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The Effects of Exercise Training on Indices of Cardiovascular Autonomic Neuropathy in STZ-Induced Type 1 Diabetic Rats Treated with Insulin

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A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

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ABSTRACT

This study investigated whether regular aerobic exercise training could prevent the dysregulation of autonomic cardiovascular (CV) control in a streptozotocin (STZ)-diabetes model designed to represent clinical type 1 diabetes mellitus (T1DM). Rats were divided into control (C), control exercise (CX), diabetic (D) and diabetic exercise (DX) groups. Baroreflex sensitivity (BRS), heart rate variability (HRV) and vascular sympathetic tone (VST) were measured following 10 weeks of exercise. Parasympathetic-mediated bradycardia BRS was reduced in D compared to C and DX ($p<0.05$). The HF (parasympathetic) HRV was reduced in D compared to CX and DX ($p<0.05$) and the LF/HF ratio (sympathetic HRV) was elevated in D compared to all other groups ($p<0.05$). The VST was increased in D compared to all other groups ($p<0.05$). Diabetes caused a CV autonomic imbalance, which was prevented by exercise training. Thus, this model paralleled clinical T1DM and demonstrated that exercise can prevent autonomic dysfunction.

Keywords: type 1 diabetes, aerobic exercise, diabetic autonomic neuropathy, cardiovascular autonomic neuropathy, sympathetic overactivity, heart rate variability, baroreflex sensitivity, parasympathetic nervous system

CO-AUTHORSHIP

Dr. Jamie Melling was involved in the design of the experiment, interpretation of the results, and in revising this thesis.

DEDICATION

To my parents and grandparents, who set before me a path of opportunity paved with unconditional support and who have been role models for living with integrity and compassion.

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"Those who learned to collaborate most effectively have prevailed" – Charles Darwin

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ACE	Angiotensin-converting-enzyme
ACTH	Adrenocorticotropic hormone
ADH	Antidiuretic hormone
AGE	Advanced glycation end-product
ANG II	Angiotensin II
ANOVA	Analysis of variance
ANS	Autonomic nervous system
AT1	ANG II type 1 receptor
BG	Blood glucose
BP	Blood pressure
BRS	Baroreflex sensitivity
C	Non diabetic sedentary control group
CAN	Cardiovascular autonomic neuropathy
ChAT	Choline acetyltransferase
CNS	Central nervous system
CRH	Corticotropin releasing hormone
CV	Cardiovascular
CVLM	Caudal ventrolateral medulla
CX	Non diabetic exercise control group
D	Diabetic sedentary group
DAN	Diabetic autonomic neuropathy

DNA	Deoxyribonucleic acid
DX	Diabetic exercise group
FFT	Fast Fourier transform
GABA	Gama-aminobutyric acid
GADA	Glutamic acid decarboxylase antibody
GLUT1	Glucose Transporter 1
GLUT4	Glucose Transporter 4
GLUT3	Glucose Transporter 3
HF	High frequency
HLA	Human leukocyte antigen
HPA	Hypothalamo-pituitary-adrenocortical
HR	Heart rate
HRV	Heart rate variability
IAA	Insulin autoantibody
IA-2A	protein tyrosine phosphatase-2 antibody
ICA	Islet cell antibody
IML	Intermediolateral
IP	Intraperitoneal
IVGTT	Intravenous glucose tolerance test
K_G	Glucose clearance rate
LF	Low frequency
MAP	Mean arterial pressure
NADPH	Nicotinamide adenine dinucleotide phosphate

NIBP	Non-invasive blood pressure
nNOS	Neuronal NOS
NO	Nitric oxide
NOS	Nitric oxide synthase
NTS	Nucleus of the solitary tract
PE	Phenylephrine
PKC	Protein Kinase C
PSNS	Parasympathetic nervous system
PVN	Paraventricular nucleus
RAAS	Renin-angiotensin-aldosterone system
RAS	Renin-angiotensin system
ROS	Reactive oxygen species
RVLM	Rostral ventrolateral medulla
SBP	Systolic blood pressure
SDNN	Standard deviation between normal pulse peaks
SNP	Sodium nitroprusside
SNS	Sympathetic nervous system
SOD	Superoxide dismutase
STZ	Streptozotocin
TPR	Total peripheral resistance
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
VO_{2max}	Maximal rate of oxygen consumption

VST	Vascular sympathetic tone

CHAPTER 1

1.1 Introduction

Type 1 diabetes mellitus (T1DM) is an idiopathic autoimmune disease that results in the destruction of pancreatic β cells and subsequently, the inability to produce endogenous insulin (4, 7, 144). This results in chronic hyperglycemia and imbalances of fat, protein, and carbohydrate metabolism that lead to progressive complications, such as: retinopathy, nephropathy, neuropathy and increased risk of microvascular and macrovascular disease (4, 7, 24). These complications can be so severe that, even with insulin therapy, a child diagnosed with diabetes at 10 years of age will lose an average of 19 years of life due to the disease (97). Epidemiological studies have shown that T1DM incidence is continuously rising in both high and low incidence populations; particularly, in the 15 years of age and younger demographic (63, 99, 163). For example, Onkamo et al. (99) reported that the global incidence rate of the under 15 demographic from 1960 to 1996 increased at 3% per year. The DiaMond Project Group (26), a multinational effort to investigate and monitor the incidence patterns of childhood type 1 diabetes, reported a yearly global increase in incidence of 3.4% for the years 1995 to 1999. In Europe it has been predicted that by 2020 the number of children under the age of 5 afflicted with T1DM will double and the number of cases for children under the age of 15 will increase by 70% (104). Clearly, this demonstrates that T1DM is a problem that is continuously growing in prevalence and importance. Also of importance are the financial costs of T1DM on patients and society at large (130). In fact, it was found that patient costs for individuals with T1DM are disproportionately higher than costs for those with type 2 diabetes mellitus (T2DM) (142). Furthermore, the societal costs of T1DM in the USA and the UK are estimated at $14.4 billion and $3 billion per year, respectively (46,

142). In light of such profound health and economic impacts, understanding the predictors, onset and treatment of T1DM has been a focus of extensive investigation; yet, a substantial amount about the disease and its complications remains to be elucidated. Although it has been long understood that exercise can generate beneficial health outcomes, more recently and increasingly, exercise is being regarded as a therapy and is being prescribed by physicians as medicine (123, 128). The benefits of exercise in the prevention and treatment of T1DM-related complications has been well documented (36, 105, 112). In particular, diabetic autonomic neuropathy (DAN) is known to be one of the earliest complications of diabetes which not only precludes a variety of related complications but is also directly linked to increased mortality (30, 40, 100, 149). Thus, the ability of exercise therapy to reduce DAN is a research focus of great importance (9, 18, 105, 150). This chapter will provide an overview of T1DM onset and its mediation of neuropathy, the function of the autonomic nervous system and its regulation of the cardiovascular system, how neuropathy leads to autonomic and cardiovascular dysfunction and the efficacy of exercise in mitigating this dysfunction.

1.2 Etiology of Type 1 Diabetes Mellitus

Susceptibility to T1DM is determined by both genetic and environmental factors (7, 31, 58, 144). The genetic basis of the disease is strongly exhibited by studies of monozygotic and dizygotic twins. It has been found that monozygotic twins can demonstrate over a 50% concordance rate of T1DM, which is far greater than the concordance between dizygotic twins (50). Due to the fact that monozygotic twins share the same DNA and developmental environment, whereas dizygotic twins have non-identical DNA but also share the same developmental environment, it can be

concluded that the difference in concordance between the two types of twins is resultant of a genetic influence (31). Through such twin studies, as well as familial linkage studies, it has been established that the human leukocyte antigen (HLA) encoding genes, which code for many different components of the immune system, are the major factors conferring genetic susceptibility to T1DM (24, 31, 48, 50). Although a high percentage of T1DM susceptibility can be attributed to a genetic component, even at 50% concordance, there remain at least equally substantial non-genetic factors that are involved in the manifestation of T1DM.

Environmental factors, including viral infections, diet, growth, toxins and stress, have been implicated in the pathogenesis of T1DM (3). D'Angeli et al. (28) provide evidence for the "hygiene hypothesis" by demonstrating that a clean environment during childhood decreases antigenic stimulation and results in a greater vulnerability to developing T1DM. Further evidence of an environmental determinant of T1DM is the apparent role of geography and climate in its prevalence. In both Europe and North America there is a north to south, high to low, gradient of incidence (58, 144). In accordance with a climatic influence, a lower incidence of T1DM during warm seasons than cold seasons has been observed within populations (58, 68). Although these genetic, regional and climatic insights provide evidence of risk factors for developing T1DM, they are not necessarily predictors of great utility. The environmental factors may be correlated with T1DM but most cannot realistically be avoided or managed, whereas the genetic factors alone do not yield disease onset; as evidenced by the finding that, at most, only 10% of individuals with the highest risk susceptibility genes actually become afflicted with T1DM (146).

The most effective predictors of T1DM are a complement of autoantibodies that can be detected years before the disease develops; in fact, they can even be

detected during infancy (12, 61). The autoantibodies that predict T1DM are: islet cell antibodies (ICA), insulin autoantibodies (IAA), glutamic acid decarboxylase antibodies (GADA) and protein tyrosine phosphatase-2 antibodies (IA-2A) (71, 80). These autoantibodies direct the infiltration of immune cells, mostly T cells, to the islets and facilitate their inflammation and the destruction of β cells (19, 25, 71). It has been observed that the detection of two or more of these autoantibodies in relatives of T1DM patients has a 90% predictive value for their eventual development of T1DM (147). In the general population, marginally elevated levels of 3 to 4 autoantibodies indicates a risk of roughly 60% for the development of T1DM (12). Despite the predictive value of autoantibody detection, no autoantibody based treatments exist to avert the onset of T1DM (25). Therefore, for now, the only way to delay or prevent T1DM associated morbidity and mortality is to understand and ameliorate the progression of the disease-related complications (4, 25, 80). However, it has been suggested that exercise could ameliorate the severity of autoantibody mediated inflammation and subsequently, reduce T1DM initiation and progression (66).

1.3 Pathogenesis of Neuropathy in T1DM

The neurological complications of T1DM are understood to arise from either a metabolic etiology, a vascular etiology or a combination of both (30, 41, 137, 156). However, the pathology of metabolism and vasculature in T1DM, as well as the wider breadth of complications that develop, arises primarily due to chronic hyperglycemia (24, 41, 149). Indeed, the duration and severity of hyperglycemia have both been indicated as predictors of the degree of diabetic neuropathy (30, 41). Fittingly then, advanced glycation end-products (AGEs), which are formed when hyperglycemia

leads to irreversible binding of glucose molecules to intracellular and extracellular proteins, have been implicated as a causative link between vascular and metabolic dysfunction in T1DM (41).

The metabolic factors, whose dysfunction primarily contributes to diabetic neuropathy, are many. For example, T1DM leads to increased polyol pathway activity and decreased myo-inositol, which are associated with decreased nerve conduction velocity (30, 41, 137). It also causes decreased nerve Na/K ATPase activity, the function of which is necessary to maintain the transmembrane ionic gradient required for electrical impulse conduction (41, 137). Further, T1DM is associated with decreased synthesis and transport of axonal proteins, which are supplied from the cell body to maintain structural and functional integrity of the axon (41, 87, 137) and diminished incorporation of glycolipids and amino acids into myelin, thereby reducing axon insulation and conduction velocity (30, 41, 139). T1DM also leads to decreased nerve protein kinase C (PKC) activity, which can further reduce Na/K ATPase activity (137). Dyslipidemia, consisting of increased triglycerides, increased low-density lipoprotein and decreased high-density lipoprotein, is a metabolic factor that contributes to neuropathy via the atherosclerotic impairment of the vasculature (30, 41, 107).

Vascular factors that contribute to diabetic neuropathy mostly involve pathology of the endoneurial capillaries (92, 152). These vascular impairments include: basement membrane thickening and endothelial cell swelling and proliferation, resulting in reduced luminal diameter, decreased nerve blood flow and endoneurial hypoxia (41, 83, 143); degeneration of pericytes, which are integral for maintaining endothelial cell function at the blood-nerve barrier (41, 129); and ultimately, endoneurial capillary atherosclerosis, which leads to the dysregulation of

nutrient exchange between axons and blood vessels across the endoneurium (30, 41, 129, 143). There is also evidence that hyperglycemia can have tissue-specific, bidirectional effects on PKC activity (138). While hyperglycemia decreases PKC activity in the nerve as mentioned above, it also becomes overactive in vascular endothelium and leads to increased endothelial permeability, the dysregulation of endothelial-derived vasodilator nitric oxide (NO) and thus, diminished blood flow (127, 138).

Despite these metabolic and vascular factors often being considered discrete etiologies of diabetic neuropathy, it is more likely that they arise concomitantly and share protein glycation as their common cause (see Figure 1.1) (30, 41). For example, glycation of antioxidant enzymes hinders their ability to remove reactive oxygen species (ROS). This results in the accumulation of ROS that damage membranes, cellular proteins and DNA, all of which contribute to neuropathic progression (41). Glycation of the nitric oxide synthase (NOS) enzyme is likely a mechanism by which NO production is decreased, driving the vascular dysfunction described above (41, 138). Also, glycated elastin and collagen in the endothelium can restrict access of NO to the smooth muscle, further inhibiting NO-mediated vasodilation, reducing nerve blood flow and nerve oxygenation (41). The manifestation of AGEs in the basement membrane, elastic lamina and endothelial cells of the blood vessels causes macrophage infiltration, which release macrophage-derived growth factors , leading to smooth muscle proliferation and atherogenesis (41). Due to evidence that inflammatory cytokines also cause vascular damage by inducing excessive ROS, it is plausible that AGE-mediated inflammation is the primary cause of neurovascular dysfunction in diabetic neuropathy (36, 134). AGE levels in the endoneurium and associated microvessels have been observed to correlate with the degree of axonal

atrophy in nerve biopsies; with AGE immunoreactivity detected in 90% of peripheral nerves of diabetics compared to a complete absence of AGEs in controls (91). Whether or not AGEs can be said to underlay both the metabolic and vascular causes of neuropathy, they certainly do represent a link between them. Ultimately, hyperglycemia-mediated AGEs, metabolic dysregulation and vascular dysfunction are all fundamental to nerve dysfunction and degeneration in T1DM.

1.4 The Autonomic Nervous System and Mechanisms of Autonomic Cardiovascular Regulation

The autonomic nervous system (ANS) is a network of efferent and afferent nerves linked to structures of the central nervous system (CNS) that, together, govern the motor control of visceral organs (40, 76). Since targets of the ANS include multiple endocrine organs, the cardiovascular (CV) system and regions of CNS, the ANS is a powerful regulator of homeostasis throughout the entire body (76, 124). The ANS differentially regulates these systems by using two antagonistic branches, the sympathetic nervous system (SNS) and the parasympathetic nervous system (PSNS), which mutually inhibit one another (116). The main neurochemical difference between the SNS and PSNS is the neurotransmitters involved in signaling to the target organs. Preganglionic neurons of both branches utilize acetylcholine as their primary neurotransmitter, whereas the postganglionic neurons of the PSNS emit primarily acetylcholine and the postganglionic neurons of the SNS release primarily norepinephrine (76, 116). The use of these different neurotransmitters underlies the antagonistic physiological actions of the two branches of the ANS (76, 116). To understand the discrete roles of the SNS and PSNS in controlling bodily functions, the mnemonics “fight or flight” and “rest and digest” are used, respectively (116, 149).

The SNS directs oxygen and energy toward organ systems that are recruited in stressful or active situations; for example, it acts to increase heart rate (HR) and decrease enteric motility. The PSNS, on the other hand, promotes conservation and restoration of energy and so, stimulates functions that are more useful at rest while inhibiting others; for example, it decreases blood pressure (BP) and facilitates the excretion of waste (76, 116). When functioning properly, these two branches maintain chronic homeostasis of visceral organ function (116).

One such autonomically-mediated mechanism of homeostasis is the regulation of CV function by the baroreflex (see Figure 1.2). Afferent arterial baroreceptors are stretch-sensitive mechanoreceptors located in the aortic arch, carotid sinus and elsewhere in the vasculature that send frequency coded signals to the brainstem in order to signal increases and decreases in BP (40). Once these signals arrive at the brainstem they are processed by several structures that govern the efferent sympathetic and parasympathetic output to the heart, the vasculature, kidneys and other visceral organs (40, 116). The four major brain regions involved in processing these signals include the hypothalamus and three regions of the brainstem: the rostral ventrolateral medulla (RVLM), the caudal ventrolateral medulla (CVLM) and the nucleus of the solitary tract (NTS) (40, 76, 116). When a rise in BP above the set physiological range occurs, the baroreceptors fire rapidly and stimulate the NTS. The NTS in turn stimulates the CVLM, which provides inhibitory input to the RVLM. Since the RVLM has excitatory input to sympathetic preganglionic neurons in the intermediolateral (IML) column of the thoraco-lumbar spinal cord, when baroreceptors fire the RVLM, and by extension sympathetic outflow, is inhibited and the autonomic balance shifts towards greater parasympathetic activity; the outcome of which is reduced HR and the restoration of BP to the physiological range (8, 40, 116).

Alternatively, when arterial pressure declines below the set physiological range, baroreceptor firing decreases, removing the stimulation of the NTS and CVLM and disinhibiting the RVLM, resulting in increased sympathetic outflow, HR and again, the restoration of BP to the physiological range (40, 116).

The baroreflex also has an important influence on total peripheral resistance (TPR); which is the resistance of all peripheral vasculature in the systemic circulation (145). Peripheral resistance and the variables that influence it can be described by Poiseuille's law, $R = \frac{\Delta P}{Q}$; where R is resistance, ΔP is the change in pressure between two points and Q is the blood flow, which is equivalent to cardiac output (90). Resistance can also be represented as $R = \frac{8\eta L}{\pi r4}$; where η is the viscosity of the fluid, L is the length of the vessel, and r is the radius (90). Blood pressure and blood flow (cardiac output) are acutely regulated by the baroreflex (77). However, beat-to-beat changes in TPR are difficult to measure and thus, most studies examine feedback control of HR as opposed to TPR when assessing the baroreflex (10).

The paraventricular nucleus (PVN) of the hypothalamus also modulates sympathetic outflow (11, 21, 40). The PVN receives inputs from circulating hormones and molecules (such as norepinephrine and glucose), visceral receptors that detect osmolarity and blood volume and other regions of the brain (11). The PVN has many complex effector subnuclei but its effects can be summed through its three major connections. The magnocellular neurons of the PVN are large cells that interact with the posterior pituitary to release oxytocin and vasopressin (also known as antidiuretic hormone, ADH; a vasoconstrictor and mediator of water absorption in the kidneys) into the blood (113). The medial parvocellular neurons synthesize corticotropin releasing hormone (CRH), which stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary, which then acts on the adrenal cortex to

stimulate aldosterone (a mediator of water retention in the kidneys)and cortisol production (which stimulates gluconeogenesis in the liver); this constitutes what is known as the hypothalamo-pituitary-adrenocortical (HPA) axis (11, 125). Another subset of parvocellular PVN neurons have direct connections to the RVLM in the brainstem and the IML in the spinal cord (8). Thus, these PVN neurons can directly and indirectly influence sympathetic nerve activity via inputs to preganglionic sympathetic neurons and by modulating RVLM activity (8). Indeed, it has been demonstrated that sympathoexcitation is elicited through the PVN in response to a drop in blood volume, without a drop in pressure, via the activation of both RVLM and ILM connected neurons (8). Conversely, the autonomic neurons of the PVN have been reported to selectively decrease renal sympathetic activity and increase cardiac sympathetic activity in response to blood volume expansion (21). This demonstrates that the PVN's influence on BP and the CV system is mediated through hormones, sympathetic neurons and interactions with the kidney. The stimulation of sympathetic activity by PVN neurons in response to increased circulating angiotensin II (ANG II), generated by the renin-angiotensin-aldosterone system (RAAS), is another example of its interaction with the kidneys (11, 54).

Renin release from juxtaglomerular cells of the kidney primarily occurs in response to hypovolemia and increased osmolarity but can also be produced by sympathetic stimulation (11, 20, 38). Once released, the renin enzyme acts locally and systemically in the blood to cleave angiotensinogen, which is constitutively secreted by the liver into circulation, to form angiotensin I (38). Angiotensin I is then cleaved by angiotensin-converting-enzyme (ACE), which is found in blood plasma, vascular endothelial cells, the kidneys, the brain, the lungs and many other tissues (38, 40). The product of ACE cleavage is the biologically active ANG II which has a multitude

of effects on numerous organs and tissues. For instance, ANG II is a potent vasoconstrictor, it acts on presynaptic receptors of sympathetic nerve terminals to potentiate norepinephrine release, it decreases glomerular filtration rate and increases reabsorption of sodium and water into the blood and it stimulates aldosterone release from the adrenal cortex, thus completing the RAAS (38). It also acts on the subfornical organ (a circumventricular organ of the brain) to stimulate PVN mediated sympathoexcitation to the heart and kidneys (20, 40, 54). However, it is very important to note that this classic, endocrine view of the RAAS has undergone profound conceptual change due to the discovery of local, tissue-intrinsic renin-angiotensin systems (RAS – note the distinct acronyms denoting local and circulatory renin-angiotensin systems) and its importance in autocrine and paracrine signalling which act independently of the circulating RAAS factors (32, 115).

Thus, both neural and humoral mechanisms interact with the ANS to modulate autonomic activity and CV function. For example, the PVN and the RAAS influence blood volume, vascular tone and BP; baroreceptors regulate BP and maintain it within a set range by modulating cardiac function; ANG II produced by the RAAS can act directly on peripheral sympathetic synapses and can modulate PVN activity; and the PVN can directly modulate sympathetic outflow and influence the brainstem circuitry that mediates the baroreflex (40). All of these systems are integrated through the ANS and cooperate to maintain CV homeostasis.

1.5 Autonomic Dysfunction and Cardiovascular Dysregulation in T1DM

Dysfunction of the ANS is a common and serious complication of T1DM that can result in dysregulation of all of the organ systems under its control (30). Known as diabetic autonomic neuropathy (DAN), prevalence of this condition has been

reported to vary widely, ranging from roughly 10-90% in diabetics depending on the assessment criteria used (30, 149). The clinical manifestations of DAN are also variable due to the systemic influence of the ANS and can include orthostatic hypotension, tachycardia, hypertension, silent myocardial ischemia, gastroparesis, xerostomia, decreased heart rate variability (HRV), anhidrosis and many others symptoms (30, 33, 69, 95, 149). However, it should be noted that experimental forms of diabetes do not always faithfully replicate the full spectrum of clinical complications; in some cases, opposing outcomes have been observed, such as resting bradycardia and hypotension (6, 47, 81)**.** However, unlike the clinical population, most of these studies do not use insulin therapy to manage hyperglycemia, which has been shown to reverse those effects (47)**.** DAN can also be a considerably deadly complication of T1DM as it underlies diabetes-mediated CV dysfunction (85, 100). Indeed, based on an analysis of the Pittsburgh Epidemiology of Diabetes Complications Study, Orchard et al. (100) found that mortality increased fourfold in patients with DAN. Similarly, a meta-analysis by Maser et al. (85) found that cardiovascular autonomic neuropathy (CAN), a subset of DAN, significantly increased the relative risk of mortality by a factor of 3.45. Another form of mortality in T1DM is the occurrence of sudden nocturnal death (100, 154). This so called “dead in bed” syndrome is thought to be exacerbated by increased susceptibility to nocturnal hypoglycemia resulting from abnormal pancreatic α-cell function and impaired glucagon release (140, 154). However, even this impaired glucagon response is thought to be mediated by autonomic dysfunction and furthermore, sudden death in T1DM is more greatly attributed to cardiac arrhythmia than hypoglycemia; thus, “dead in bed” syndrome is likely resultant of DAN (140, 154). DAN, therefore, has a significant impact on the quality of life, morbidity and mortality of patients with

T1DM; the most deadly consequences of which are mediated by loss of CV control via CAN (30, 101, 149, 151).

As described previously, the etiology of general diabetic neuropathy and thus, DAN, in T1DM is linked to chronic hyperglycemia (127, 155). Evidence of this was reported by the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group (98) who observed that maintenance of near normoglycemia via intensive insulin therapy reduced neurologic complications of T1DM and resulted in a 42% decrease in the risk of any CV disease event, such as myocardial infarction, stroke or death. The neuropathic effects of hyperglycemia that mediate DAN usually affect longer nerves first and since the single longest ANS nerve, the vagus nerve, facilitates 75% of all parasympathetic activity, a sympathovagal imbalance leading to sympathetic overactivity is purported to be an early cause of CAN (30, 149, 151). Furthermore, hyperglycemia can lead directly and indirectly to increased sympathetic outflow, indicating peripheral and central mechanisms are behind ANS and CV dysfunction in T1DM (108, 148). It is pertinent then to understand how the regulation of autonomic activity and CV function are affected by T1DM.

Although there has been some evidence for increased RAAS activity in T1DM and hyperglycemia, most of the literature has demonstrated that systemic components of the RAAS, such as plasma renin, aldosterone and ANG II, are suppressed (13, 22, 73, 89). When considering this in the context of the physiological action of circulating ANG II (vasoconstriction, volume expansion and sympathoexcitation), it should follow that it is not involved in the increased BP and sympathetic tone observed in T1DM; however, this is not the case (115, 148). More recent evidence implicates the role of local, tissue-intrinsic RAS systems in the pathology of diabetes-related

complications (109, 115). Hyperglycemia has been shown to increase local RAS activity in numerous tissues and this process is being connected to the onset of many downstream complications of T1DM; including, nephropathy, retinopathy and cardiac myopathy (27, 34, 67, 110). This widespread deleterious effect of local RAS overactivity in diabetes is likely interconnected to oxidative damage mediated by locally produced ANG II (34, 115). Thus, RAS activation in diabetes is believed to contribute to microvascular complications and has a probable role in the vascular etiology of neuropathy (86, 109). Due to the presence of RAS in the brain and the known ability of ANG II in the subfornical organ to elicit PVN-mediated increases in sympathetic activity, there exists a plausible mechanism by which local RAS may be driving increased sympathetic outflow in T1DM (11, 148).

The PVN of the hypothalamus is known to influence the basal level of sympathetic outflow (60). In T1DM there are increases in both basal PVN activity and sympathetic activity (35, 56). It has been demonstrated that the excitation of the PVN by ANG II is enhanced in rats with streptozotocin (STZ)-induced diabetes; an experimental form of T1DM produced by STZ-mediated destruction of β-cells (103). This suggests that diabetes induced elevations of sympathetic outflow could be due to increased levels of ANG II in the brain produced by local RAS system activation or increased sensitivity to ANG II in the PVN (103). Another previously described diabetes-related deficit that is also a mechanism contributing to central sympathetic overactivity in T1DM is the loss of NOS activity and NO (138, 161). NO is a known inhibitor of PVN activity that acts by increasing GABAergic input to the PVN (160). Zheng et al. (161) found that STZ-diabetic rats had fewer neuronal NOS (nNOS) positive cells and lower NO release in the PVN compared to controls. Thus, there is a reduction of GABA inhibition on the PVN in T1DM that facilitates its overactivity

and thereby, contributes to sympathetic overactivity (114). Furthermore, overstimulation of the PVN would also cause excitation of magnocellular neurons, resulting in the release of vasopressin, which would contribute to increased BP via direct vasoconstriction and volume expansion (70). However, the aberration of sympathetic tone in diabetes is more directly influenced by the parvocellular neurons of the PVN due to their association with the RVLM and IML; hence, PVN dysfunction can dysregulate sympathetic outflow and CV parameters by modulating neural control of HR, BP and the baroreflex (20, 60, 103).

The baroreflex itself is an important regulator of sympathetic and parasympathetic outflow, BP, HR and TPR (84). Baroreflex dysfunction, in both sensitivity and set point, is an established consequence of T1DM, with reductions in sensitivity observed to occur as early as 5 days after STZ-diabetes induction (15, 23, 81). Thus, studies have investigated how each aspect of the baroreflex arc are uniquely affected in T1DM; this arc consists of baroreceptors, baroreceptor afferents, central regulators and sympathetic and parasympathetic efferents (74). Salgado et al. (122) found that aortic baroreceptor neurons have reduced activity in STZ-diabetic rats. Further, they determined that the aortic depressor nerves, the afferent nerves between baroreceptor neurons and the brainstem, showed morphological signs of axonal atrophy by measuring the *G* ratio; the ratio between axon diameter and whole fiber diameter (122). Li et al. (72) observed that the excitability of aortic baroreceptors, which mediates the set point of baroreceptor firing, was blunted in STZ-diabetic rats and that later, this reduced excitability was due to up-regulated, ANG II-NADPH oxidase derived, superoxide within the baroreceptor ganglion (73). They also found that microinjection of an ANG II receptor antagonist into the nodose ganglia restored aortic baroreceptor excitability, making the case for diabetes-induced

local RAS activity being responsible for dysfunction of the afferent portion of the baroreflex arc (73). Evidence for the dysfunction of central regulation of the baroreflex was presented by Gu et al. (39) using OVE26 T1DM mice; a transgenic model of T1DM that acquires β-cell damage through an overexpressed calmodulin regulated by an insulin promoter. They found that these mice, compared to controls, had a less prominent bradycardia response to aortic depressor nerve stimulation but that the bradycardia response to vagal efferent stimulation was increased; suggesting a central mechanism was responsible for the blunted response to baroreceptor stimulation (39). Also, Chen et al. (16) documented a reduced firing rate in the NTS, both basally and after phenylephrine injection, in STZ-induced diabetic rats compared to controls. This decreased activity was implicated in their observation of decreased baroreflex sensitivity (BRS) and could possibly indicate chronic disinhibition of the RVLM; and therefore, increased sympathetic outflow (16). Of the two efferent arms of the ANS, the PSNS appears to be the one primarily impaired in T1DM. Maeda et al. (81) reported significant decreases in both basal PSNS tone and responsiveness to hypertensive drugs in STZ-induced diabetic rats; but non-significant changes to similar parameters in the SNS. Lund et al. (79) found that choline acetyltransferase (ChAT, the enzyme that produces the chief neurotransmitter of the PSNS, acetylcholine), had reduced activity in the hearts of diabetic rats as early as 4 weeks post-STZ induction. After 8 weeks of diabetes, they measured a reduction in cardiac ganglion size and number, prompting the conclusion that these PSNS deficits may contribute to the impaired parasympathetic control of the heart, sympathetic overactivity and baroreflex blunting that is common in T1DM (79).

A non-invasive functional measure capable of detecting such impaired autonomic control of the heart in T1DM is the assessment of HRV (75). HRV can be

measured by time domain analyses, measuring the changes in the standard deviation between R-R intervals or by frequency domain analyses, using Fourier analysis to disintegrate HR variance into underlying high and low frequencies (136). Regardless of which method is chosen, HRV has been described as one of the earliest indicators of autonomic dysfunction in T1DM (126, 162). A variable HR has been known to be a characteristic of a healthy heart for roughly a century (37). In frequency domain analysis, high frequency (HF) variation (0.15-.4Hz) is considered to be mediated by parasympathetic activity, whereas low frequency (LF) variation (0.04-0.15Hz) is considered to be entrained by the oscillation of baroreceptor firing; and so, it is modulated by both parasympathetic and sympathetic activity (37, 136). As described above, T1DM results in baroreflex dysfunction that is largely attributed to damage of the PSNS (29). Since PSNS dysfunction occurs first, a reduction in HF variability is usually the first sign of autonomic dysfunction (52). Thus, in general, a reduction in HRV in T1DM is associated with the onset of autonomic dysfunction and more specifically, the loss of parasympathetic tone and baroreflex dysfunction (37, 126, 157). Now used as a standard indicator in the diagnosis of cardiac autonomic neuropathy, reduced HRV is associated with cardiac arrhythmias that can have mortal consequences for diabetics and is therefore an important marker of autonomic function in T1DM (151).

1.6 The Effects of Exercise on Neuropathy, Autonomic Regulation and Cardiovascular Dysregulation in T1DM

Exercise training is a well-established and widely prescribed non-pharmacological therapy capable of ameliorating a wide breadth of diseases and their complications (98, 105, 141). For example, exercise has been demonstrated to

improve heart disease, pulmonary disease, muscle disease, bone disease, joint disease, cancer, depression, asthma, obesity, hypertension, dyslipidemia, insulin resistance, type 2 and type 1 diabetes (18, 105, 123, 128). Beyond just treating disease, exercise is also associated with preventing disease risk factors and manifestation. It has been shown to improve cognitive performance, increase longevity and has a strong negative correlation with all-cause mortality (1, 64, 78). One of the underlying principles likely facilitating the benefits of exercise is the concept of “hormesis”; wherein low level stress or damage causes a disruption of homeostasis and results in an adaptive overcompensation and increased fitness of a physiological system (14, 36). In the case of exercise, the acute induction of low-level oxidative stress and inflammation results in an increase of endogenous antioxidant enzymes and anti-inflammatory mediators (59, 132). Continuous exercise training results in the chronic upregulation of antioxidants and anti-inflammatory agents which confers constitutive resistance to other sources of pathogenic insult (132, 133). Another outcome of exercise, which is especially important in diabetes, is increased insulin sensitivity and glucose tolerance (43). Exercise training increases the amount and function of proteins in the insulin signalling cascade, which facilitates the translocation of glucose transporter 4 (GLUT4) into the skeletal muscle membrane (53). Furthermore, muscle contraction can upregulate GLUT4 translocation to the plasma membrane via an insulin-independent mechanism (118). In T1DM, hyperglycemia mediated ROS act to decrease insulin sensitivity by impairing the insulin cascade and GLUT4 translocation (44). Therefore, the antioxidant, anti-inflammatory and glucoregulatory effects of exercise make it a potent therapy for T1DM and many of its complications (9, 36).

The influence of exercise on insulin sensitivity and glucose tolerance both directly and indirectly affects nerve health. This is because neither nerves nor

endoneurium express GLUT4 or utilize it to transport glucose; instead, they use GLUT1 and GLUT3 which do not require insulin for translocation and are constitutively active (82, 131). Thus, exercise-mediated sensitization to insulin does not occur within neural tissue, yet through improving glucose tolerance in other tissues, exercise reduces exogenous insulin requirements and neural hyperglycemic insult in T1DM (105, 112, 158). This moderated hyperglycemia reduces the level of AGEs, which are known to be major contributors to neuropathy through ROS formation and inflammation in the neurovasculature (36, 41, 117). Furthermore, exercise-induced insulin sensitivity and AGE reduction also means increased NO production and bioavailability, resulting in enhanced function of endoneurial capillaries and oxygen delivery to peripheral nerves (117, 135, 138). Exercise training also increases endothelial Cu/Zn-superoxide dismutase (SOD) and mitochondrial Mn-SOD antioxidant enzymes independently of hyperglycemic status, while simultaneously decreasing inflammatory cytokines and increasing anti-inflammatory mediators (51, 59, 93). Thus, exercise training is capable of ameliorating neuropathy through the alleviation of both its metabolic and vascular etiologies by improving glucose metabolism and upregulating antioxidant and anti-inflammatory mediators (36, 112, 158). Indeed, exercise training has been shown to both, prevent diabetic neuropathy from occurring and improve nerve function in individuals previously diagnosed with diabetic neuropathy (9, 62).

Another mediator of T1DM complications that is influenced by exercise is the local RAS (115, 121). RAS overactivity has been shown to be mediated by hyperglycemia and is implicated in causing insulin resistance (45, 148). As previously described, exercise can reduce hyperglycemia and so can decrease hyperglycemia-mediated RAS stimulation. Furthermore, exercise results in the down-regulation of

components of the RAS system, such as ACE, ANG II, ANG II type 1 receptor (AT1), and NADPH oxidase (43, 57, 106, 121). Indeed, exercise has been shown to decrease plasma ANGII and local RAS activity in cardiac muscle, blood vessels and the brain (2, 121, 159). For example, Kar et al. (57) found that 3 weeks of treadmill running decreased ACE mRNA in the NTS, RVLM, PVN and decreased sympathetic outflow in rabbits with induced heart failure. Similarly, Pan et al. (102) found that non-T1DM Sprague-Dawley rats had decreased AT1 and NADPH oxidase protein expression and decreased superoxide production in the PVN following 3 to 4 weeks of exercise training. As aforementioned, the PVN is activated in T1DM through increased ANGII and decreased NO and this leads to sympathetic overactivity (103, 161). Thus, inhibiting RAS activity and ANGII production, while increasing NO bioavailability, are likely mechanisms by which exercise reduces PVN mediated sympathetic outflow (102, 117). This influence of exercise on RAS activity in the PVN, NTS and RVLM makes it likely that exercise will also influence baroreflex function in T1DM (55, 57, 102).

Since exercise can influence both vascular and neural function, both must be taken into account when assessing the influence of exercise on BRS and baroreceptor set point (65, 120). Indeed, both the neural structures involved in the baroreflex arc and the carotid artery compliance can affect BRS and baroreceptor firing in diabetes and exercise (65, 94, 120). Monahan et al. (94) found that both age-related and exercise induced changes in the BRS corresponded to differences in carotid artery compliance in healthy men. However, Komine et al. (65) determined that endurance training increased BRS through alterations to the neural circuitry. In this vein, Miki et al. (88) reported that exercise has been demonstrated to cause a shift in baroreflex control of sympathetic nerve output to visceral organs. Further, Ruiz et al. (120)

reported that diabetic neuropathy is a more significant determinant of BRS than carotid artery elasticity in T2DM. Whether mediated by carotid artery compliance, neural circuitry or a combination of both, exercise has been shown to increase BRS after even a single bout of aerobic exercise in STZ-diabetic rats (55). Furthermore, the benefits of aerobic exercise on baroreflex function have been shown to persist after 3 weeks of detraining subsequent to 10 weeks of moderate intensity training (50-70% maximal running speed) in STZ-diabetic rats (96). Thus, exercise can be an effective means to rescue or prevent baroreflex dysfunction and mitigate aberrations of CV function in T1DM (42, 65, 102).

Since baroreflex function is directly linked to the neural control of HR, fittingly, HRV is also influenced by exercise (119). In T1DM, there is generally a reduction in HRV; an indication of autonomic dysfunction (17). However, in individuals with T1DM there are inconsistent changes in HRV in response to exercise. Chen et al. (17) reported that children with T1DM with moderate to high physical activity did not differ in HRV compared to controls, whereas children with low physical activity had reduced HRV, suggesting only higher intensity exercise prevented a decline in HRV. On the other hand, Zoppini et al. (164) observed no change in total HRV following twice-weekly exercise over 6 months but did find that it increased HF power and lowered the LF/HF ratio, meaning there was a shift toward more parasympathetic output in the sympathovagal balance. Howorka et al. (49) tested HRV responses to 12 weeks of bicycle training in diabetic subjects who had differing levels of cardiovascular autonomic neuropathy. They found that patients without CAN had the greatest increases in HRV, patients with early CAN had lesser increases in HRV and patients with severe CAN did not improve following training; suggesting the level of neuropathy can dictate the outcomes of exercise (49).

Therefore, the intensity of exercise and stage of disease both factor in to the ability of exercise to modulate HRV in T1DM.

Some inconsistency in the effectiveness of exercise modulation of HRV is reflected by the somewhat mixed outcomes for the influence of exercise on autonomic function in T1DM. For example, De Angelis et al. (5) found that exercise training in STZ-diabetic rats did not modify sympathetic tonus nor parasympathetic function. In the same model, Wegner et al. (153) reported that training did not induce changes in markers of parasympathetic innervation of the heart. However, vagal tonus and function have been observed to improve after acute and prolonged training in the STZ-diabetic model (96, 111). Indeed, the majority of literature supports the role of exercise as an effective means to improve and maintain autonomic function in diabetes (17, 150).

1.7 Summary

The pathogenesis of neuropathy in T1DM is a product of hyperglycemia-mediated AGEs, oxidative stress, metabolic dysfunction, inflammation and microangiopathy (41). Diabetic neuropathy affects peripheral and central aspects of the autonomic nervous system, alters the homeostatic maintenance of the sympathovagal balance and leads to a variety of systemic complications (30, 40). One of the most common and serious complications of DAN is cardiovascular dysregulation (85). The local RAS, PVN of the hypothalamus and baroreflex circuitry all interact to regulate autonomic cardiovascular control (40, 41). In T1DM, hyperglycemia causes an increase in local RAS, which in turn generates local ANGII mediated oxidative stress that can decrease insulin sensitivity, blunt the excitability of baroreceptor neurons and increase PVN activity (45, 72, 103, 148). This altered PVN activity interacts with the central

mediators of the baroreflex and directly with preganglionic neurons to influence sympathetic and parasympathetic outflow (8, 56). Thus, the baroreceptors, afferent nerves, central neural structures and efferent nerves of the baroreflex arc are all affected by T1DM (74). Together, dysfunction of these circuits underlie many of the serious functional CV complications associated with DAN (40, 100). Exercise is not only known to improve CV function directly but is also capable of improving ANS regulation of CV control in diabetics (17, 105, 150). Through improving insulin sensitivity and glucose metabolism, as well as the hormetic induction of antioxidant and anti-inflammatory factors, exercise is capable of decreasing local RAS, reducing PVN activity and improving the baroreflex and overall autonomic function in T1DM via both molecular and physiological mechanisms (36, 43, 65, 102, 159). Therefore, despite some inconsistencies between clinical and animal trials examining the effectiveness of exercise in the improvement of autonomic control of CV function, exercise remains an established, promising intervention for improving the prognosis of those with T1DM.

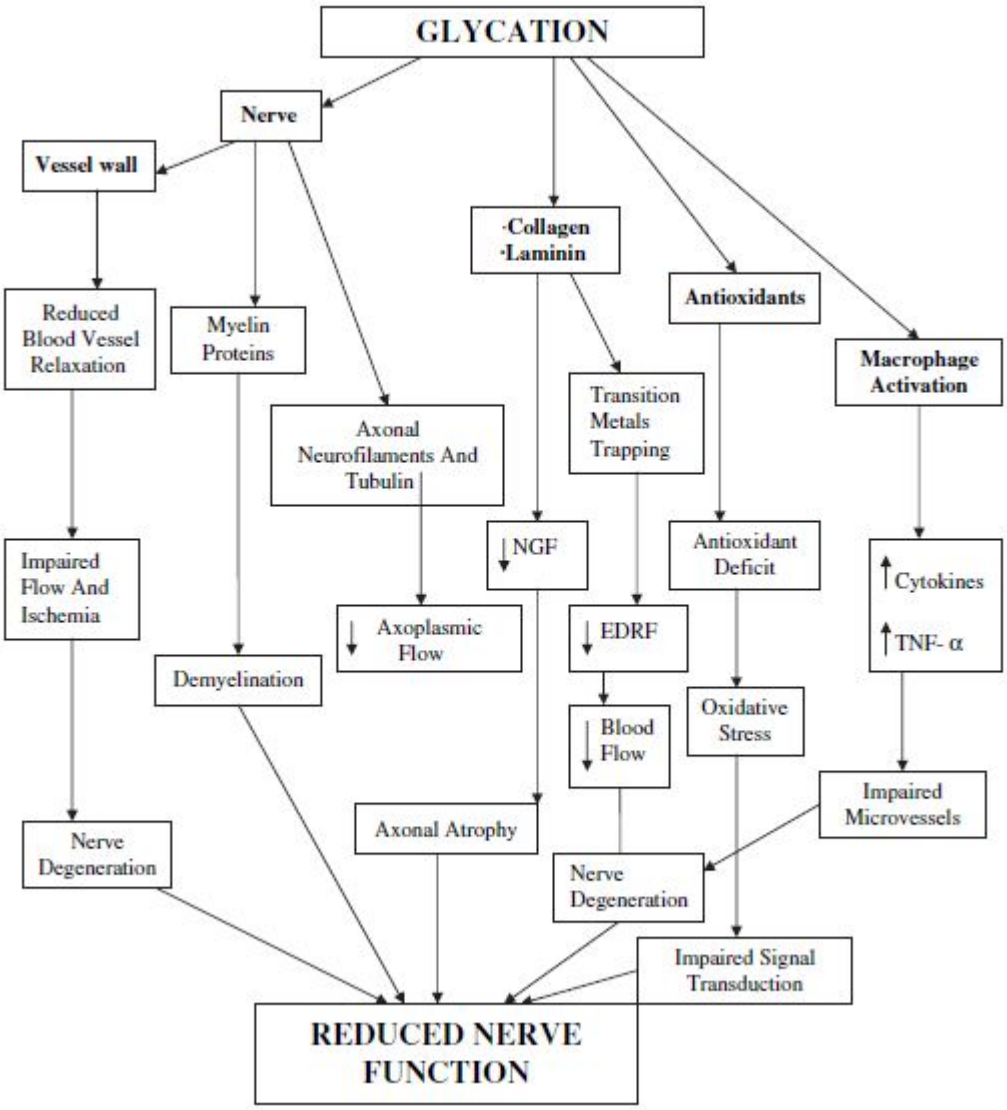

Figure 1.1. Protein glycation as the common cause of neuropathy; from Harati 2007 (41).

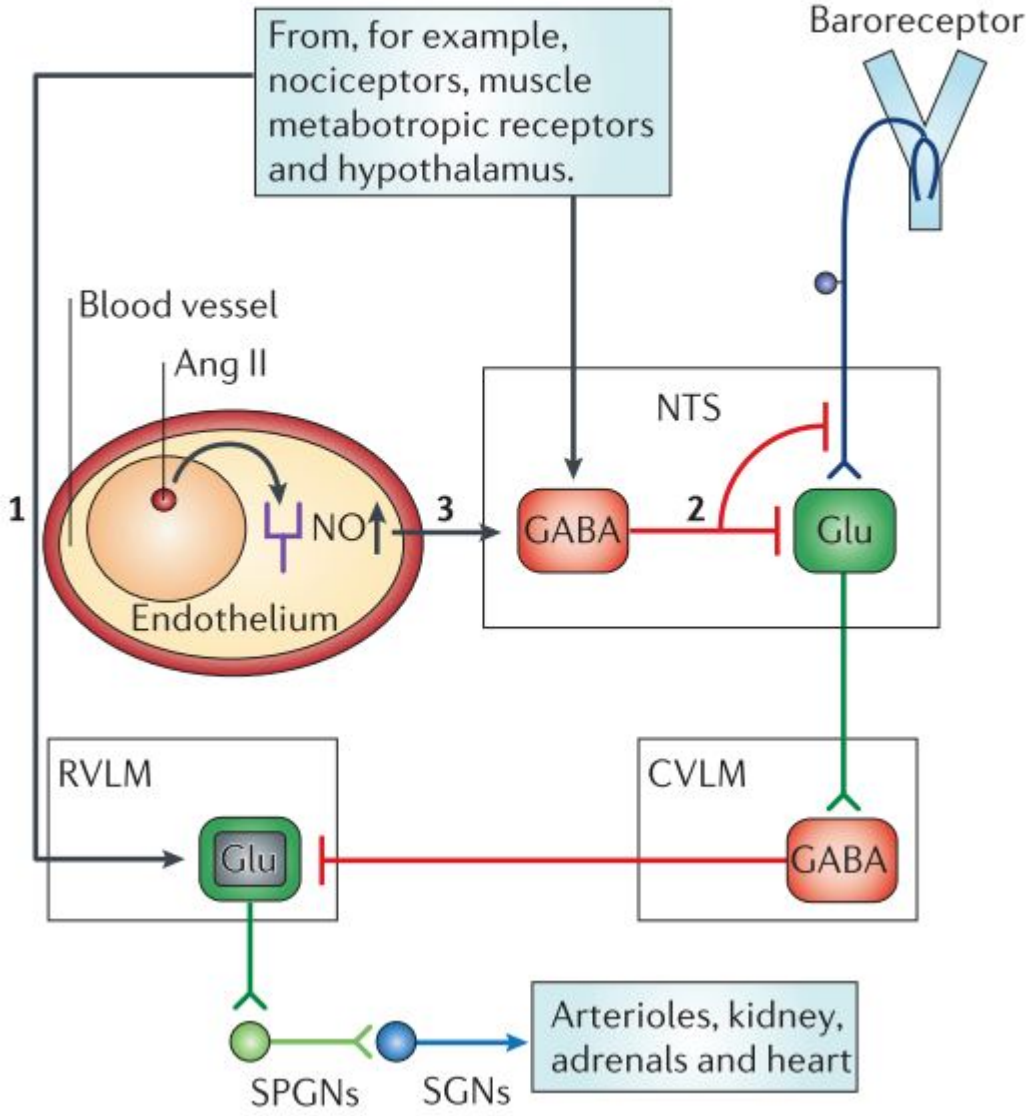

Figure 1.2. The baroreflex circuit; from Guyenet 2006 (40).

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CHAPTER 2

2.1 Introduction

One of the most common and serious complications of type 1 diabetes mellitus (T1DM) is diabetic autonomic neuropathy (DAN) (20, 95). The prevalence of DAN can range from roughly 10% in those recently diagnosed with T1DM, up to 90% in patients who are awaiting pancreas transplantation (20, 43, 95). Due to the breadth of the autonomic nervous system (ANS) throughout the body, DAN can influence the dysfunction of virtually any organ system (95). The involvement of the ANS in the homeostasis of so many systems is likely implicated in the four-fold increase in mortality observed in T1DM patients with DAN (64). One of the most critical system affected by DAN is the cardiovascular (CV) system (57). Cardiovascular autonomic neuropathy (CAN) is a subset of DAN characterized by impaired autonomic control of the CV system (46). CAN is consistently associated with increased mortality. For instance, CAN has been reported to increase mortality of diabetics by a factor of 3.45 and further, symptomatic CAN 5 years after the onset of type 2 diabetes has been shown to predict mortality 5 years later, even after adjusting for other cardiovascular disease risk factors (48, 57, 97).

The etiology of DAN and thus, CAN, stems from both metabolic and vascular pathologies that arise as consequences of sustained hyperglycemia (84, 100). Hyperglycemia leads to the irreversible binding of glucose to proteins, forming what are known as advanced glycation end-products (AGEs) (30). These AGEs exacerbate the formation of reactive oxygen species (ROS) by inhibiting the function of antioxidant enzymes and impair vascular dynamics by inhibiting nitric oxide synthase (NOS) function, promoting inflammation and atherosclerosis of endoneurial microvessels (30, 83, 89). This leads to further metabolic dysfunction, decreased

nerve conduction and reduced oxygen delivery to nerve tissue and ultimately, neuropathy (30, 81, 84).

When this neuropathy occurs in the ANS it leads to an imbalance between the sympathetic (SNS) and parasympathetic (PSNS) nervous systems and dysregulates their control of cardiac and vascular dynamics (20, 97). Often, this balance shifts towards greater sympathetic activity in early T1DM as longer nerves are affected first and the longest efferent ANS nerve, the vagus nerve, facilitates 75% of all parasympathetic activity (20, 95). However, peripheral receptors, afferent nerves and central regions regulating autonomic activity are also implicated in the imbalance of the ANS during DAN (42, 50, 78). Together, the dysfunction of these ANS components leads to the sympathetic overactivity, hypertension, tachycardia and cardiac arrhythmias characteristic of CAN in T1DM (21, 44, 46, 68, 80, 97). However, it has been observed that as the duration of T1DM progresses, so does neuropathy of the SNS, which can reduce sympathetic overactivity and its associated symptoms (46, 80).

The most common methods for assessing CAN are heart rate variability (HRV) analysis and baroreflex sensitivity (BRS) measurement (5, 46, 52). In T1DM, it has been shown that all aspects of the baroreflex arc can be impaired (51). For instance, both baroreceptor activity and excitability have been shown to be blunted (50, 78); the aortic depressor nerves have been observed to undergo axonal atrophy (78); central regions have been implicated as the limiting factors of BRS (9, 27); and autonomic efferents, primarily of the PSNS, have been reported to have decreased tone, reduced responsiveness and decreased neurochemical activity in the heart (54, 55). Each of these changes can act to reduce the sensitivity of the baroreflex independently and in conjunction. However, reduced HRV is often the earliest

symptom of CAN (80). Whether measured by time domain analysis or by frequency domain analysis and whether in clinical or experimental T1DM, HRV is consistently reported to be reduced in T1DM, with significant decreases observed as early as 10 days after diabetes induction in the experimental streptozotocin (STZ) model of T1DM (34, 52, 79, 80, 102).

Exercise has been demonstrated to be an effective means of improving these deficits of HRV and BRS in both clinical and experimental T1DM (11, 45, 61, 67). Such improvements have been attributed to improved insulin sensitivity, increased antioxidant and anti-inflammatory mediators and improved autonomic control of the CV system (23, 32, 96). However, despite clinical and experimental forms of T1DM demonstrating similar reductions of HRV and BRS in response to diabetes and improvement with exercise, there are marked differences in the initial changes to other CV parameters in their early stages (33). More specifically, in clinical T1DM, increases in heart rate (HR) and blood pressure (BP) are commonly reported in early DAN (12, 14, 46, 47, 80, 95, 96). In contrast, experimental, STZ-induced T1DM is regularly associated with decreases in BP and HR beginning shortly after diabetes induction (16, 19, 34, 79, 91). Due to these opposing initial changes in BP and HR, exercise training is observed to effect contrasting outcomes on these CV parameters in experimental and clinical T1DM; namely, it results in an increase in BP and HR in experimental T1DM and a decrease in BP and HR in clinical T1DM (1, 31, 33, 53, 67). As a result, both the increase and decrease of those CV factors are concurrently cited as exercise mediated improvements to CAN with little consideration of the fact that the changes are reversed between these types of diabetes (33).

This may be due to a discrepancy between clinical and experimental T1DM in the disease progression. The most common method of STZ-diabetes induction is a

single bolus injection of STZ typically ranging from 50-100mg/kg (18, 74, 104). Aside from causing rapid pancreatic β-cell destruction and severe hyperglycemia, high doses of STZ have been associated with bone loss, increased lipid peroxidation, liver and kidney oxidative stress and even immunosuppression (41, 62, 63, 69). However, increasingly, a multiple low-dose treatment of STZ is being demonstrated to more closely represent the gradual pathogenesis of clinical T1DM and has even been demonstrated to induce inflammation (74, 99). Indeed, Wei et al. (99) found that 5 consecutive days of STZ injection at 25mg/Kg resulted in the generation of autoantibodies against insulin and islets of Langerhans and induced T cell infiltration in the β-cells of primates. Thus, this model may more closely replicate clinical parameters of the progression of DAN and CV dysfunction in T1DM by incorporating an immune response and more gradually introducing cytotoxicity and hyperglycemia (74, 96, 99).

Another important difference between experimental and clinical T1DM is the common omission of insulin treatment in experimental diabetes. Indeed, of the experimental studies listed above that showed reductions in HR and BP, not one utilized insulin therapy and all animals presented severe hyperglycemia ranging from roughly 17-25mM (16, 19, 34, 79). As the severity and duration of hyperglycemia have been shown to influence the degree of diabetic neuropathy and with evidence that intensive insulin therapy can reduce neurologic complications of T1DM, it has been suggested that such acute and steep elevations of blood glucose levels in STZ-induced diabetes are causing early-onset neuropathy to not only the PSNS but to the SNS and the sinoatrial node as well; which is likely mediating the observed reduction in BP and HR (2, 20, 30, 59, 64). Indeed, intensive insulin therapy has been shown to restore BP and HR to control levels in STZ-diabetic rats (33, 35). Therefore, the

disparity of insulin therapy in clinical and experimental diabetes can have a significant impact on the progression of CAN and the CV dysfunction that is observed. However, even in clinical T1DM, it is often the case that chronic, moderate hyperglycemia is maintained as a result of difficulties in regulating blood glucose in response to dynamic influences on glycemic control, such as food intake and exercise, combined with the tendency to err on the side of hyperglycemia in order circumvent the discomfort and danger associated with hypoglycemic episodes; which occur more frequently with DAN due to the impairment of the glucagon response (15, 70, 86, 94).

Thus, whereas many diabetic complications and their modification by exercise training are similar between clinical and experimental diabetes, important inconsistencies exist that warrant resolution (2, 33). As such, the objectives of this study are to mirror the moderate hyperglycemic state common to poorly controlled human T1DM by employing a model of multiple low-dose STZ-induced diabetes in combination with insulin therapy, while examining the capacity of high intensity aerobic training to improve indices of CAN. Specifically, it is hypothesized that: a) this model of T1DM will prevent the decline in HR and BP typical of other STZ models, result in the hypertension and tachycardia typical of clinical T1DM and induce reductions in HRV and BRS; and b) exercise training will improve or prevent deficits in HRV and BRS.

2.2 Materials and Methods

Ethics approval

The protocols used in this investigation were approved by the Research Ethics Board of the University of Western Ontario (London, Ontario, Canada) and conformed to the guidelines of the Canadian Council on Animal Care (Appendix B.1).

Animals

Eight-week-old male Sprague-Dawley rats were obtained from Charles River Laboratories Canada (Saint-Constant, Quebec). The rats were housed in pairs and maintained on a 12-hour dark/light cycle at a constant temperature (20 ±1°C) and relative humidity (50%). Rats were allowed access to standard rat chow and water *ad libitum.*

Experimental groups

Sixty-four rats were randomly assigned to one of four groups as follows: 1) Non-diabetic sedentary control (C; n=16), 2) control exercise (CX; n=16), 3) diabetic sedentary control (D; n=16), 4) diabetic exercise (DX; n=16). A subset (n=8) of each group was used for tests of autonomic cardiovascular control at the end of the experiment. However, the total n used for testing varied based on animal availability at the time of the test.

Experimental procedures

Diabetes Induction

Upon arrival rats were acclimatized to the laboratory setting for 5 days. T1DM was induced over 5 consecutive days by multiple intraperitoneal (IP) injections of 20mg/kg streptozotocin (STZ; Sigma-Aldrich) dissolved in a citrate buffer (0.1 M, pH 4.5). Diabetes was confirmed by blood glucose measurements greater than or equal to18mM on two consecutive days. If necessary, subsequent 20mg/kg STZ injections were administered until diabetes was confirmed (Appendix A1). Following the

confirmation of diabetes, insulin pellets (1 pellet; 2U insulin/day; Linplant, Linshin Canada, Inc., Toronto, Ontario, Canada) were implanted subcutaneously in the abdominal region. Insulin pellet dosages were then monitored for 1 week and adjusted (± 0.5 pellet) in order to obtain daily non-fasting blood glucose concentrations in the moderate hyperglycemic range of 9-15mM (Appendix A2).

Exercise Protocol

Prior to the initiation of the exercise training program, rodents were familiarized with the exercise equipment on two consecutive days. The familiarization consisted of two 15 minute sessions of running at progressive treadmill speeds up to 30 meters per minute. The exercise training program consisted of 1 hour of motor-driven treadmill training per day at 27 m/min with a 6 degree incline, 5 days per week, for 10 weeks. The exercise intensity was determined based on earlier research that investigated oxygen uptake in rats at various treadmill speeds. The chosen intensity was found to represent approximately 75-85% VO_{2max} (90).

Experimental measures

Body Weight and Blood Glucose

Body weights and non-fasting blood glucose concentrations were obtained weekly. Blood was obtained from the saphenous vein by venous puncture with a 30 gauge needle and measured via Freestyle Lite Blood Glucose Monitoring System (Abbott Diabetes Care Inc., Mississauga, Ontario, Canada).

Insulin Dose

Insulin dose was determined by multiplying the total quantity of pellet implanted (0.5 pellet increments) by the amount of insulin secreted per pellet (2 units of insulin/day/pellet)

divided by the body weight (Kg) of the rat. Insulin pellet dosages were then monitored and adjusted throughout the course of the study in order to obtain daily non-fasting blood glucose concentrations in the moderate hyperglycemic range of 9-15 mM (Appendix A2).

Intravenous Glucose Tolerance Test

Intravenous glucose tolerance tests (IVGTT) were performed on all animals immediately prior to week 1 and at the end of week 10 of the exercise-training period. Rats were fasted for approximately 8 to 12 hours prior to the assay and did not perform exercise on the day of their IVGTT. A sterile-filtered dextrose solution (50% dextrose, 50% ddH_20) was injected (1g/kg) into the lateral tail vein of the conscious rat. Following dextrose infusion, blood glucose was measured at 5 minutes, 10 minutes and then at 10-minute intervals thereafter until blood glucose levels plateaued (Appendix A3).

Non-invasive Blood Pressure

Non-invasive blood pressure (NIBP) recording was performed on all animals immediately prior to week 1 and at the end of week 10 of the exercise-training period. NIBP was recorded using the CODA non-invasive blood pressure system – tail cuff method (Kent Scientific Corp., Torrington, Connecticut, USA). Rats were restrained in the provided restraining tubes and tails were warmed with the included electrical heating pad. The occlusion cuff was fitted at the base of the tail while the volume-pressure recording (VPR) cuff was fitted 5cm distally. The occlusion pressure of the tail cuff was set to 180mmHg.
Data were recorded using the bundled CODA acquisition system software in 3 sets of 5 cycles. The first set consisted of 5 acclimation cycles, whereas sets 2 and 3 recorded 5 blood pressure measurements each.

Surgery and Instrumentation

To achieve a surgical plane of anesthesia, rats were placed in an induction chamber circulating 4% isoflurane (96% O_2). Once motor reflexes were undetectable, rats were

transferred to a nosecone delivering 3% isoflurane (97% O_2) and placed on a hot water pad (37°C). Immediately following transfer, rats were injected intraperitoneally (IP) with a 25mg/Kg "cocktail" of urethane (16mg/mL) and α-chloralose (100mg/mL). A total of 10mL of urethane/α-chloralose was made, 5mL of which was diluted with 5mL of ddH_2O and was used as needed to maintain anesthesia throughout data collection. Isoflurane anesthesia continued during instrumentation but was gradually removed near the end of surgery to gauge the depth of urethane/α-chloralose mediated anesthesia.

Rats were cannulated with saline-infused polyethylene (PE90) catheters in the right jugular vein and carotid artery and each catheter was attached to a three-way stopcock. The jugular vein catheter was used for drug infusions and the carotid artery catheter was connected in series with a pressure transducer (PX272, Edwards Life Sciences, Irvine, California, USA) for arterial blood pressure measurements. Once cannulation was complete, rats were transported to a new location for data acquisition, a process that required roughly 20-30 minutes. Heart rate (HR), systolic blood pressure (SBP) and mean arterial pressure (MAP) were determined from the blood pressure pulse waveform and were collected while the rats were under urethane/α-chloralose anesthesia in the supine position. The pressure transducer was calibrated using a standard analog manometer. Data were obtained using a PowerLab data acquisition system, digitized and recorded at1kHz using the bundled Lab Chart 7 Pro software (ADInstruments, Colorado Springs, Colorado, USA).

Heart Rate Variability

Five minutes of resting, spontaneous BP and HR data were selected and analyzed for HRV parameters prior to drug infusions using the HRV module included with the Lab Chart software. Time domain analysis of the standard deviation between normal peak pulses of the pressure pulse waveform (SDNN) was quantified as a measure of the total variability of the HR. Frequency domain analysis of the high frequency (HF) band of the Fast Fourier Transform (FFT) of the data was assessed as an index of parasympathetically mediated HRV.

The ratio between low frequency (LF) and HF bands was measured to determine the contribution of sympathetic activity to the sympatho-vagally mediated LF HRV.

Baroreflex Sensitivity

Baroreflex Sensitivity (BRS) was assessed using the modified Oxford technique (25, 37). The slope of the linear regression line (see Figure 2.1) was taken for the linear portion of the systolic blood pressure-heart rate relationship (BPM $mmHg^{-1}$) after rapid bolus injections of phenylephrine (PE) and sodium nitroprusside (SNP) dissolved in ddH_2O. For each drug, the catheter was first filled with a 0.2mL volume to ensure the following dosage was accurate. After a stable baseline was obtained, a bolus injection of SNP (60ug/Kg, 110ug/mL) was rapidly infused (~5s) and the reflex SNS mediated tachycardia response was measured. The analysis began at the onset of SBP decrease after SNP infusion and ended when SBP reached its nadir. This was followed by a saline flush to washout any remaining SNP in the catheter. This same procedure was then followed using PE (12ug/Kg, 10ug/mL) to measure PSNS mediated reflex bradycardia, except that analysis began at the onset of SBP increase and ended when SBP reached its zenith (See Table 1). Exclusions of data points in the threshold and saturation regions of the sigmoid relationship were done manually in order to determine linear gain. Responses to PE and SNP were plotted separately and only regression lines (slopes) with correlation coefficients (r) ≥ 0.70 and $p<0.05$ were accepted (76, 85).

Vascular Sympathetic Tone

To measure the sympathetic tone of the peripheral vasculature, a rapid bolus injection of the α-adrenergic receptor blocker, prazosin (85ug/Kg, 500ug/mL), was infused at the end of the baroreflex testing and the change in MAP was assessed. Following this protocol, animals were euthanized via exsanguination while still under urethane/α-chloralose anesthesia.

Data Analysis

Body weight, blood glucose concentrations, NIBP and IVGTT data were compared using a two-way repeated measures analysis of variance (ANOVA) test, where the two factors were time (week) and specific treatment group (C, CX, D, DX). When significance was found, pairwise comparisons were made using the Fisher LSD post-hoc test. Endpoint measures were compared by one-way ANOVA between treatment groups. When significance was found, pairwise comparisons were made using the Fisher LSD post-hoc test. If initial statistical tests indicated significance, secondary analysis was performed using a two-way ANOVA to determine the main effects of and interaction between diabetes and exercise. If a significant interaction was found, pairwise comparisons were made using the Fisher LSD post-hoc test.

The insulin dosages were compared using a one-tailed t-test. In the event that both the normality and equal variance assumptions were not met and significantly reduced the power of the parametric one-way ANOVA, a non-parametric Kruskal-Wallis one-way ANOVA on ranks was used and pairwise comparisons were made with a Dunn’s post-hoc test.

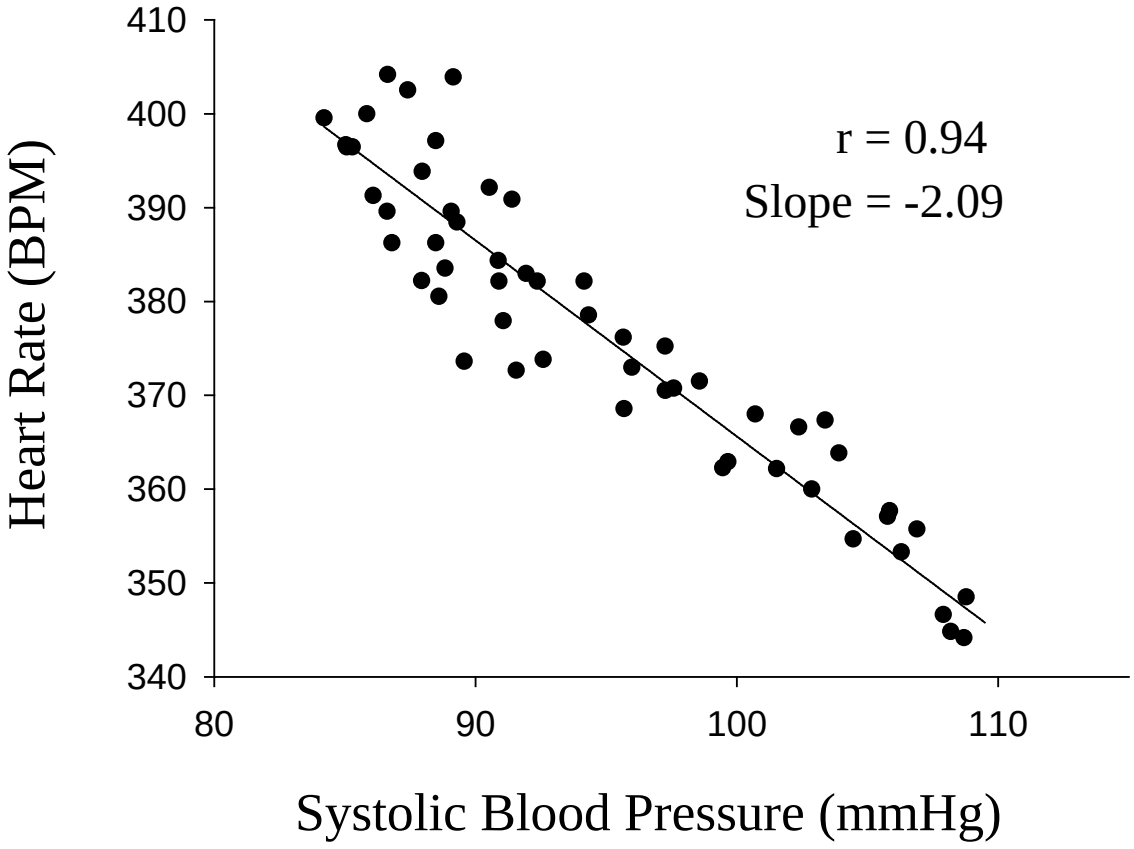

Figure 2.1. Example of a typical regression line used to calculate the slope of the systolic blood pressure-heart rate response to SNP for a single rat.

	C		CX		D		DX	
	SBP	HR	SBP	HR	SBP	HR	SBP	HR
Zenith	160.07	288.17	166.02	297.27	150.07	311.11	171.51	268.72
Baseline	116.73	357.83	123.17	340.32	107.20	354.66	111.48	333.15
Nadir	84.45	407.00	87.438	379.50	75.96	398.97	88.58	360.87

Table 2.1. Coincident average systolic blood pressure (SBP, mmHg) and heart rate (HR, BPM) at the baseline, zenith post-PE infusion and nadir post-SNP infusion. C, sedentary control, n=7; CX, control exercise, n=6; D, sedentary diabetic, n=8; DX, diabetic exercise, n=10.

2.3 Results

Body Weight

All groups (C, n=16; CX, n=16; D, n=15; DX, n=12) significantly gained weight over the course of the study ($p<0.05$). From Week 0 to Week 10, group C was significantly different from all other groups ($p<0.05$). From Week 1 to Week 10, group CX had significantly lower weight than C and significantly greater weight than D and DX ($p<0.05$), with the exception of group D at week 4. From Week 4 to Week 10, with the exception of Week 6, group D had a significantly greater weight than DX ($p<0.05$). Overall, rats in the diabetic conditions were significantly lower weight than those in the non-diabetic conditions ($p<0.001$) and exercise conditions had significantly lower weight than their sedentary counterparts ($p<0.001$). However, there was not an interaction between diabetes and exercise on body weight ($p=0.528$) (Figure 2.1).

Blood Glucose

The C (n=16) and CX (n=15) groups did not change nor significantly differ in blood glucose throughout the study. The blood glucose of D (n=15) and DX (n=13) increased significantly following STZ treatment ($p<0.05$) and remained significantly elevated compared to C and CX throughout the study ($p<0.05$). Groups D and DX were significantly different from week 2 to week 6 and at week 9 ($p<0.05$). Rats in the diabetic conditions had significantly greater blood glucose concentrations than those in the non-diabetic conditions ($p<0.001$). However, there was not a significant interaction between diabetes and exercise on blood glucose ($p=0.27$) (Figure 2.2).

Intravenous Glucose Tolerance Test

The glucose clearance rate (K_G) of D (n=16) and DX (n=8) decreased from Week 1 to Week 10 ($p<0.05$) whereas the K_G of CX (n=15) increased ($p<0.05$). Both diabetic groups (D, DX) were significantly different from both control groups (C, n=16; CX) at Week 10 ($p<0.05$). Indeed, there was a significantly lower K_G for rats in the diabetic conditions than for those in the non-diabetic conditions at Week 10 ($p<0.05$). However, there was not a significant interaction between diabetes and exercise ($p=0.906$) (Figure 2.3).

Insulin Dosages

The amount of insulin supplementation that DX (n=18) received was significantly less than D (n=16) received at Week 10 ($p<0.001$) (Figure 2.4).

Non-Invasive Blood Pressure

At week 1, the diabetic groups (D, =14; DX, n=11) had significantly higher BP than the control groups (C, n=15; CX, n=16) ($p<0.05$). All groups (C, CX, D, DX) increased in BP from week 1 to week 10 ($p<0.05$). At week 10, CX, D and DX had significantly greater BP than C ($p<0.05$). At week 1 there was significantly higher BP in rats in the diabetic conditions in comparison to those in the non-diabetic conditions ($p<0.05$), however, there was not a significant interaction between diabetes and exercise ($p=0.076$). Within non-diabetic groups at week 10, there was a significantly higher BP in the exercise than sedentary conditions ($p<0.05$). Within sedentary groups, there was significantly greater BP in the diabetic than non-diabetic conditions at week 10 ($p<0.05$). Indeed, there was a significant interaction between diabetes and exercise on BP at week 10 ($p<0.05$) (Figure 2.5).

Heart Rate Variability and Heart Rate Variability

Resting heart rate (BPM) under anesthesia was not significantly different between any groups (C, n=7; CX, n=7; D, n=8; DX, n=11) at week 10 ($p=0.122$) (Figure 2.6). Total HRV, as measured by the standard deviation of the normal pulse wave peaks (SDNN), was not significantly different between any groups (C, n=5; CX, n=7; D, n=8; DX, n=8) at Week 10 ($p=0.369$) (Figure 2.7). The parasympathetic, high frequency (HF) contribution to HRV had a significantly greater power for CX (n=7) and DX (n=8) in comparison to D (n=8) at Week 10 ($p<0.05$). Further, there was significantly greater HF HRV in exercise than sedentary conditions ($p<0.05$). However, there was not a significant interaction between diabetes and exercise on HF HRV ($p=0.281$) (Figure 2.8). The low frequency to high frequency (LF/HF) ratio, an index of sympathetically mediated HRV, was significantly greater in the D (n=7) group compared to all other groups (C, n=6; CX, n=6; DX, n=7) ($p<0.05$). The LF/HF ratio was significantly greater in the diabetic than non-diabetic conditions and in the sedentary than exercise conditions ($p<0.05$), however, there was not a significant interaction between diabetes and exercise ($p=0.068$) (Figure 2.9).

Vascular Sympathetic Tone

The decrease in MAP in response to prazosin infusion was significantly greater in the D (n=5) group compared to all other groups (C, n=8; CX, n=7; DX, n=7) ($p<0.05$). Within the diabetic groups (D, DX), there was a significantly greater decrease in MAP in the sedentary condition compared to the exercise condition ($p<0.05$). There was a significant interaction between diabetes and exercise ($p<0.05$). Within the sedentary groups (C, D), there was a significantly greater decrease in MAP in the diabetic than non-diabetic condition ($p<0.05$) (Figure 2.10).

Baroreflex Sensitivity

The slope of the tachycardia response to sodium nitroprusside infusion was not significantly different between any groups (C, n=7; CX, n=6; D, n=6; DX, n=10) ($p=0.86$) (Figure 2.11). The slope of the bradycardia response to phenylephrine was significantly greater in the C (n=7) and DX (n=10) groups compared to the D (n=7) group ($p<0.05$) but not CX (n=5). Within diabetic groups, there was a significantly greater bradycardia response in the exercise than sedentary group ($p<0.05$). There was a significant interaction between diabetes and exercise on the slope of the bradycardia response ($p<0.05$) (Figure 2.12).

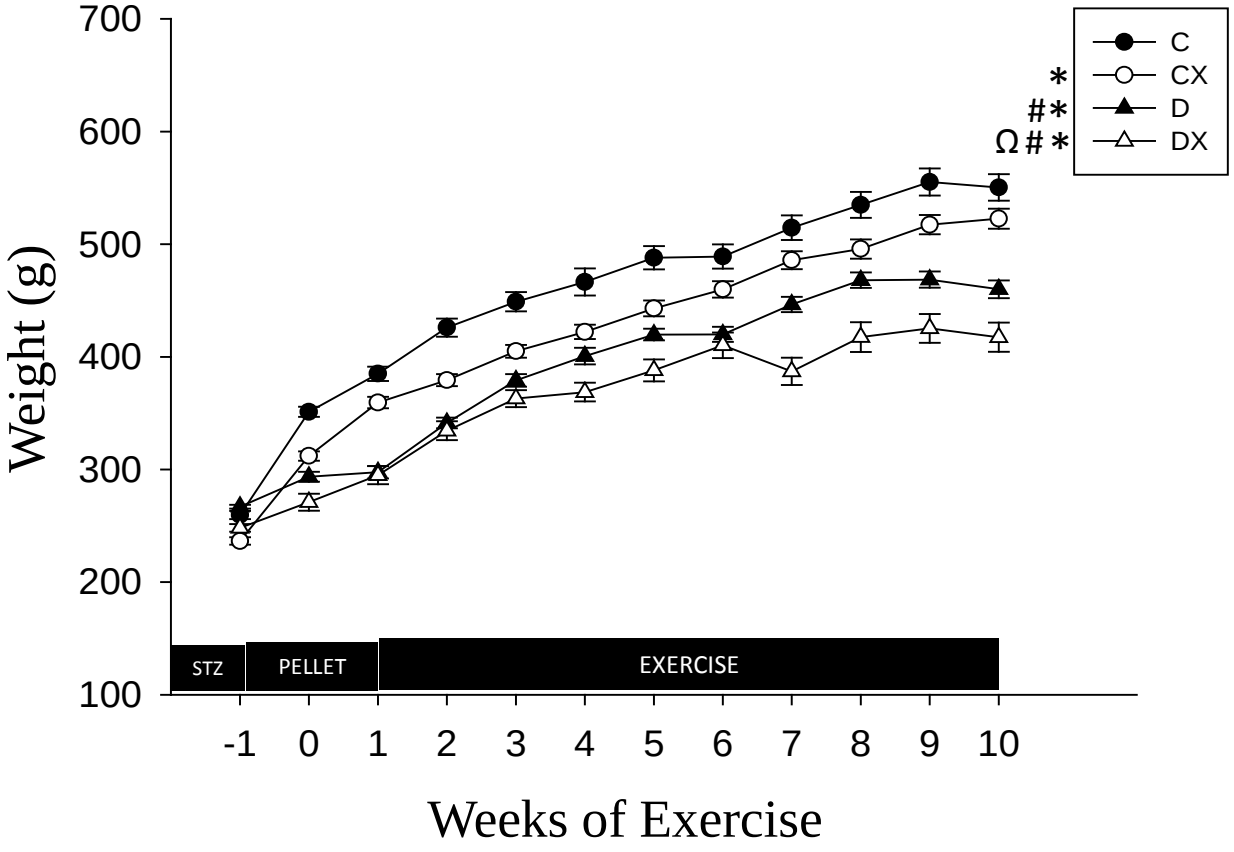

Figure 2.2. Weekly body weights. Data are mean ± SE. C, sedentary control (n=16); CX, control exercise (n=16); D, sedentary diabetic (n=15); DX, diabetic exercise (n=12). * = significantly different from C ($p<0.05$). # = significantly different from CX ($p<0.05$). Ω = significantly different from D ($p<0.05$). There is a statistically significant difference between diabetic and non-diabetic conditions ($p<0.001$). There is a statistically significant difference between exercise and sedentary conditions ($p<0.05$).

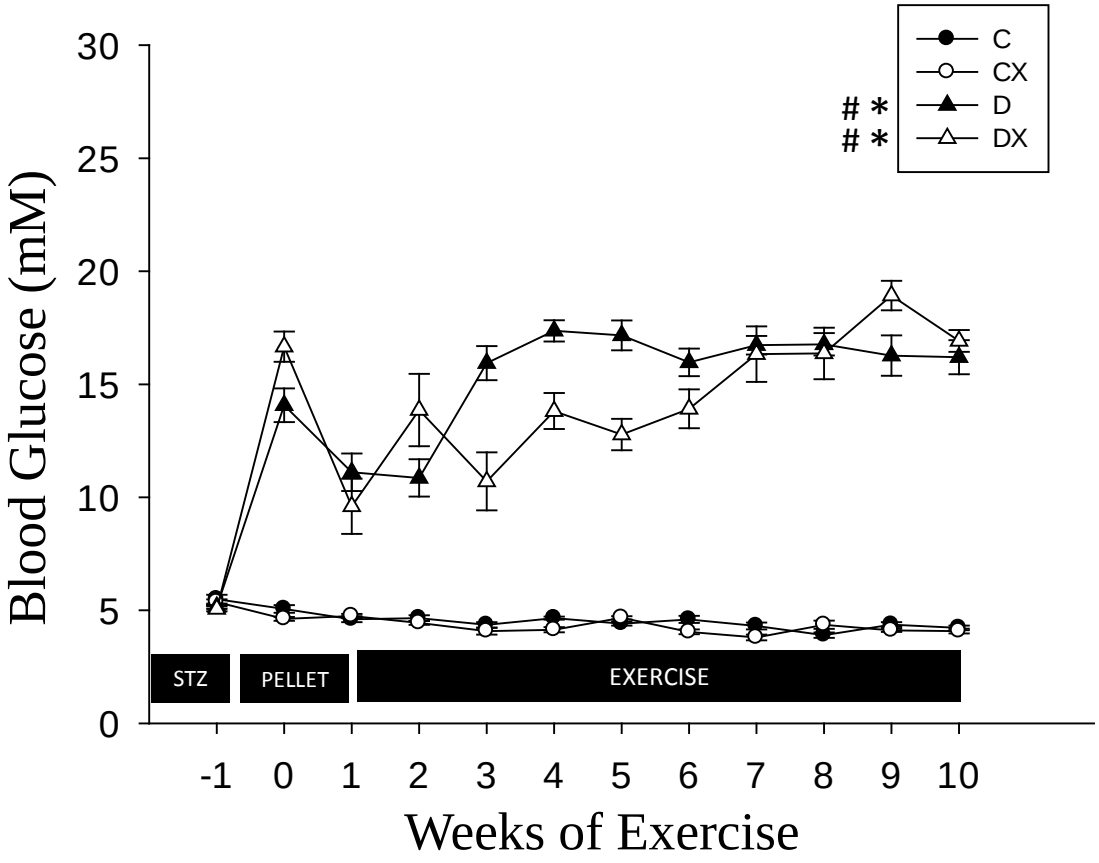

Figure 2.3. Weekly blood glucose concentrations. Data are mean ± SE. C, sedentary control (n=16); CX, control exercise (n=15); D, sedentary diabetic (n=15); DX, diabetic exercise (n=13). * = significantly different from C ($p<0.05$). # = significantly different from CX ($p<0.05$). There is a significant difference between diabetic and non-diabetic conditions ($p<0.001$).

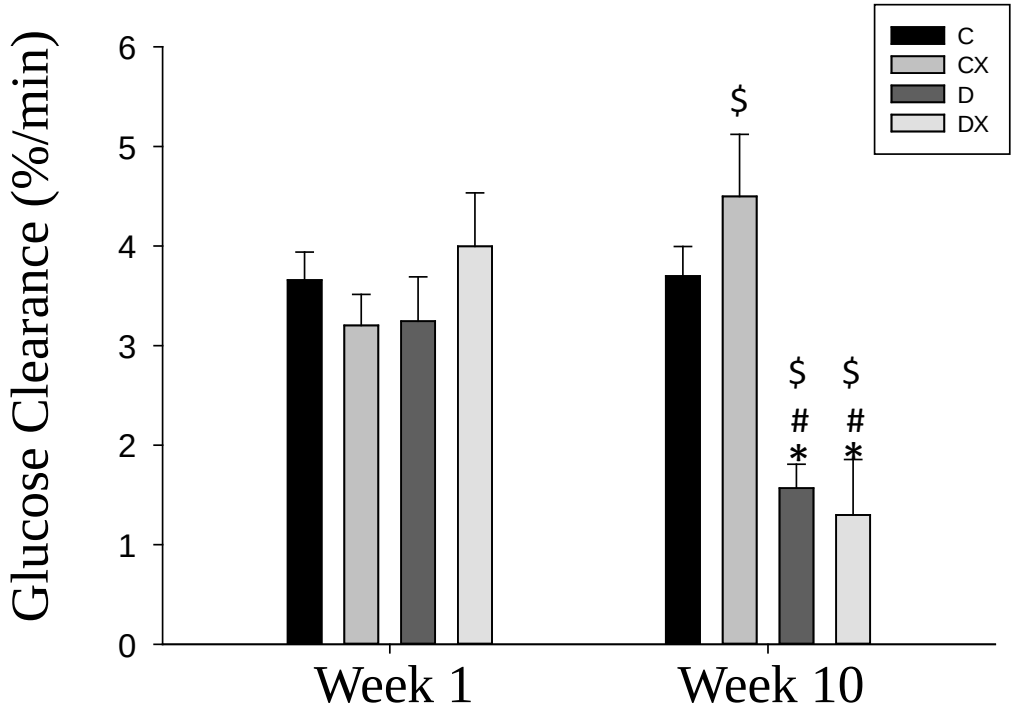

Figure 2.4. IVGTT glucose clearance (K_G) values at week 1 and week 10. Data are mean ± SE. C, sedentary control (n=16); CX, control exercise (n=15); D, sedentary diabetic (n=16); DX, diabetic exercise (n=8). * = significantly different from C ($p<0.05$). # = significantly different from CX ($p<0.05$). $ = significantly different from Week 1 ($p<0.05$). There is a statistically significant difference between diabetic and non-diabetic conditions at week 10 ($p<0.001$).

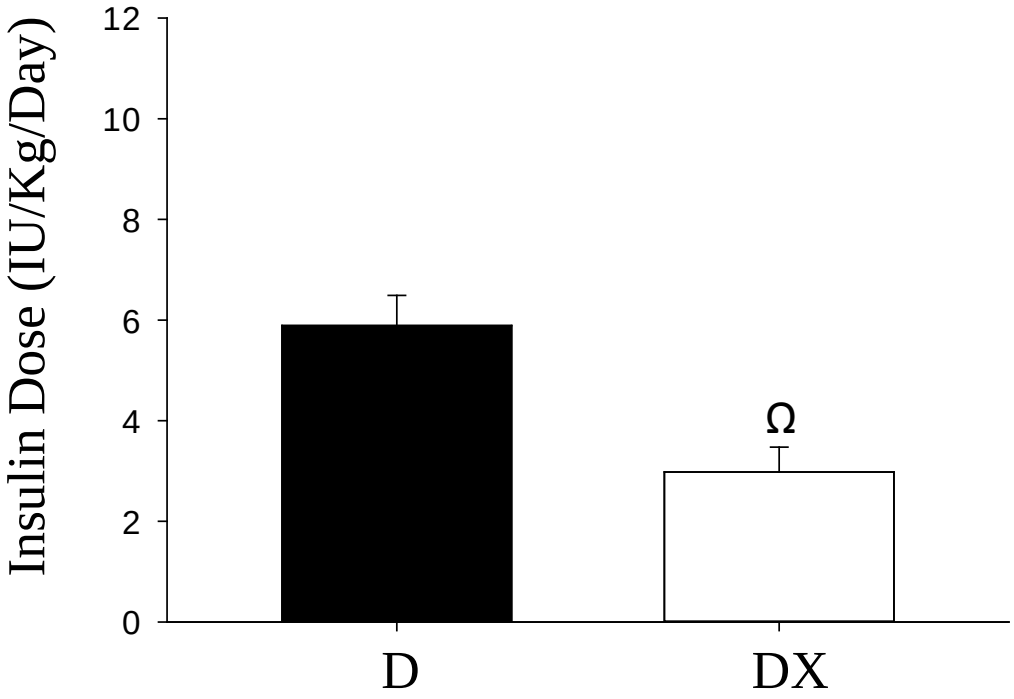

Figure 2.5. Insulin dosages at Week 10. Data are mean ± SE. D, sedentary diabetic (n=16); DX, diabetic exercise (n=18). Ω = significantly different from D ($p<0.001$).

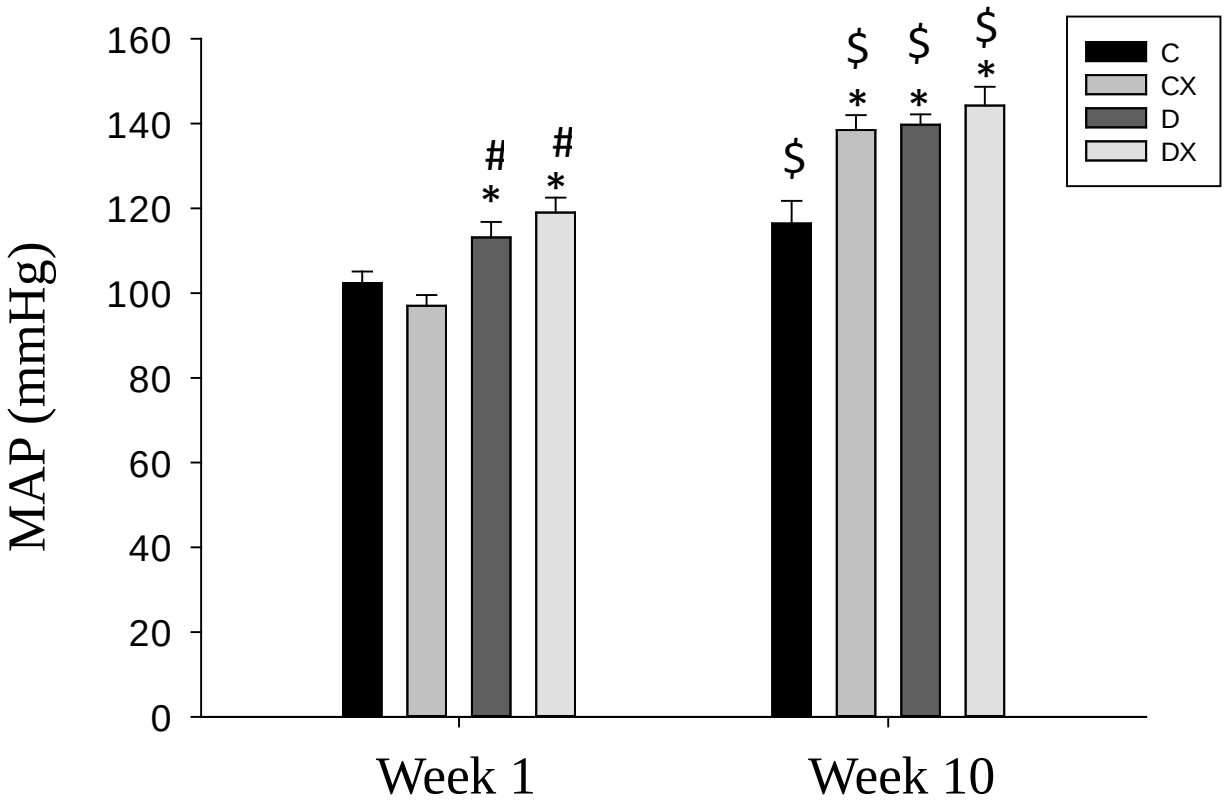

Figure 2.6. NIBP values at week 1 and week 10. Data are mean ± SE. C, sedentary control (n=15); CX, control exercise (n=16); D, sedentary diabetic (n=14); DX, diabetic exercise (n=11). * = significantly different from C ($p<0.05$). # = significantly different from CX ($p<0.05$). $ = significantly different from Week 1 ($p<0.05$). There is a statistically significant interaction between diabetes and exercise at week 10 ($p<0.05$).

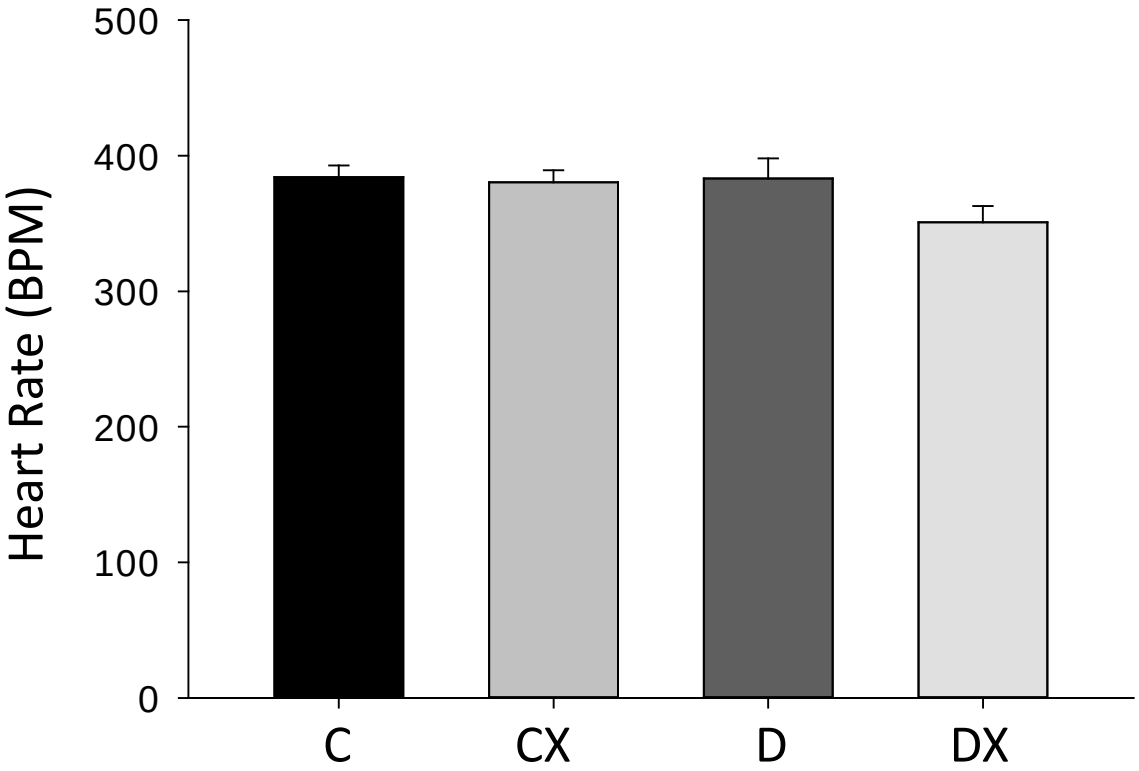

Figure 2.7. Heart Rate (beats per minute) at Week 10. Data are mean ± SE. C, sedentary control (n=7); CX, control exercise (n=7); D, sedentary diabetic (n=8); DX, diabetic exercise (n=11). There is not a statistically significant difference between groups (p=0.122).

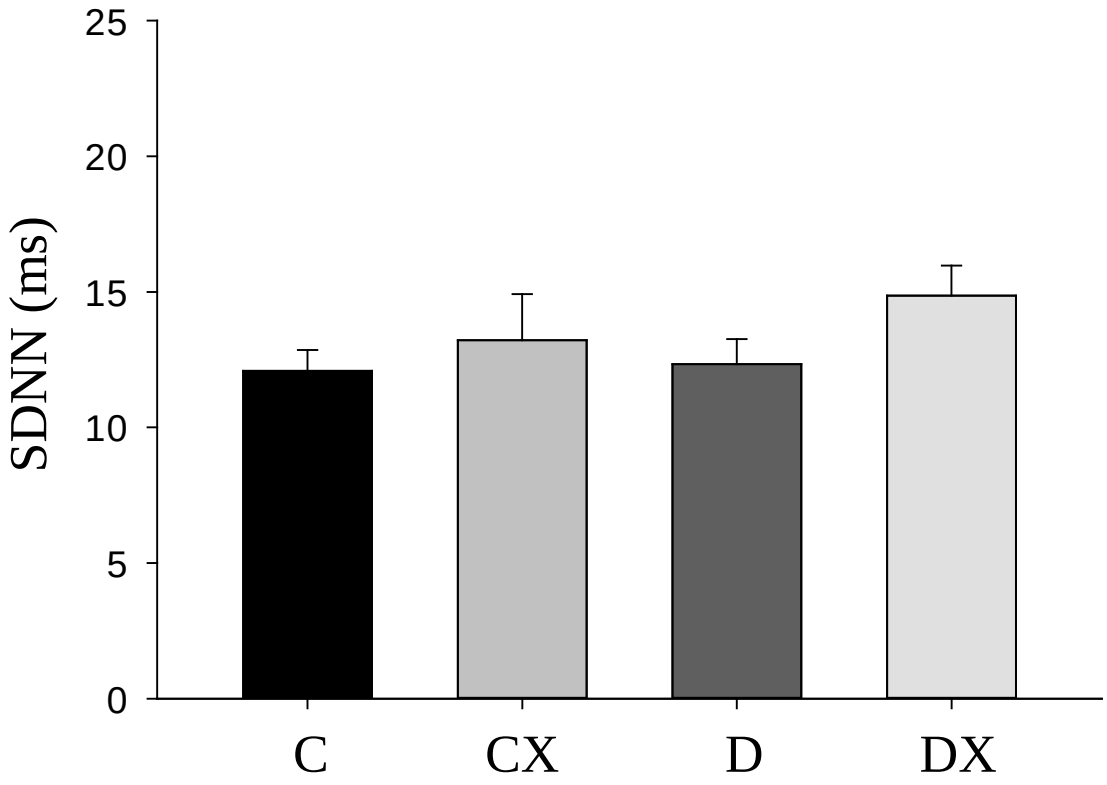

Figure 2.8. Total HRV (SDNN) at Week 10. Data are mean ± SE. C, sedentary control (n=5); CX, control exercise (n=7); D, sedentary diabetic (n=8); DX, diabetic exercise (n=8). There is not a statistically significant difference between groups (p=0.369).

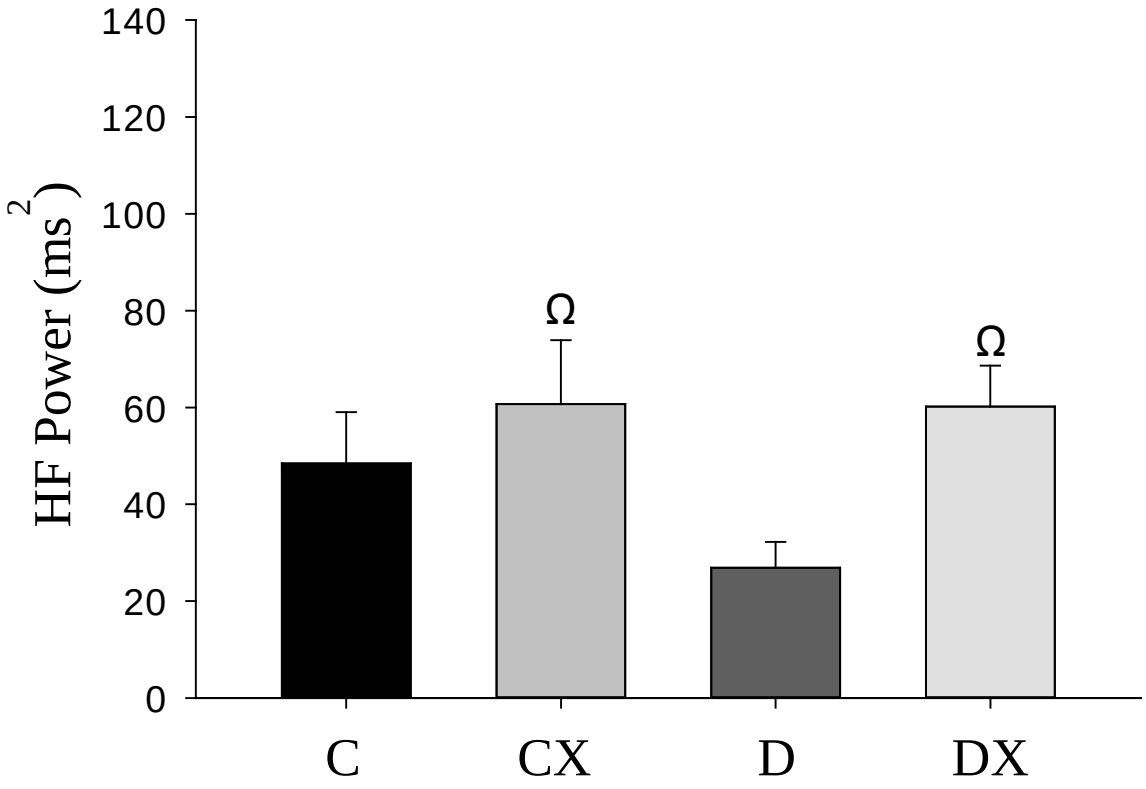

Figure 2.9. High Frequency (parasympathetic) HRV component at Week 10. Data are mean ± SE. C, sedentary control (n=6); CX, control exercise (n=7); D, sedentary diabetic (n=8); DX, diabetic exercise (n=8). Ω = significantly different from D ($p<0.05$). There was a statistically significant difference between exercise and sedentary conditions ($p<0.05$).

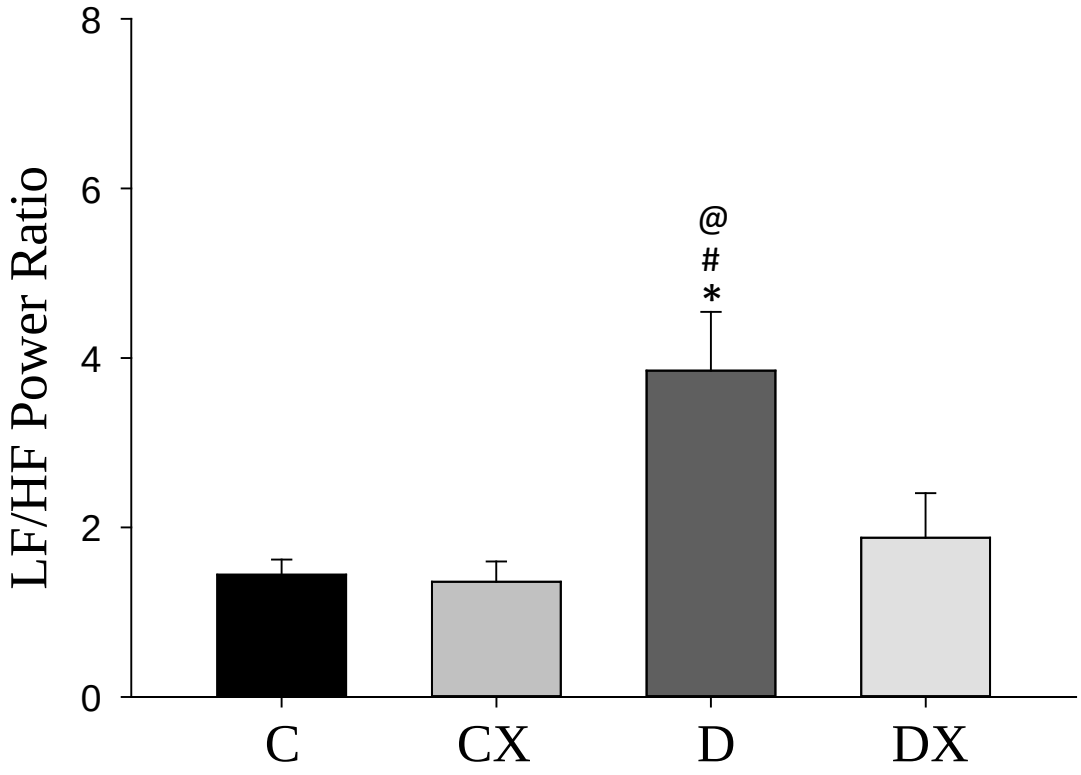

Figure 2.10. Low frequency to high frequency ratio (sympathetic) HRV component at Week 10. Data are mean ± SE. C, sedentary control (n=6); CX, control exercise (n=6); D, sedentary diabetic (n=7); DX, diabetic exercise (n=7). * = significantly different from C ($p<0.05$). # = significantly different from CX ($p<0.05$). @ = significantly different from DX ($p<0.05$). There was a statistically significant difference between diabetic and non-diabetic and exercise and sedentary conditions ($p<0.05$).

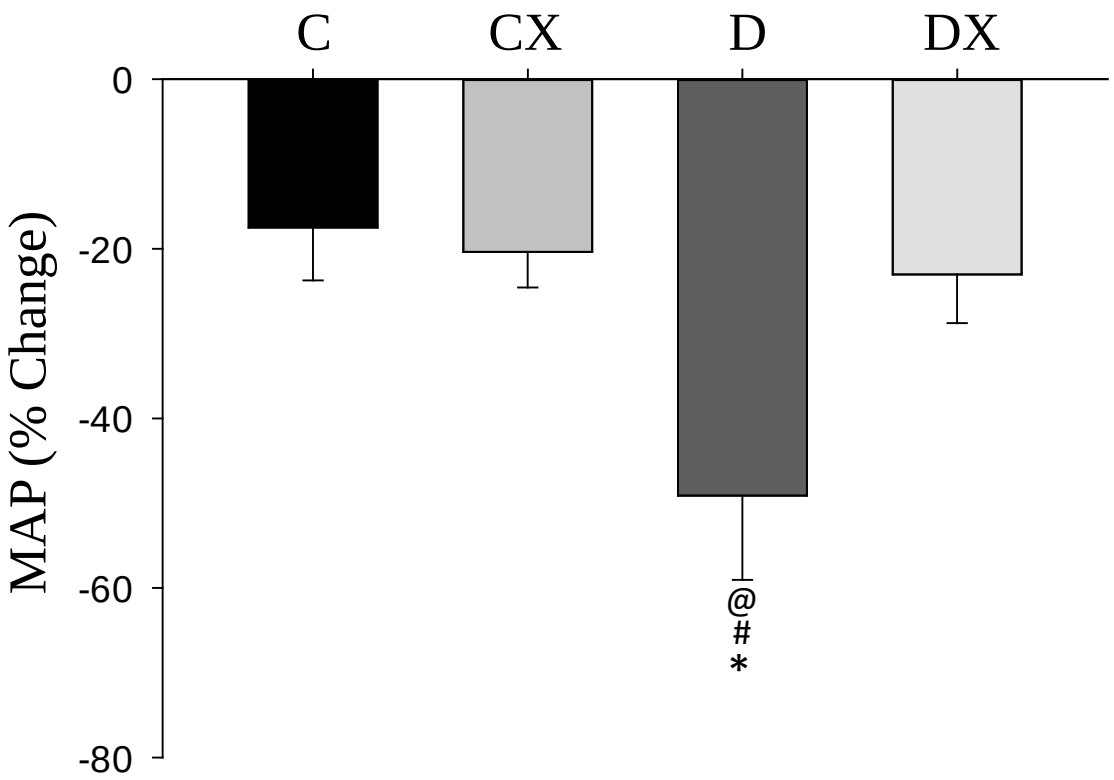

Figure 2.11. Vascular sympathetic tone at week 10. This was determined by measuring the percent change in MAP after prazosin treatment at Week 10. Data are mean ± SE. C, sedentary control (n=8); CX, control exercise (n=7); D, sedentary diabetic (n=5); DX, diabetic exercise (n=7). * = significantly different from C ($p<0.05$). # = significantly different from CX ($p<0.05$). @ = significantly different from DX ($p<0.05$). There was a statistically significant interaction between diabetes and exercise ($p<0.05$).

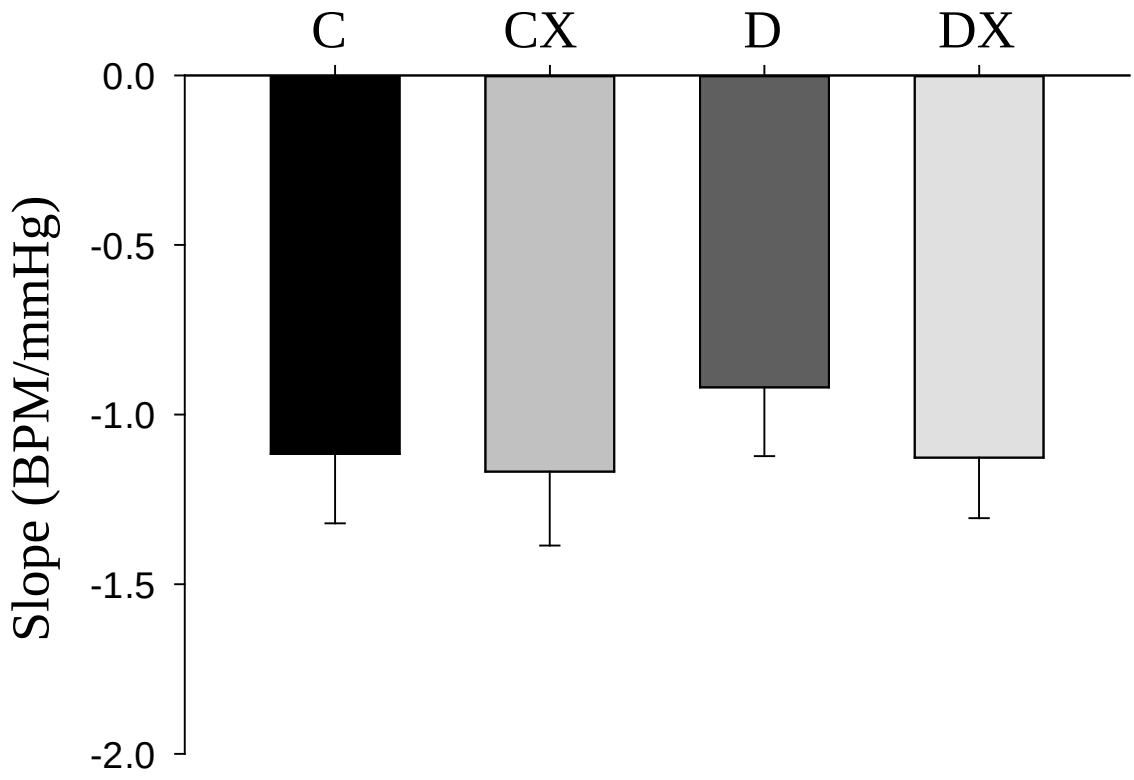

Figure 2.12. Tachycardia baroreflex response sensitivity to sodium nitroprusside at Week 10. Data are mean ± SE. C, sedentary control (n=7); CX, control exercise (n=6); D, sedentary diabetic (n=6); DX, diabetic exercise (n=10). There is not a statistically significant difference between groups (p=0.86).

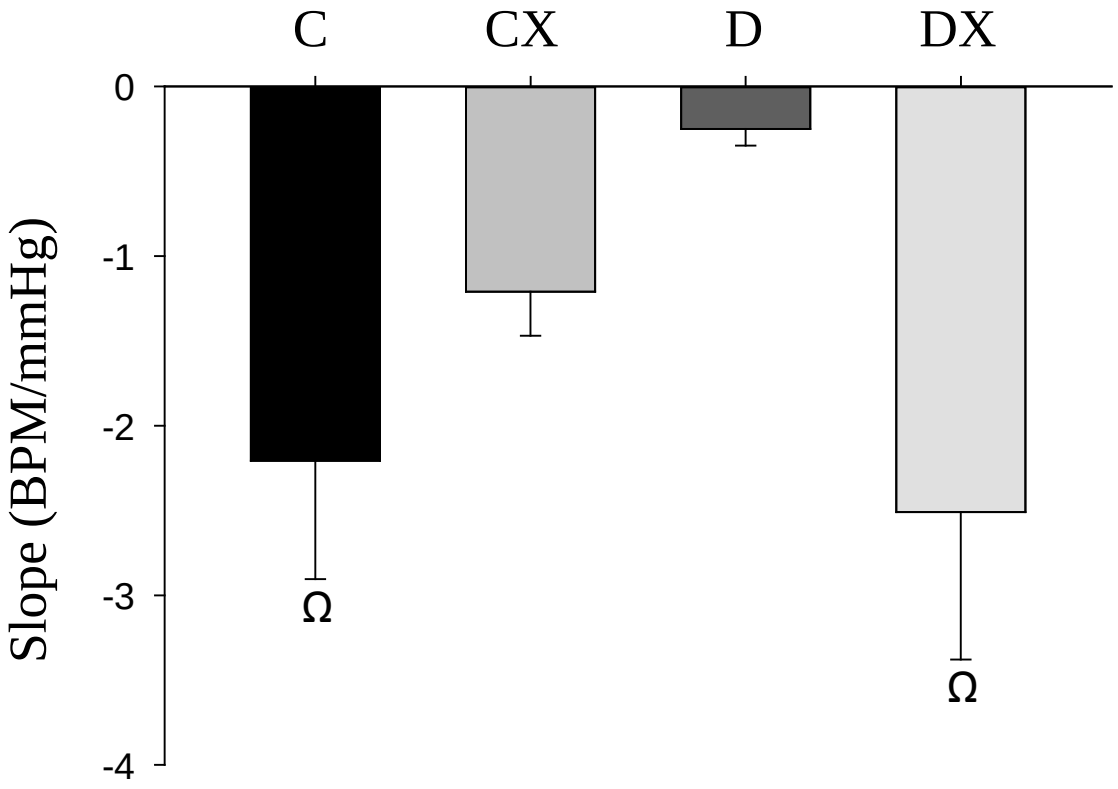

Figure 2.13. Bradycardia baroreflex response to phenylephrine at Week 10. Data are mean ± SE. C, sedentary control (n=7); CX, control exercise (n=5); D, sedentary diabetic (n=7); DX, diabetic exercise (n=10). Ω = significantly different from D ($p<0.05$). There was a statistically significant interaction between diabetes and exercise ($p<0.05$).

2.4 Discussion

This study demonstrated that a multiple low-dose STZ model with moderate hyperglycemia, maintained using insulin therapy, produced deficits in cardiovascular autonomic function without inducing the resting bradycardia or hypotension typical of other STZ models. Furthermore, this study showed that exercise training can prevent the alteration of cardiovascular autonomic function caused by diabetes in this model.

Although time domain analysis of total HRV, as measured by the SDNN, did not demonstrate differences between groups, frequency domain analysis of the distinct contributions of the autonomic branches to HRV did reveal significant differences. For instance, there was a significant difference in the HF power between the D group and both exercise groups. Furthermore, the LF/HF ratio was significantly higher in the D group compared to all other groups. Since the HF power corresponds to the level of vagally-mediated (parasympathetic) HRV and the LF/HF ratio is a measure of sympathovagal balance, these results indicate that there was significantly lower parasympathetically-mediated HRV in the D group compared to the exercise groups as well as an overall shift toward greater sympathetic activity in the sympathovagal balance of the D group (75, 103). Thus, exercise training prevented the dysregulation of the sympathovagal balance caused by diabetes. These findings are similar to those of other experiments of both clinical and experimental diabetes. A study by Zoppini et al. (103) demonstrated an increase in HF power and a decrease in the LF/HF ratio but no change in total HRV, after 6 months of exercise in T2DM patients. Mostarda et al. (61) found that STZ-induced diabetes reduced the HF component of HRV which was improved by exercise. Also comparable to the present study, they found that the vagal tonus of the control exercised rats did not differ from sedentary controls (61). Chen et al. (11) reported that children with type 1 diabetes

who performed high physical activity did not differ from controls in HRV; however, they did find that children with type 1 diabetes who had low physical activity had significantly reduced HRV compared to both active T1DM and control children. Indeed, most studies report that diabetes decreases HRV even from control levels, yet it is important to note that the timing of HRV decline is variable (2). Schaan et al. (79) measured HRV at 5-7, 14, 30, 45 and 90 days post-STZ-induction of diabetes in rats and found that there were no significant differences from control in HRV until the 30 or 45 day time points. Moreover, in this STZ model no insulin therapy was used, which could indicate that significant decreases in HRV in the present study would have taken even longer due to the reduced hyperglycemic insult. Indeed, the authors reported that there was a negative correlation between plasma glucose levels and reductions in HRV (79); an observation also noted by Chen et al. (10) in a study of children with T1DM, in which they found that glycemic control had a greater negative correlation with HRV than did disease duration. However, regardless of severity or duration of hyperglycemia, it has been demonstrated that exercise is an effective means to improve HRV as long as pre-existing CAN is not too severe (36, 75).

In fact, the current study provides evidence that the ability of exercise to ameliorate cardiovascular autonomic dysfunction may be independent from its ability to reduce blood glucose, which contradicts such an inference suggested by previous studies (13, 87, 98). The IVGTT performed at the end of the 10 week exercise period demonstrated an increased glucose clearance (K_G) in the CX group compared to week 1 of training, which indicates there was an increase in glucose tolerance in the exercise control group. In both diabetic groups there was a decrease in K_G to nearly the same level that was significantly different from their week 1 values and the week 10 values of the C and CX groups. While this would normally indicate that both of the

diabetic groups had equally impaired glucose tolerance, it was also the case that the DX group received approximately half of the dosage of exogenous insulin than the D group received. If this entails that the area under the curve for insulin over a given time during IVGTT would have been greater in the diabetic group, then with the K_G being equal, this would indicate there was a greater insulin sensitivity in the diabetic exercise group (29, 92). Together, these IVGTT results indicate improved glucose metabolism and insulin sensitivity as a result of exercise training (87). However, since the BG concentration in this study was held in a relatively constant range, any exercise-induced improvements of CV function that resulted from improved glucose metabolism would not have been mediated through an insulin sensitivity-induced reduction in systemic blood glucose but could still be the result of tissue-specific improvements in glucose utilization (6, 66). This should be borne in mind when considering the effects of diabetes and exercise on BRS.

The sensitivity or "gain" of baroreflex-mediated tachycardia and bradycardia were examined in this study by measuring the slope of the regression line of the ΔHR/ΔSBP in response to the vasodilator SNP and the vasoconstrictor PE, respectively. The slope of the tachycardia response was not significantly different between any of the groups. The slope of the bradycardia response was not significantly different from C in either exercise group; nor was there a significant difference between the exercise groups. However, the slope of the bradycardia response in the D group was significantly reduced compared to the C and DX groups. The tachycardia elicited by SNP is mediated by baroreflex-induced disinhibition of the RVLM, resulting in sympathoexcitation (28, 72). Thus, neither diabetes nor exercise affected the function of the sympathetic arm of the baroreflex. Conversely, the bradycardia induced by PE is mediated by baroreflex-induced inhibition of the

RVLM, resulting in depressed sympathetic activity and increased parasympathetic activity (28, 72). Therefore, diabetes reduced the function of the parasympathetic arm of the baroreflex, a decline which was prevented by exercise. These results are directly in line with a study by Jorge et al. (40) that investigated BRS after 30 days of STZ-diabetes, with and without aerobic exercise during the final 5 days. They found that neither diabetes nor exercise had any effect on the tachycardia reflex to SNP. Further, they found that control exercised rats did not differ from the control group before or after exercise training in either response. However, similar to the current study, they found that diabetes decreased the bradycardia response to PE and that this decrease was ameliorated by exercise (40). This indicates that the acute effect of exercise in a more severe, yet shorter duration, model were similar to the outcomes of the model employed in this experiment, giving credence to the postulation that the multiple low-dose STZ, insulin supplementation approach reduces the speed of neuropathic progression. However, it should be noted that Maeda et al. (55) observed a decreased tachycardia response and unchanged bradycardia response after only 5 days post-STZ treatment. This suggested that in the very short-term, there may be some neural toxicity of STZ directly on the SNS (24, 82).

An explanation of why short-term exercise in the study by Jorge et al. (40) appears to have been as effective as endurance training in the current study may be explained by the findings of Bernardi et al. (3), who elucidated the importance of tissue oxygenation in T1DM. They demonstrated that blunted parasympathetic BRS in T1DM patients was improved by both oxygen supplementation and deep breathing to the same degree, which implicated the increased respiration and oxygen delivery resultant of exercise could have been mediating increases in BRS (3). This led the authors to suggest hypoxia in T1DM functionally restrains parasympathetic activity

(3). Most of the studies discussed thus far, including this current experiment, are all in line with the observation that parasympathetic dysfunction precedes sympathetic dysfunction (38). However, reduced BRS can be attributed to defects in the baroreceptors, baroreceptor afferent nerves, CNS structures or efferent fibres of the baroreflex circuit (40, 51, 78). The observation in the present study that the tachycardia response of the baroreflex was unimpaired by diabetes, while the bradycardia response was, suggests that the afferent arm and central regulators of the baroreflex were not dysfunctioning and that the observed decrement of baroreflex bradycardia was due to efferent parasympathetic neuropathy (16, 78). Since the maintenance of the tachycardia response to SNP indicates the functionality of the baroreceptors in response to stretch was unhindered, it can also be inferred that arterial stiffening was not a likely cause for the decreased bradycardia response (77). It has been reported that arterial stiffening results in reduced arterial compliance, which limits the magnitude of arterial stretch and thereby, the magnitude of the baroreceptor firing (58). In fact, arterial stiffness has even been associated with decreased HRV in type 1 diabetics (39). However, Ruiz et al. (77) reported that neuropathy is a greater determinant of BRS than carotid artery elasticity in diabetes. Further, Komine et al. (45) demonstrated the effects of exercise on BRS are mediated through neural alterations, as opposed to changes in vessel wall compliance of the carotid artery. Thus, the alteration in BRS of the bradycardia response in this study was most likely due to the dysfunction of parasympathetic efferents, which was prevented via exercise. This is corroborated by the VST observed in this study, which was measured through the changes in MAP in response to prazosin treatment.

Prazosin injection resulted in a decrease in MAP in all groups. This decrease in MAP, relative to baseline, was not significantly different between the C, CX and

DX groups. Conversely, the drop in MAP from baseline in the D group was significant compared to all other groups; the reduction being approximately twice as large. Prazosin is an α1-adrenergic receptor antagonist that blocks the sympathetic contribution to peripheral resistance at the level of the vascular smooth muscle (56, 71). Thus, a larger reduction in blood pressure in response to prazosin treatment indicates a greater sympathetic tone in the maintenance of the baseline MAP (17, 73). Therefore, the significantly greater decrease in the MAP of the D group in this study indicates that diabetes resulted in a significant elevation of vascular sympathetic tone, which was prevented by exercise training. Martinez-Nieves and Dunbar (56) reported a similar finding that male diabetic rats had a greater decrease in MAP after a bolus injection of prazosin compared to their control cohorts. They postulated that an elevated prazosin response could be the result of increased α1-adrenergic receptor sensitivity (56). However, the finding that treatment with PE, an α1-adrenergic receptor agonist, did not have a greater impact in the diabetic group makes this an unlikely explanation in this experiment (26). The result of prazosin treatment is in concordance with both the HRV and BRS results in this study. HRV demonstrated a similar shift toward sympathetic activity in the sympathovagal balance, as determined by the LF/HF ratio. The BRS tests demonstrated a significant reduction in parasympathetic modulation of the HR. In conjunction, these findings point toward a diabetes-induced reduction in parasympathetic function, the consequent loss of PSNS-mediated sympathetic inhibition and resultant sympathetic overactivity (95, 97). This may explain the outcome of the NIBP measurement in this study.

The NIBP measurement of MAP in conscious rats showed a significant increase in blood pressure at week 10 compared to week 1in all groups. At week 1, the D and DX groups both had higher BP than the C and CX groups. At week 10, D,

DX and CX all had similarly elevated blood pressures compared to C. The elevation of BP in the diabetic groups at week 1 was likely resultant of their recent, acute elevation in BG in response to STZ treatment, as acute elevations in BG have been shown to increase BP in both normal and type 1 diabetic individuals, as well as animals (4, 8, 22, 49). It is also possible that BP was elevated due to the osmotic pressure caused by hyperglycemia; however, pre-treatment with the free radical scavenger, glutathione, has been shown to prevent any acute hemodynamic changes associated with hyperglycemia, suggesting its effects are more likely mediated by the impairment of endothelial-derived relaxing factors, such as NO (22, 65). Also, stress could have played a role in elevating the BPs of the diabetic groups at week 1. Restraint alone has been observed to dramatically increase heat shock protein expression in both the adrenal cortex and the vasculature (93). In fact, the stress of training is probably why both exercise groups had BP values similar to the D group at week 10. Moraska et al. (60) showed that 8 weeks of forced treadmill training caused adrenal hypertrophy, decreased corticosterone-binding globulin, suppressed lymphocyte proliferation and other factors indicative of chronic stress. Likewise, Brown et al. (7) reported elevated heat shock proteins, serum corticosterone and decreased spleen weights after up to 10 days of treadmill training. Forced treadmill exercise has also been demonstrated to mediate increases in BP and HR that exceed the values of voluntary wheel running rats, which is indicative of a stress response (60, 101). This is particularly relevant in the present study as the NIBP measurements were taken during the week. Therefore, both the chronic stress of long-term training and acute effects of forced training could have contributed to elevated BP in the exercise groups. The D group, however, demonstrated increased BP without exposure to acute or chronic training stress, suggesting their BP values were most likely the

result of chronic diabetes. As a result of the previous findings that indicated sympathetic overactivity in the D group and the strong association between sympathetic tone and BP, it is plausible that the observed hypertension is driven by sympathetic overactivity in this model (73). However, urine albumin excretion was not measured in this study, so it is not possible to rule out that diabetic nephropathy contributed to the increased BP (88). Unfortunately, HR extrapolations from the NIBP recordings were sporadic and deemed unreliable. However, while at rest under urethane/α-chloralose anesthesia, there was no significant difference between any of the groups in HR. Although it is unknown if non-invasive recording would have demonstrated diabetes-induced tachycardia, this observation still demonstrates that the commonly reported decrease in HR in STZ diabetes was prevented in the present model (91).

2.5 Conclusion

Cardiovascular autonomic neuropathy is a common form of autonomic dysfunction in T1DM that is highly associated with mortality (80). HRV and BRS are common indices of CAN that have been observed to improve with exercise training, highlighting why exercise is such an important nonpharmcological intervention in the prevention of T1DM complications. However, experimental models of T1DM often demonstrate fundamental differences from clinical T1DM in the nature and progression of alterations to CV parameters in response to diabetes and exercise (33, 51). The objective of this study was to explore the potential of a multiple low-dose STZ model of T1DM, paired with insulin therapy to maintain moderate hyperglycemia, as a means to more closely represent the conditions of human T1DM.

In this study it was found that the only measures that differed from C in the CX group were the IVGTT and NIBP. Thus, training did result in metabolic improvements, as indicated by increased glucose tolerance; however, exercise did not appear to elicit any advantages in HRV, BRS or BP over the non-diabetic sedentary state. This suggests that the chronic stress of forced treadmill training may have prevented many of the benefits normally conferred by exercise to the CX group (7, 60, 101). In the D group, a blunted baroreflex bradycardia response, a shift in LF/HF toward sympathetic activity and a significant drop in BP in response to prazosin compared to C indicated diabetes-induced parasympathetic withdrawal and sympathetic overactivity (28, 75, 103). This was supported by the significantly higher NIBP compared to C. Similarly to CX, the only differences between DX and C in this study were in IVGTT and NIBP. However, unlike CX, this implies that exercise training resulted in significant adaptations. Indeed, exercise training in the DX group was able to prevent many of the defects listed above that occurred in the D group as a result of diabetes. Specifically, exercise prevented the sympathovagal imbalance in the modulation of HRV, prevented the overactivity of the vascular sympathetic tone and maintained the sensitivity of the parasympathetically-mediated baroreflex bradycardia. Therefore, the present study demonstrated that STZ models of T1DM can be augmented to more accurately reflect clinical T1DM by implementing progressive induction and insulin treatment. Further, this study reinforces the importance and effectiveness of exercise as a means to prevent the progression of cardiovascular autonomic neuropathy.

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APPENDIX A

A1. Streptozotocin Induction Protocol (Rat)

REVISION DATE: JUNE 13, 2012

PURPOSE:
To induce Type I diabetes in rats

MATERIALS:
Gloves
Lab Coat
Streptozotocin (STZ)
5X Stock Citric Acid/Citrate Buffer
- Anhydrous Citric Acid
- Sodium Citrate Dihydrate
- MilliQ Deionized Water

13M HCl
3 Falcon Tube
Sterile Filter

EQUIPMENT:
Biological Safety Cabinet
Weigh Scale
pH Meter

PROCEDURE:
Preparing 5X Citric Acid/Citrate Buffer

1. For a pH 4.6 buffer at 765 mM (5X stock solution), in a beaker, Add
   i. 13.8g Anhydrous Citric Acid (Sigma) or 15.1g Citric Acid Monohydrate
   ii. 23.8g Sodium Citrate Dihydrate (Sigma)
   Mix into iii. 175mL of MilliQ water
   The pH should be at 4.6, Add HCl or NaOH to adjust (do not over-shoot pH)
2. Once the proper pH is obtained, add MilliQ water until you are close to the 200 ml mark (pH will move slightly). If satisfied with the pH, adjust volume in a 250 ml graduated cylinder and filter in a 0.2µm filter.

3. Store at room temperature. This is your 5X stock solution.

Making up Streptozotocin (STZ) for Injection

**NOTE Animals should be pre-weighed prior to making up STZ to ensure accurate amounts of STZ to be prepared.

1. Using pre-made buffer, put 1 mL of buffer in a 50 mL Falcon Tube and add 4 mL of distilled water filtered through a 0.2µm syringe filter. Check the pH. This gives you a working concentration of 153 mM

2. The desired pH is between 4.5-4.7. Under the fume hood, add 1 drop at a time of concentrated HCl to the buffer, checking pH in between until desired pH is reached.

3. Once pH is reached, add 1 mL distilled water (sterile filtered through a 0.2µm syringe filter as before). If pH is below 4.5, restart.

4. Weigh out an appropriate amount of STZ for the number of animals (see calculations below) that will be injected in a 15 minute time frame.

   Ex. Rats will be injected at 20mg/kg, so for 10 animals at an ideal weight of 200g (avg. weight of rats to be injected), you will require a minimum of 40mg.

   20mg/kg X 0.2kg = 4mg per animal

   The amount of STZ weighed out should be more than the minimum as some solution will be lost in filtering. (4mg (per animal) X 12 rats = 48mg total (0.048g)

5. Dissolve the STZ into buffer (keeping in mind a comfortable injection volume). Shake to dissolve powder (approx. 1min). Sterile filter using a 0.2µm syringe filter.

   Ex. 48mg STZ ÷ 3 mL buffer = 16mg/mL solution

   4mg ÷ 16mg/mL solution = 0.25mL

6. STZ is time dependent and must be used within 15 minutes

Injecting and Follow-Up of the Animals

1. Promptly inject each rat with the solution (intraperitoneal) at a dosage rate of 20mg/mL (in this example, 0.25mL). Do not use anymore STZ solution more than 15 minutes after it has been dissolved in the sodium citrate buffer.

2. Dispose of any container having come into contact with the STZ (in either powder or dissolved form) into a biohazardous waste receptacle. Dispose of needles into a sharps container.

3. Return injected rats to their cage. Record the date of STZ injection and add a biohazard label to the cage (leave biohazard label on cage for at least 3 days following the last injection).

4. Repeat this procedure the following day.

5. Check blood glucose daily. Diabetes is achieved with two non-fasting blood glucose readings of >18 mmol Diabetes should be achieved after 5-8 injections (i.p. 20mg/kg).

Reference:

Low dose STZ induction protocol. Animal Models of Diabetic Complications Consortium AMDCC Protocols.2003

A2. Insulin Pellet Implantation Protocol

MATERIALS:
LinShin LinPlant Insulin Pellet
Rat anesthetic - Isoflurane
Ampicillin
Sterile water
1ml syringe with 25 g needle
10% providone-iodine solution
gauze (or swab)
Tissue forceps
Scalpel handle and blades (or scissors)
Silk suture
Needle drivers

EQUIPMENT:
IsofluoraneAnaesthetic Machine
Hair clippers
Heat lamp

Special Safety:

Must don lab coat and gloves before handling rodents. Any bite or scratch that breaks the skin must be thoroughly scrubbed with soap and water (report to Occupational Health and Safety).

Procedure:

Pellet implantation (for a rat):

1. Anesthetize the animal using the isofluorane machine by placing it in the induction chamber. Set isoflurane to 4-5% with an O2 flow rate of 1L/min. Open the stopcock valve so gas reaches the chamber. Keep in chamber until the animal is unconscious.

2. Remove the animal and place its nose in the nose cone, reduce the isofluorane to 3% to maintain the plane of anesthesia.

3. Shave the area where the pellet is to be implanted.

4. Using gauze (or a swab), apply 10% providone-iodine solution to the skin, followed by 70% ethanol, to disinfect the site of insertion.

5. Hold the skin with forceps and make a subcutaneous incision.

6. Cleanse a 12g trocar with 10% providone-iodine solution and insert it through the puncture site to a depth of at least 2 cm.

7. Using forceps, briefly immerse the pellet in 10% providone-iodine solution, rinse with saline and insert into the subcutaneous region.

8. Use 1 pellet for the first 350g of body weight.

9. Pinch the skin closed after the last pellet is inserted. Place a drop of 10 % providone-iodine solution over the opening.

10. Close the incision by suturing.

11. Place the animal under a heat lamp and monitor until it recovers from anesthesia.

12. Record on the cage card that insulin pellets have been implanted.

Pellet removal:

1. Anesthetize the animal as described above for implantation.

2. Shave and palpate the area of implantation to locate pellets. Sterilize this area by applying 10% providone-iodine solution followed by 70% ethanol.

3. Using a scalpel (or scissors), make an incision through the skin superficial to the location of the pellets.

4. Using forceps, remove the pellet. Some connective tissue may need to be cut away using scissors. Discard the pellet.

5. Close the incision by suturing.

6. Place the animal under a heat lamp and monitor until it recovers from anesthesia.

7. Record on the cage card that the pellets have been removed.

References:

http://www.linshincanada.com

A3. Intravenous Glucose Tolerance Test Protocol (Rat)

MATERIALS:
15 ml Falcon tube
D-glucose
Distilled water
0.2 um syringe filter
5 ml vacutainer
Lidocaine Cream
3 ml syringe
Restraining towel
Microcentrifuge tubes
Vaseline
27 G needles
30 G needles
Gauze squares

EQUIPMENT:
Mettler balance
Biological safety cabinet
Heat lamp with 175 watt bulb
Glucometer (Freestyle mini)
Timer

Special Safety:

Must don lab coat and gloves before handling rats. Any bite or scratch that breaks the skin must be thoroughly scrubbed with soap and water (report to Occupational Health and Safety).

PROCEDURE:

Fasting Rats:

1. Fast animals for 8-12 hours prior to the tolerance test

Glucose preparation:

1. On the morning of the IVGTT, dispense 5ml distilled water into a clean 15 ml Falcon tube.

2. Weigh out 5 grams of D-glucose, and add it to the tube containing the sterile water. Dissolve by vortexing to result in a 50% glucose solution.

3. In a biological safety cabinet, sterilize the glucose solution using a 0.2 um syringe filter.

4. Transfer the sterile glucose solution to a new sterile 15mL Falcon tube.

Intravenous Glucose Tolerance Test (IVGTT):

1. Place the cage containing the rat under a heat lamp. Remove the tube from the cage, and provide water for the rat. Warm the rat in this manner for 10 minutes.

2. Remove the rat from the cage and scrub the tail using soap and water.

3. Measure and record the rat's weight.

4. Obtain a baseline blood glucose reading from saphenous vein and collect a baseline serum sample (keep blood sample on ice).

5. Return the rat to the cage and warm for an additional 20 minutes.

6. Place the rat's tail in a container of warm tap water for 30-60 seconds.

7. One person should dry the tail and restrain the rat by wrapping it in a green drape so the tail is exposed. Occlude the lateral tail vein by applying pressure.

8. Using a 3 ml syringe and a 27 G needle, a second person will draw up the 50% glucose solution at a dose of 1g/kg (2 ml/kg). Locate the lateral tail vein and draw back on the plunger to ensure that the needle is within the vein.

9. Release the occlusion while the glucose solution is injected. Draw back the plunger a couple of times during the injection to ensure that the needle remains located within the vein.

10. Start the timer and return the rat to its cage. Continue warming it for the duration of the IVGTT.

11. Check and record the blood glucose values for the rat at 5, 10, 20, 30, 40, 50 and 60 minutes post glucose challenge. If by 60 minutes, the blood glucose value hasn't returned to baseline, continue to check every 30 minutes until it does so (up to a maximum of 180 minutes).

12. Obtain a blood serum sample 10 minutes following D-glucose injection (store at -70°C until insulin analyses).

13. Upon completion of the IVGTT, remove the rat from under the heat lamp and return the tube to the cage. Place food on the wire lid of the cage, and ensure that the rat has access to water.

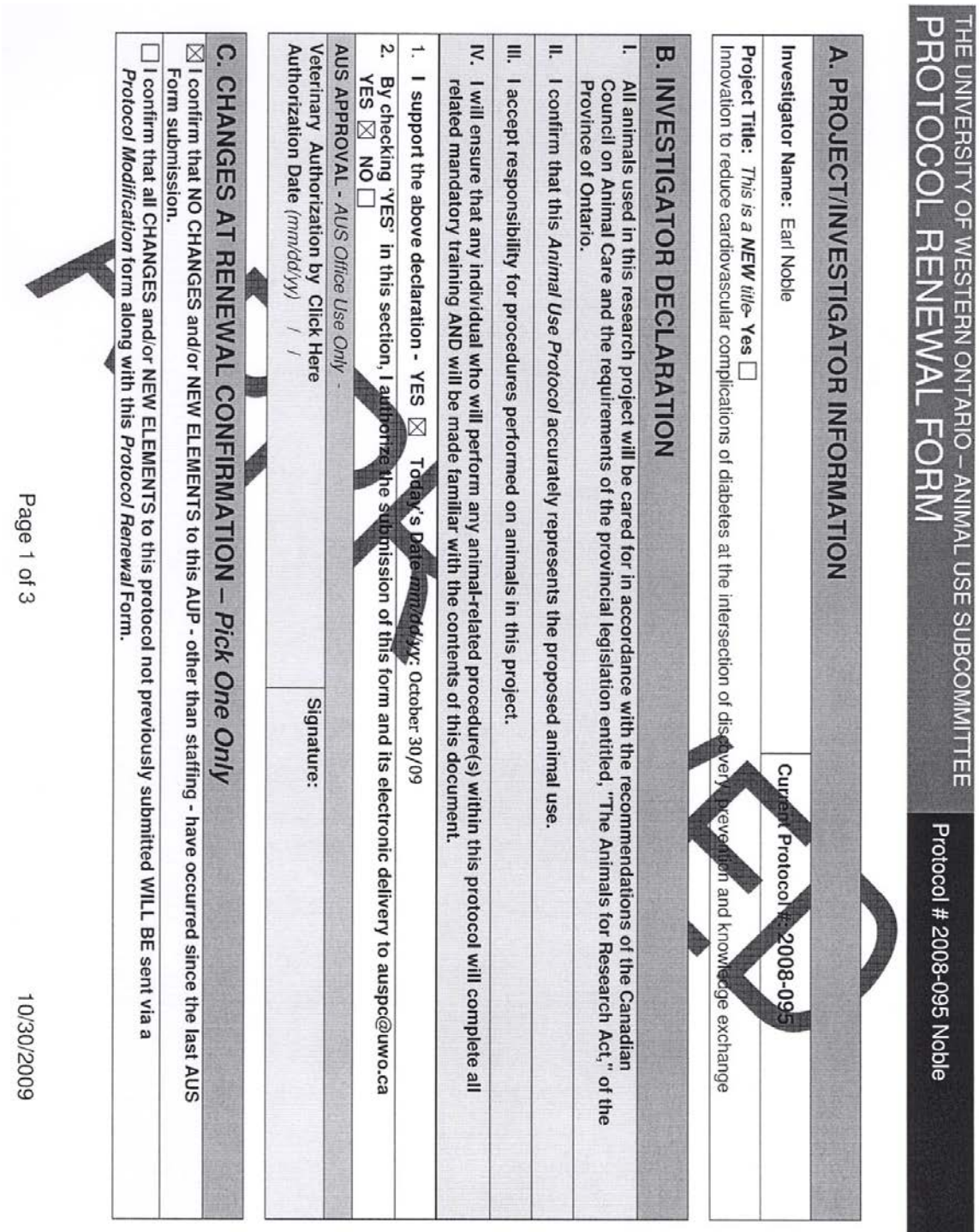
14. Note on the cage card that an IVGTT has been performed.

Reference:

Straczkowski M et al, The effect of exercise training on glucose tolerance and skeletal muscle triacylglycerol in rats fed with a high-fat diet. Diabetes and Metabolism (2001) 21 p19.

APPENDIX B

B.1 Ethics Approval



THE UNIVERSITY OF WESTERN ONTARIO – ANIMAL USE SUBCOMMITTEE
PROTOCOL RENEWAL FORM
Protocol # 2008-095 Noble

A. PROJECT/INVESTIGATOR INFORMATION

Investigator Name: Earl Noble	**Current Protocol #:** 2008-095
Project Title: *This is a* ***NEW*** *title*- **Yes** ☐ Innovation to reduce cardiovascular complications of diabetes at the intersection of discovery, prevention and knowledge exchange	

B. INVESTIGATOR DECLARATION

I. **All animals used in this research project will be cared for in accordance with the recommendations of the Canadian Council on Animal Care and the requirements of the provincial legislation entitled, "The Animals for Research Act," of the Province of Ontario.**

II. **I confirm that this *Animal Use Protocol* accurately represents the proposed animal use.**

III. **I accept responsibility for procedures performed on animals in this project.**

IV. **I will ensure that any individual who will perform any animal-related procedure(s) within this protocol will complete all related mandatory training AND will be made familiar with the contents of this document.**

1. **I support the above declaration - YES ☒** **Today's Date** *(mm/dd/yy)*: October 30/09
2. **By checking 'YES' in this section, I authorize the submission of this form and its electronic delivery to auspc@uwo.ca YES ☒ NO ☐**

AUS APPROVAL - *AUS Office Use Only* -	
Veterinary Authorization by Click Here	**Signature:**
Authorization Date *(mm/dd/yy)* / /	

C. CHANGES AT RENEWAL CONFIRMATION – *Pick One Only*

☒ **I confirm that NO CHANGES and/or NEW ELEMENTS to this AUP - other than staffing - have occurred since the last AUS Form submission.**

☐ **I confirm that all CHANGES and/or NEW ELEMENTS to this protocol not previously submitted WILL BE sent via a *Protocol Modification* form along with this *Protocol Renewal* Form.**

Page 1 of 3

10/30/2009

D. THREE R's PROGRESS REPORT

Describe any progress made with respect to the Three R's of replacement, reduction and refinement of animal use during the PAST PROTOCOL YEAR. ***See the CCAC 3 R's Microsite: http://www.ccac.ca/en/alternatives/***

We have used less animals than anticipated in the past year because we have made more use of the tissues generated by the animals that we employed.

☒ ***No additional animals were used during the past Protocol Year.***
☒ ***No additional animals were requested during the past Protocol Year.***

E. ANIMAL USE COMPLICATIONS PROGRESS REPORT

Describe any complications beyond the expected experimental outcomes and end points encountered relative to animal use – e.g. unpredicted outcomes and any unexpected animal pain, distress, or mortality - AND indicate measures being undertaken to resolve these complications for the PAST PROTOCOL YEAR.

☒ **No complications were encountered relative to animal use during the past Protocol Year.**

F. ANALGESIA ADMINISTRATION UPDATE

Choose One of the Following Statements:

1. **Analgesics were administered as outlined within the *Animal Use Protocol*** ☐ ***If checked, please list all analgesics currently used below:**

2. **Analgesics are not indicated in this *Animal Use Protocol*** ☒

G. PROTOCOL PERSONNEL UPDATE

Complete for ALL Staff Working Under This Protocol for This Renewal Year

TRAINING INFORMATION

CCAC Mandated Training Requirements – All personnel working with live animals require CCAC mandated training including the '*Basic Animal Care & Use* Web-CT *Course*' and related hands-on '*Workshops*'. Completion of the '*Basic Animal Care & Use* Web-CT Course' once every 5 years is mandatory for ALL personnel, including the Principal Investigator. The Animal Use Subcommittee will be informed of all personnel with incomplete training beyond 1 month of notice.

Workshop Enrolment Detail – The '*Workshop*' requirements are determined by the species and procedures associated with each individual

listed below. All personnel listed below will be contacted directly via the email address listed below for auto-enrolment in all '*Workshop*' requirements. Previous hands-on '*Workshops*' attended at another research institution may be accepted; please submit training documentation with this form. For additional training requirement detail and associated costs, go to http://www.uwo.ca/animal/website/VS/Content/Teaching_and_Courses.htm

COMPLETE *ALL* COLUMNS BELOW PER PERSON					If '*YES*' to HANDS-ON ANIMAL WORK, Complete This Section PER SPECIES										
FIRST NAME *Repeat For Each* ***Species*** *Used*	**LAST NAME** *Repeat For Each* ***Species*** *Used*	**ROLE** *(Role within this Protocol)* *Researcher Staff Student*	**EMAIL Address *Mandatory Field*** *UWO & Affiliated Institution Emails Preferred*	**HANDS ON Animal Work? YES** *or* **NO**	**SPECIES One Species Per Row** *Use an Additional Row for Each Species*	**Expected START DATE** *mm/dd/yy*	**PROCEDURES PER SPECIES** 1=Basic Handling 2=Health Monitoring 3=Blood Collection 4=Injections 5=Anaesthesia 6=Surgery-Recovery 7=Surgery-Non-Recovery 8=Euthanasia/Post Mortem 9=Other, *Provide Detail Below, e.g. suturing*								
							1	2	3	4	5	6	7	8	9
Earl	Noble	Researcher	enoble@uwo.ca	Yes	Rat	11/01/09	☒	☒	☒	☒	☒	☒	☒	☒	☒
Tomasz	Dzialoszynski	Staff	tdzialos@uwo.ca	Yes	Rat	11/01/09	☒	☒	☒	☒	☒	☐	☒	☒	☒
Oscar	Campos	Student	ocampos@uwo.ca	Yes	Rat	11/01/09	☒	☒	☒	☒	☒	☐	☒	☒	☐
Jordan	Silver	Student	jsilve4@uwo.ca	Yes	Rat	11/01/09	☒	☒	☒	☒	☒	☐	☒	☒	☐
Mehrbod	Estaki	Student	mestaki@uwo.ca	Yes	Rat	11/01/09	☒	☒	☒	☒	☒	☐	☒	☒	☒

PROCEDURE #9 DETAIL	**suturing after implantation of insulin pellets is necessary (EN and TD only); gavage for ginseng**	
EMERGENCY *AFTER HOURS* CONTACT NAMES & NUMBERS - NO LAB PHONE NUMBERS -	**PRIMARY** EMERGENCY CONTACT LAST NAME & INITIAL : **Noble E**	**PRIMARY** EMERGENCY CONTACT NUMBER (HOME OR CELL) : **519-472-8399**
	SECONDARY EMERGENCY CONTACT LAST NAME & INITIAL : **Dzialoszynski T**	**SECONDARY** EMERGENCY CONTACT NUMBER (HOME OR CELL) : **519-472-7371**
If applicable, provide POST-OP CARE personnel detail	☒ **Same as Emergency Contact, or Name:**	**Emergency #:** 519-472-8399 **Lab #:** 88373 **Email:** enoble@uwo.ca
If applicable, provide MONITORING personnel detail	☒ **Same as Emergency Contact, or Name:**	**Emergency #:** 519-472-8399 **Lab #:** 88373 **Email:** enoble@uwo.ca

CURRICULUM VITAE

Kenneth N. Grisé

EDUCATION

2010-Present **Master of Science Candidate, Exercise Physiology and Biochemistry**
Exercise Biochemistry Laboratory, School of Kinesiology, The University of Western Ontario
Thesis: The effects of exercise training on indices of cardiovascular autonomic neuropathy in STZ-induced type 1 diabetic rats treated with insulin
- *Expected Completion Date:* August, 2012
- *Supervisor:* Dr. C.W. James Melling

/2008-2010 **Honours Bachelor of Science, Double Major Physiology and Psychology**
2004-2007/ *Faculty of Science, The University of Western Ontario*

HONOURS/AWARDS

2011-Present Queen Elizabeth II Graduate Scholarship in Science and Technology ($15000)

2010-Present Western Graduate Research Scholarship ($8000)

2012 First Place, Western Research Forum, Health and Psychological Sciences – Oral ($125)

2011 Second Place, Western Research Forum, Social and Biosciences – Poster ($50)

RESEARCH EXPERIENCE

2010 **Summer Research Assistant**
Exercise Biochemistry Laboratory, School of Kinesiology, The University of Western Ontario
- Trained students on rat anatomy and dissection
- Surgically implanted telemeters for measurement of electroneurophysiology and blood pressure
- *Supervisors*: Dr. Earl G Noble, Dr. C.W. James Melling

2009-2010 **Work-Study Student**
Exercise Biochemistry Laboratory, School of Kinesiology, The University of Western Ontario
- Trained students on immunohistochemistry
- Performed western blot protein quantification

- Attended three-day training seminar at Michigan State University on telemeter implantation for recording of electroneurophysiology and blood pressure
- *Supervisors*: Dr. Earl G Noble, Dr. C.W. James Melling

2009 **Summer Research Assistant**
Exercise Biochemistry Laboratory, School of Kinesiology, The University of Western Ontario
- Executed 10 week, type 1 diabetes and exercise training study; performed injections, exercise training, blood glucose measurements, intravenous glucose tolerance tests, insulin pellet implantation, dissection and tissue collection
- Performed immunohistochemistry
- *Supervisors*: Dr. Earl G Noble, Dr. C.W. James Melling

2008 **Consultant**
London Health Sciences Centre, Human Islet Transplantation Program, London, Ontario
- Trained incoming intern on all aspects of the position (see below)
- Performed pancreatectomies and isolated rat islets for allogeneic transplantation within biocompatible cell delivery devices

2007-2008 **Intern**
London Health Sciences Centre, Human Islet Transplantation Program, London, Ontario
- Ordered and tracked inventory for the research lab, the clinical lab and two surgery rooms
- Prepared solutions and equipment for islet cell isolation, culture and transplantation
- Procured pancreata from pigs and rats; assisted with human pancreas procurement
- Performed surgery on mice, rats and pigs
- Isolated human, pig and rat islets; prepared for co-transplantation with sertoli cells
- On-call twenty-four hours a day, seven days a week to respond to human pancreas donation
- Assays performed: ELISA, fluorescence microscopy, islet oxygen consumption, protein quantification, intravenous glucose tolerance test

TEACHING EXPERIENCE

2010-2012 **Teaching Assistant**
Systemic Approach to Functional Anatomy (Kin 2222), Department of Kinesiology, The University of Western Ontario
- Ran independent tutorial section in anatomical model laboratory
- Held office hours for individual tutoring
- Proctored and marked exams
- *Supervisor*: Dr. C.W. James Melling

ADMINISTRATIVE EXPERIENCE

2011-Present **Vice President Student Services**
Kinesiology Graduate Board, School of Kinesiology, The University of Western Ontario

- Duties include: liaison between student body and graduate board, event coordination, budgeting, committee decision-making
- Position required nomination and election by graduate student vote

ORAL PRESENTATIONS

2012 *Comparing renal sympathetic nerve activity to sciatic neurovascular morphology in type 1 diabetic rodents*
25th Annual Western Research Forum, The University of Western Ontario, London, Ontario, Canada (March 27, 2012

2011 *The measurement of sympathetic nerve activity in rodents using telemetry*
2nd Annual Biochemistry of Exercise Conference, Collingwood, Ontario, Canada (July 14, 2011)

2010 *Physiological adaptations to exercise in STZ-induced diabetic rodents*
3rd Annual Cardiovascular Complications of Diabetes Initiative Annual Reporting and Advisory Session, CIHR-Tekes Collaboration, Helsinki, Finland (August, 26, 2010)

POSTER PRESENTATIONS

2011 **Grise, K.N.** and Melling, C.W.J. The long-term measurement of sympathetic nerve activity in diabetes using telemetry. Lawson Health Research Institute 2nd Annual Diabetes Research Day, St. Joseph's Hospital, London, Ontario (November 15, 2011)

2011 **Grise, K.N.**, Hall, K.E., McDonald, M.W., Noble, E.G., Melling, C.W.J. The *cardioprotective effects of insulin therapy and exercise in type 1 diabetic rodents.* Faculty of Health Sciences Research Day, The University of Western Ontario, London, Ontario (March 25, 2011)

2011 **Grise, K.N.**, Hall, K.E., McDonald, M.W., Noble, E.G., Melling, C.W.J. *Physiological adaptations to exercise in STZ-induced diabetic rodent.* Western Research Forum, The University of Western Ontario, London, Ontario (February 26, 2011)

2010 **Grise, K.N.**, Hall, K.E., McDonald, M.W., Noble, E.G., Melling, C.W.J. *Moderate-hyperglycemia mediated organ damage in exercise-treated STZ-induced diabetic rodents.* Lawson Health Research Institute 1st Annual Diabetes Research Day, St. Joseph's Hospital, London, Ontario (November 16, 2010)

PUBLISHED ABSTRACTS

2012 Murias, J.M., Campos, O.A., Hall, K.E., McDonald, M.W., **Grise, K.N.**, Melling, C.W.J., Noble, E.G. *High-intensity endurance training but not vitamin c changes vascular responsiveness in diabetic rats.* To be presented at American College of Sports Medicine 59th Annual Meeting, San Francisco, U.S.A., May 2012.

2012 **Grise, K.N.**, Hall, K.E., McDonald, M.W., Noble, E.G., Melling, C.W.J. *Early-stage changes to neurovascular morphology and the telemetric measurement of sympathetic nerve activity in streptozotocin-induced diabetic rats.* Presented at the Experimental Biology Annual Meeting 2012, San Diego, California, U.S.A. Published in *The FASEB Journal*. 2012;26:lb802

2012 Olver, T. D., Mattar, L.M., **Grise, K.N.**, Twynstra, J., Lacefield, J.C., Noble, E.G., Shoemaker, J.K. *Glucose infusion (3g/kg) increases nerve vascular conductance through an insulin-mediated nitric oxide mechanism in rats.* Presented at the Experimental Biology Annual Meeting 2012, San Diego, California, U.S.A. Pubished in *The FASEB Journal*. 2012;26:853.19

2011 McDonald, M. W., **Grise, K. N.**, Noble, E. G., Melling, C. W. J. *Exercise training does not attenuate the exercise mediated reduction in blood glucose levels in streptozotocin-induced rats.* Canadian Society for Exercise Physiology 2011: Exercise and Health for Everybody, Quebec City. Québec, Canada, October 2011. (Abstract published CSEP's, *Applied Physiology, Nutrition and Metabolism* (APNM) September, issue No. APNM 36(S2))

2011 Murias, J. M., Campos, O. A., Hall, K. E., McDonald, M. W., **Grise, K. N.**, Melling, C. W. J., Noble, E. G. *Short-term high intensity continuous endurance training results in a faster rate of vasorelaxation in diabetic rats.* Canadian Society for Exercise Physiology 2011: Exercise and Health for Everybody, Quebec City. Québec, Canada, October 2011. (Abstract published CSEP's, *Applied Physiology, Nutrition and Metabolism* (APNM) September, issue No. APNM 36(S2))

2009 Vilk, G.J.A., Kriz, J., Mazzuca, D.M., **Grise, K.**,Leushner, J., White, D.J.G.*Islet transplantation in pre-vascularized collagen chambers placed subcutaneously or in the omentum: a comparative study*. Joint Meeting of the International-Pancreas-and-Islet-Transplant-Association/International-Xenotransplantation-Association, Venice, Italy, October 12-16, 2009. Published in *Xenotransplantation* 16 (5): 406, 2009.

2009 Vilk, G.J.A., Kriz, J., Mazzuca, D.M., **Grise, K.**,Leushner, J., White, D.J.G.*Direct comparison of syngeneic islet survival and function*

between portal vein delivery and islets transplanted either sub-cutaneously or in the omentum using a polypropylene device. Canadian Society of Transplantation–Abstracts, 2009.

2009 Pepper, A.R., Mazzuca, D.M., Hasilo, C.P., Jakob, J., Verheyden, J., **Grise, K.**, Negrave, T., White, D.J.G. *The function of isolated islets of Langerhans can be predicted prior to transplantation by measuring oxygen consumption rates / islet index ratio*. Canadian Society of Transplantation–Abstracts, 2009.

2009 Hasilo, C.P., Melling, C.W.J., Pepper, A.R., Mazzuca, D.M., Verheyden, J., Newell-Kraus, H., Gall. C., **Grise, K.**, Sverzhinsky, A., Jakob, J., Vilk, G.J.A., White, D.J.G. *Effective retrofit cooling of a cobe 2991 cell processor enables isolation of functional human and porcine islet preparations for diabetes reversal – the London modifications.* Canadian Society of Transplantation–Abstracts, 2009.

2008 Hasilo, C.P., Vilk, G.J.A., Melling, C.W.J., Pepper, A.R., MacGillivary, A., Sverzhinsky, A., Gall, C., Siroen,D.M., **Grise, K.**, and White, D.J.G. *Enhancement of Diabetes Reversal in BalbCAthymic Nude Mice With Optimal Ratios of Adult Porcine Islets of Langerhans To Adult Porcine Sertoli Cells.* Supplement to Transplantation July 27, 86(25), 2008.

MANUSCRIPTS IN PREPARATION

2012 Melling , C.W. J., **Grise, K.N.**, Hasilo, C.P., Fier, B., Hall, K.E., Milne, K.J., Karmazyn, M. and Noble, E.G. *Cardiovascular adaptations to exercise in moderately hyperglycemic T1-diabetic rodents.* (Planned submission to *Diabetes*)

2012 Hall, K.E., McDonald, M.W., **Grisé, K.N.**, Campos, O.A., and Melling, C.W.J. *The effect of resistance and aerobic exercise on insulin receptor and GLUT4 protein content and glucose tolerance in type 1 diabetic rodents.* (Planned submission to *Journal of Applied Physiology*)

2012 Vilk, G**.**, Mazzuca, D.M., Kriz, J., Jirak, D., MacGillvray, A., Pepper, A.R., **Grise, K.**, Melling, C.W.J., Toleikis, P.M., Saudek, F, Hajek, M. and White, D.J.G. *Islet transplantation in pre-vascularized collagen pouches placed subcutaneously or in the omentum: A comparative study.* (Planned submission to *Transplant International*)