibrate for 10 minutes. This process was repeated twice. Following the experiment the developed force was calculated (peak contractile force – resting tension) to reflect the change in contractile properties of the vessel.

Tissue rings were then exposed to phenylephrine 10^{-9} to 10^{-4} mM concentrations to induce a contraction 70% of the KCl max contraction is obtained. Tissues were rinsed thoroughly between concentration level testing and allowed to rest 2 minutes. Once the

phenylephrine concentration producing 70% of the response to KCl, Phenylephrine (10^{-7} mM) was used to evaluate the contractile response.

The tissue baths were then infused with phenylephrine (10^{-7} mM) along with increasing concentrations of acetylcholine (10^{-9} to 10^{-4} mM) to test the relaxant properties of the tissue. Concentration curves were obtained for acetylcholine concentrations (10^{-9} to 10^{-4} mM).

Measurements obtained in this study included the following: baseline resting tension, peak contraction, and developed force (calculated). The optimal baseline resting tension was determined prior to the start of each experiment and was adjusted to be maintained constant throughout the experiment. The peak contraction was determined as the maximum force (gms) produced by the pulmonary arterial vascular ring when exposed to either KCL, phenylephrine or phenylephrine/acetylcholine solutions. The developed force was calculated as difference between the peak contraction and the baseline resting tension and was used as the measure of contractile change with each measurement. Using these measurements, the effectiveness of the various concentrations of phenylephrine and phenylephrine/acetylcholine was gauged.

Results

A one-way analysis of variance (ANOVA) was used to examine if there were statistically significant differences between the means of the three groups in relation to weight, height, blood glucose, KCl contractions, and heart weight and heart ratio. Second, one-way repeated measures ANOVA was used to examine if differences existed between groups with respect to developed force at varying concentrations of phenylephrine and phenylephrine/acetylcholine solutions.

An initial alpha level of 0.05 was selected as the criterion for statistical significance. This level of significance was then adjusted to an alpha level of 0.01 using an adjustment in the Bonferroni procedure to control for an overall type I error rate due to performing multiple analyses. To account for deviations from sphericity, the Greenhouse-Geisser Epsilon correction factor was used to adjust the univariate test degrees of freedom for each test involving with-in subject effects.

Weight and Length. Three groups of rats, two groups with induced pulmonary hypertension (PH) and one control group, were used for these experiments and included control lean rats (N=9), lean PH rats (N=6) and type II diabetic PH rats (N=4). An ANOVA demonstrated there was a statistically significant difference between groups with respect to weight [$F_{(2, 18)} = 87.25$, $p < .005$]. Post hoc tests revealed there was a statistically significant difference between the diabetic PH rats and the lean control rats ($p < .005$) and lean PH rats ($p < .005$) but there was not a statistically significant difference in weight between the lean PH and lean control rats (Figure 1). An ANOVA did not reveal a significant difference in body length measurements between the groups (Figure 2)
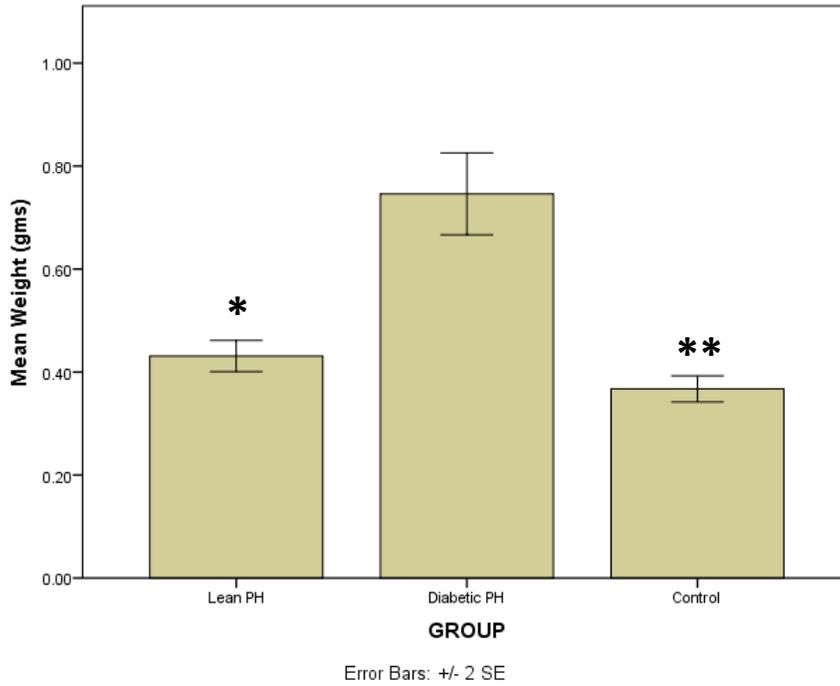
Figure 1 – Comparison of mean weights (gms) between lean PH, Diabetic PH and control rats. No statistically significant difference was noted between Lean PH and Control rats.
 *Denotes a significant difference between Lean PH and Diabetic PH rats.
  **Denotes a significant difference between Diabetic PH rats and Control rats.
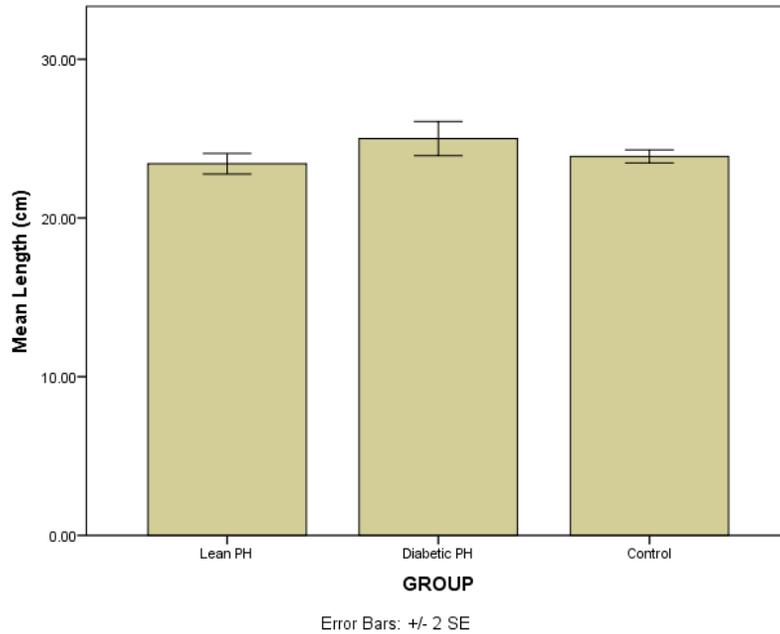
Figure 2 – Comparison of the body lengths (cm) of the three groups. No statistically significant differences were noted between groups in relation to length

Blood Glucose Levels. Blood glucose levels were monitored weekly and analyzed using ANOVA. A statistically significant difference in blood glucose was noted between groups [$F_{(2, 18)} = 243.94$, p < .005]. A post hoc test revealed a statistically significant higher blood glucose levels in the diabetic PH than the lean PH rats ($p < .005$) and lean control rats ($p < .005$). There was not a statistically significant difference in blood glucose levels between the lean PH and lean control rats (Figure 3).
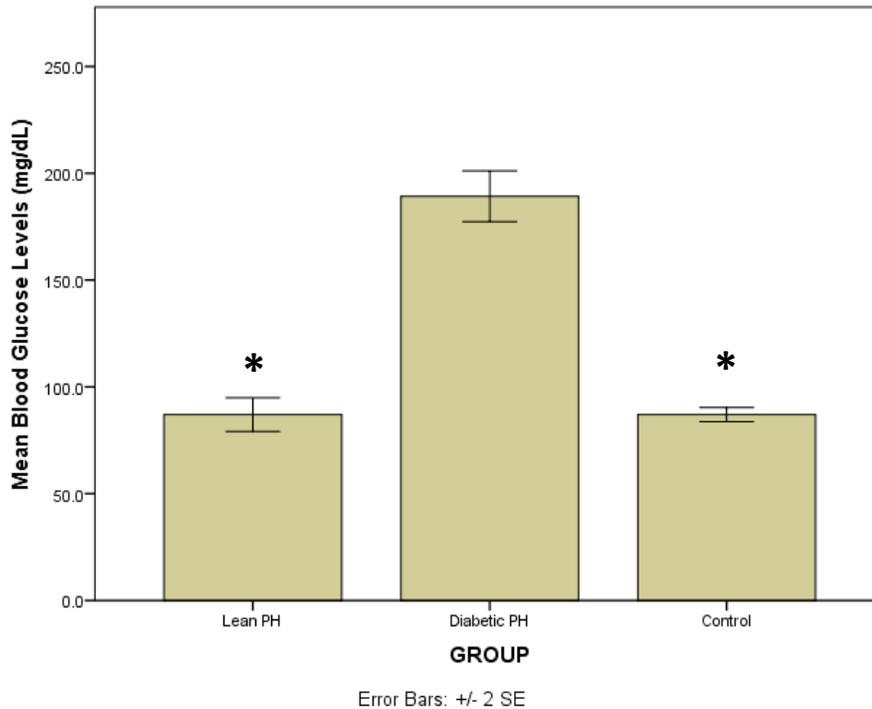
Figure 3 – Comparison of the mean blood glucose levels (mg/dL) of the three groups.
* Denotes statistically significant difference between Lean PH rats and Diabetic PH rats and between Diabetic PH rats and Control rats. No statistically significant difference in blood glucose between Lean PH and Control rats.

Right Mean Ventricular Pressures. An ANOVA revealed a statistically significant difference in the mean right ventricular pressures between the three groups [$F(2,18) = 14.46$, $p < .005$]. Post hoc tests revealed there was not a statistically significant difference between the right mean ventricular pressures in the lean PH and diabetic PH rats. However, the control rats had statistically significant lower right ventricular pressures than either the lean PH rats ($p = .001$) or the diabetic PH rat ($p = .002$) (Figure 4).
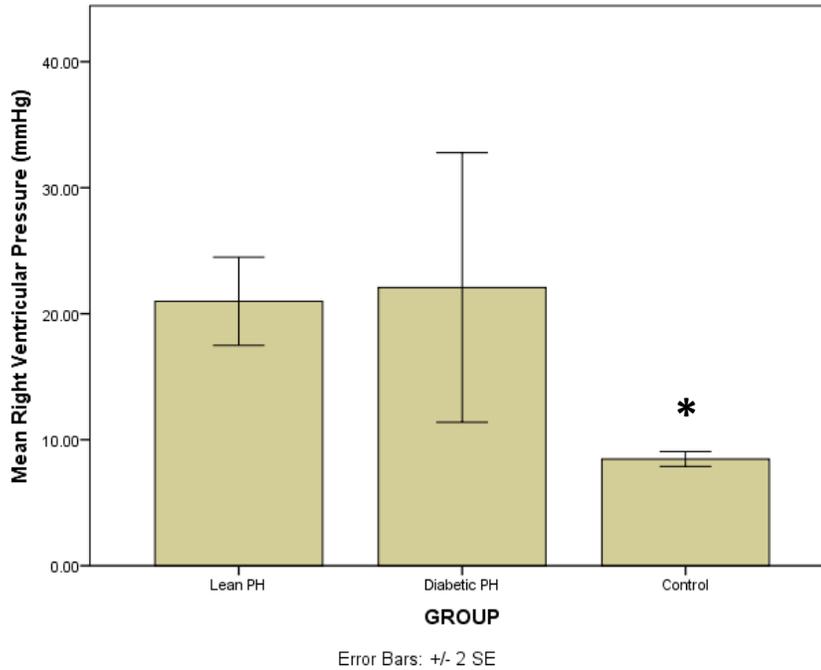
Figure 4 – Mean right ventricular pressures between the three groups
* Denotes statistically significant difference in right ventricular pressure between Lean
PH and Diabetic PH with regard to Control rats.

Mean Heart Weight. An ANOVA determined there was a statistically significant

difference between the three groups with regard to mean heart weight [F (2, 18) =16.86,

$p<.005$]. Post hoc comparisons indicated a statistically significant increase in the lean PH

($p=.001$) and diabetic PH ($p<.005$) rate mean heart weights as compared to the control rat

mean heart weights (Figure 5).

Heart weight Ratio. Heart weight ratios were calculated by the following

formula: (left ventricular heart weight – septum)/right ventricular weight. An ANOVA

was used to determine if there were statistically significant difference between the three

groups of rats. The analysis did not demonstrate a statistically significant difference

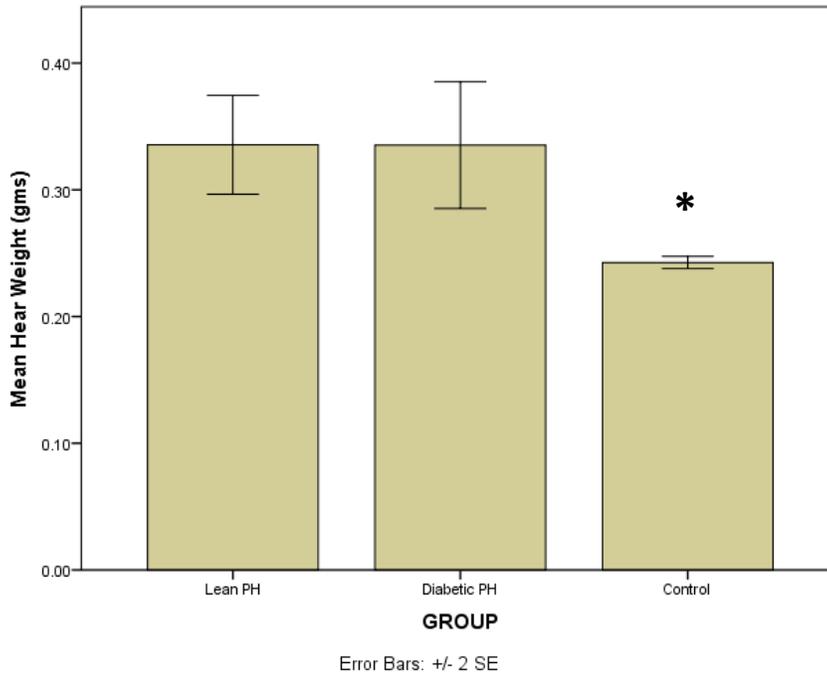between the groups of rats [F (2, 18) = .34, $p= .71$].

Figure 5 – Mean Heart Weight comparisons between the three groups.
*Denotes a statistically significant difference between Lean PH and Diabetic PH with regard to Control rats.

Potassium Chloride Infusion. Potassium chloride (KCl) infusions (0.096 mol/L) were used to test the contractility of the pulmonary vascular arterial rings for viability. It also was used to determine the concentration of Phenylephrine HCl infusion (a concentration that produced 70% peak contraction generated by the KCl test) to be used for the experiment. An ANOVA determined there was no statistically significant difference in peak contraction between the groups with the first contraction test [F (2, 17) = 1.96, p= .18] or the second contraction test [F (2, 17) = 3.28, p= .06] (Figure 6). Additionally there was not statistically significant difference in developed force between the groups [F (2, 14) = .63, p= .55].
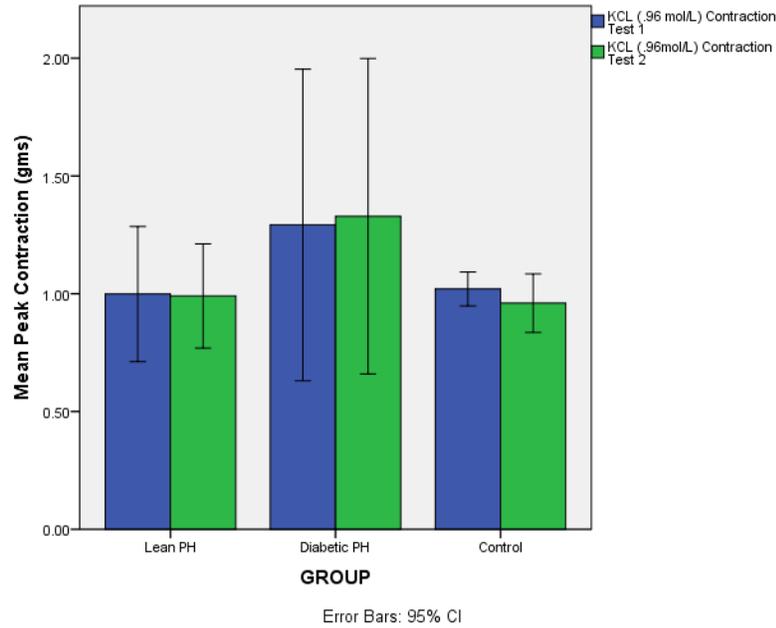
Figure 6 – Mean Peak Contraction (gms) with infusion of KCL. No statistically significant difference was noted between groups with either KCl test.

Phenylephrine HCl Infusion. Following the KCl contraction tests, the magnitude of developed force produced to achieve 70% of the peak KCl contraction through stimulation of the pulmonary artery vascular ring was determined through exposure of the tissues to phenylephrine HCl concentrations of -9 mOm to -4mOm successively added to the tissue wells. A concentration of phenylephrine HCl -7mOm was used as the constant infusate for the acetylcholine phase of the experiment based on the peak contractions of the 3 groups (Figure 7).
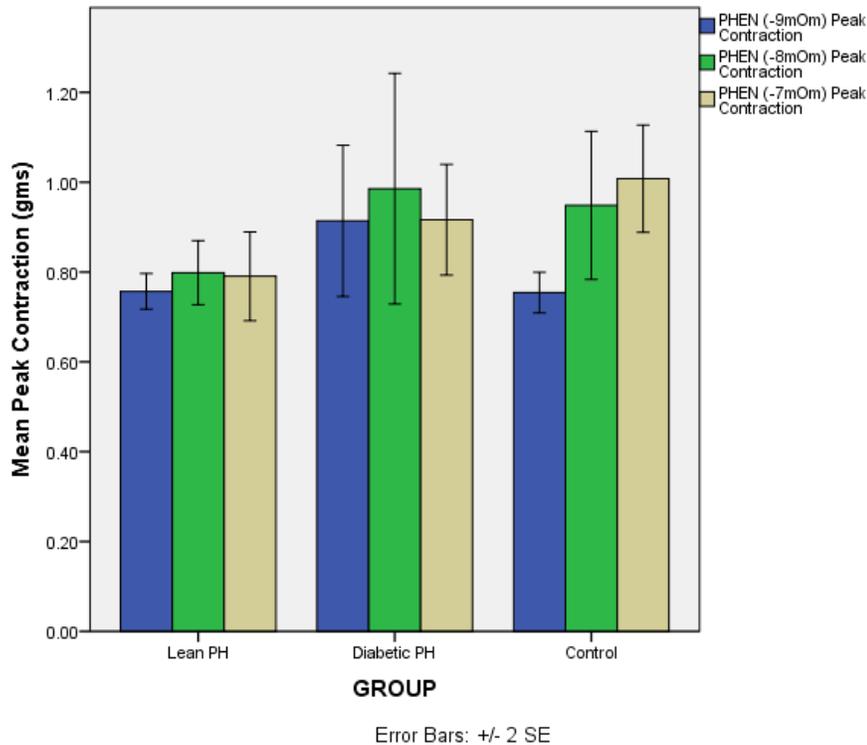
Figure 7 – Mean peak contraction at varying levels of phenylephrine

A one-way repeated measures ANOVA was used to determine if there was a statistically significant difference in developed force at phenylephrine concentrations of -9 mOm, -8 mOm, and -7 mOm between the Control group, and the Lean PH and Diabetic PH groups. The assumption of sphericity was not met, as assessed by Mauchly's test of sphericity, $x^2 (2) = 8.92$, $p= .01$. Epsilon (ε) was .61 as calculated by Greenhouse & Geisser (1959), and was used to correct the one-way repeated measures ANOVA. The change in phenylephrine concentrations did not elicit statistically significant changes in developed force over time [$F (1.23, 12.28) = 2.93$, $p = .12$] (Figure 8).
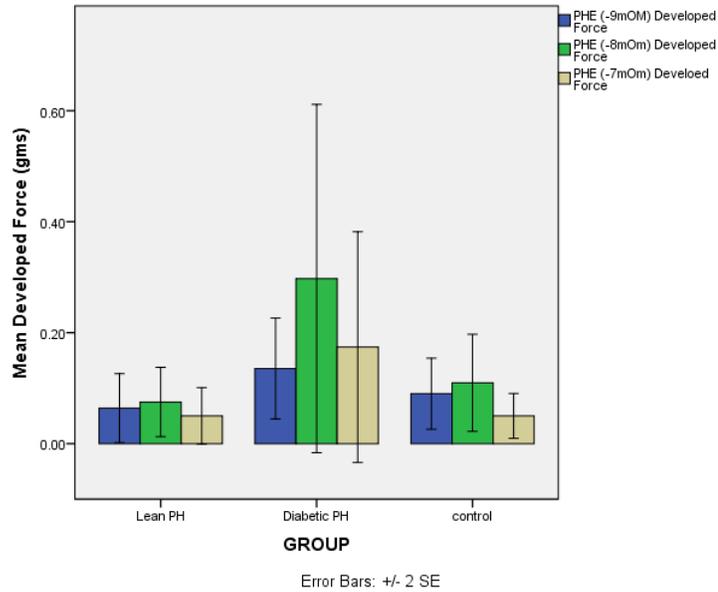
Figure 8. Developed force of pulmonary arterial vascular contraction (gms) at varying levels of Phenylephrine

Acetylcholine/phenylephrine (-7mOm) infusion. Phenylephrine HCl

concentration (-7mOm) produced a peak contraction of 70% of the maximum contraction

obtained with the KCL infusion was infused in the tissue bath in combination with

acetylcholine concentrations from -9mOm to -4mOm. Following each successive

concentration of acetylcholine pulmonary arterial vascular contractility measures were

obtained. An ANOVA was used to determine if there were statistically significant

differences between the Control group, and the Lean PH and Diabetic PH groups at the

tested concentrations of phenylephrine/acetylcholine -9 mOm to -4 mOm. Mauchly's test

of sphericity indicated that the assumption of sphericity had been violated, $x^2(9) = 38.57$,

$p< .005$. Therefore the Greenhouse & Geisser (1959) was calculated and used to correct

the one-way repeated measures ANOVA. There was no statistically significant

difference in peak contraction [F (2.22, 33.31) = 2.32, $p= .11$] or developed force [F

(1.68, 25.14) = 2.17, p= .14] of the pulmonary arterial vascular tissue between any
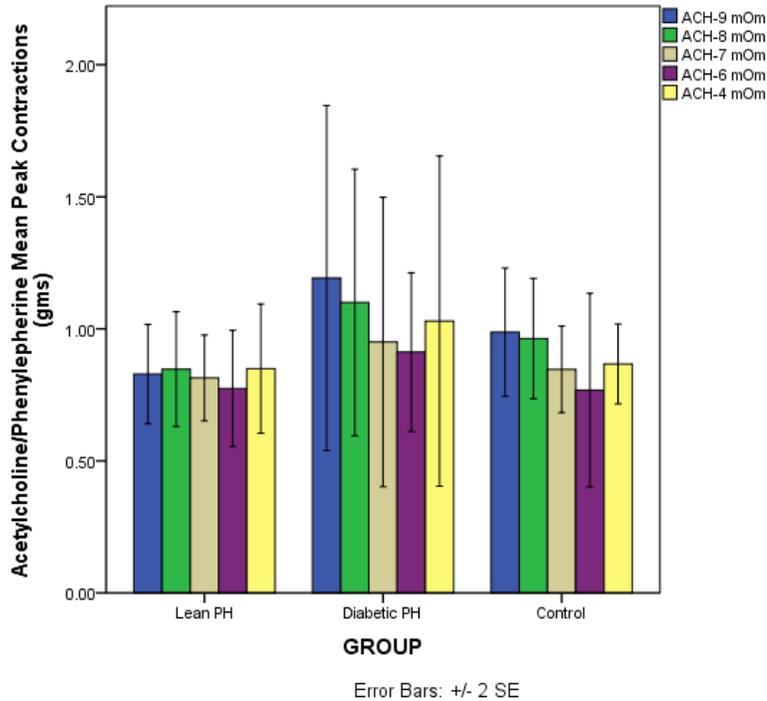
concentrations of acetylcholine (Figure 9).

Figure 9. Acetylcholine/Phenylephrine Mean Peak Concentration between groups at varying concentrations of acetylcholine. No statistical significant difference noted.

A one-way repeated measures ANOVA was computed to compare the developed force by groups. Data from the acetylcholine/phenylephrine -5mOm concentration was deleted from the observations due to an outlier. Mauchly's test of sphericity indicated that the assumption of sphericity had been violated, x^2 (9) = 33.96, p< .005. Epsilon (ε) was .415 as calculated by the Greenhouse & Geisser (1959), which was used to correct the one-way repeated measures ANOVA. There was no statistically significant difference in developed force [F (1.66, 21.59) = .964, p= .12] of the pulmonary arterial vascular tissue between any concentrations of phenylephrine/acetylcholine (Figure 10).
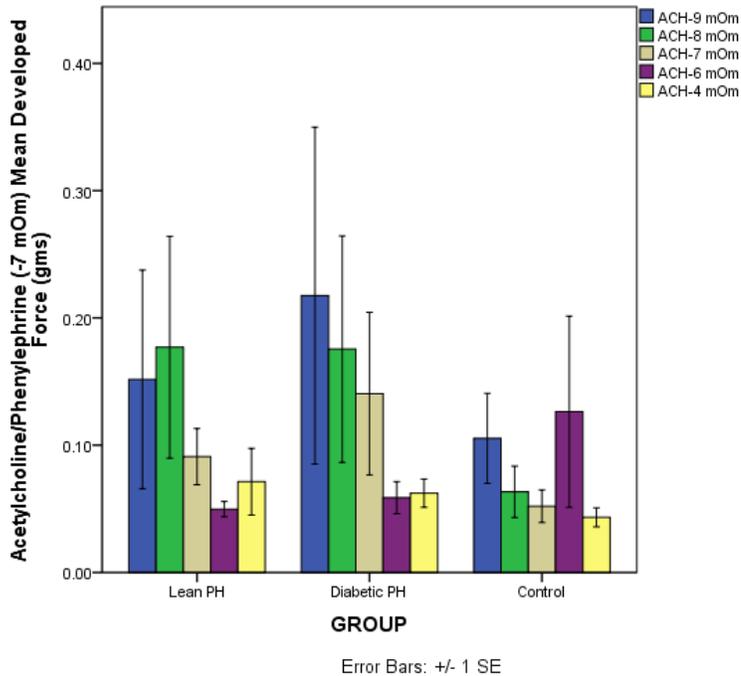
Figure 10 – Comparison of the Acetylcholine/Phenylephrine (-7mOm) Mean Developed Force (gms) between the three groups. No statistically significant differences were noted between groups at any of the acetylcholine concentrations.

Discussion

The body weights and lengths of the diabetic PH rats in our study were significantly heavier than both the Lean PH and Control rats, which is consistent with the literature (Moral-Sanz, et al., 2014). Additionally blood glucose levels of type II diabetic Zucker rats were significantly higher (mean = 189.25 mg/dl) than the lean rats (mean = 87.0) or the control rats (mean = 87.0) confirming the development of type II diabetes. This development which is consistent with insulin resistance in the Zucker rats (fa/fa) is consistent with other studies (Schmidt, Dorsey, Beaudet, & Peterson, 2003).

Pulmonary hypertension is characterized by elevated pulmonary pressures resulting in compensatory right ventricular hypertrophy. The weight of the right ventricle can be used as an indirect index of pulmonary artery pressure. Increased right ventricular

weight compared to the left ventricle plus the septum weight has been described in the literature as a measure of the hypertrophy (Moral-Sanz et al., 2012). Additionally, increased right ventricular pressure has also been noted in PH (Csiszar et al., 2009). Statistically significant increases in right ventricular pressures seen in the lean PH and diabetic PH rats were also consistent with the literature. Our data highlights the drastic differences between the two experimental groups and the control group. Heart weights and the heart weight ratio were determined in all animals to evaluate right ventricular hypertrophy. The heart weights indicated that both experimental groups of rats had greater heart weights than the control rats suggesting that pulmonary hypertension existed in these groups. Hearts from rats affected with PH were hypothesized heavier than rats that do not have PH which was consistent with the literature (Moral-Sanz et al., 2012). However, we did not find an increase in heart weight ratio (left ventricular heart weight – septum/right ventricular weight) in the PH experimental groups which came as a surprise after significance was found in right ventricular pressures and heart weights. Although measures to maintain reproducibility of heart dissection was observed by having the same researcher dissecting each heart, some variation in tissue landmarks may have impacted this latter measurement.

Potassium chloride was used to establish a maximum contraction of the tissues, confirmed viability of the tissue samples, and provided a consistent level of contraction to be achieved with the phenylephrine infusion. Although not statistically significant, it is interesting to note that the diabetic PH group had a slightly higher mean peak contraction in response to KCL infusion. The findings of the phenylephrine contractions correspond

to results found in other studies that used similar concentration-response curves for phenylephrine (Gomart et al., 2014).

It has been noted in numerous studies (Dietrich et al., 2010; Vanhoutte, Rubanyi, Miller, & Houston, 1986) that acetylcholine induces smooth muscle dilation in pulmonary arteries. We observed dose-related dilation effects and were able to compare the differences between lean PH rats, diabetic PH rats, and a control group with neither morbidity. The results of the study indicate that there were no significant differences in the pulmonary artery contractile function between the three groups of rats. Additionally, we found no statistically significant changes to the relaxant properties with increased concentrations of acetylcholine. Although no significance was found in pulmonary artery contractile function in this study, there may be alternative mechanisms that could be tested instead of the dilation effects of acetylcholine. One such mechanism could be the vasodilatory effects of nitric oxide (NO) on pulmonary artery contractile function (Hampl, Tristani-Firouzi, Hutsell, & Archer, 1996). Future studies should aim to test these alternative mechanisms.

The identified limitations encountered during the course of the research may have impacted the results of the study. The laboratory experienced death of a large number of obese Zucker rats. Additionally, we experienced death of one of the tissues after extraction. Both factors limited the amount of data available to four rats in that group. Possible causes of death for the rats may have included complications from the MCT injection and the increase in severity of PH progressing to death in the animal. Although the dosage of MCT used (40 mg/kg) has been found to produce stable PH in other studies (Handoko et al., 2009) it is unclear why some animals experience a more severe disease

progression. The total experimental time of these experiments was longer than other

studies, which include some ending as soon as 28 days post injection of MCT

(Dumitrascu et al., 2008). Our longer study period could have contributed to the higher

mortality from PH complications. Alternatively, a virus may have infected the rats and

been spread through their respiratory tract, although preventive measures were taken with

the micro-filter tops and autoclaved pine litter. The quick nature in loss of the animals

lends to an infection. In future research, extra measures should be taken to ensure that

fewer rat deaths are recorded.

Another limitation is that we induced pulmonary hypertension artificially through

MCT injection, and this is not the way it develops in vivo. The development of

pulmonary hypertension in vivo could create significant deviations from the results found

through artificially mimicking it with injection of MCT. Induction of pulmonary

hypertension with MCT happens in a matter of weeks, whereas development in vivo

takes much longer. During the experimental phase of the study, some variation in the

tissue extraction may have taken place. However, this limitation was addressed during the

experiments by having the same researcher extract all hearts for consistency. Although

the pH of the all solutions was carefully measured prior to injection into the tissue bath,

some change in pH may have occurred during the measurement phases of the experiment

which could affect the viability of the arteries. Both alkalosis and acidosis have a critical

influence on pulmonary artery viability.

A final limitation of the study may be the size of the isolated arteries used in the

study. The targeted tissues for dissection were the first branch of the right and left branch

of the pulmonary artery. Large conducting arteries contribute little to pulmonary vascular

resistance. Resistance arteries are technically difficult to isolate and some degree of error may have occurred with the dissection process of these arteries.

Future studies should aim to mimic a slower progression of PH such as hypoxia and determine if there are ways to conduct a study similar to this on older rats since the rats used in our study were young. The age of the animal can influence vascular function. Aging is associated with structural changes and functional response of arteries. In addition, it may help to extend the time each tissue is exposed to the dosages of KCl, phenylephrine, and acetylcholine. When measuring the baseline for the tissues during experimentation, ten minute time intervals were allowed between each dose of the three chemicals for equilibration. Additional time may impact the measurements.

In summary, we have shown that there were no significant differences in the pulmonary artery contractile function between the three groups. Additionally, pulmonary arterial vascular relaxant responses were not significantly decreased in the PH and PH plus type II diabetic rats compared to the control rats as hypothesized.

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