The Affect of Low Intensity, Progressive, Aerobic Exercise Training on Cardiovascular Function in the Diabetic Rat

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By

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Accepted by the faculty of the College of Science and Technology, Morehead State University, in partial fulfillment of the requirements for a Masters of Science degree.

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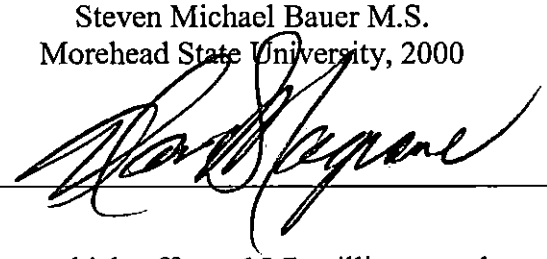
Morehead State University, 2000

Director of Thesis: 

Diabetes mellitus, which affects 15.7 million people worldwide and is the sixth leading cause of death by disease, causes pathological changes in heart tissue that affect cardiovascular function. Exercise appears to have a significant influence on the reduction of these affects. The purpose of this study was to determine if low intensity, progressive, aerobic exercise could alter body weight, heart weight, and cardiovascular function in diabetic rats. Twenty-four male Sprague-Dawley rats were divided in to four groups; Control Exercise (CE), Control Sedentary (CS), Diabetic Exercise (DE), and Diabetic Sedentary (DS). A type 1 diabetic state was induced by a single injection of 65 mg/kg of streptozotocin. After a five-day training period, the exercise treatment groups began a nine-week experimental protocol of increasing activity. The speed was increased 1 m/min/week and the incline was increased 1° per week (experimental range: 15 m/min and a 5° incline and increased to 24 m/min with a 14° incline). After the training period the animals were anesthetized, electrocardiograms were recorded and heart rate, systolic and diastolic blood pressures were determined using the Digi-Med transducers and software evaluation procedures. The animals were sacrificed and blood samples, heart, and pancreases were removed from each subject. Body weight was significantly decreased in both

diabetic groups. Exercise increased body weight in the Diabetic Exercise rats (DE: 279.5±21.4 g > DS: 216.8±49.1 g), but decreased body weight in the Control Exercise group (CE: 437.7±3.2 g < CS: 504.2±10.1 g). Heart weight was also significantly decreased in the sedentary and exercised diabetic groups in comparison to the respective Control Sedentary and Control Exercised animals (CE: 1.55±0.02 g, CS: 1.56±0.03 g, DE: 1.13±0.07 g, DS: 1.01±0.07 g). The diabetic rats did not have a significantly different heart rate when compared to non-diabetic rats. Mean systolic and diastolic blood pressures in the diabetic rats were not significantly different from non-diabetic animals. These results indicate that diabetes mellitus decreases body weight and heart weight, but the cardiovascular functions of heart rate and blood pressure were not significantly affected.

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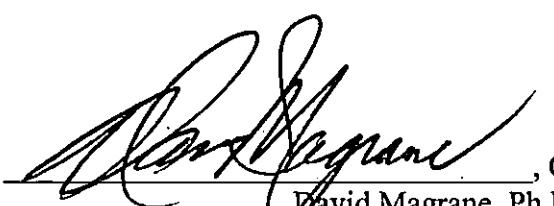

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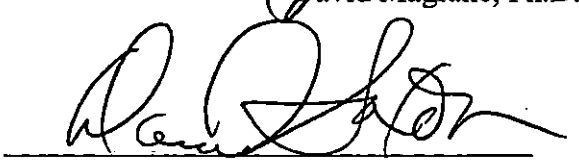

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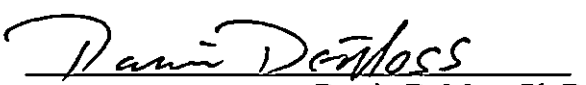

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Introduction

Diabetes mellitus affects 15.7 million people in the United States, (5.9 percent of the population) and accounts for 200,000 deaths per year, making it the seventh leading cause of death in the United States. It is considered one of the most expensive diseases in United States, costing 98 billion dollars a year in treatment, research, and lost productivity. (American Diabetes Association, 2000)

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (ECDCDM, 1997) described the disease as a group of metabolic disorders that are characterized by hyperglycemia. This condition can range in pathology from an autoimmune destruction of the insulin secreting β cells of the islets of Langerhans in the pancreas to increased tissue resistance in the target tissue. (ECDCDM, 1997) Symptoms and signs of diabetes mellitus include hyperglycemia, polyuria, polyphagia, blurred vision, and weight loss. Diabetes mellitus is associated with diseases in major organ systems of the body that include the eyes, kidneys, heart, nerves, and blood vessels. This may lead to a loss of vision, peripheral and central neuropathy, cardiovascular disease, hypertension, and atherosclerosis. (ECDCDM, 1997) The long-term affects of diabetes mellitus include renal dysfunction due to nephropathy and risk of amputation due to peripheral neuropathy. Degeneration of autonomic nerves leads to sexual, gastrointestinal, urogenital, and cardiac dysfunctions. (ECDCDM, 1997)

The pancreas, the most important organ associated with diabetes mellitus has both exocrine and endocrine functions. The endocrine cells of the pancreas produce

hormones that are utilized by all the major cell types. The production of these hormones takes place in cells located in the islets of Langerhans. The islets of Langerhans have three distinctive cell types (Figure 1). The D cells produce somatostatin. Somatostatin is produced in a variety of tissues including the hypothalamus and cells lining the digestive tract. The function of pancreatic somatostatin is to inhibit digestion of nutrients and to decrease nutrient absorption. A second cell type is the alpha (α) cell, which is responsible for the production of glucagon. The main site of action for glucagon is the liver and glucagon is responsible for raising glucose levels in the body by regulating the conversion of glycogen to glucose. The third islet cell type is the beta (β) cell. These are the most abundant cells in the islets of Langerhans and in the rat are located in the center of the islets. (Figure 1) (Sherwood, 1997)

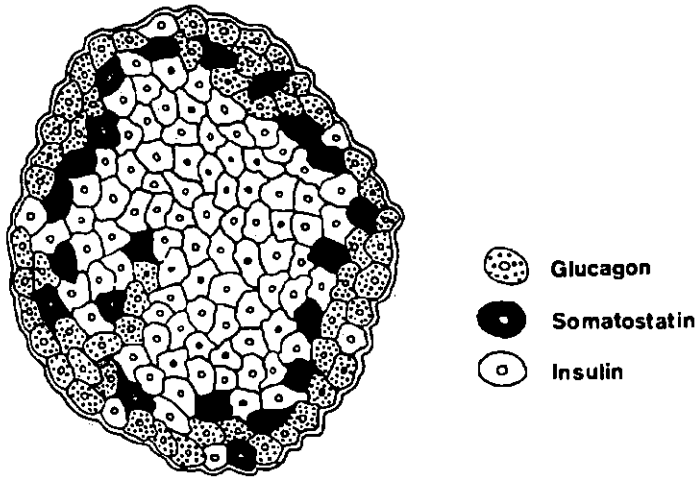

Figure 1: Schematic diagram of the islets of Langerhans in the pancreas of the rat. (Norman and Litwack, 1997)

Insulin production has direct effects on carbohydrate, fat, and protein metabolism. It also lowers plasma fatty acid, glucose and amino acid concentrations by promoting the storage of these compounds (Sherwood, 1997). Insulin is heavily involved in a number of biological processes that include metabolism, growth, nutrient transportation, and is the only hormone that can directly lower blood glucose concentrations (Vestergaard, 1999).

The insulin receptor is a dimeric tyrosine receptor in which the β subunit is phosphorylated when insulin binds. These events cause adapter proteins called the insulin receptor substrate (IRS) to become phosphorylated. These proteins function as docking proteins for molecules downstream in the signal transduction pathway (Figure 2). There are four subtypes of the IRS proteins (IRS-1, IRS-2, IRS-3, and IRS-4), but only IRS-1 and IRS-2 are involved in the biological action of insulin (Whitehead, et al, 2000). However, Hagaki, et al (1999) showed that IRS-2 is not needed for either insulin or exercise stimulated glucose transport. One of the molecules involved in the insulin pathway is phosphatidylinositol-3-kinase (PI3K), which is the key signal transducer in GLUT-4 translocation (Chibalin, et al, 2000). GLUT-4 vesicles are translocated to the plasma membrane and the surfaces of T-tubules and by using facilitated diffusion brings glucose into the cell. Research suggests that there are two separate intracellular pools for GLUT-4, a insulin sensitive pool and a exercise sensitive pool (Douen, et al, 1990; Coderre, et al, 1995). Hexokinase phosphorylates glucose after it is transported into the cell by GLUT-4. The phosphorylated glucose is then sent to the major biological pathways, such as

oxidative glucose metabolism, or stored as glycogen (Vestergaard, 1999). Defects in the IRS-1/ PI3K signal transduction pathway, decreased GLUT-4, and decreased glucose transport are associated with type 2 diabetes mellitus (Chibalin, et al, 2000).

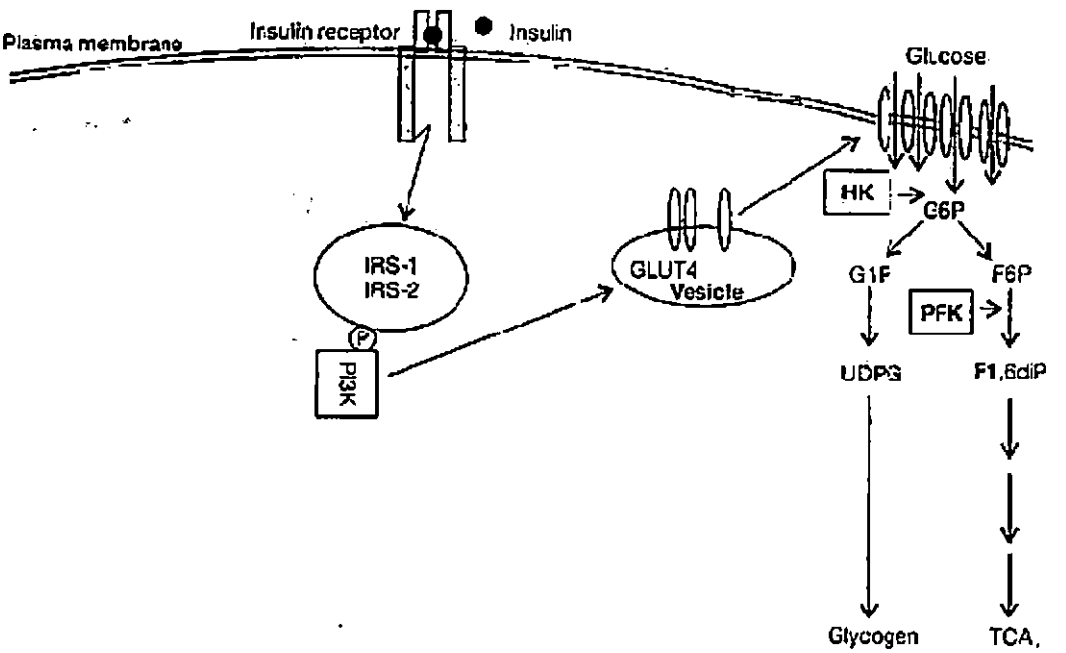

Figure 2: Flow diagram of the biological action of insulin at the target tissue. (Vestergaard, 1999)

The two most prevalent types of diabetes mellitus are Type 1 and Type 2 . Type 1 diabetes mellitus was formerly known as insulin dependent or juvenile diabetes mellitus but was reclassified by the ECDCDM in 1997 because of a growing prevalence of adult onset insulin dependent diabetes. Type 1 results from an autoimmune destruction of the insulin producing β cells of the islet of Langerhans in the pancreas. (Sherwood, 1997) This destruction is caused by release of β cell antigens, which are processed by macrophages and presented by helper T-cells. The

helper T-cells cause a response that leads to apoptosis (Figure 3) of the β cells (Mandrup-Poulsen, 1998). Type 2 diabetes mellitus occurs when the target tissues or the insulin receptor becomes insulin resistance, which may be through decreased receptor affinity of down regulation of the insulin receptor. (Sherwwod, 1997).

There are major differences between individuals with type 1 and type 2 diabetes mellitus. A type 1 diabetic is usually average or below average in weight, prone to ketosis, and produce little or no insulin. Type 1 diabetes mellitus is usually diagnosed before the age of eighteen and accounts for 10 to 20% of the diabetics worldwide. Type 1 diabetes can only be treated with regular injections of insulin. A type 2 diabetic on the other hand, is usually overweight and produces normal levels or in some cases over produces insulin. Insulin production in type 2 diabetics does start to decrease as the individual ages. (ECDCDM, 1997) Type 2 diabetes is the most common form of diabetes mellitus, accounting for 80 to 90% of all diabetics. Type 2 diabetes mellitus is usually diagnosed in adults over the age of 45 and is usually controlled through their diet and exercise in younger individual, but insulin is usually need be older individuals. (Sherwood, 1997; ECDCDM, 1997).

Diabetes mellitus has major effects on the body as a whole. Since the insulin receptor is expressed in most areas of the body and is important in fuel uptake and transport, any defect or deficiency in the insulin receptor could have detrimental effects on the individual. Insulin has marked affect on glucose transport, glycogen synthesis, cell proliferation, and gene expression. (Figure 2, Vestergaard, 1999)

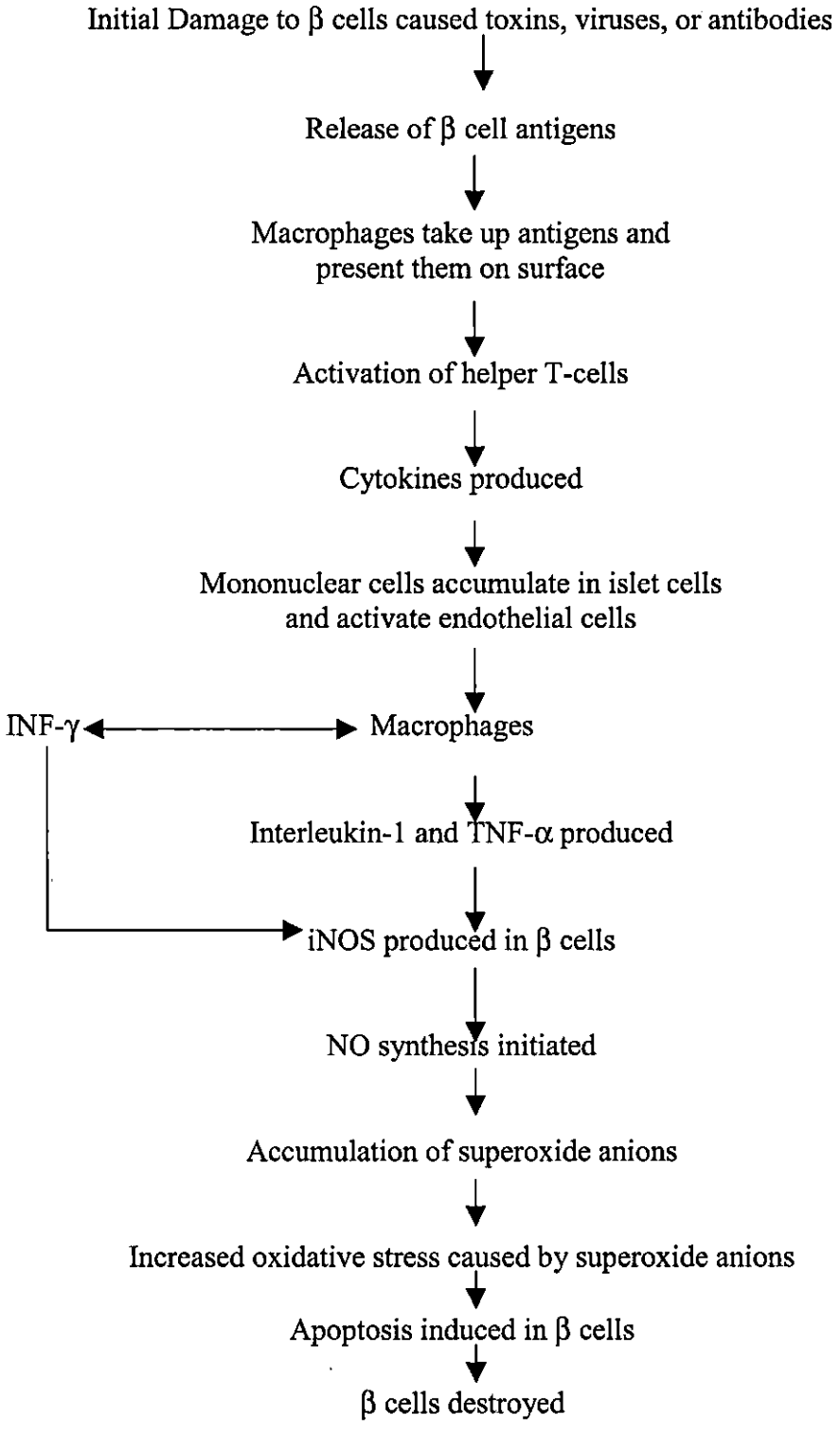

Figure 3: Autoimmune destruction of β cell in the islets of Langerhans. (Mandrup-Poulsen, 1998)

Decreases in protein synthesis in cardiac and skeletal muscle has been linked to decreases in insulin production and sensitivity, which are characteristic of type 1 diabetics. (Flaim, et al, 1980; Jefferson, 1980; Fluckey, et al, 1996). GLUT-4 concentrations have been shown to increase in the brain (Vannucci, et al, 1998), but decrease in the heart (Osburn, et al, 1997). Hall, et al (1995) showed as much as a 70% decrease in GLUT-4 and a 19% decrease in GLUT-1 concentration in diabetic rats.

The most common and widely researched complication of diabetes mellitus is cardiac dysfunction. The cause is due to major alterations in the composition and function of the heart. The myocardium of the diabetic heart has less contractile ability due to a build up of the free collagen and glycosylated proteins of collagen (Woodiwiss, et al, 1996; Riva, et al, 1998). These glycosylated fibers are more rigid and decrease the contractility of the heart muscle (Woodiwiss et al, 1996). Other studies have shown a significant rise in normal collagen in the diabetic ventricular wall and in the myocardial septum (Riva, et al, 1998). The myocardium has decreased levels of ATP, oxidative phosphorylation, creatine kinase, and phosphocreatine (Mohkar, et al 1993; Mohkar et al, 1992). Diabetic heart tissue also shows increased levels of glycogen, triacylglycerol, and free fatty acids (Paulson et al, 1992; Shehadeh and Regan, 1996).

Calcium utilization and transport in the cardiac and skeletal muscle is also compromised in diabetic animals. Decreases in calcium uptake, transport, and binding have previously been reported (Tsuchida and Watajima, 1997). There is a debate on

the effects of diabetes mellitus on sacroplasmic concentration of calcium in the left ventricle. Magyar, et al (1992) showed a 3-fold increase in calcium levels. Wang, et al, (1997) noted that intracellular calcium levels in the heart decreased significantly. The diabetic heart experiences longer action potentials, which are caused by an increased plateau phase and delayed repolarization of the papillary muscles (Sauviat and Feuvray, 1986). These increased action potentials have been reported in both the atriums and ventricles of the heart (Shigematsu, et al, 1994; Wang, et al, 1995).

The electrocardiogram (ECG) can be used to suggest cardiac dysfunction and autonomic nerve dysfunction (McEwen and Sima, 1989). The ECG waveform represents one cycle of electrical activity in the heart (Figure 4). The ECG can be used to determine whether the heart is functioning properly or if automonic neuropathy is present and how the disease is progressing. A direct relationship has been established between cardiac autonomic neuropathy and the elongation of the QT interval (Kahn, et al, 1987). Lengthening of the QT interval has been associated with diabetes mellitus (Chambers, et al, 1990) and increases the risk of sudden death because of the links to ventricular arrhythmia. Alterations in function include elongation of the QT interval (Chambers, et al, 1990), decreased cardiac reserve (Deblieux, et al, 1993), and hypertension (Weidmann, et al, 1993).

The RR interval is the time between two consecutive PQRST complexes (Figure 4). This interval is used to calculate heart rate or to monitor autonomic neuropathy. RR intervals are increased due to vagal denervation which is characteristic of diabetes mellitus (McEwen and Sima, 1989). These measurements

are a valuable index of autonomic involvement and a sensitive, noninvasive method to assess vagus nerve dysfunction. Abnormal autonomic nerve function is one of the most common complications of diabetes mellitus (McEwen and Sima, 1989). Vagal dysfunction is responsible for cardiovascular, respiratory, gastrointestinal, and genito-urinary dysfunction. Scaramuzza, et al (1998) showed that RR interval was significantly decreased in adolescent type 1 diabetics. This study also showed that there the significant difference in RR interval after an 18-month follow up was still present. This result suggests that a decrease in cardiac function is present even in the early stages of type 1 diabetes mellitus.

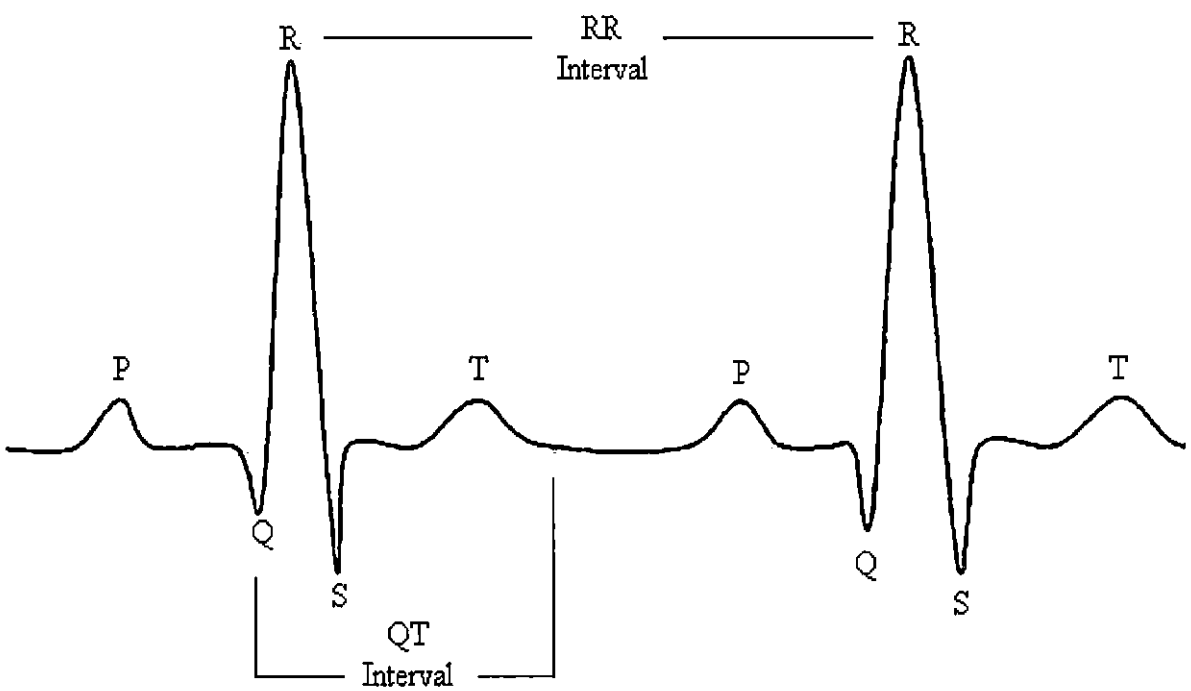

Figure 4: Representation of an Electrocardiogram (ECG)

Experimentally induced diabetes mellitus in animals is the best way to study the effects of the disease. Numerous studies have been done to show that diabetes

mellitus affects all tissues of the body. The most common way to induce diabetes in rodents is through injections of streptozotocin. Streptozotocin is a member of a group of chemicals that are said to be diabetogenic (Figure 5). Therefore, it is one of the most common ways to experimentally induce diabetes mellitus even though it is a known carcinogen, causing tumors in the kidneys, lung, and liver (ARC, 1998). Streptozotocin is a nitrosourea compound produced by the fungus *Streptomyces achomogenes.* The action of streptozotocin was thought to be through the nitroso group damaging DNA directly. Takasu, et al in 1991 discovered that streptozotocin actually upregulated the production of hydrogen peroxide which lead to DNA fragmentation resulting in apoptosis in the β cells of the islets of Langerhans in the pancreas. Therefore, streptozotocin conforms to Okamoto's model, which states oxygen radicals cause DNA fragmentation, which leads to β cell destruction (Figure 6). (Takasu, et al, 1991)

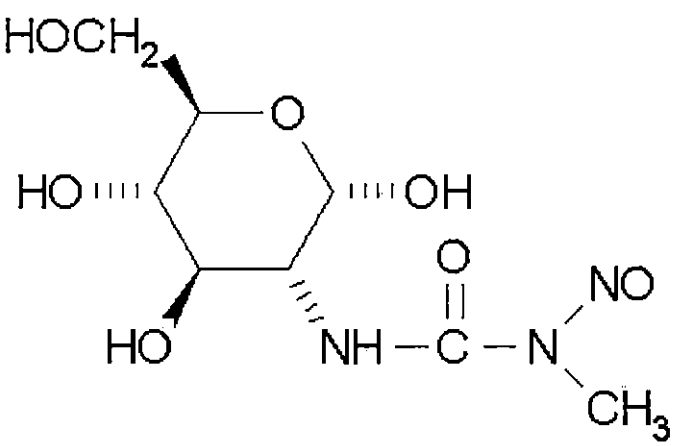

Figure 5: Chemical structure of streptozotocin (N-(Methyl nitrosocarbamoyl)-D-glucosamine) (Sigma Chemical Co.)

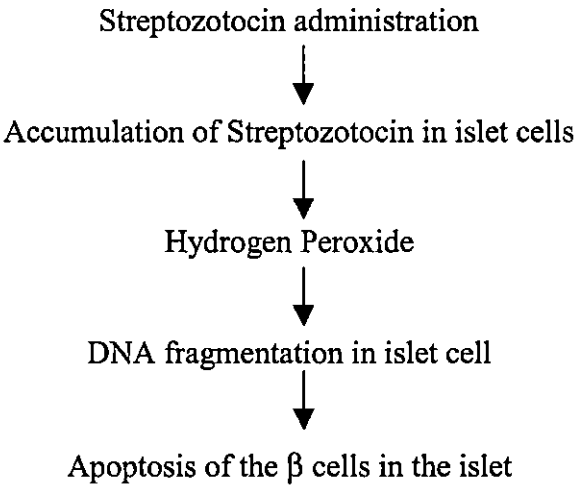

Figure 6: Okamoto's Model for induction of experimental diabetes mellitus (Okamoto, 1985)

After injection of streptozotocin, increases are seen in CD8 cells, macrophages, apoptotic cells, and IFN-γ produced by helper T-cells and cytotoxic T-cells. This is the typical type 1 diabetic immune response in the β cells of the islets of Langerhans. The effectiveness of streptozotocin is dependent on animal size and gender. Males are more susceptible than females are to its diabetogenic affects (Rodrigues and McNeill, 1987).

Hypertension is one of the most common complications of diabetes mellitus affecting humans. Hypertension occurs twice as often in diabetics than in normal individuals (ADA, 2000). Experimental blood pressure analysis in animal models has given mixed results. Several factors may be involved in this conflict, which include length of the diabetic state, the severity of the disease, and the equipment used to analyze blood pressure readings. Several studies have shown decreases in both

systolic and diastolic blood pressures (Pfaffman, 1980; Somani, et al, 1979). Other reports have shown that experimentally induced diabetes mellitus causes hypertension in the test subjects (Woodiwiss, et al, 1996; Riva, et al, 1998). Other studies show no change in blood pressure in experimental animals (Susic, et al, 1990; Oliveira, et al, 1999;Scaramuzza, et al, 1998). Susic, et al (1990) states that any decrease in blood pressure could be caused by vasodialation induced by streptozotocin. The experimental diabetic state has shown to have a marked decrease on heart rate, which could cause a decrease blood pressure (Davidoff and Rodgers, 1990; Pfaffman, 1980; and Woodiwiss, et al, 1996; Oliveira, et al, 1999).

The study of the effects that exercise have on the structure and function of the body has been extensively research. This area, referred to as Exercise Science, has grown so much that exercise has almost become a scientific discipline of its own (Baldwin, 2000). Research has focused on two main types of exercise. The first type is resistance exercise. This type of exercise is performed by the subject lifting weight, and is used mainly to study muscular changes and changes in protein synthesis (Farrell, et al, 1998; Farrell, et al, 1999a; Farrell, et al, 1999b; Fedele, et al, 2000). Diabetics showed a significant increase in protein synthesis after resistance exercise (Farrrell, et al, 1998; Farrell, et al, 1999b). Both of the latter studies showed that severe resistance exercise in individuals had no effects. Farrell, et al, (1999a), showed that chronic exercise can increase muscle mass in diabetic animals. Fedele, et al, (2000), showed that a severe diabetic state inhibits this increase in protein synthesis.

The second type of exercise is aerobic exercise, which consists of running or swimming. In normal individuals, exercise can produce a lower resting heart rate, and larger stroke volumes (Fuller and Nutter, 1981). Aerobic exercise can lower blood lactate concentrations, increase peripheral insulin sensitivity, and lower muscle glycogen utilization (Henriksson, 1992). Numerous studies have shown that aerobic exercise improves both cardiac performance and composition in the diabetic individual. Experimental models have shown that exercise improves systolic function, left ventricular diastolic chamber compliance by decreasing stiffness (Woodiwiss and Norton, 1995), and increases cardiac compliance (Woodiwiss and Norton, 1996). Deblieux et al (1993) showed that exercise increased cardiac output, but only at high preload volumes.

Aerobic exercise improves glucose homeostasis (Horton, 1988; Tancrede, et al, 1982; and Tan, 1982) and decreases glycosylated collagens and collagen subtypes (Woodiwiss and Norton, 1996). This form of exercise also decreases plasma lipids (Paulson, et al, 1987 and Tan, et al, 1982) and decreases maximal oxygen consumption (Wegner, et al, 1987). Deaths by myocardial infarctions can be decreased by aerobic exercise training (Dompierre, et al, 1990; Nadeau, et al, 1988). It also increases ATP, phosphocreatine, and oxidative phosphorylation in the diabetic heart (Mokhar et al, 1993). Swim training improves reduced glucose metabolism by increasing contractility, energetics, and catecholamine responsiveness in the myocardium of diabetic rats. (Takeda, et al, 1988). Exercise also increases GLUT-4 transporters in the plasma membrane (Douen, et al, 1990). Myocardial GLUT-4

concentrations are increased in diabetic rats after moderate intensity aerobic exercise (Hall, et al, 1995).

Aerobic exercise has been shown to have beneficial affects on blood pressure and heart rate. Aerobic exercise has been shown to increase lowered resting heart rates commonly seen in experimental diabetic animals (Mohkar, et al, 1993; De Angelis, et al, 2000; Riggs, et al, 1992). It also decreases cardiac output and normalizes hypertension in rats that are spontaneously hypertensive (Veras-Silva, et al, 1997). De Angelis, et al (2000) showed that aerobic exercise attenuates hypotension caused by diabetes mellitus induced by streptozotocin.

The effect of exercise is dependent on a variety of factors which include sex, age, type and intensity of the training, and severity of the diabetic state. Females show less myocardial improvement than males when given the same training regiment (Schaible, et al, 1981). Swim training in female rats lead to a higher increase in cardiac mass than running regiments produced (Schaible, et al, 1985). Myosin ATPase activity changed only when the subjects were undergoing intense exercise regiments (Baldwin, et al, 1977).

Exercise training has shown to have normalizing effects on the diabetic condition. According to the University of Pennsylvania Alumni study, exercise is important in the maintenance and prevention of high-risk individual for type 2 diabetes mellitus (Helmrich, et al, 1991). It has also been shown to decrease a diabetic risk of developing certain forms of heart disease (Woodiwiss, et al, 1996).

For these reasons, exercise should be a critical part of the treatment and maintenance of both type 1 and type 2 diabetics.

This study had three goals. The first goal was to evaluate the affect of diabetes mellitus on morphometric measurements such as body weight, heart weight, and a relative comparison of heart weight to body weight. The second goal was to evaluate the affect of diabetes mellitus on the cardiovascular functions of heart rate, systolic blood pressure, and diastolic blood pressure. The third goal was to evaluate the affect of a low intensity, progressive aerobic exercise regiment on those morphometric and cardiovascular parameters.

Materials and Methods

Twenty-four male Sprague-Dawley rats (Harlan Sprague Dawley Inc.) were housed in the Lappin Hall Animal Care Facility beginning at the age of twenty-eight days. These animals were housed two per cage under a lighting regime of twelve hour of light and twelve hours of dark and fed Purina Lab chow and water *ad libitum.* The animals were divided into four treatment groups with six animals per group. The treatment groups were labeled Control Exercise (CE); Control Sedentary (CS); Diabetic Exercise (DE); and Diabetic Sedentary (DS).

Induction of the Diabetes State

A type 1 diabetic state was induced in the diabetic treatment groups on age day thirty-five. Streptozotocin (Sigma Chemical Co.) was dissolved in sodium citrate buffer at pH 4.5 to a concentration of 65 mg/kg. The streptozotocin was administered in the tail vein using a 1cc syringe with a 27-gauge needle according to the procedure of Maygar, et al, 1992; Shigematsu, et al, 1994; Hall, et al, 1995; and Wang, et al, 1995.

Blood Glucose Determination

On age day forty-two, blood was drawn from each animal in the two diabetic treatment groups by clipping the end of the tail and collecting the blood in a 1.5-ml microcentrifuge tube. The clotted blood was then centrifuged at 7,000 rpm (Fischer Scientific, Micro V) for twenty minutes and the serum was drawn off the blood with a 1cc disposable transfer pipette. The serum was used to perform a spectrophotometic analysis for blood glucose levels using the 510-A Glucose Determination Kit (Sigma

Diagnostics). Briefly, 25µl of serum was mixed with 0.5 ml of deionized water in a 15 ml glass cuvette. A 500 mg/dl glucose standard was made by mixing 25-µl of the glucose standard solution with 0.5-ml of deionized water in a 15-ml glass cuvette. The tubes were incubated at 37°C. for 30 minutes (Fisher Scientific, Isotemp Waterbath). Absorbancies were measured on a spectrophotometer (Fisher Scientific, Spectro-Master 415) blanked with water at 450 nm. Blood glucose levels were calculated using the following calculations:

$$\text{Serum Glucose Concentration (mg/dl)} = \frac{\text{Absorbance of the sample}}{\text{Absorbance of the standard}} \times 100$$

$$\text{Blood Glucose Concentration (mmol/dl)} = \frac{\text{Serum glucose concentration (mg/dl)}}{18}$$

Any animal that had a blood glucose level of 20 mmol/ dl or higher was considered diabetic and kept in the study (Sauviat and Feuvray, 1986; Shigematsu, et al, 1994).

Aerobic Exercise Protocol

On age day forty-two, the exercise treatment groups (DE and CE) were started on an exercise-preconditioning regime. This period lasted five-days and consisted of the animals running on a rodent treadmill (Columbus Scientific Instruments). The speed was increased from 10 m/min to 15 m/min at a rate of 1 m/min/day. The incline of the treadmill was increased from 0° to 5° at a rate of 1°/day. The animals were encouraged to run using an electrical grid at the posterior end of the treadmill.

On age day forty-nine, the exercise treatment groups started the exercise protocol, which consisted of exercise for fifteen minutes a day for five consecutive

days and two days of rest. The speed of the treadmill was increased from 15 m/min to 24 m/min at a rate of 1 m/min/week. The incline of the treadmill was increased from 5° to 14° at a rate of 1°/week. The exercise protocol lasted for nine weeks.

Anesthesia

After the end of the nine-week aerobic exercise regiment, the animals were housed in a quiet room for twenty-four hours. The animals were placed in an anesthesia chamber for small animal anesthesia (ANESCO Inc.), which was then filled with 100% O_2 at 4 L/min for three minutes. Then the chamber was filled with isoflurane (Mallinckrodt Veterinary Inc.) at a final concentration of 3 percent in O_2 at a rate of 3 L/min. The rats were transferred to the surgical bench where the anesthetic plane was maintained using a small rodent nose cone secured to the animal.

Blood Pressure Analysis Setup

A cannula was formed from a 25 cm section of polyethylene tubing (PE-50) with one edge beveled with a pair of fine scissors. A 22-gauge needle was cut, leaving a non-crimped 1-cm section extending from the hub of the needle. This was inserted into the non-beveled end of the tubing. The cannula was soaked overnight in 100 units/ml heparinized saline. The transducer was prepared by connecting three-way stopcock at each end and filled with 0.9% saline so that no air bubbles were evident. A three-way stopcock was placed on the hub end of the cannula, both were flushed with heparinized saline, and connected to the transducer.

The Digi-Med Blood Pressure analyzer (Micro-Med Inc., Louisville, KY) was used to measure blood pressure *in vivo*. A mercury manometer was used to calibrate

the analyzer after it had been turned on and warmed up for a least fifteen minutes. The manometer was connected to the transducer and the pressure was raised to above 100 mmHg. Then the Blood Pressure Analyzer was set to match the manometers reading.

Isolation of the Carotid Artery

After the animal was at the proper plane of anesthetization, the carotid artery was isolated using a mid-ventral cervical approach. A scalpel with a number 10 blade was used to open a two-inch incision through the skin of the neck over the trachea. With two pairs of pointed end forceps, the incision was pulled open, extending the incision to about one-inch wide in length. The same forceps were used to separate the fascia and overlaying muscle layers. Once the trachea was exposed, the area to the right of the trachea was dissected so that the right common carotid artery and vagus nerve were visible (Figure 7). A one-half inch section of the carotid artery and vagus nerve were dissected and isolated away from the rest of the tissue. Then, the artery was isolated away from the nerve. A small pair of forceps was placed under the carotid so that the artery was held above the opening. Three sutures were placed around the carotid artery as shown in Figure 8. The first suture was placed at the anterior-most end of the exposed carotid. This suture was secured and tied tight to prevent the back flow of blood from the head. The next suture was placed under the artery posteriorly, closest to the heart, but was not tied. The last piece of suture was placed between the two others and tied with one loop very loosely. The ends of the

posterior suture were grasped with a pair of small hemostats and the suture was pulled tight to occlude the flow of blood from the heart. A small hole was cut in the right common carotid artery with a pair of Vanna type vascular scissors. The beveled end of the cannula was inserted into the hole and pushed posterior toward the suture that was being used to stop the blood flow from the heart. The two remaining sutures were secured around the artery and tied to hold the cannula in place. After the cannula was secured, it was flushed with heparinized saline to prevent clot formation.

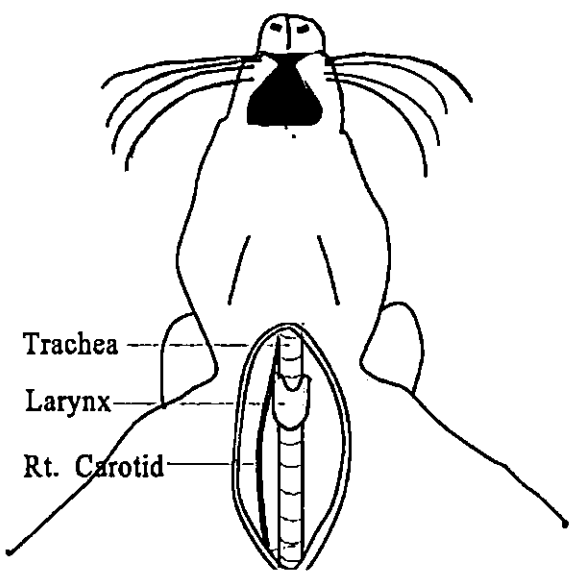

Figure 7: Representation of the incision site and visualization of the carotid artery and vagus nerve.

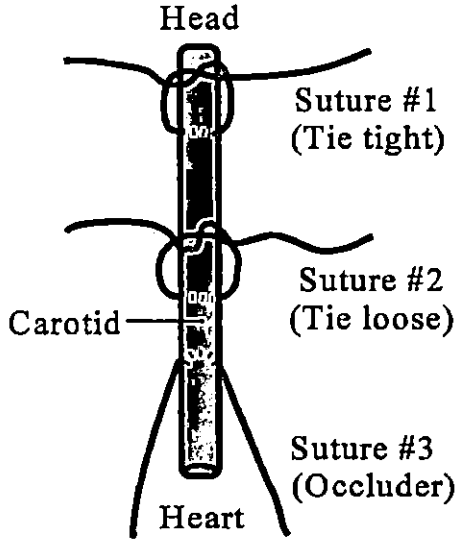

Figure 8: Representation of the carotid artery and the placement of the three sutures.

Electrocardiograph Analysis

While under anesthesia, the electrocardiograph (ECG) electrodes were connected to the animal in a Lead II setup. This consisted of the blue alligator clip (negative lead) being attached to a T-pin in the right front leg; the red alligator clip (positive lead) being attached to a T-pin in the left rear leg; and the white alligator clip (ground lead) being attached to a T-pin in the right rear leg. The ECG electrodes were connected to the Digi-Med Sinus Rhythm Analyzer (Micro-Med Inc., Louisville, KY), which was connected to a computer (Gateway Inc.). Using the Digi-Med System Integrator 200-8 (DMSI) software (Micro-Med Inc, Version 1.7), the data were recorded every 0.5 seconds for about one-minute. The data were saved as a text file and a wave form file. Both were converted to an ASCII format using WaveForm Converter (Micro-Med Inc.) and were examined using Microsoft Excel

(Microsoft Inc.). The waveform file was used to graph the ECG and the text file was used to calculate RR intervals and heart rates.

Blood Pressure Analysis

After the cannula was secured and flushed with heparinized saline, the transducer was connected to the Digi-Med Blood Pressure Analyzer (Micro-Med Inc.). The data were collected using the DMSI software every 0.5 seconds for about one-minute. The data were stored as text and wave form files. These files were converted to ASCII format and were examined with using Microsoft Excel, as described previously.

Morphometric Analysis

Before anesthetizing the animals, all individuals were weighed in grams on a rodent triple beam balance. After data collection, the animals were euthanized by decapitation using a rodent guillotine. Blood samples were taken from the animals and the hearts were removed. The blood samples were placed in micro-centrifuge tubes and blood glucose levels were determined using a colormetric assay (Sigma Diagnostics) as described previously. The hearts were weighed and frozen on dry ice and acetone. The pancreases from representative animals in each group were removed, fixed in 10% formalin, and sent to St. Claire Medical Center in Morehead, KY. The samples were stained with hemotoxylin and eosin stain, sectioned at 10 μm, and mounted on microscope slides for evaluation of the islets of Langerhans.

Statistical Analysis

Statistical analysis was performed using Sigma Stat statistical package (Jandel Scientific Software Inc.). Descriptive statistics were performed for all treatment groups. Then, the mean for each studied parameter from each treatment was used to run a One Way Analysis of Variance (ANOVA). For any parameter found to be significant, pairwise comparisons were performed using a Student-Neuman-Kuels test. All significance was tested to the $p<0.05$ level.

Results:

Blood Glucose Determination

Blood samples were taken from the diabetic animals at age day 35 and at autopsy blood used to run a colormetic analysis for blood glucose concentration. Samples taken at 35 days of age, one week after the injection of streptozotocin showed that the all rats in the diabetic groups had serum glucose levels greater than 20 mmol/dl and averaged 25.5 mmol/dl. The results of the serum glucose determination taken from the rats at autopsy are shown in Table 1. Table 1 indicates that the mean blood glucose concentrations of the diabetic groups are above 20 mmol/ dl and were significantly higher than those of the controls.

Table 1. Mean Blood glucose concentrations of all the treatment groups at time of autopsy. Values expressed as a mean ±SEM. A different superscript letter within columns represents significance at $p<0.05$.

Treatment Group	Mean Blood Glucose Concentration (mmol/ dl)
Control Exercise	15.3 ± 1.0^{a}
Control Sedentary	12.3 ± 2.2^{a}
Diabetic Exercise	23.6 ± 0.7^{b}
Diabetic Sedentary	23.7 ± 0.5^{b}

Microscopic Evaluation

In Figure 9, two islets are present in each 100X microscopic field. This is typical for a normal rat pancreas section. This is in direct contrast to the diabetic animals which have very few islets. The islets of Langerhans of the non-diabetic rat at 400X magnification are shown in Figure 10. A single measurement with an ocular

micrometer indicates that this islet had an area of 140 μm^2, and contained a large cell population with many light staining cells present that had large nuclei. These lighter staining cells are the insulin producing β cells of the islets of Langerhans. In contrast, Figure 11 shows the Islets of Langerhans for the diabetic rats, which have a greatly reduced number of these cells. The size of the Islet is dramatically smaller with an area of 35 μm^2.

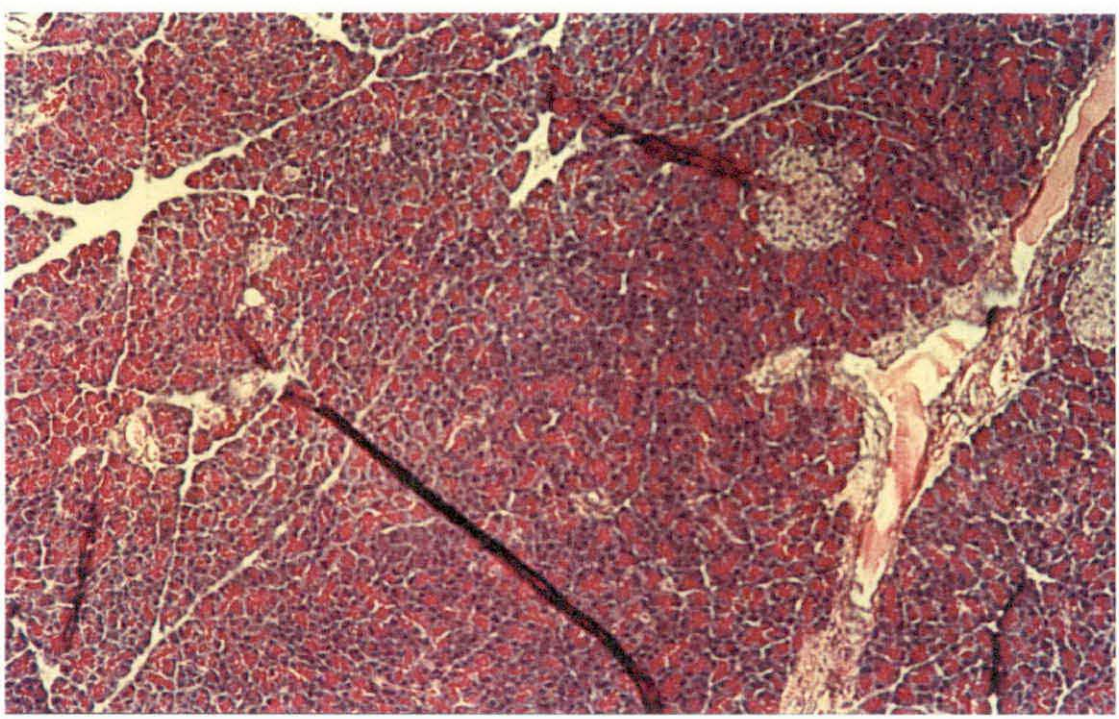

Figure 9: Islet of Langerhans of a non-diabetic rat stained with H&E, 100X total magnification

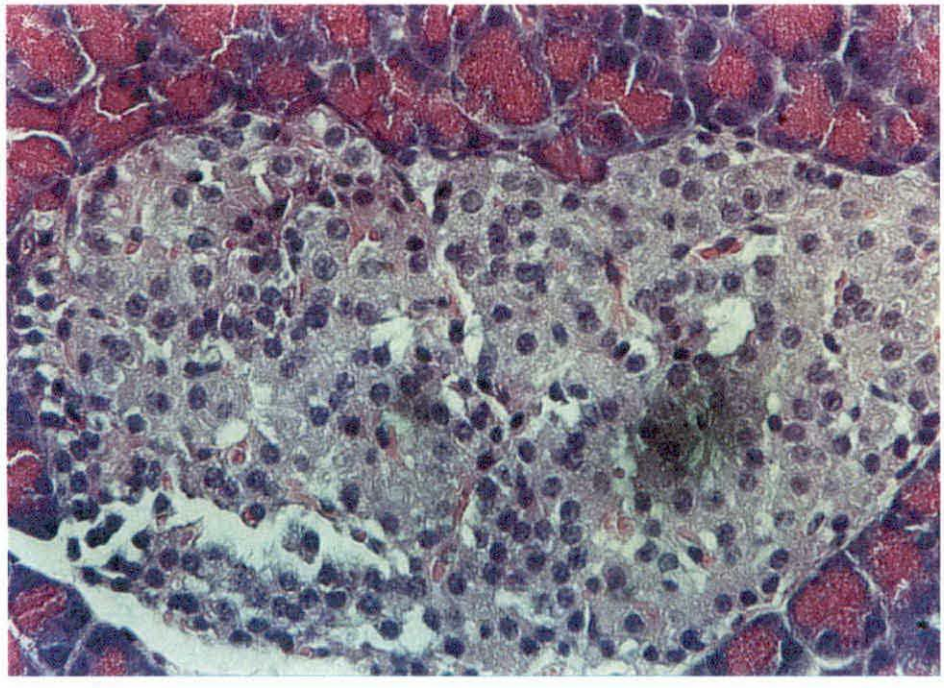

Figure 10: Islets of Langerhans of a non-diabetic rat stained with H&E, 400X total magnification.

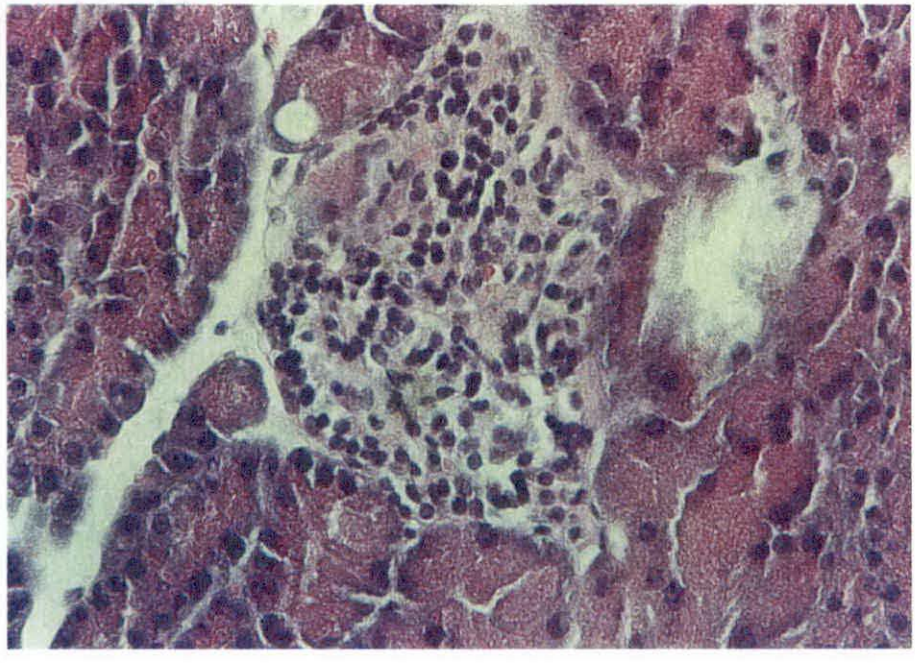

Figure 11: Islet of Langerhans of a diabetic rat stained with H&E, 400X total magnification.

Morphological Data:

Morphological data are presented in Table 2. Absolute body weight decreased significantly in both of the diabetic groups when compared to the control group. Exercise caused a significant increase in the absolute body weight in the diabetic animals and a significant decrease in the control animals. These results are seen graphically in Figure 12. Absolute heart weight is also significantly decreased in the diabetic animals. Exercise had a non-significant effect on heart weight in the diabetic heart (Table 2, Figure 13). Relative heart weights for the diabetic animal significantly increased compared to the control animals. (Table 2, Figure 14).

Table 2. Absolute body weight, absolute heart weight, and relative heart weight. All data are expressed as the mean ± SEM. A different superscript letter within columns denotes significance at the $p<0.05$

Treatment Groups	Body Weight (g)	Heart Weight (g)	Relative Heart Weight (g/kg)
Control Exercise	437.7 ± 2.9^{a}	1.55 ± 0.023^{a}	3.55 ± 0.63^{a}
Control Sedentary	504.2 ± 8.9^{b}	1.56 ± 0.034^{a}	3.11 ± 0.12^{a}
Diabetic Exercise	279.5 ± 21.4^{c}	1.13 ± 0.067^{b}	4.08 ± 0.22^{b}
Diabetic Sedentary	216.8 ± 20.1^{d}	1.01 ± 0.067^{b}	4.72 ± 1.35^{b}

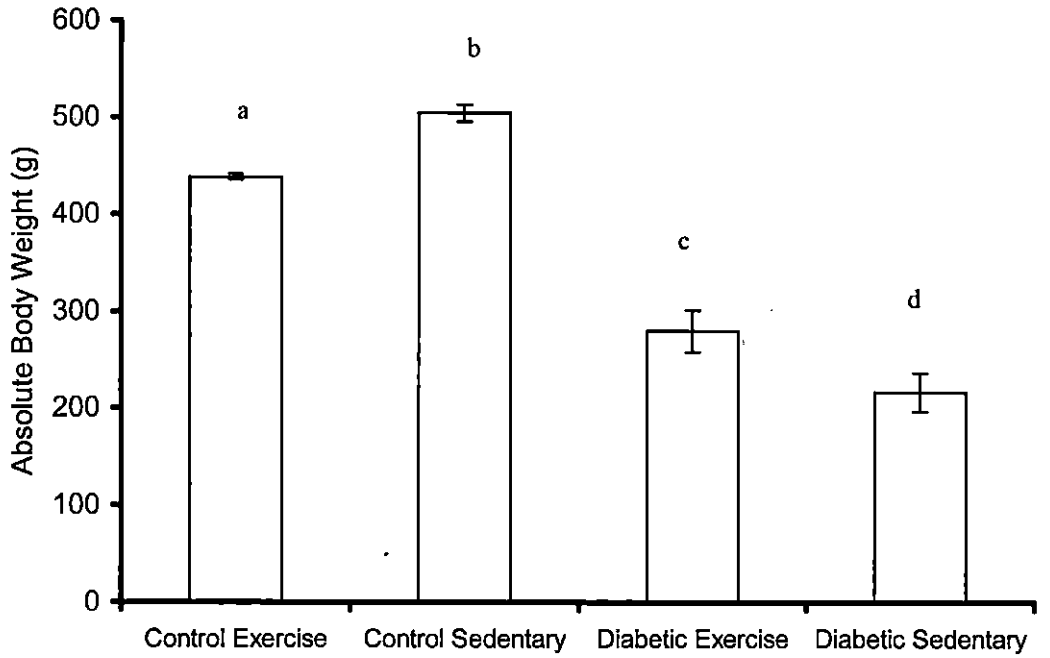

Figure 12: Absolute body weight for the four treatment groups. All data are expressed, as mean ± SEM. Groups with a different letter above the bars are significant at $p < 0.05$.

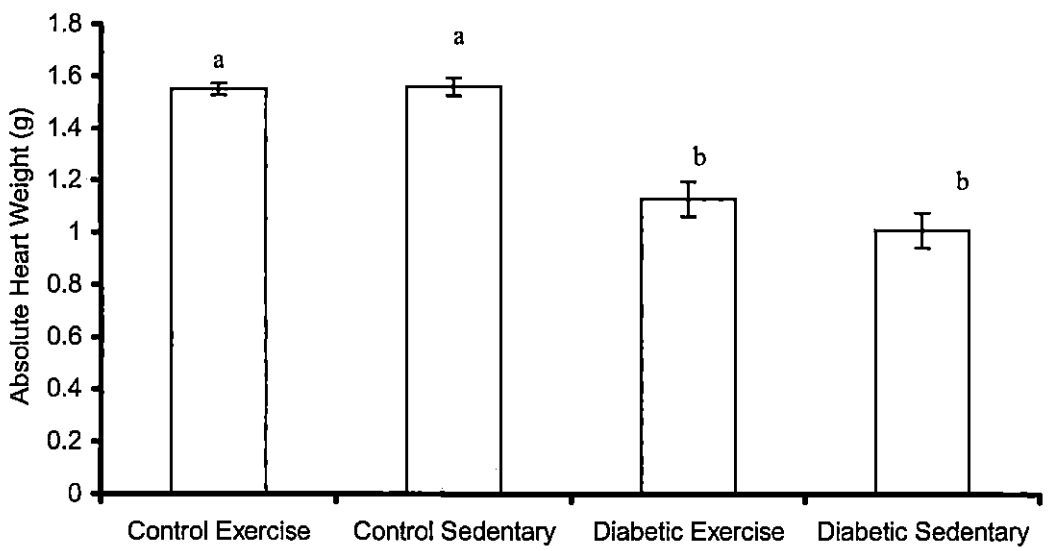

Figure 13: Absolute heart weight for all the treatment groups. All the data are expressed as mean ± SEM. Groups with a different letter above the bars are significant at $p<0.05$

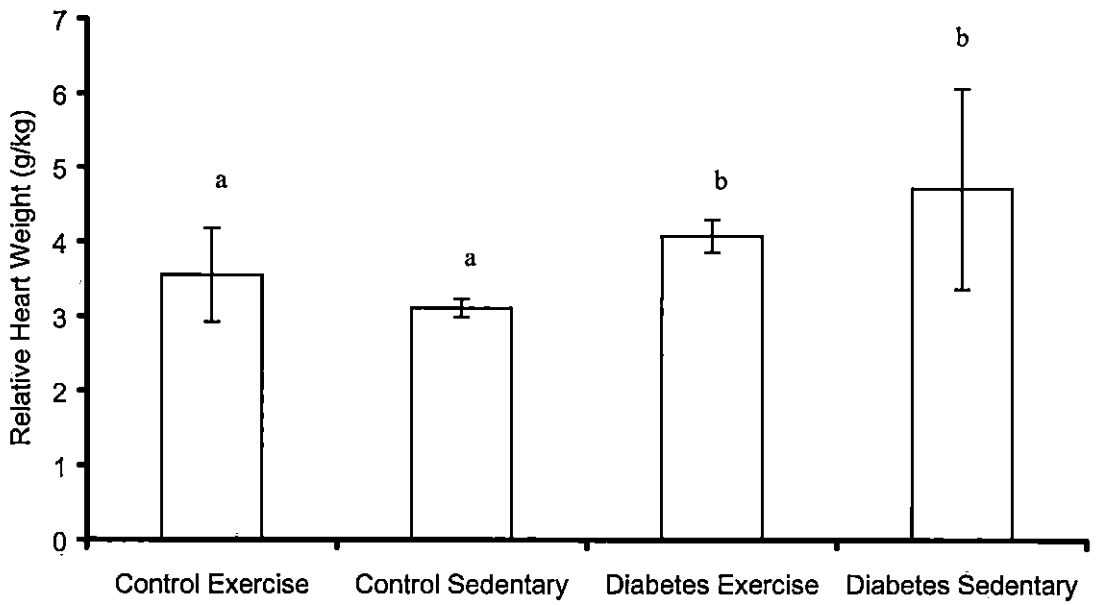

Figure 14: Relative heart weight for all the treatment groups. All data are expressed as mean ± SEM. Groups with a different letter above the bars are significant at $p<0.05$.

Electrocardiograph Analysis:

RR intervals were measured from the electrocardiographs and an average RR interval was calculated. There is no significant difference between the control groups and the diabetic animals. There is also no significant difference in the exercise animals compared to the sedentary groups (Figure 15). Average RR interval was used to calculate mean resting heart rate. There is no significant difference between any of the treatment groups in mean resting heart rate. (Figure 16)

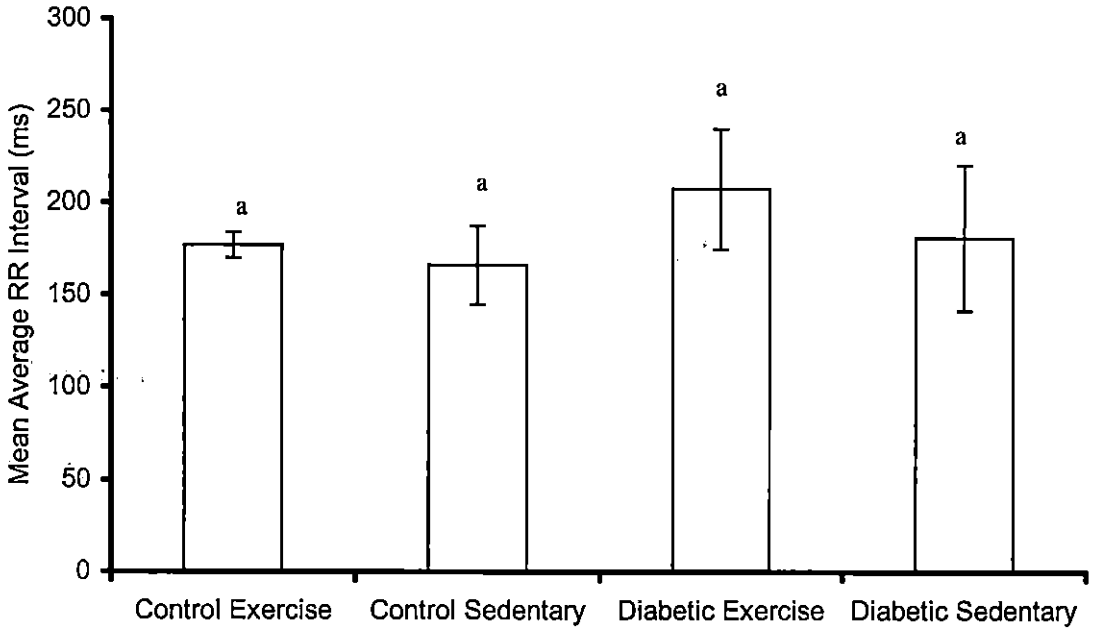

Figure 15: RR intervals for all the treatment groups. All data are expressed as a mean ± SEM. Groups with a different letter above the bars are significant at $p<0.05$.

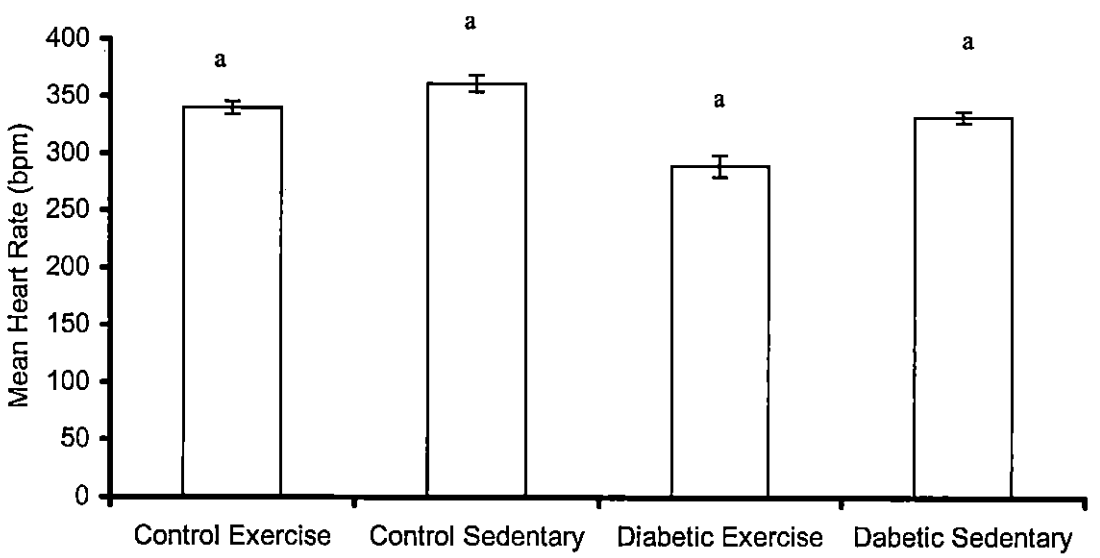

Figure 16: Mean heart rate for all the treatment groups. All data is expressed as the mean ± SEM. Groups with a different superscript letter above the bars are significant at $p<0.05$.

Blood Pressure Analysis:

Mean systolic blood pressures were not significant when the diabetic animals are compared to both respective control groups. (Figure 17). Mean diastolic blood pressure was significantly decreased in the diabetic exercise group compared to the control exercise treatment group. (Figure 18).

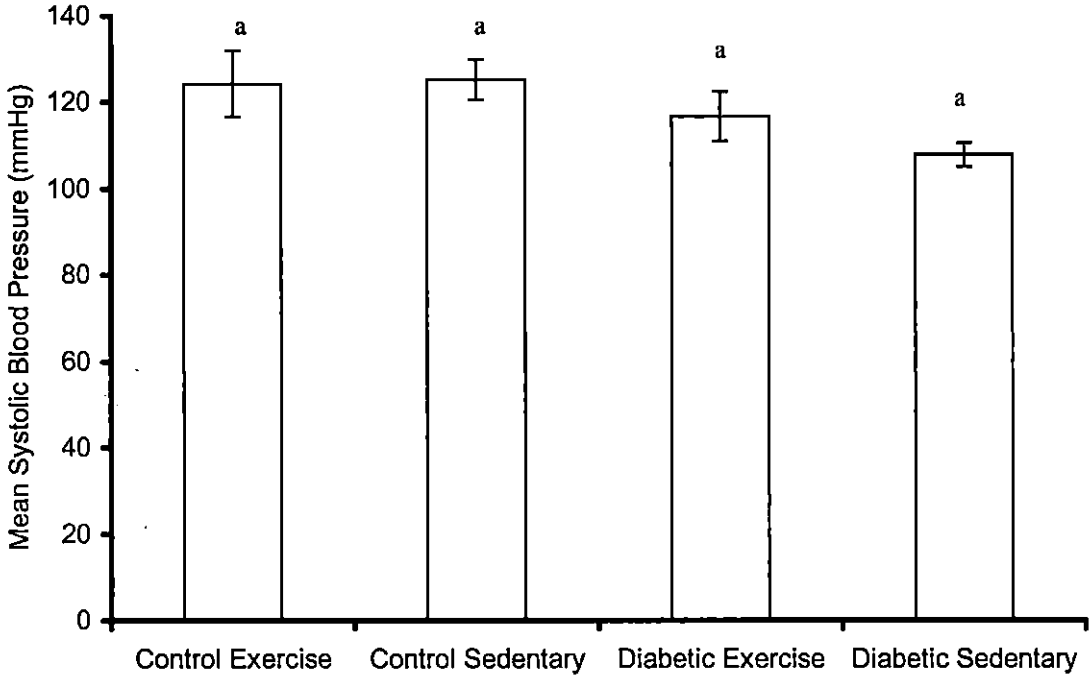

Figure 17: Mean systolic blood pressure for all treatment groups. All data expressed as the mean ± SEM. Groups with a different letter above the bars are significant at $p<0.05$.

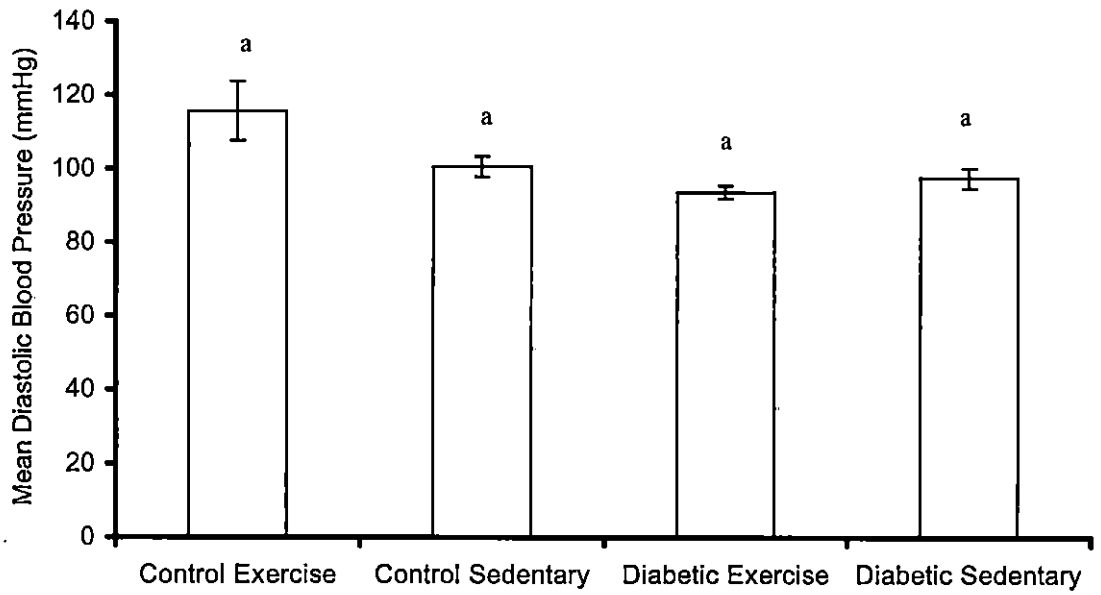

Figure 18 Mean diastolic blood pressure for all treatment groups. All data are expressed at the mean ± SEM. Groups with a different letter above the bars are significant at $p<0.05$.

Discussion

This study was performed to determine if exercise has an influence on the streptozotocin induced diabetic condition. Morphometric comparisons of body weight, heart weight, and relative heart weight (body/ heart weight) were included in this study. The cardiovascular parameters of electrocardiograms, heart rate, and systolic and diastolic blood pressures were also evaluated. Streptozotocin induced diabetes has been used in a number of experimental diabetic studies, and the rat model produced from streptozotocin injection is the most widely used model to study Type 1 diabetes mellitus in humans. Streptozotocin-induced diabetes is also an excellent model for studying the alteration the heart goes through during the progression of the disease. Figures 10 and 11 provide histological evidence of the establishment of the diabetic state. This is evident by a decrease in area of the islet of Langerhans and by the decrease in the lighter staining insulin-producing β cells. Table 1 also shows that all the rats injected with streptozotocin had blood glucose concentrations above 20-mmol/ dl, which has been shown by other studies to experimentally represent the diabetic condition (Sauviat and Feuvray, 1986; Shigematsu, et al, 1994). The greater blood glucose concentration is a direct result of decreased insulin production caused by the streptozotocin induced damage of the β cells in the pancreas. This also indicates that a single injection of streptozotocin at a concentration of 65-mg/ kg is sufficient to induce diabetes in the murine model.

Numerous studies have shown that both aerobic and resistance exercise have a beneficial effect on the diabetic state. Different exercise regiments have been shown

to increase contractile function of the heart (Osburn et al, 1997), decrease plasma lipids (Paulson, et al, 1987; Tan, et al, 1982) and improved contractile function (Woodiwiss and Norton, 1996). Exercise did not produce a decrease in blood glucose concentration in the diabetic animals (Table 1). Previous reports have shown conflicting results in regards to blood glucose concentrations. Dall'agilo, et al (1983) and Tancrede, et al (1982) showed significant decreases in blood glucose concentration in diabetics that were on a exercise training regiment.

A decrease in body weight in diabetic animals has been shown in previous studies (Mohkar, et al, 1996; Susic, et al, 1990; Oliveira, et al, 1999). Table 2 and Figure 12 illustrates that there is a significant reduction in weight in both of the diabetic treatment groups when compared to the control groups. Exercise did increase body weight in the diabetic animals and decrease body weight in the control animals. The decrease in body weight of the control group could be a trained effect for loss of body fat. The exercise protocol was comparatively mild and may have not been sufficient to increase skeletal muscle mass, causing an overall decrease in body weight. The increase in body weight of the diabetics who were exercised could be due to increased insulin sensitivity in tissue. (Dall'agilo, et al 1983; and Tancrede, et al 1982) This increased insulin sensitivity coupled with increased protein synthesis seen with certain forms of exercise (Farrell, et al, 1999a; Farrell, et al, 1999b) could result in increased muscle mass and body weight. The decrease in body weight in diabetic rats is accompanied by a significant decrease in heart weight (Table 2 and Figure 13). Exercise did not produce an increase in the heart weights of diabetic groups, a trend

seen in other studies. (Fuller and Nutter, 1981; Susic, et al, 1990). Table 2 and figure 14 also shows that relative body weight (also called cardiac weight index in research) significantly increased in the diabetic animals when compared to the control animals. This was also seen by Oliveira, el al (1999), who suggested that this was due to the loss of body weight rather than changes in heart morphology.

The present study support the conclusions of Susic, et al (1990) and Oliveira, et al (1999) that the diabetic state causes no significant changes in blood pressure. Figures 17 and 18 indicate that there was no significant change in systolic and diastolic blood pressure in the diabetic groups when compared the control groups. However, these results were contrary to what was expected as most studies show that streptozotocin induced diabetes mellitus causes hypotension in rats (Woodiwiss, et al, 1996; Davidoff and Rodgers, 1990). Woodiwiss, et al (1996) noted that diabetes cause a decrease in blood pressure that was normalized by exercise. This normalization in blood pressure could be due to a decrease in glycosylated collagens in the myocardium of the heart, which causes an increase in cardiac muscle compliance. Other studies show that decreases in adrenergic stimulation and in thyroid function could explain a decrease in blood pressure (Davidoff and Rodgers, 1990). The significant weight loss could also explain a decrease in blood pressure (Susic, et al, 1990).

RR interval was used to calculate heart rate and an increased RR interval represents a decrease in heart rate. Eventhough a decrease in RR interval is commonly seen, it was not apparent in this study (Figure 15). This decrease

represents an increase in abnormal autonomic nerve function (McEwen and Sima, 1989). This study showed a no significant change in heart rate (Figure 16) which is in direct contrast to Fuller and Nutter, 1981; Wegner, et al 1987; and Susic et al, 1990. All of these studies showed that diabetes caused a significant decrease in heart rate. This decrease has been associated with a decrease in adrenergic stimulation (Davidoff and Rodgers, 1990), hyperglycemia, insulinopenia, and myocardial stiffness. (Pfaffman, 1980; Woodiwiss, et al, 1996) Aerobic exercise training is known to cause bradycardia with increased cardiac output. (Fuller and Nutter, 1981)

The exercise intensity, duration, and the severity of the diabetic state and the low number of rats per experimental group could explain why these data conflicts with the published data. Other factors could have added to experimental error. The ECG and blood pressure analyses were performed while the animals were under gas anesthesia. There has been a shift in thinking in the way that anesthetic procedures are being used during these types of experiments. The shift is away from gas anesthesia (Isoflurane) to injectable anesthesia, such as sodium pentabarbitol. (Oliveira, et al, 1999) The reason for the switch is that injectable anesthesia are easier to control anesthetic plane than with a gas anesthesia.

Another factor is the equipment used to collect the data. The blood pressure analyzer (BPA) is connected to a transducer that is then connected to the animal through the cannula. If the setup has any air left after filling with saline, the bubble will absorb the pressure and cause false blood pressure readings. The sinus rhythm analyzer SRA has a set of wire electrodes that have alligator clips at the end. This

setup is prone to picking up background noise and losing contact with the subject. Both of which will cause the reading to be false. The largest contribution to experimental error was that during the surgery portion of this study, four animals died before ECG and BPA measurements were taken.

Conclusions

The specific aims of this study were to determine if streptozotocin induced diabetes mellitus produced any changes in morphology and cardiovascular parameters in rats and to assess if exercise could normalize these changes. This study provides evidence that streptozotocin-induced diabetes mellitus produces a decrease in body weight and that exercise can cause the body weight to normalize in streptozotocin-induced diabetics. Diabetes mellitus did produce a reduction in heart weight that was not normalized by exercise. No significant changes were seen in RR interval, heart rate, systolic blood pressure, and diastolic blood pressure between streptozotocin-induced diabetic rats that exercised and rats that did not. This indicates that streptozotocin-induced diabetes mellitus and exercise has no effect on these cardiovascular parameters.

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