THE HEART IN TYPE 1 DIABETES: CHARACTERIZATION OF STRUCTURE, FUNCTION AND EXERCISE-INDUCED BENEFITS IN DIABETIC CARDIOMYOPATHY

By
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Submitted to the graduate degree program in Rehabilitation Science and the Graduate Faculty of the University of Kansas In partial fulfillment of the requirements for the degree of Doctor of Philosophy

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ABSTRACT

Diabetes mellitus compromises the structure and function of the cardiovascular system. We have characterized the structural and functional abnormalities of the diabetic myocardium using streptozotocin-induced (generic) and autoimmune-intolerant (specific) rat models of type 1 diabetes. In addition, we have identified potential structural, functional, and molecular correlates of exercise-induced benefits in the diabetic myocardium. The experimental models demonstrated highly compromised structure and function of myocardium in the diabetic state. Using structural magnetic resonance imaging, we were able to demonstrate the abnormal heart wall dynamics resulting from myocardial stiffness; a characteristic of the fibrotic heart in diabetes. Furthermore, the diabetic left ventricle manifested cardiac cycle abnormalities detectable via functional magnetic resonance imaging. Systolic and diastolic left ventricular functions were compromised in the diabetic heart. Microscopically, increased accumulation of interstitial collagen and decreased distribution of mitochondria were identifiable as the cardinal features of the diabetic myocardium. Endurance training, however, attenuated the structural and functional defects of the diabetic heart. Training prevented the development of myocardial fibrosis and loss of viable mitochondria in the diabetic heart. Training also ameliorated the systolic and diastolic dysfunctions of the ventricular pump in diabetes. Moreover, training-induced benefits were evident at the molecular level as decreased expression of myocardial protein kinase c (β II isoform); a critical protein implicated in the pathogenesis of diabetic cardiomyopathy. In summary,
these investigations demonstrate the vulnerability of the heart for failure and the efficacy of exercise in attenuating the major cardiac abnormalities in type 1 diabetes.
To

Mom, Dad and Teachers
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Preface

In the series of investigations that follow, we have attempted to characterize the pathologic features of diabetic cardiomyopathy (DCM) in a generic and specific rat model of type 1 diabetes. Further, we have also characterized the benefits of exercise training on these models during DCM. We have used the following methodology to accomplish our objectives: a) structural and functional imaging, b) light and electron microscopy, c) cardiac surgical procedures and d) biochemical assays.

The first chapter was attempted to gather evidence, prior to the experimental work, for exercise-induced benefits on major cardiac complications: a critical theme of this dissertation.

The major objective of the second chapter was to characterize the structural manifestations of DCM in a generic model of type 1 diabetes using MRI.

The aim of the third chapter was to characterize the functional manifestations of DCM in a generic model of type 1 diabetes using MRI.

The fourth chapter describes our attempt to characterize the exercise induced benefits on the heart with DCM in a generic model of type 1 diabetes.

The major aims for the fifth chapter were to verify a) the presence and manifestations of DCM in a specific model of type 1 diabetes and b) the possibility of exercise-induced benefits on the diabetic heart in a specific model of type 1 diabetes.
The sixth chapter concludes the dissertation by presenting the implications and future directions, in the author’s opinion, of the abovementioned work.
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CHAPTER 1
Exercise Induced Benefits in Individuals with Type 1 Diabetes

ABSTRACT

Individuals with type 1 diabetes are exposed to the pathologic effects of chronic hyperglycemia at an early age. Type 1 diabetes is associated with a variety of complications, including microvascular complications like diabetic retinopathy, nephropathy, and neuropathy. Conditions like diabetic cardiomyopathy also cause significant mortality and morbidity. The most common macrovascular complications include coronary heart disease, cerebrovascular disease, and peripheral arterial disease. Aerobic exercise is a critical component in the management of individuals with type 1 diabetes. Results from diabetic animal models reveal a variety of cellular mechanisms in response to exercise, producing beneficial effects on diabetic tissue. These results suggest that exercise training leads to improved tissue function by targeting specific adaptive mechanisms in diabetes, especially when initiated at the early stages of the disease. Hence, long-term aerobic exercise produces beneficial effects beyond insulin and nutrition therapy alone, as indicated by epidemiological and clinical studies in type 1 diabetics.

Key terms: Type 1 diabetes, aerobic exercise, complications.
Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (1). Type 1 and Type 2 are the two most common forms of diabetes. Type 1 diabetes is the result of an autoimmune process that specifically destroys the insulin producing pancreatic cells (beta cells), resulting in absolute insulin deficiency. Type 2 diabetes results from a combination of tissue insulin resistance and relative insulin deficiency; the insulin resistance of tissues in type 2 diabetes is attributed to abnormalities of insulin-signaling pathways.

Over the long-term, the chronic hyperglycemic microenvironment in both types of diabetes leads to damage, dysfunction and failure of multiple organs (eyes, kidneys, nerves, heart, and blood vessels). This review focuses on recent developments in our understanding of diabetic complications with a special emphasis on exercise-induced benefits at the cellular level in type 1 diabetes.

Aetiopathology of Type 1 Diabetes

Type 1 diabetes includes all the cases of diabetes mellitus that are primarily due to pancreatic beta cell autoimmune destruction and are prone to ketoacidosis. Type 1 diabetes is associated with HLA (Human Leucocyte Antigen), a class of molecules responsible for presenting antigens on the cell surface to promote immunocyte interaction. The autoimmune destruction of pancreatic beta cells in type 1 diabetes is a cell-specific process (see Figure 1 for details). The autoimmune reaction involves the synergistic interaction
between immunocytes and beta cells culminating in the death of insulin producing beta cells (51). The average age of onset for type 1 diabetes is 14 years (22). The type 1 diabetic is usually a child or adolescent, often thin or of normal weight, and prone to ketoacidosis, an acute complication of type 1 diabetes resulting in the acidification of blood and can be fatal. Hence the type 1 diabetic is dependent on exogenous insulin for survival.

A variety of complications occur as a result of chronic hyperglycemia in type 1 diabetes. These could be broadly classified in to microvascular and macrovascular complications. The early onset of hyperglycemia in type 1 diabetes exposes this group to the risk of vascular complications early in their life (40). The most common microvascular complications result from the damage of the vasculature in the retina, kidneys, and nerves, termed diabetic retinopathy, nephropathy, and neuropathy respectively. In addition a diabetes-specific cardiomyopathy also occurs as a result of chronic hyperglycemia. The common macrovascular complications result from the damage of coronary arteries, vasculature of the brain, and the periphery leading to myocardial infarction, stroke, and peripheral arterial disease respectively.

**Exercise in the Management of Type 1 Diabetes**

Classic management approaches to type 1 diabetes utilized the therapeutic interventions of insulin and nutrition therapies. The goal of the classic therapeutic regime in type 1 diabetes has been to maintain blood glucose levels within the normal range (euglycemia) to prevent life-threatening vascular complications of chronic hyperglycemia. A shift in the
management strategy of type 1 diabetes was made possible with the use of short, intermediate, and long-acting insulin combinations to improve hourly glucose levels (17). The success of islet cell transplantation techniques has opened the possibility of a future without daily insulin injections (66). However, formidable barriers need to be overcome before the therapeutic strategy toward a cure can be fully realized.

Exercise has long been considered as beneficial in the amelioration of diabetes; (44, 73) however this has not been without uncertainties due to early reports of impaired exercise capacity in diabetic individuals (5, 79). Recent results have challenged this idea as the cardiovascular responses of individuals with uncomplicated type 1 diabetes have been shown to be similar to their non-diabetic control counterparts (53). As a result, exercise has become the cornerstone of management in type 1 diabetes (24). Exercise benefits in these individuals are not only limited to improving glucose tolerance: regular exercise is critical for the prevention of the “diabetic disuse syndrome” which compromises the functional capacity of patients with diabetic complications (26).

In the past, many physicians were reluctant to believe that exercise was beneficial for individuals with type 1 diabetes; some even surmised that exercise might exaggerate complications in type 1 diabetes. A justification for this belief was the lack of experimental evidence. Even though exercise was cautiously included as a key component in the management of individuals with type 1 diabetes, the mechanisms of exercise-induced benefits in various diabetic tissues were unclear. But evidence has accumulated over years on the
efficacy of exercise in the management of type 1 diabetic individual with complications. The practical and ethical considerations associated with human experimentation have been largely overcome by the results of research on animal models of type 1 diabetes that are reminiscent of human type 1 diabetes. A closer look into some of these studies reveals the cellular mechanisms by which exercise might exert its beneficial effects on various diabetic tissues. A combination of experimental strategies on both human and animal models of diabetes has provided valuable clues on the mechanism of exercise-induced benefits. However, available information is restricted to selective components of diabetic complications, and elaborate studies including all aspects of hyperglycemic complications and their response to exercise are required in the future.

The following is a brief summary of the major diabetic complications that have been studied with relevance to exercise-induced benefits.

**Diabetic Retinopathy**

In adults with diabetes for 30 years, the prevalence of retinopathy increases to an alarming 95% of all cases(37). The spectrum of presentation ranges from *background retinopathy* (with normal vision) to *proliferative retinopathy* with retinal detachment (bilateral loss of vision). The retina is comprised of a patch of brain, lining the interior of the eye ball (Figure 2A). It is the site of phototransduction with the following major cellular components (Figure 2B):

(i) neural cells (rods, cones, bipolar cells, amacrine cells, horizontal cells, and ganglion cells);
(ii) glial (Muller cells);
(iii) epithelial (pigment epithelium); and
(iv) vascular (endothelial cells).

The vascular endothelial cells are the key players in the pathogenesis of diabetic retinopathy.

After prolonged exposure to hyperglycemia, the retinal microvessels are damaged. During the initial stages, the damage manifests itself as microaneurysms, hemorrhages, and other abnormalities of the microvascular structure and function that are collectively referred to as background retinopathy. If untreated, the background retinopathy may transform into a pre-proliferative retinopathy, which is characterized by microangiopathic changes resulting in severe retinal ischemia and hypoxia. During advanced stages of the condition, proliferative retinopathy ensues. Proliferative retinopathy is characterized by new vessel formation (neovascularization) and growth of non-functional fibrous tissue into the eye (fibrous proliferation). Figure 2C is a simplified representation of the neovascularization that characterizes proliferative diabetic retinopathy. However, the new vessels are susceptible to spontaneous hemorrhage and subsequent fibrosis. Contracture of the fibrous tissue leads to traction on the retina and retinal detachment (detachment of the neural components from the pigment epithelium), resulting in vision loss (65).

For many years, the predominant clinical concern centered on the safety and limitations of the individual with diabetic retinopathy who underwent exercise training. Therapists were discouraged by the possibility of exacerbation of retinopathy as a result of increased blood pressure with
exercise training. However with regular aerobic exercise, there appears to be no increased risk of progression to proliferative retinopathy;(12); in fact the risk may be reduced in individuals with type 1 diabetes(13). Albert and Bernbaum suggested that if exercise should be encouraged for individuals with diabetes, it should not be restricted from those with visual impairment(2). Modified exercise training protocols tailored to the needs and limits of a diabetic individual with visual impairment appear to be without any significant risk or side-effects(7, 26). Exercise training results in systemic improvements in glycemic control and exercise tolerance in individuals with diabetic retinopathy; the improved maintenance of normal plasma glucose levels might reduce the risk of progression of background retinopathy to proliferative retinopathy in type 1 diabetes.

**Diabetic Nephropathy**

Diabetic nephropathy is more common in type 1 than type 2 diabetic individuals(21). The primary renal target of chronic hyperglycemia is the functional units of kidneys called the glomeruli (Figure 3), which are the sites of filtration of blood in order to produce urine. An afferent arteriole brings blood into the glomerulus for filtration (Figure 3A); following filtration in the capillary loops, the arterial blood leaves the glomerulus via the efferent arteriole. As shown in the cross-sectional representation (Figure 3B), each glomerulus is an intricately woven network of renal capillaries, mesangial matrix, basement membrane, mesangial cells, and epithelial cells. In diabetes mellitus, thickening of the glomerular basement membrane occurs with expansion of the mesangial matrix, and proliferation of mesangial cells
(Figure 3C). The resulting proteinuria marks the diabetic nephropathy and progresses rapidly to end-stage renal failure in the absence of therapeutic intervention (28).

As with diabetic retinopathy, the predominant issue in the training of individuals with nephropathy has been the safety and extent of benefits of the exercise protocol. The latter concern was predicated on reports of increased albumin excretion rate after a single exercise challenge in diabetic individuals(25, 27, 39). Although increased albumin excretion rate is an important predictor of impaired glomerular function, it is important to consider that such increases following exercise is a normal physiologic renal response in healthy individuals as well. However, concerns about worsening the albumin excretion rate in already damaged diabetic kidneys may have prevented the therapist/clinician from engaging individuals with diabetic nephropathy on an exercise protocol.

Meanwhile clinical studies on individuals with end stage renal disease (of various causes including diabetes) are highly suggestive of the positive effects of a long-term exercise program on their physical and mental well being (8, 47, 56, 75). Type 1 diabetic individuals with adequate glycemic control were able to tolerate exercise intensities of 50-70% VO\textsubscript{2max} without any adverse effects of increased albumin excretion rates (45). Importantly, experiments on animal models of type 1 diabetes revealed that the beneficial effects of an aerobic exercise program on renal function occurs in the form of a reduction of glomerular mesangial volume, attenuation of the increase in albumin excretion rate, reduction in levels of lipid peroxidation, and increased levels of antioxidants (e.g. glutathione peroxidase, vitamin E) (3,
42). Hence it is evident that exercise targets the principal renal mechanisms that fail in diabetes, and thus ameliorates the condition. Perhaps the most important conclusion that could be derived from these animal studies is that a long-term aerobic training program initiated during the early stages of diabetic nephropathy is highly effective in impeding the progression of renal pathology.

Even though the detailed mechanisms of exercise-induced benefits on improved function are not completely clear in diabetic retinopathy and nephropathy, ample evidence from both human and animal studies suggest that an exercise intervention does not worsen diabetic complications. In particular, the low drop-out rates in the clinical trials described above show the ability of individuals with renal disease to sustain training protocols, and should encourage therapists/clinicians to include exercise therapy in the management of individuals with diabetic nephropathy. As pointed out by Tawney et al. (75), a combination of traditional cardiovascular conditioning programs and modified physical activity protocols (e.g. life readiness program) may provide optimal physical and psychological benefits in individuals with renal failure due to various causes including diabetes.

**Diabetic Neuropathy**

Diabetic neuropathy is the most common form of neuropathy in the western world; and includes a number of mono and poly neuropathies, as well as plexopathies and radiculopathies (67). From a clinical perspective, sensorimotor and autonomic neuropathies are extremely important due to the profound morbidity associated with these conditions. The most common presentation of diabetic sensorimotor neuropathy is one with a “stocking and
glove distribution” that symmetrically affects distal limb segments. Meanwhile diabetic autonomic neuropathy is a subtype of peripheral polyneuropathies, and is among the least recognized and poorest understood complications of diabetes. The ubiquitous distribution of the autonomic nervous system renders virtually every body tissue susceptible to autonomic dysfunction(80). The most common life-threatening complication of diabetic autonomic neuropathy is cardiac autonomic neuropathy.

Both metabolic and vascular factors are involved in the pathogenesis of diabetic neuropathy. Metabolic changes lead to oxidative stress and impaired mitochondrial function with resultant apoptosis of neurons and Schwann cells. Vascular changes lead to ischemic microvascular injury of endoneurium, resulting in structural damage to nerve fibers. Although positive sensory symptoms (prickling, tingling, electrical sensations, throbbing, etc.) dominate the clinical concerns of patients(67) with sensorimotor neuropathy, subclinical motor deficits are also present in these cases(61). Type 1 subjects with peripheral neuropathy are more likely to report injury and feel unsafe with their gait and posture in unusual conditions (11). Meanwhile the earliest indicator of cardiac autonomic neuropathy is reduced heart rate variation (84).

Stretching, massage, and transcutaneous electrical nerve stimulation have been employed to treat painful diabetic neuropathy(60, 71). Muscular pain due to chronic painful neuropathy in individuals with both type 1 and 2 diabetes decreases maximally in response to stretching exercises, with muscle relaxant and non-steroidal anti-inflammatory drugs used only as adjunctive agents(58). Interestingly, the decrease in muscular pain score is associated
with improvements in sleep, indicating the enhanced quality of life achieved by the exercise intervention(58). Although evidence is available for a possible interaction between endogenous oxytocin and opioid-based anti-nociceptive mechanisms in the relief of thermogenic and mechanogenic pain by massage in healthy animals, their role in diabetic sensorimotor neuropathy awaits exploration(48). Transcutaneous electrical stimulation is effective in the relief of neurogenic pain, perhaps by decreasing the levels of excitatory neurotransmitters (e.g. glutamate) and increasing inhibitory neurotransmitters (e.g. GABA) in the spinal-cord dorsal horn region(49). The role of therapeutic modalities in the alleviation of neuropathic pain by utilizing endogenous pain modulatory systems in type 1 diabetes merits further study.

Abnormal exercise tolerance in individuals with cardiac autonomic neuropathy should not be a sufficient reason to preclude this group from exercise training(7, 80). During the development of exercise protocols for individuals with cardiac autonomic neuropathy, the most important consideration is the adaptation of traditional aerobic exercise regimens to individual tolerance. This is often achieved on the basis of self-reported tolerance levels rather than standard tests, due to broad variations in the degree of perceived physiological challenges in this group(26). A low intensity exercise program significantly increases the heart rate variability in insulin dependent diabetics with early cardiac autonomic neuropathy abnormalities(35). Meanwhile, individuals with severe cardiac autonomic neuropathy do not benefit from exercise-induced increase in heart rate variability, although their maximal performance capacity increases with
exercise. The report by Howorka et al., (35) is evidence that diabetic individuals with cardiac autonomic neuropathy are able to tolerate low grade aerobic training, reaping the maximum benefits of exercise with early initiation. The benefits of low intensity aerobic training in a rat model of type 1 diabetes with cardiac autonomic neuropathy included reversal of hypotension, bradycardia, and left ventricular contractile abnormalities (15). With exercise training there is an improvement in intrinsic cardiac pacemaker regulation, vagal tonus, and myocardial glucose metabolism in these animals. The abovementioned cardiac changes may represent the adaptive response of cardiac tissue to the demands posed by early and extended training.

**Diabetic Cardiomyopathy**

In addition to the damage exerted by cardiac autonomic neuropathy, the diabetic heart is also a target of a distinct form of myopathy known as diabetic cardiomyopathy. Diabetic cardiomyopathy leads to cardiac failure even without coronary artery disease,(6, 70) and is manifested by early diastolic dysfunction, small-vessel disease, interstitial fibrosis, myocardial hypertrophy, and eventual loss of cardiac contractility(30). Accumulation of excessive amounts of collagen in myocardium is a consistent marker of interstitial fibrosis in diabetes (Figure 4). The accumulation of collagen in the diabetic myocardium in Figure 4B is indicated by the dark red profiles (arrows) on the micrograph. Similar to diabetic nephropathy, the interstitial fibrosis in diabetic cardiomyopathy significantly limits the parenchymal tissue function (18). In the case of the heart, excessive amounts of collagen
deposition in the myocardium may alter the normal dynamics of cardiac contractility.

Early reports of exercise induced benefits in type 1 diabetes primarily focused on the improved cardiovascular endurance attained with aerobic protocols in subjects(73). Meanwhile, exercise-induced benefits can occur on specific functional components of diabetic myocardium that may improve the contractile function of the diabetic heart; this has been shown in various animal models of type 1 diabetes, in which early exercise training prevented abnormalities that compromised cardiac function in the sedentary (non exercised) animals(9, 16). At the cellular level, exercise-induced amelioration of cardiac dysfunction occurred through a variety of metabolic and structural adaptations; training lead to improved myocardial glucose oxidation and glycolytic rates(9). Training also prevented the decrease in mitochondrial and myofibril area that occurred in the cardiac tissue of sedentary diabetic animals. Most importantly, training impeded the myocardial interstitial fibrosis, detected as a decrease in collagen fiber circumference with electron microscopy (63). These reports indicate that aerobic training can be used as a valuable tool to prevent myocardial deterioration in type 1 diabetes.

**Coronary Artery Disease**

Coronary artery disease is the result of cardiac macrovascular pathology. Atherosclerosis of the coronary arteries is a chronic inflammatory condition that can also manifest as an acute clinical event by plaque rupture and thrombosis. The stages of diabetic atherogenesis are outlined in Figure 5.
During the early stages, abnormal vascular endothelial cell function coupled with rheological factors lead to impaired vessel wall permeability, resulting in low density lipoprotein transport into the macrovascular intima (Figure 5B). Oxidation of the low density lipoproteins in the intimal layer stimulates the endothelial cells to produce cell adhesion molecules on their surface; this in turn triggers the migration of circulating monocytes and T-cells into the intima (Figure 5C). Following the maturation of monocytes into macrophages in the intima, the macrophages initiate their scavenging function to clear the intimal lipids. By the end of this scavenging process, the macrophages become foam cells (Figure 5D). During the later stages, the death of foam cells leads to the accumulation of cellular and lipid debris in the intima (Figure 5E). In the final stages of atherogenesis, vascular smooth muscle cells migrate from the media into the intima. The smooth muscle cells proliferate and produce extracellular matrix into the space that is already filled with cellular and lipid debris, to form the fibrous plaques characteristic of atherosclerosis (Figure 5F). During advanced stages of atherogenesis, the plaques may calcify or destabilize, resulting in the formation of a thrombus. The descriptions in Figure 5 of atherosclerotic events are applicable to both diabetic and non-diabetic individuals; however, the former group is at a higher risk due to the overlap of events that characterize diabetes with the processes of atherogenesis.

The loss of vascular homeostasis in diabetic individuals can worsen atherosclerosis, which already accounts for about 50% of all deaths in westernized societies (50). Both hyperglycemia and insulin resistance (lack of tissue insulin sensitivity) contribute toward coronary artery disease in type 1
diabetic individuals(22, 54, 68). It has been suggested that insulin resistance, which is commonly associated with type 2 diabetes, might be the result of “glucotoxicity” (chronic tissue exposure to hyperglycemia) in type 1 diabetes(82). In fact, with respect to coronary artery disease, the fine line between both types of diabetes vanishes(69). As a result, individuals with type 1 diabetes have similar or even higher risk of coronary artery disease than their type 2 diabetic counterparts and the general population(83).

Under normal conditions of insulin sensitivity both during rest and physical challenge, an increase in peripheral glucose levels is countered by normal tissue glucose uptake mechanisms, chiefly in the skeletal muscle. Skeletal muscle glucose uptake is mainly mediated by insulin binding to its receptor followed by a series of molecular signals that end ultimately with the mobilization of a glucose transporter (glut-4) from the interior to the cell membrane, facilitating the diffusion of glucose. During insulin resistance states, abnormalities of the insulin-responsive molecular signals in both types of diabetes prevent glut-4 translocation resulting in hyperinsulinemia. For the same reason, there is a failure of insulin-mediated glucose uptake that results in hyperglycemia. Meanwhile, insulin-independent glucose uptake occurs in skeletal muscle in response to exercise (32). To be more specific, skeletal muscle glucose uptake is facilitated by molecular signals culminating in the translocation of glut-4 in response to contraction-induced decrease in cellular energy charge (i.e. an increase in AMP:ATP), that acts as a stimulus (instead of insulin) for glucose uptake (31). Hence a contractile stimulus (e.g. exercise) could potentially be employed to attenuate insulin resistance, thus reducing the risk of coronary artery disease.
A significant change in the lipid profile, with a considerable increase in insulin sensitivity, occurs in type 1 diabetic subjects with well-controlled hyperglycemia(45). The degree of chronic hyperglycemia predicts the risk of coronary artery disease better than recent hyperglycemic events in type 1 diabetes;(74) thus, a long-term training protocol is most effective for cardio-protection in this group. An individualized aerobic exercise program, along with intense education, can positively influence lipid profiles and physical fitness in individuals with type 1 diabetes(57). Through its influence on lipid metabolism, exercise training improves the mass of high density lipoproteins, although exercise may have little benefit on the reduction of low density lipoproteins(29). Interestingly, exercise training has been shown to prevent deterioration of cardiac function by mechanisms independent of blood glucose and total cholesterol levels in a pig model of type 1 diabetes with dyslipidemia(38). Although it is unclear whether exercise could independently produce similar positive effects in coronary arterial function in type 1 diabetes, it has been pointed out that exercise training induces adaptations in perfusion characteristics of the cardiac muscle itself in non-diabetic subjects with coronary artery disease(46). These benefits might occur in the form of improved coronary vasodilation(64, 72) and collateral circulation(62), as shown with animal models.

**Cerebrovascular Disease**

The risk of stroke in individuals with type 1 diabetes is increased relative to their age-matched non-diabetic counterparts, and compared to type 2 diabetic subjects(41). In particular, ischemic stroke in type 1
individuals greatly increases their risk of death (43). In fact, the risk for a cerebrovascular event is well predicted by decreased levels of serum high density lipoprotein levels (14). In type 1 diabetic individuals there is also an adaptive decrease of vasoconstrictive factors with an increase of hypoperfused brain regions compared to controls (78). In addition to the vasoconstrictive changes, changes in the levels of vascular relaxation may also occur depending on the duration of diabetes, as shown by experiments using rat blood vessels (59). Hence, the extent of tissue damage and recovery following a stroke might be determined by a variety of glucose dependent and independent factors in type 1 diabetes. Meanwhile in animal models of type 1 diabetes, the severity of tissue damage following a stroke is determined by the availability of glucose-independent factors, such as sex steroids (76).

A regular exercise program designed to reduce the detrimental effects of the diabetic disuse syndrome (26) could be beneficial as a neuroprotective tool, along with its benefits on other diabetic complications discussed above. The generalized benefits of exercise training on the prevention of atherosclerosis and subsequently on stroke (23, 29) may benefit individuals with type 1 diabetes as well. Although information is scarce on the specific mechanisms of prevention and attenuation of stroke in both humans and experimental models of type 1 diabetes, a reasonable speculation is that exercise benefits on cerebrovascular disease in this group might occur in the form of activity-induced angiogenesis, nerve growth factor expression, and production of vasodilatory mediators (19, 20). In fact it has been suggested that the effects of physical activity in reducing stroke risk might be equivalent to the effects of
anti-dyslipidemic pharmacotherapy and both interventions might act via a same vasodilatory mechanism (20). In addition exercise training may also induce expansion of cerebral microvasculature to provide a cerebral preconditioning effect (19). The role of the above mechanisms in individuals with/animal models of type 1 diabetes remains to be verified, although exercise is an attractive choice for reduced stroke risk in individuals with type 1 diabetes (14).

**Peripheral Arterial Disease**

Yet another manifestation of atherosclerosis, peripheral arterial disease, includes those entities which result in obstruction to blood flow in arteries exclusive of coronary and intracranial vessels (55). The most intensively studied peripheral arterial disease is associated with the lower extremity. The pain in the lower extremities associated with ambulation (intermittent claudication) is a severe disabling symptom. Diabetes mellitus is an important risk factor for peripheral arterial disease and increases the risk of limb loss. As with other macrovascular complications discussed above, the ratio of very low density lipoproteins to high density lipoprotein is a key determinant of peripheral arterial disease in individuals with type 1 diabetes (81). Abnormal lipid profiles acting in concert with impaired fibrinolytic activity (i.e. reduced degradation of fibrin/clots) place individuals with type 1 diabetes at a higher risk for peripheral arterial disease complications (10).

Aerobic exercise training can be used to improve functional benefits in subjects with peripheral artery disease (4, 33, 55). Exercise has been shown to directly affect the activity of key molecules like tissue plasminogen activator
and plasminogen activator inhibitor-1, which are involved in the prevention/dissolution of thrombus. Six months of treadmill training has been shown to result in an increase in the time of onset of claudication pain and maximal walking time in subjects with intermittent claudication; such functional benefits were accompanied by an increase in the activity of tissue plasminogen activators, and decreases in the activity of plasminogen activator inhibitor, both of which are essential for fibrinolysis(36). Meanwhile, maintenance of functional benefits requires continuation of exercise training in subjects with peripheral arterial disease(52). It can be speculated that the mechanism of functional benefits of exercise in type 1 diabetics with peripheral arterial disease is similar to type 2 diabetics(36), since the adaptation of factors that regulate fibrinolysis during aerobic training remains intact in individuals with type 1 diabetes without major complications(77).

**Summary**

A carefully designed exercise training protocol has the potential to improve the health-related quality of life of individuals with type 1 diabetes, by supplementing the beneficial effects of nutrition and pharmacotherapy. Evidence has been accumulating describing the mechanisms of exercise-induced benefits on various micro- and macro-vascular complications in type 1 diabetes. Most importantly, the availability and increased utilization of appropriate animal models has proved to be useful for understanding the benefits of exercise (aerobic) training in type 1 diabetes at the cellular and molecular level. The information obtained from animal models and human
studies, in elucidating the response of various diabetic tissues to exercise training, will provide valuable insights into the exercise physiology of type 1 diabetes.

In addition to its beneficial effects in terms of overall cardiovascular effects, aerobic training in type 1 diabetes results in tissue specific effects that impede diabetic pathology. Aerobic training increases the levels of renal antioxidants(3, 42), reverses left ventricular contractile abnormalities(15), and improves myocardial glucose oxidation and glycolytic rate,(9) to positively influence microvascular complications in animal models. Although not confirmed, it seems reasonable to hypothesize that some of the exercise-induced benefits on diabetic macrovascular disease (e.g. atherosclerosis) in individuals with type 1 diabetes(57), might occur in the form of improved vasodilation, collateral circulation, fibrinolysis and rheological changes by modulation of critical molecules involved in these adaptation in non-diabetic individuals/ animal models(20, 34, 62, 64, 72). The impact of training on the cellular mechanisms of adaptation in type 1 diabetic macrovascular complications merits further exploration in animal models. Another important aspect of both animal and human studies that requires our attention is the fact that subjects were able to tolerate the training protocols. Hence tolerance to aerobic protocols may not be a sufficient reason to preclude type 1 diabetics, with or without complications, from exercising, provided the training protocol is tailored to meet the needs and limits of the particular individual(4, 26, 80).

There has been a radical shift in perspective with respect to management of type 1 diabetes since the report of long-term insulin
independence with islet transplant protocols(66). A cure for type 1 diabetes remains the ultimate goal of such protocols; however, the conventional management triad of insulin, exercise, and nutrition will remain the practical approach until the challenges to islet transplants (such as availability of donor cells, reliable islet-isolation procedures, and availability of expertise) are overcome. Hence, promptly initiated and prolonged exercise training designed to meet specific individual demands, along with insulin and nutrition therapy, will play a key role in the management of individuals with/without complications from type 1 diabetes.

Conclusion

Aerobic exercise training remains the cornerstone in the management of individuals with type 1 diabetes with and without complications. Training in certain cases may utilize the same cellular substrates used by pharmacotherapy to mediate its positive effects on the diabetic tissues. The effects of a regular exercise program greatly expand the benefits of insulin and nutrition therapy to improve the health-related quality of life in individuals with type 1 diabetes.
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Figure Legends

Figure 1:

(A) Illustration of the pancreas.

(B) The major component of pancreas is the exocrine tissue (brown cells). Masses of endocrine tissue (yellow) called the islets of langerhans are interspersed within the exocrine tissue. Each islet is a collection of 4 kinds of endocrine cell masses and vascular components (red). The cellular component includes beta cells (yellow), alpha cells, delta cells, and F cells (last three cell types not illustrated), each producing a single hormone. The beta cells are the predominant cell type and produce insulin.

(C) During the early stages of type 1 diabetes, T-cells (violet) invade the beta cell mass prompted by an autoimmune reaction that involves both the beta cells and the cells of immune system. This phase of the disease is called insulitis.

(D) Later when most beta cells have been killed off, absolute insulin deficiency ensues, marking type 1 diabetes.

Figure 2:

(A) Illustration of the three layers of the eye. The outer layer is comprised of both the cornea (a) and the sclera (b). The middle layer is called the uvea (c) and it consists of the choroid, the iris, and the ciliary bodies (not shown). The inner retinal layer is composed of both the pigment epithelium (d) and the sensory retina (e).
(B) The major cellular components of normal retina. a) Pigment epithelium, b) Cone photoreceptor, c) Rod photoreceptor, d) Bipolar cell, e) Amacrine cell, f) Ganglion cell, g) Muller cell, and h) Endothelial cell. The horizontal cells (see text) have been omitted from the cartoon for better clarity.

(C) The diabetic retina. Note the increased vascularization (H) characteristic of proliferative diabetic retinopathy. The corresponding region of the normal retina contains vascular endothelial cells (h) only.

Figure 3:
(A) Illustration of a glomerulus. a) Capillary loops, b) Mesangium, and c) Urinary space. “x” denotes the plane of cross-section for (B) and (C). The arrows denote the inflow and outflow of blood through the afferent and the efferent arterioles respectively.

(B) Cross-section of a normal glomerulus representing the glomerular components: a) Parietal epithelium, b) Visceral epithelium, c) Basement membrane, d) Renal capillary, e) Mesangial cell, and f) Mesangial matrix.

(C) Cross-section of a diabetic glomerulus. Note the thickening of basement membrane (a), proliferation of mesangial cells (b), and expansion of mesangial matrix (c), all features of diabetic glomerulosclerosis.

Figure 4:
Histological sections from a normal (A) and diabetic (B) rat left ventricular myocardium, stained with collagen specific dye (picrosirius red). Note the accumulation of collagen in the diabetic myocardium (arrows), a characteristic feature of diabetic cardiomyopathy. The duration of diabetes was 7 weeks. Scale bar in right lower corner represents 50 microns.

**Figure 5:**

(A) Structure of a normal large artery.

(a) The intima (inner layer) is made up of endothelial cells (red), extracellular matrix consisting mostly of collagen and proteoglycans (violet stipples), and the internal elastic lamina (turquoise).

(b) The media (middle layer) is made up of smooth muscle cells.

(c) The adventitia (outer layer) is made up of connective tissue interspersed with fibroblasts and smooth muscle cells.

(x) Represents the plane of section for (B) through (F) describing the stages of atherogenesis.

(B) Stage 1

Rheological factors and abnormal endothelial function increase the intimal permeability to allow low density lipoprotein (LDL) transport (blue) into the intima. LDL interacts with intimal matrix components, and reactive oxygen species to be retained in the intima.

(C) Stage 2

Oxidized LDL stimulates the overlying endothelium to produce adhesion molecules that attract immunocytes like monocytes that later
become macrophages (green), and T-cells (violet) to mark the inflammatory stage.

(D) Stage 3
In the intima, macrophages scavenge the accumulated oxidized LDL to become foam cells (arrowhead), which later undergo apoptosis (cell death), leaving behind lipids and cell debris.

(E) Stage 4
Factors secreted by immunocytes in the intima trigger smooth muscle cell (SMC) migration and proliferation (green). In the intima, foam cell derived lipids and cell debris along with SMC derived extracellular matrix accumulate (arrows), forming the fibrous plaques characteristic of atherosclerosis. Calcification of plaque ensues later.

(F) Stage 5
Thrombus formation (brown) follows plaque destabilization at advanced stages of atherogenesis.
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CHAPTER 2

Characterization of alterations in diabetic myocardial tissue using high resolution magnetic resonance imaging

ABSTRACT

Cardiovascular complications, including diabetic cardiomyopathy, are the major cause of fatalities in diabetes. Diabetic cardiomyopathy is expressed in part through fibrosis and left ventricular hypertrophy, increasing myocardial stiffness leading to heart failure. In order to search for curative interventions, precise evaluation of the diabetic heart pathology is extremely important. Magnetic Resonance Imaging (MRI) is ideally suited for the assessment of heart disorders due to its high resolution, three-dimensional properties and dimensional accuracy. In this study streptozotocin injected Sprague-Dawley rats were used as a model of type 1 diabetes to characterize abnormalities in the diabetic left ventricle (LV). High resolution MRI using a 9.4 T horizontal bore scanner was performed on control and 7 weeks diabetic rats. In the diabetic rats as compared to controls, we found increased LV wall volume to body weight ratio, suggestive of LV hypertrophy; increased LV wall mean pixel intensity, and decreased T2 relaxation time, both suggestive of changes in the diabetic tissue properties, perhaps due to the presence of fibrosis which was detected through increase in the collagen fractional area. In addition, changes in the LV cavity area were observed and quantified in postmortem diabetic hearts indicative of stiffer and less resilient LV myocardial tissue with diabetes. Together the data suggest that LV hypertrophy and fibrosis may be a major factor underlying structural and
functional abnormalities in the diabetic heart, and MRI is a valuable tool to non-invasively monitor the pathological changes in diabetic cardiomyopathy.

**KEY WORDS:** Collagen, Fibrosis, Heart, MRI, Type 1 Diabetes
ABBREVIATIONS:

gems - gradient echo multi-slice
LV - left ventricle
mems - multi echo multi-slice
MRI - magnetic resonance imaging
sems - spin echo multi-slice
INTRODUCTION

Approximately 150 million patients worldwide suffer from diabetes mellitus, with 16 million in the United States alone, among them 1 million afflicted with type 1 diabetes (8). Diabetes mellitus profoundly affects the cardiovascular homeostasis of an individual. Cardiovascular complications account for the most number of mortalities in diabetic patients. Even though the incidence of cardiovascular disease events has reduced in diabetic individuals over the last several decades, the absolute risk of the cardiovascular disease is 2 fold greater in people with diabetes compared to healthy subjects (11). Diabetic cardiomyopathy is a chronic complication of diabetes independent of coronary heart disease (9, 39). Diabetic cardiomyopathy is a distinct entity commonly prevalent in the diabetic population contrary to earlier beliefs (5) that it is a rare condition.

Experimental models such as the streptozotocin diabetic rat model imitate functional impairment (41) and the structural abnormalities of the diabetic cardiovascular system (35). Features such as interstitial fibrosis and left ventricular diastolic dysfunction mark the cardiac pathology that ultimately leads to cardiac failure (30). Effective intervention strategies can only be facilitated by a detailed understanding of the mechanisms underlying this chronic cardiac pathology. Currently available methods are not only invasive, but also provide an incomplete profile of the internal cardiac features (4, 17). As a result these methods limit the availability of valuable information that might provide clues to the clinician about the nature of the tissue pathology. There is an urgent need to characterize diabetic
cardiomyopathy due to the minimal diagnostic information currently available to screen patients (16). Magnetic Resonance Imaging (MRI) is an invaluable non-invasive method in accurate assessment of myocardial mass and ventricular function compared to echocardiography. MRI is a better alternative due to its capacity to evaluate all myocardial segments with equal accuracy (38). It provides precise numerical information concerning tissue characteristics, allowing differentiation between normal and pathological states. Here we report the MRI characterization of the diabetic left ventricle (LV) in the streptozotocin rat model of experimental type 1 diabetes.

MATERIALS AND METHODS

Experimental Model

All animal procedures were approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee. Sixteen male Sprague-Dawley rats (n = 8 per group) were given unlimited access to chow and water for the entire duration of study. At 2 months of age (with body weight of approximately 250 g), the rats were randomly assigned to one of the two experimental groups, control and diabetic. The rats in the diabetic group were given a single intraperitoneal injection of streptozotocin (65 mg/kg, Sigma, St. Louis, MO) in 10 mM sodium citrate buffer, pH 4.5. The controls were injected with the same volume of vehicle. Diabetes was confirmed in the former group by measuring the non-fasting blood glucose level (≥ 200 mg/dL) two days post-injection. Body mass and blood glucose levels were monitored throughout the study on a weekly basis. Hemoglobin
A1C levels were measured before the termination of the experiment using antibody based A1CNow monitor from Metrika Inc. (Sunnyvale, CA).

**MRI Procedure**

At the end of 7 weeks of diabetes, cardiac MRI was performed using a 9.4 T horizontal bore scanner (Varian Inc., Palo Alto, CA). Rats injected with heparin (100 U/100 g body weight) and subjected to an overdose of anesthetic (Nembutal), were positioned supine on a custom built plexiglass sled and inserted in a 60 mm radio frequency coil. A water filled 15 ml plastic tube that served as internal reference for MRI was placed over the chest of the animal within the field of view during the scans. A cardiovascular physiological monitoring system (SA Instruments Inc., New York, NY) was connected to monitor heart rate and rhythm, respiratory status and body temperature. The sled was positioned in the magnet bore for optimal signal detection from the heart as ascertained with an initial gradient echo multi-slice (gems) multi-plane sequence in all three planes. Immediately following the cessation of the heart beat (designated time 0 min), T1 weighted images were acquired repetitively every 5 minutes from the transverse sections of the heart using spin echo multi-slice (sems) sequence with the following parameters: TR/TE = 800/12 ms, average = 2, matrix = 128 pixels x 128 pixels, field of view = 60 mm x 60 mm, number of slices = 10, slice gap = 0 mm and slice thickness = 2 mm. The acquisition continued for 40-50 min. Next, T2 weighted images were acquired using a multi echo multi-slice (mems) sequence under the following conditions: TR/TE1, TE2= 2500/12, 36 ms (2 echoes for each slice), while the other parameters remained the same as in the T1 weighted images.
Image Analysis

The acquired images were analyzed using Adobe Photoshop (Version 7.0) software. For precise delineation of the area of interest, images were analyzed at 400% zoom. The T1 weighted images were used to quantify LV wall volume, LV cavity area and LV wall mean pixel intensity. The T2 weighted images were utilized for measuring the transverse relaxation time, of the LV wall.

For the estimation of LV wall volume in each animal, the final set of T1 weighted images (40-50 min after cessation of the heart beat) were chosen. Starting with the first slice of the LV apex, the boundaries of the LV wall were traced manually in 5 consecutive cranial slices (those covering the entire LV of rat), and the pixel count within the selected region of interest was used to estimate the area via the conversion factor (1 pixel/0.22 mm²). LV volume was calculated as a sum of volumes of these five 2-mm thick neighboring slices. The LV cavity area in all hearts was calculated from the 2nd cranial slice from the apex. As mentioned above for the LV wall volume, the cavity area was determined using the same conversion factor to convert the pixel count to the LV cavity area.

The mean pixel intensity of the LV wall was determined in the 2nd cranial slice from apex, after normalization of pixel intensity of the slice to the internal reference standard.
Finally, the T2 relaxation time of the LV wall was determined from the analysis of the 2nd cranial slice from apex. The mean pixel intensity values of LV wall were calculated as described above for the T1 weighted images. The T2 relaxation time was calculated according to the formula $T_2 = \frac{24}{\ln(I_{12}/I_{36})}$ in ms, where $I_{12}$ is mean pixel intensity of area in echo #1 acquired at TR/TE1 = 2500/12, and $I_{36}$ is mean pixel intensity of area in echo #2 acquired at TR/TE2 = 2500/36.

**Ex vivo Heart Tissue Analysis**

Immediately following MRI scan, the chest wall of the animal was opened and the heart was removed and weighted for gravimetrical analysis. In order to collect LV myocardium for histological staining immediately after the heart stopped beating, additional groups of 3 control and 3 diabetic rats were used. Anesthetized rats were perfused with 10% formaldehyde in phosphate buffered saline. Hearts were excised, and LV slices at approximately 3 mm above the apex were removed and transferred to 4% paraformaldehyde for further fixation, followed by paraffin embedding, sectioning and staining with picrosirius red to detect collagen deposits under light microscopy. Collagen fractional area was calculated using Adobe Photoshop (Version 7.0) software and was expressed as percentage of collagen area per total area.

**Statistical Analysis**

The data was analyzed using SigmaPlot 2000 software. Values presented in the table and figures were expressed as means ± SEs. The
difference in means between the groups was detected by independent sample Student’s t-test and significance was defined at \( p \leq 0.05 \).

RESULTS

Animal Characteristics

The mean values for blood glucose levels, glycated hemoglobin, A1C levels, body mass, and heart mass to body mass ratio measured at the termination of the experiment, for both control and diabetic groups are presented in the Table. The blood glucose levels of the diabetic group was significantly increased compared to control group and remained greater than 350 mg/dL throughout the duration of the study. In addition, the hemoglobin A1C levels were significantly higher in diabetic rats compared to controls, and were similar to those reported by others using the same animal model (22). In fact, the A1C levels exceeded the maximum value measurable by the A1CNow monitor. This confirms that the diabetic rats underwent long term hyperglycemia (18), and that the animal model used in this study represents a model of uncontrolled diabetes. The diabetic group also showed decreased gain in weight compared to controls over the duration of the study that was in agreement with other reports (26). The heart mass to body mass ratios measured at the time of sacrifice after MRI scanning procedures showed a significant increase in diabetic animals suggesting that they developed heart hypertrophy.

Changes in the Left Ventricular Wall Volume to Body Mass Ratio in Diabetes
There was no significant difference in the LV wall volume between control and diabetic rats, collected at 35 min after the heart stopped beating. When LV wall volume to body mass ratios were compared, diabetic rats displayed a significant increase of 39% compared to control rats (Figure 1). This finding is in agreement with the gravimetric data (Table), where heart mass to body mass ratio was increased in diabetes compared to control, and suggests that the diabetic rats in this study developed heart hypertrophy at 7 weeks of diabetes.

**Closure of Left Ventricular Cavity in Postmortem Heart**

The LV cavity is fully open at the time when the heart stops beating (0 time, Figure 2). The reduction in LV cavity area was noticeable in control animals at approximately 20 min after the heart stopped beating, and the LV wall completely closed on itself within 30-35 min after the last heart beat (Figure 2, top panel). In diabetic rats the LV cavity closure followed a strikingly different time-course. Not only was the rate of closure decreased, but also it was incomplete, preventing the LV cavity from total obliteration (Figure 2, bottom panel).

Figure 3 shows decrease of the LV cavity areas with time after the cessation of heart beat in both groups. The average time taken for complete closure of the LV cavity in the control group was approximately 20 minutes, while in diabetic rats the LV cavity failed to close completely even at 50 minutes. At 35 min we compared the areas of the LV cavities between the two groups. Figure 4 demonstrates significant differences in the mean values for
the LV cavity area of control and diabetic rats. The LV cavity area measured immediately after the heart stopped beating, was 19% greater in diabetic rats compared to controls, however, the difference was not significant. These patterns of the LV cavity closure in postmortem rat hearts suggest that the diabetic myocardial tissue may be stiffer and less resilient than control tissue.

**Pixel Intensity Differences of Diabetic Left Ventricular Myocardium**

The contrast provided by the high field MRI, allowed us to accurately and reliably evaluate LV wall pixel intensity on the high resolution images. This characteristic of the tissue is influenced by the structural properties of the tissue. The mean pixel intensity values showed a significant increase of 31% in the diabetic group compared to control (Figure 5). This data is in accordance with other reports suggesting that increased myocardial tissue pixel intensity on MRI scans is associated with fibrotic depositions in the tissue (6).

**Changes in T2 Relaxation Time in Diabetic Left Ventricular Myocardium**

T2 relaxation time is another characteristic of the magnetic properties of the tissue. To minimize the effects of any imperfect 180° focusing pulses on the T2 estimates, we normalized the T2 estimates by those of the water reference. We found that control LV tissue had T2 relaxation time (~35.6 ms) in agreement with values published by others for the myocardial tissue (6, 15, 34). Diabetic LV wall showed a 69 % decrease in T2 relaxation time when compared to the controls (Figure 6) suggesting structural changes in myocardium brought about by diabetic disease (see Discussion).
Histological Detection of Collagen Deposition in the Diabetic LV Myocardium

In addition to MRI derived measures of fibrosis as mentioned above, we evaluated the level of fibrosis in the diabetic LV by estimating the accumulation of fibrotic protein collagen. Profiles of the LV myocardium with the collagen specific stain, picrosirius red (27), showed widespread areas of collagen accumulation in the diabetic LV compared to controls as illustrated in the representative photomicrographs on Figure 7. Upon quantification, the diabetic LV myocardial sections showed a 95% increase of collagen deposits compared to control (Figure 8). This suggests that the diabetic rats in our study developed fibrotic changes in the myocardium after 7 weeks of diabetes.

DISCUSSION

We used high field MRI to detect disparity in structural properties between the diabetic and control age matched rat myocardium. The animal model used in this study is a widely accepted model for probing the pathological signaling events that are specific to diabetic cardiomyopathy (8, 24). The diagnosis of the different kinds of cardiomyopathy has been greatly facilitated by the recent developments in non-invasive cardiac imaging (32). Yet, in type 1 diabetes, the clinical investigation for diabetic cardiomyopathy is not routinely performed (10) largely due to the limits on available technology for optimal screening. To differentiate this condition from other forms of cardiac muscle pathology during a routing cardiac screening, the
structural features of diabetic heart need to be characterized. As a non-invasive technique, MRI could be a valuable tool in characterizing the pathological changes in diabetic heart during the progression of the disease allowing follow up imaging and to monitor treatments and/or interventions. This study was designed to determine the structural changes in the diabetic rat left ventricle 7 weeks after the development of diabetes that may provide valuable information for prospective clinical imaging studies.

A very limited number of MRI studies have been performed to evaluate the pathology of the diabetic heart (1, 2, 29). To the best of our knowledge, this is the first report on the structural characteristics of the diabetic LV wall obtained using the MRI technique. The incomplete closure of the non-beating diabetic LV wall (Figure 2) and the resultant increase in the LV cavity area (Figures 3 and 4) compared to controls suggest that the resiliency of the LV wall is decreased in diabetes. This may result from the stiffness of myocardium due to an increase in passive stretch properties brought about by the accumulation of myocardial collagen (25) that occurs in diabetes (Figures 7 and 8) (28, 36). The decreased resilience of the diabetic myocardium is complemented by the differences in structural (Figure 5) and relaxometric measures (Figure 6) suggesting a stiff myocardium in diabetes. The non-compliance that results from a stiff, fibrotic myocardium may compensate for poor contractility by increased pressure (20) though limiting LV filling during cardiac cycle. Though the mechanism responsible for the myocardial fibrotic changes is not clear, the induction of an intracardiac renin-angiotensin system in streptozotocin diabetic model that ultimately
leads to interstitial fibrosis and impaired myocardial contractility (1) has been proposed.

Changes in magnetic resonance image intensity and relaxation times have been associated with fibrosis in different tissues (15, 19, 40, 43). In this study, the reference normalized mean pixel intensity of the LV wall, was taken as a measure of the fiber content of myocardium. Our result shows that LV wall mean pixel intensity of the diabetic myocardium is increased compared to control (Figure 5) supporting the notion that diabetic myocardium is fibrotic (Figures 7 and 8) (7, 13, 23, 42).

Studies on correlation between T2 relaxation time and tissue fibrosis level or collagen content produced controversial results. Increase in hydroxyproline concentration (as a measure of collagen) were linked to longer T2 relaxation times in spontaneously hypertensive rats (15). Likewise, T2 values were decreased in fibrotic lungs (43), and tumors with abundant collagen accumulation (40). The tissue T2 relaxation time of diabetic LV wall is in concordance with an earlier report (6) suggesting that decreased T2 values are characteristic of tissue fractions with increased surface area in the form of extracellular or intracellular filaments or fibrillar macromolecules. We attribute the decreased T2 relaxation time of diabetic LV wall to increased ventricular wall surface area in the form of interstitial collagen as indicated also by other evidence in our study (Figures 7 and 8). This increased collagen deposition in LV supports the notion that the changes in resiliency and other
structural properties of the diabetic myocardium are, perhaps, due to a direct result of collagen accumulation (31).

One of the cardiac phenotypic manifestations of the streptozotocin rat model of diabetes is LV hypertrophy (12). Diabetes leads to an increase in LV myocardial mass both in the presence (21) and absence of hypertension (33). MRI is currently the technique of choice for precise measurements of ventricular volume (3). As an intrinsically three-dimensional method and therefore independent of geometrical assumption, MRI permits precise non-invasive measurement of LV volume in human (14) and rodents (37). The ratio of LV wall volume to body mass was increased in diabetic heart compared to control in our study, in agreement with previous reports (1) thus suggesting that after 7 weeks of diabetes, the rats developed LV hypertrophy. A Recent MRI study on diabetic individuals reported thicker LV walls compared to healthy subjects (29).

Our study has limited clinical interpretation, since the data were collected on postmortem tissue. However, the described pathologic changes in diabetic rat heart may have a physiological significance as they are related to the biomechanical tissue properties, which may be verified in the beating heart with EKG gated imaging technology.

In summary, this study suggests that high resolution MRI might be an invaluable tool in characterizing the LV myocardial changes in the diabetic animals. Poor resilient properties of the diabetic LV myocardium
complemented with the presence of the hyperintensive areas and decreased T2 relaxation time support evidence of fibrosis in the diabetic heart. In addition, increased LV wall volume to body mass ratio in diabetic rats compared to control suggests the development of heart hypertrophy in diabetes. Overall, high resolution MRI findings in this study corroborate other reports on similar pathological changes of diabetic myocardium measured by other means in this diabetic model. As the-state-of-the-art method, MRI may invaluably contribute to our understanding of the pathology of the diabetic heart.

ACKNOWLEDGMENTS

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**TABLE**

Characteristics of control and 7 week diabetic rats.

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Blood Glucose (mg/dL)</th>
<th>Hemoglobin A1C (%)</th>
<th>Body Mass (g)</th>
<th>Heart Mass to Body Mass Ratio (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (8)</td>
<td>95 ± 1</td>
<td>4.4 ± 0.6</td>
<td>367 ± 11</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td>Diabetic (8)</td>
<td>460 ± 20*</td>
<td>&gt;13*</td>
<td>287 ± 14*</td>
<td>5.3 ± 2.2*</td>
</tr>
</tbody>
</table>

Values presented as means ± SEs; * p < 0.05.
FIGURE LEGENDS

Figure 1. Increase in LV wall volume to body mass ratio in diabetes

Mean LV wall volume to body mass ratio was significantly increased (p=0.0004) in diabetic rats (n=8) compared to control (n=8).

Figure 2. Time series of T1 weighted images of control and diabetic rat hearts.

Representative images of the same transverse section of control (top panel) or diabetic (bottom panel) rat were obtained at 0, 20 and 35 min after the heart stopped beating. Arrowheads point towards the LV. Note that diabetic LV wall failed to close completely (arrow) while control LV had no noticeable cavity at the corresponding time point (image above). The bright filled circle on the top of each image represents a cross section of a plastic tube fill with water that served as an internal reference standard.

Figure 3. Time dependent decrease in LV cavity area in control and diabetic hearts.

LV cavity area was calculated from images similar to those presented in Figure 2. Dotted line corresponds to LV of representative control rat, and solid line corresponds to LV of representative diabetic rat. The control tissue tended to take a rapid course of decrease in LV cavity area whereas the diabetic tissue demonstrated a slower rate. On average, complete closure of control LV occurred within 20 min, and maximal decrease of diabetic LV cavity area was observed 30-35 min after the hearts stopped beating.
Figure 4. Difference in LV cavity area in control and diabetic rats in closed state

Mean LV chamber area was significantly increased (p=0.03) in diabetic rats (n=8) compared to control (n=7). The control LV cavity was almost completely obliterated while a significant portion of diabetic LV cavity remained open. These measurements were done on images taken 35 min after heart beat cessation as the LV cavity areas remained constant at this time point.

Figure 5. Increase in mean pixel intensity of diabetic LV wall

Mean pixel intensity values of LV wall were calculated after adjustment to the pixel intensity values of internal reference standard. The mean pixel intensity values of LV wall were significantly greater (p=0.01) in diabetic rats (n=8) compared to control (n=8).

Figure 6. Decrease in T2 relaxation time of diabetic LV wall

Diabetic rats (n=8) showed significantly lower (p=0.01) T2 relaxation time when compared to control (n=6). Two animals from the diabetic group were not included for image analysis due to a technical failure during the acquisition of T2 images.

Figure 7. Accumulation of collagen in diabetic LV myocardium

Representative histological sections from control and diabetic LV stained with picrosirius red show increased collagen deposition (fibrosis) in the diabetic heart. Control myocardium (left) displays minimal collagen
profiles compared to diabetic (right) which is interspersed by large bundles of collagen (*dark red*) such as the one marked by arrows. Scale bar – 50 μm.

**Figure 8.** Quantitative analysis of collagen fractional area in diabetic and control LV

Collagen fractional area is significantly increased (p=0.02) in diabetic LV (n=3) when compared to control (n=3). The collagen fractional area was calculated as described in methods using images of histological sections stained with picrosirius red, similar to those presented in Figure 7.
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Control

Diabetic

0 min  20 min  35 min
Figure 4

LV cavity area (mm²)

Control

Diabetic

*
Figure 5

LV wall mean pixel intensity (arbitrary units)

Control  Diabetic

*
Figure 7

Control  Diabetic
Figure 8

Collagen area per total area, %

Control  Diabetic

*
CHAPTER 3
Characterization of cardiac cycle abnormalities in diabetic cardiomyopathy using high resolution magnetic resonance imaging

Abstract
Diabetes is a major risk factor for cardiovascular disease. In particular, type 1 diabetes compromises the cardiac function of individuals at a relatively early age due to the protracted course of abnormal glucose homeostasis. The functional abnormalities of diabetic myocardium have been attributed to the pathological changes of diabetic cardiomyopathy. In this study, we used high field magnetic resonance imaging (MRI) to evaluate the left ventricular functional characteristics of streptozotocin treated diabetic Sprague-Dawley rats (8 weeks disease duration) in comparison with age/sex matched controls. Our analyses of EKG gated cardiac MRI scans of the left ventricle showed a 28% decrease in the end-diastolic volume and 10% increase in the end-systolic volume of diabetic hearts compared to controls. Mean stroke volume and ejection fraction in diabetic rats were decreased (48% and 28%, respectively) compared to controls. Further, dV/dt changes were suggestive of phase sensitive differences in left ventricular kinetics across the cardiac cycle between diabetic and control rats. Thus, the MRI analyses of diabetic left ventricle suggest impairment of diastolic and systolic hemodynamics in this rat model of diabetic
cardiomyopathy. Our studies also show that in vivo MRI could be used in the evaluation of cardiac dysfunction in this rat model of type 1 diabetes.

**KEY WORDS:** MRI, type 1 diabetes, cardiac cycle

**ABBREVIATIONS**

DCM – Diabetic cardiomyopathy
LV - Left ventricle
MRI - Magnetic resonance imaging
STZ- Streptozotocin
Introduction

Diabetic cardiomyopathy (DCM) is characterized by a cascade of myocardial changes that occurs in diabetes mellitus with fibrosis, hypertrophy and microcirculatory abnormalities. These cardiovascular complications compromise cardiac performance ultimately resulting in cardiac failure. A high prevalence of cardiac failure is seen in individuals with diabetic cardiovascular complications, with DCM as one of the key determinants (32). DCM is marked by diastolic dysfunction early in the disease progression (3, 5, 7), with its reported occurrence even in patients with well-controlled diabetes in the absence of clinically detectable cardiac disease (19). In addition reports also suggest subtle systolic dysfunction later during the course of diabetes that evades detection with echocardiography (7). Meanwhile it has been suggested that detection of systolic dysfunction might require highly sensitive techniques (3).

Magnetic resonance imaging (MRI) has proven to be a powerful and robust noninvasive imaging modality for structural and function evaluation of the rat heart (33). However, in vivo cardiac MRI studies using diabetic rat models are very limited. For example, Al-Shafei and colleagues (1, 2) performed elaborate MRI studies with a 2 T magnet on streptozotocin- (STZ) diabetic Wistar rats to assess abnormalities of myocardial structure and cardiac cycle events in diabetes.

Understanding the course of pathological events in an appropriate model is the key for developing therapeutic strategies aimed at preventing
the heart failure. In order to evaluate the cardiac performance in vivo we used MRI, a robust technique for resolving cardiac functional information and the reference standard for real time three dimensional visualization of myocardial structure (20, 34). In a previous study, we demonstrated the merits of high resolution MRI in visualizing the diabetic heart and characterized the structural properties of non-beating myocardial tissue in the STZ-diabetic Sprague-Dawley rat (15). As an extension of our previous study, we have characterized the cardiac dysfunction associated with diabetes in this model. In particular, we report quantitative measurements on left ventricular end-diastolic and end-systolic volumes and demonstrate that these parameters are different for STZ-diabetic Sprague-Dawley rats compared to control rats.

**Methods**

**Experimental model of type 1 diabetes**

All procedures on rats were approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee. Twelve male Sprague-Dawley rats aged 2 months with an initial body mass of approximately 250 g were used for the study. The rats were randomly assigned to control or diabetic groups (n = 6 per group). The rats in the diabetic group were given a single intraperitoneal dose of streptozotocin (65 mg/kg, Sigma, St. Louis, MO) in 10 mM sodium citrate buffer, pH 4.5. The control rats were injected with the same volume of vehicle. Diabetes was
confirmed in the former group by measuring the non-fasting plasma glucose levels (≥ 300 mg/dL) two days following the injection. Body mass and plasma glucose levels were recorded once weekly. All rats were given unlimited access to chow and water for the entire duration of study.

MRI procedures

At the end of 8 weeks of diabetes, MRI scans were performed on rats using a 9.4 T horizontal bore scanner (Varian Inc., Palo Alto, CA) and a 60 mm radio frequency volume coil while the rats were under 1.5% isoflurane anesthesia delivered via a nose cone in a mixture of air and oxygen (60% and 40% respectively). A cardiovascular physiological monitoring system (SA Instruments Inc., New York, NY) was used to monitor electrocardiogram (EKG), respiratory status, and body temperature. The physiological status of the rats was continuously monitored to ensure stable heart and respiratory rates during the imaging session. The rats were positioned in the magnet bore for imaging the left ventricle (LV). After confirmation of position with scout images, EKG gated gradient-echo based sequence was used to acquire cine images of cardiac cycle from a short axis view of the heart over 10 equally incremented intervals (labeled phase 1 through 10) with the following parameters: TR/TE = 25/2.44 ms, number of averages = 1, image matrix = 128 pixels x 128 pixels, field-of-view = 60 mm x 60 mm, frame rate = 10, number of slices = 1, and slice thickness = 2.0 mm. The image acquisition was repeated for a total of six times by moving the slice location to completely encompass the LV cavity from the base to the apex.
Image analyses

Images were analyzed using the Image J software (http://rsb.info.nih.gov/ij/) at 300% precision zoom. For the purpose of graphical representation and discussion, the cardiac cycle was apportioned into ten phases. The blood filled LV appeared hyper-intense on images, thus providing excellent contrast for manually tracing the boundary of LV endocardium. For each LV slice, the slice volume of the particular phase was computed by the product of slice thickness and area of the manually traced blood disc using the pixel to area conversion factor 1pixel/0.22mm². The volumes from all six slices acquired during the same phase delay were integrated to obtain the volume of LV at the corresponding phase. These computations were repeated for all ten phases of the cardiac cycle. The phases corresponding to the largest and smallest LV volume were chosen to be representative of end-diastole and end-systole, respectively. The difference between the LV end-diastolic and the LV end-systolic volume was expressed as the stroke volume. The ratio of stroke volume to the end-diastolic volume was expressed as the ejection fraction (%).

The LV wall volume was calculated from the phase 1 reconstruction of all six slices. Briefly, the LV wall was manually traced to obtain the pixel count within the region of interest, and the abovementioned pixel to area conversion factor was used to estimate the LV wall area. The wall volume for each slice was obtained from the product of slice thickness and estimated area (15). The sum of wall volumes from all six slices was expressed as the total LV wall volume.
Glucometry and gravimetry

Plasma glucose, body mass, and glycated hemoglobin (HbA1c) levels were measured at the end of 8 weeks, one day prior to MRI scans. Plasma glucose levels were measured using AccuCheck Active (Roche Diagnostics Co, Indianapolis, IN) meter. HbA1c was determined using antibody based A1CNow meter (Metrika Inc, Sunnyvale, CA). After MRI procedures, rats were euthanized with an overdose of sodium pentobarbital. The hearts were excised, washed in cold phosphate buffered saline, blotted, and weighed.

Statistical analysis

The data were analyzed using SigmaPlot 2000 software. All the values were presented as group means ± SDs. One-sided independent sample Student’s t-test was used to assess the difference between group means. The difference between groups was considered significant when P ≤ 0.05.

Results

Animal model characteristics

The animal glucometric and gravimetric characteristics measured at the termination of experiment, for both control and diabetic groups, are presented in Table 1. Diabetic rats displayed dramatically elevated plasma glucose level when compared to controls. Glycated hemoglobin increased beyond the level of measurable range (>13 %), confirming long-term uncontrolled hyperglycemia in the diabetic rats. The mean body mass value was significantly decreased in diabetes. The mean heart to body mass ratio
was significantly higher in the diabetic group compared to controls (P < 0.05). All these parameters suggested that the rat model used in this study displayed features characteristic of type 1 diabetes.

Left ventricular characteristics

The entire cardiac cycle of all rats was partitioned into 10 equi-duration phases. There was an insignificant (P > 0.05) increase in the mean R-R interval of diabetic rats (242.5 ± 15.0 ms) compared to controls (216.7 ± 28.7 ms).

Gating the data acquisition with strong R wave on the EKG signal resulted in the LV attaining maximum volume at phase 1 of the cardiac cycle in both control and diabetic rats. Hence this maximum was taken as the LV end-diastolic volume (Fig. 1). The mean LV end-diastolic volume in the control group was 579.7 ± 8.4 µl, while the diabetic group showed a significantly (P < 0.01) decreased value of 419.4 ± 5.4 µl (Fig. 2).

The LV end-systolic volume was taken as the lowest cardiac cycle phase volume which occurred at phase 6 in both control and diabetic rats (Fig.1). The mean LV end-systolic volume was 206.7 ± 7.0 µl in the control group and it was significantly (P < 0.01) increased in the diabetic group (226.3 ± 5.3 µl) (Fig.2).

Subsequently, the mean stroke volume was 373.1 ± 8.8 µl in the control rats. Diabetic rats showed a significantly (P < 0.01) decreased value of 193.2 ± 4.5 µl. The mean ejection fraction remained significantly (P < 0.01) lower in diabetic group compared to the controls (46.1 % vs 64.4 %, respectively).
The body mass normalized mean end-systolic and stroke volumes (Table 2) were significantly (P < 0.01) different between control and diabetic rats, while the normalized end-diastolic volume values demonstrated no difference between groups (P > 0.05). The body mass normalized mean LV wall volume however showed an increase (P < 0.01) with diabetes, suggesting LV hypertrophy in the diabetic rats.

The first derivatives of the LV volume with respect to time (dV/dt) during the cardiac cycle phase transitions are presented in Figure 3. The dV/dt values remained significantly different (P < 0.05) between the control and diabetic groups at all but the phase 6-7 (the end-systolic phase) transition suggesting a phase sensitive flow velocity difference between control and diabetic LV in this particular model of DCM.

**Discussion**

The STZ induced diabetic rats used in our experiments are reminiscent of a model of uncontrolled hyperglycemia due to absolute insulin deficiency. The later feature closely captures the metabolic condition of type 1 diabetes. The STZ rat model has been used to study both tissue pathology (22, 25) and therapeutic interventions (18, 26) in type 1 diabetes. There has been a growing interest in the application of MRI to obtain structural and functional information from a variety of tissues including the eye (16), the kidney (23) and the heart (1, 2, 12) that are targeted by diabetic complications.

Functional sensitivity of imaging modalities poses a major challenge for delineation of abnormalities of cardiac function in DCM (3). However,
limitations on functional sensitivity might be lowered with the use of robust non-invasive techniques such as MRI. MRI has evolved as a powerful tool for the evaluation of cardiac function in both humans and experimental animal models of cardiovascular pathology (14, 33). Hence MRI can be applied to the study of cardiac structure and function in DCM. In particular, the MRI study of cardiac abnormalities in DCM provides unique insights into cardiac dynamics that may remain undetected otherwise, with the use of other techniques. For example, echocardiography fails to capture the real state of the tissue due to intrinsic assumptions of tissue geometry (8). Our gravimetric finding of higher heart to body mass ratio in the diabetic group when compared to the control (Table 1) is suggestive of cardiac hypertrophy and altered ventricular geometry in this rat model at 8 weeks of diabetes. The LV wall volume, calculated from MR images of diabetic rats was not significantly different from that of controls. However with body mass normalization, the mean LV wall volume of the diabetic group became significantly higher than the control group. This supports our gravimetric results and indicates LV hypertrophy in this model of DCM, and is in agreement with our earlier findings reported on non-beating diabetic hearts (15).

In this study we utilized EKG gating to correlate the image acquisition with electromechanical end diastole to obtain functional information on the diabetic LV. The use of cine MRI to image the LV along the cardiac short axis provided excellent temporal resolution to delineate volume changes. The high contrast between the blood and endocardium allowed us to perform the planimetry on LV cavities from all images representing the ten phases of cardiac cycle. LV volume calculations showed a significant reduction of 28%
in the mean end-diastolic volume of the diabetic group compared to controls. It has been suggested that the reduction of end-diastolic volume might be the undesirable consequence of an adaptive mechanism of stiff myocardium, in an effort to compensate for poor contractility by increased pressure during experimental cardiomyopathy (13). A stiff myocardium is characteristic of STZ induced diabetes of similar duration (15). However the difference in mean end-diastolic volume between groups disappeared when normalized for their body mass suggesting that the role of abovementioned early diastolic adaptive mechanism is plausible in DCM. Meanwhile the end-systolic volume of the diabetic rats increased 10% compared to controls. This difference between groups was also present after body mass normalization suggesting systolic volume dysfunction in this model. As a consequence of disparity between control and diabetic rats in phase volumes, the stroke volume and ejection fraction declined (48% and 28%, respectively) in the diabetic group compared to controls.

The LV end-diastolic volume, stroke volume and ejection fraction displayed significant changes with diabetes in this study, in accordance with a previous report (2). However, in contrast to our finding of an increase in LV end-systolic volume with diabetes (8 weeks diabetes duration), the previous study (9 weeks diabetes duration) observed no change in this parameter (2). In addition, the difference in body mass normalized end-diastolic volume between groups was insignificant in our study. Meanwhile recent MRI analyses of cardiac function in 8 weeks STZ-diabetic Wistar-Kyoto rats showed no significant difference in the LV end-diastolic volume, end-systolic volume, stroke volume and ejection fraction from age matched controls (10).
These results may reflect the difference in the strain of rats, since this factor has been shown to clearly influence DCM in the STZ model of type 1 diabetes (24). Strain differences exist in their susceptibility to DCM with STZ induced diabetes in rodent models (21, 24) even though the diabetic cardiovascular complications closely imitate the human condition (30). In addition, echocardiographic differences in performance have been detected in the two widely used diabetic rat models, viz. STZ-diabetic Wistar (9) and STZ-diabetic Sprague-Dawley (17) rats. The differences in DCM susceptibility and cardiac performance may underlie the manifestation of cardiac functional abnormalities in these models of type 1 diabetes. Meanwhile stroke volume and ejection fraction were decreased in the STZ-diabetic Sprague-Dawley model used in our study, a finding in agreement with results from the STZ-diabetic Wistar model (2), suggesting that the overall cardiac performance is compromised in both models of type 1 diabetes. Interestingly, in a rat model of type 2 diabetes with DCM, the LV end-diastolic volume remained comparable to the age-matched controls while the end-systolic volume was increased due to poor longitudinal contractility of LV (12). In addition to compromised myocardial contractility in diabetes, the hemodynamic consequences of increased vascular resistance and compromised isoflurane induced vessel wall relaxation may also affect the cardiac cycle systole in these diabetic models (10, 11).

The derivatives of volume with respect to time (dV/dt) of the end-diastole to systolic transition and end-systole to diastolic transition were also significantly different between the diabetics and controls, substantiating the pathological changes involving both active (myocytes) and passive (matrix)
components, respectively, in the dysfunction of diabetic LV (3, 7). The complex pathology of DCM that limits normal ventricular function involves both cardiomyocyte loss (6, 29) and interstitial collagen accumulation (15, 26, 31). The loss of force-producing myocytes may underlie contractile dysfunction whereas the accumulation of interstitial collagen might produce difficulties with passive stretch of myocardium during diastole thereby compromising ventricular relaxation in DCM (4, 6). Accordingly, we speculate that a compromised systolic function in this rat model, suggesting loss of contractility may have been the result of myocyte loss due to apoptosis/necrosis that characterize the middle stage of human DCM (3). Cardiomyocytes demonstrate both impaired contractility and delayed relaxation in mice models of DCM as well (27, 28). Interestingly, the flow velocity of the diabetic group (dV/dt) in this study was not different from the control group at the 6-7 phase transition. This indifference in dV/dt at the end-systolic phase transition suggests that the compromised compliance of the diabetic myocardium is not global, encompassing the entire cardiac cycle. This unexpected result also demonstrates the ability of MRI to provide unique insights that may fail detection otherwise by methods both invasive and non-invasive. However the insignificant difference in dV/dt between the diabetic and control end-systolic phase transition requires cautious interpretation since dV/dt measures are restricted as an indirect index of flow velocity only under assumptions of linear relationship between the variables concerned.

Although our choice of division of the cardiac cycle into equi-duration phases in this study was arbitrary, thus facilitating the evaluation of LV
volume with respect to time as a perfectly smooth function between phases, our results nevertheless agree to a substantial degree, with previous reports on normal and diabetic left ventricular function utilizing a slightly different methodology (2, 11).

**Limitations of the study**

In this study we did not investigate right ventricular dynamics in diabetes. However convincing evidence suggests the impairment of right ventricular function as early as at 6 weeks of diabetes (2). In addition it may be noted that the LV planimetry in our study was accomplished manually which limits quantitative accuracy. The later limitation could be overcome however in future studies by tailoring software suitable for cardiac functional evaluation. Further, isoflurane has been reported to enhance the ejection fraction of rat hearts (10, 11). The latter needs to be taken into account during quantitative cardiac evaluation. However, our study utilized identical anesthetic regimen for both control and diabetic animals to overcome this limitation on the ejection fraction. Finally, in this study we used a single time point of diabetes (8 weeks), although cardiac dysfunction was manifested at this duration of diabetes. Longitudinal investigations will be needed to further characterize the progression of DCM in order to search for effective interventions.
Conclusions

In conclusion, the results from our investigations indicate that the functional manifestation of DCM in the STZ rat model of subchronic diabetes include early diastolic flow adaptation, systolic volume dysfunction and cardiac cycle phase dependent diminution of LV kinetics. Our study also demonstrates that in vivo MRI is capable of evaluating the cardiac dysfunction in this model of diabetes.
Acknowledgments

We are grateful to Dr. Yong-Yue He and Ms. Eileen Roach for excellent technical assistance. We thank Drs. Ken Fischer and Weishi Liu for their valuable comments on the work.
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34. **van den Bosch AE, Robbers-Visser D, Krenning BJ, McGhie JS, Helbing WA, Meijboom FJ, and Roos-Hesselink JW.** Comparison of real-time three-dimensional echocardiography to magnetic resonance
Figure legends

Figure 1- Representative end-diastolic and end-systolic cine MR images of left ventricles (LV) from control and diabetic rats
Typical slices of LV (arrowhead) along the cardiac short axis obtained during end diastole and end systole from age-matched control and diabetic rats (8 weeks diabetes duration) are shown. The blood and the endocardium are clearly distinguished during both phases by the contrast provided by high resolution MRI.

Figure 2 - Left ventricular (LV) volume profiles of control and diabetic rats obtained from MRI reconstruction of LV slices collected throughout the complete cardiac cycle
Graphical representation of LV volumes corresponding to ten equally incremented phases of the rat cardiac cycle is provided. The LV volumes for control (open circles) and diabetic (filled circles) rats were computed from the corresponding MRI scans as described in methods. End-diastole and end-systole correspond to phase 1 and phase 6, respectively, in both the control and diabetic group. LV volumes in all but phases 4 and 5 were significantly different (*, P < 0.05) between groups. Note that the actual cardiac cycle duration was 216.7 ± 28.7 ms in control and 242.5 ± 15.0 ms in diabetic rats, with an insignificant difference (P > 0.05). Hence the cardiac cycle was divided into phases 1 through 10 as discussed in the methods section.
Figure 3 - Cardiac cycle left ventricular (LV) dV/dt values for control and diabetic rats

First derivatives of LV volume with respect to time for control (open bars) and diabetic (filled bars) rats obtained from slopes of secant lines connecting the subsequent phases of cardiac cycle are presented. The x-axis labels refer to phase transitions during the cardiac cycle (for example, ‘1’ corresponds to phase 1-2 transition). The negative dV/dt values correspond to systole and positive values correspond to diastole. The dV/dt values corresponding to all transitions except 6-7 (the end-systolic phase transition) were significantly different between control and diabetic rats (*, P < 0.05).
Table 1 - Glucometry and gravimetry data obtained at 8 weeks of diabetes

<table>
<thead>
<tr>
<th>Rat group (n)</th>
<th>Plasma glucose (mg/dL)</th>
<th>HbA1c (%)</th>
<th>Heart mass (mg)</th>
<th>Body mass (g)</th>
<th>Heart to body mass ratio (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (6)</td>
<td>110 ± 15</td>
<td>4.7 ± 0.2</td>
<td>1,305 ± 80</td>
<td>420 ± 20</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>Diabetic (6)</td>
<td>545 ± 45*</td>
<td>13*#</td>
<td>1,132 ± 81</td>
<td>292 ± 35*</td>
<td>3.9 ± 0.5*</td>
</tr>
</tbody>
</table>

* P < 0.05 when diabetic rat values were compared to controls.

# - since all diabetic rats had HbA1c levels higher than detectable by the method used, we used the highest detectable value (13%) for statistical purposes.
Table 2 - Left ventricular (LV) characteristics normalized to body mass

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV wall volume (mm³/g)</td>
<td>1.12 ± 0.30</td>
<td>2.00 ± 0.30*</td>
</tr>
<tr>
<td>End-diastolic volume (µl/g)</td>
<td>1.38 ± 0.10</td>
<td>1.46 ± 0.20</td>
</tr>
<tr>
<td>End-systolic volume (µl/g)</td>
<td>0.49 ± 0.01</td>
<td>0.79 ± 0.10*</td>
</tr>
<tr>
<td>Stroke volume (µl/g)</td>
<td>0.89 ± 0.01</td>
<td>0.67 ± 0.10*</td>
</tr>
</tbody>
</table>

* P < 0.05 when diabetic rat values were compared to controls.
List of Figures

Figure 1

<table>
<thead>
<tr>
<th></th>
<th>End diastole</th>
<th>End systole</th>
</tr>
</thead>
<tbody>
<tr>
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<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>Diabetic</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Figure 2

Cardiac cycle phase

LV volume (µl)

Control
Diabetic

* *
Figure 3

![Cardiac cycle phase transition graph]

- dV/dt (µl/ms)
- Control
- Diabetic

Cardiac cycle phase transition
CHAPTER 4

Characterization of exercise-induced benefits in diabetic cardiomyopathy using high resolution magnetic resonance imaging

ABSTRACT

Diabetic cardiomyopathy is a distinct myocardial complication of the catabolic state of untreated insulin-dependent diabetes mellitus in the streptozotocin-induced diabetic rat. Exercise training has long been utilized as an effective adjunct to pharmacotherapy in the management of the diabetic heart. However the in vivo functional benefit(s) of the training programs on cardiac cycle events in diabetes are poorly understood. In this study, we used three groups of Sprague-Dawley rats: sedentary control (SC), sedentary diabetic (SD), and exercised diabetic (ED), to assess the effects of endurance training on the left ventricular (LV) cardiac cycle events in diabetes. At the end of 9 weeks of exercise training, non-invasive cardiac functional evaluation was performed by using high resolution MRI (9.4 T). An EKG gated cine imaging protocol was used to capture the LV cardiac cycle events through 10 equally incremented phases. The cardiac cycle phase volumetric profiles showed favorable functional changes in ED group including a prevention of decreased end-diastolic volume and attenuation of increased end-systolic volume that accompanies sedentary diabetes. This MRI study confirms the prevailing evidence from earlier in vitro and in vivo invasive procedures that exercise training benefits cardiac function in this model of diabetic cardiomyopathy despite the extreme catabolic state of the animals.
KEY WORDS: Cardiac cycle, Diabetic cardiomyopathy, Exercise training, Left ventricle, MRI.

ABBREVIATIONS:
DCM – Diabetic cardiomyopathy
EKG - Electrocardiogram
LV – Left ventricle
MRI – Magnetic Resonance Imaging
STZ – Streptozotocin
INTRODUCTION

The diabetic heart is targeted by both coronary and non-coronary pathology that eventually result in cardiac failure (2, 10). The failure to maintain tissue glucose homeostasis compromises cardiac structure and function in humans and experimental animal models of diabetes mellitus (16, 31). The myocardial damage due to chronic hyperglycemia is a key feature of diabetic cardiomyopathy (DCM) and occurs as a result of abnormal metabolic and cell signaling pathways (9, 23, 34). In addition to myocardial damage, DCM is manifested by deposition of interstitial collagen in the myocardial tissue (20, 32). These pathological features compromise the normal contractility and compliance of the diabetic heart (1, 21, 37). DCM is also accompanied by worsening of the recovery of heart function after an ischemic insult (39). Meanwhile, the cardiac dysfunction in diabetes is amenable to therapeutic interventions, for example, pharmacotherapy and exercise therapy (11, 12, 23).

Exercise has long been used as an effective cardioprotective agent in diabetes (18, 27, 33, 36). The structural and functional abnormalities of the diabetic heart respond favorably to exercise training. For example, at the ultrastructural level, benefits of exercise training on the diabetic myocardium manifest as attenuation of a) mitochondrial swelling and disruption b) increase in cytoplasmic area and c) increased collagen fiber cross-sectional area (33). Endurance training increases the cardiac output in diabetic rats under high preload conditions (8). Training prevents the cardiac autonomic nervous dysfunction in diabetes (7) and improves cardiac function in diabetes without benefiting plasma glucose and cholesterol levels (17). Although these
results suggest a favorable role for exercise training on the diabetic heart, the exercise induced benefits on cardiac cycle events are not apparent from earlier in vitro or invasive in vivo studies. The importance of obtaining information on the effects of exercise training on the diabetic cardiac cycle events is underscored by the evidence that DCM is associated with both diastolic and systolic left ventricular (LV) dysfunction resulting in abnormalities of cardiac cycle events (1, 19).

Thus the primary objective of this study was to characterize the effects of exercise training on the profound cardiac dysfunction noted in the catabolic state of untreated insulin-dependent diabetes. To overcome the interpretive limitations inherent to in vitro and invasive in vivo procedures, and study the cardiac cycle events under the relevant biomechanical constraints of the highly dynamic chest cavity, we utilized high resolution magnetic resonance imaging (MRI) for the evaluation of exercise induced effects on the diabetic heart. The use of MRI in our investigation was further motivated by the following advantages: a) the confounding effects of thoracotomy and deep anesthesia that often accompany an invasive approach can be avoided (29); b) MRI can capture the cardiac pump function in small animals despite their intrinsically high heart rates (38); c) being intrinsically three dimensional, MRI computations overcome the limitations posed by geometric assumptions that facilitate volumetry in other commonly used non-invasive procedures, for example, echocardiography (13). In addition to these unique advantages the versatility of use and accuracy of measurements made possible by MRI make it ideally suited for cardiac volumetric measurements in heart failure (28). MRI has also been proposed as the
technique of choice for the assessment of treatment effects in clinical studies due to its accuracy, low inter-observer variability and the potential to reduce sample size substantially (3).

METHODS

Animal model of diabetes

All procedures on animals were approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee. Twelve male Sprague-Dawley rats (Harlan, Indianapolis, IN) aged 2 months with an initial mean body mass of 250 g were used for the study. The rats were randomly assigned to one of the following three groups (n = 4 per group): 1) sedentary non-diabetic control (SC); 2) sedentary diabetic (SD); and 3) exercised diabetic (ED). The rats in the diabetic groups were given a single intraperitoneal injection of streptozotocin (STZ; 65 mg/kg, Sigma, St. Louis, MO) in 10 mM sodium citrate buffer, pH 4.5. The SC group was treated with the same volume of vehicle. Diabetes was confirmed in the SD and ED groups by measuring the non-fasting plasma glucose level (≥ 300 mg/dl) two days following the injection. Body mass and plasma glucose levels were recorded weekly. All rats were given unlimited access to chow and water for the entire duration of the study.

Exercise training protocol

The details of the treadmill endurance training protocol used in this study were presented in a previous report (33). Briefly, the rats in the ED group underwent pre-training for a period of 2 weeks prior to diabetes
induction followed by 9 weeks of exercise with diabetes. The training intensity and duration began at 15 m·min⁻¹ for 5 minutes on day 1 and progressed to 20 m·min⁻¹ for 50 min by the end of week 2. After the induction of diabetes all rats in the ED group maintained the intensity and duration of 20 m·min⁻¹ for 60 min per day for the remaining 9 weeks. No electric shock was used to stimulate animals to run. Instead, uncooperative rats were encouraged to run by occasional gentle manual brushing on their backs. To rule out the confounding effects of non-training factors in the training environment, all animals in the SC and SD groups were handled everyday and subjected to the noise of running treadmill by placing their cages next to the exercising animals. We have shown in earlier reports that this training intensity is sufficient to induce favorable structural changes in the diabetic cardiac muscle (33), although it is inadequate to produce an increase in skeletal muscle citrate synthase levels (35).

Physical activity challenge

After 9 weeks of training, the efficiency of metabolically active lean tissue (skeletal and cardiac muscles) to sustain a physical challenge was tested in all three groups, by scoring the animals on a 25 minutes running challenge. The run intensity was increased at the rate of 1 m·min⁻¹ in five steps until the speed of 25 m·min⁻¹ was reached (Step 1: 5 m·min⁻¹ at time zero; Step 2: 10 m·min⁻¹ at 5 minutes; Step 3: 15 m·min⁻¹ at 10 minutes; Step 4: 20 m·min⁻¹ at 15 minutes, Step 5: 25 m·min⁻¹ at 20 minutes). The running duration at each step was maintained at 5 minutes. The following activity capacity scores were assigned: 0 - no run; 0.5 – complete run for 5 min; 1.0 – complete run for 10
min; 1.5 – complete run for 15 min; 2.0 – complete run for 20 min; 2.5 – complete run for 25 min. In case of incomplete runs between any two of the 5 steps, a full score corresponding to the lower step was assigned. The electrical shocker was used during this procedure and a run was scored incomplete when the animal spent more than 30 s on the shocker.

**MRI procedures**

At the end of 9 weeks of diabetes, cardiac MRI was performed using a 9.4 T horizontal bore scanner (Varian Inc., Palo Alto, CA) and 60 mm radio frequency volume coil. The anesthetic dose of 1.5% isoflurane in a mixture of air and oxygen (60% and 40%, respectively) was estimated as the minimum amount required to prevent body motion while administered via a nose cone (19). Stable electrocardiogram (EKG), respiration, and temperature profiles were ensured during scanning sessions via a dedicated small animal vital signs monitoring system (SA instruments Inc., New York, NY). Inside the magnet bore, the rats were placed on a custom built plexiglass sled designed for optimal imaging of the LV. After confirming heart position with an initial set of scout images, EKG gated gradient echo based cine images of LV were captured from a short axis view of the heart. The LV was spatially resolved into 6 slices (Figure 1). The cardiac cycle was temporally resolved into 10 equally incremented phases. The following settings were used for image acquisition: TR/TE = 25/2.44 ms, number of averages = 1, field of view = 60 x 60 mm, image matrix = 256 x 256, slice thickness = 2.0 mm.

**Image analyses**
Images were analyzed with Image J, a freely-downloadable Java-based environment (http://rsb.info.nih.gov/ij). For each LV, the phase stereometry was performed by integrating the area of the sliced blood disc (bright region enclosed by the endocardium) with respect to slice thickness from the apex to base using the Cavalieri principle (22) (see Figure 1). The pixel to area conversion factor of 1 pixel/0.11 mm² was used for all computations. These computations were repeated for all ten phases of the cardiac cycle. The phases corresponding to the largest and smallest LV volume were chosen to be representative of end-diastole and end-systole, respectively (19). The difference between the end-diastolic and end-systolic volume was expressed as the stroke volume. The ratio of stroke volume to the end-diastolic volume was expressed as the ejection fraction (%). The product of stroke volume and heart rate was expressed as the LV output. In addition to the cardiac cycle volumetry, the LV myocardial volume was also estimated in all the three groups from the reconstruction of all six slices from the end-diastolic phase.

**Glucometry and gravimetry**

Plasma glucose levels were measured using the AccuCheck Active meter (Roche Diagnostics Co, Indianapolis, IN). Glycated hemoglobin (HbA1c) levels were measured one day prior to MRI scans using antibody based A1cNow meter (Metrika Inc, Sunnyvale, CA). After MRI procedures, rats were killed with an overdose of sodium pentobarbital. The hearts were excised, washed in cold phosphate buffered saline, blotted, and weighed.

**Statistical analyses**
Statistical analyses were performed with SPSS (version 11.0). Significant differences between the groups on measures of cardiac function were tested with a one-way analysis of variance (ANOVA). When prompted by group differences, post-hoc pair-wise multiple comparisons were performed using Tukey’s honestly significant difference (HSD) test with the level of significance held at $P \leq 0.05$. The following identifiers were designated for pair-wise comparisons: $a =$ significantly different from SD group, $b =$ significantly different from ED group, $c =$ significantly different from SC group. All results were presented as means ± standard errors of the mean (SEM).

**RESULTS**

**Glucometry, gravimetry, and physical activity challenge**

Table 1 summarizes the results of glucometry and gravimetry at 9 weeks diabetes duration, prior to MRI. Glucometric evidence showed uncontrolled hyperglycemia in both diabetic groups, SD and ED. The HbA1C levels were no different between the SD and ED groups. Training did not produce a significant change in body mass of the ED group compared to the SD group. Meanwhile the heart to body mass ratio was significantly increased in both SD (25%) and ED (26%) groups compared to the SC group suggesting the possibility of cardiac hypertrophy in the former groups. The confounding effect on cardiac hypertrophy, however, due to the loss of body mass in the diabetic groups should be taken into consideration.

The mean activity capacity scores were 2.5 for the SC and ED groups. The mean activity capacity score of the SD group was 1.0, thus indicating
compromised ability to sustain physical activity due to the diabetic state in the absence of training.

Cardiac cycle events

The mean R-R interval (ms) of the three groups was $210.0 \pm 10.0$ for SC; $212.5 \pm 12.5$ for SD; and $235.0 \pm 15.0$ for ED. The cardiac cycle cine image acquisition gated with EKG strong R wave resulted in a maximum LV volume at phase 1 in all the groups. Hence it was taken as the end-diastole (Figure 2). Meanwhile the minimum LV volume occurred at phase 6 in all the groups, and was labeled as end-systole (Figure 2). The cardiac cycle was apportioned into 10 phases of similar duration in all three groups thus allowing comparison of LV volume changes across the cardiac cycle (Figure 3). The statistical significance of difference between groups in the specific phase volumes of the LV cardiac cycle phase volumetric profiles is presented in the table accompanying Figure 3. Overall, the time dependent LV volumetric profile of the exercise trained diabetic animals closely followed the profile of sedentary controls. Diabetes accompanied by a sedentary lifestyle triggered a major shift in the LV volumetric profile compared to the non-diabetic sedentary lifestyle.

There was a 35% decrease in mean LV end-diastolic volume in the SD group compared to the SC group. Exercise training prevented this decrease in diabetes to a considerable extent with only 6% decrease compared to the non-diabetic sedentary lifestyle (Figure 4a). In addition to the decrease in end-diastolic volume, the LV end-systolic volume was increased 8% in the SD group compared to the SC group. Meanwhile, the end-systolic volume in the
ED group was decreased 4% compared to the SC group (Figure 4b). Accordingly the LV stroke volume and ejection fraction were decreased (55% and 32%, respectively) in the SD group with a decrease of (8% and 2%, respectively) in the ED compared to the SC group (Figure 4c and 4d, respectively). The LV output decreased by 58% in SD group and 25% in the ED group compared to the SC group, indicating that exercise training was able to prevent the decline in LV output that accompanies a sedentary lifestyle in diabetes (Figure 4e). It should be noted that the difference between groups in the LV output was the result of differences in the stroke volume since the heart rate was not different between groups as mentioned earlier.

Table 2 summarizes the body mass normalized LV cardiac cycle parameters along with myocardial volume for all three groups of animals. The body mass normalization however abrogated the difference in all the aforementioned cardiac cycle parameters except the end-systolic volume between the SC and SD groups.

**DISCUSSION**

The major findings of this series of noninvasive cardiac functional evaluation in diabetes is that the deterioration of cardiac cycle events at the sub-chronic stage of DCM could be prevented (i.e., parameters returning toward control levels yet with a significant difference from controls) and some aspects of cardiac function could be improved (i.e., parameters indistinguishable from control values) with long-term exercise training. Hence exercise training might prove to be an effective adjunct to other
possible modes of prevention/treatment of DCM. Specifically, the decrease in end-diastolic volume that accompanies DCM was prevented by endurance training. In addition, exercise training also improved the increase in end-systolic volume that occurs in sedentary diabetes. Accordingly, the deterioration of stroke volume was prevented and ejection fraction was improved with exercise training in diabetes. The decrease in LV output also improved with exercise training in diabetic animals. These results were suggestive of an overall improvement of the abnormalities of cardiac cycle events in the diabetic animals with exercise training. This first MRI evaluation of exercise induced benefits also confirms the previous results of other invasive procedures. For instance, cardiac functional deficits including a reduced cardiac output under the baseline working mode of an isolated LV working heart preparation attenuated with exercise training in the spontaneously diabetic bio-breeding rats (39).

The major advantage offered by the animal model used in this study is that it allows us to study the effect of therapeutic interventions on the diabetic heart disease chiefly due to DCM without the overlapping effects of cardiac macrovascular disease (15). However, we must recognize that, in addition to hyperglycemia, the extreme catabolic state of the animals is likely to contribute to the myocardial dysfunction in this model. Hence the exercise training induced changes in the diabetic LV in this study can be interpreted as the effects of training on the functional complications of DCM. The insignificant difference in the mean R-R interval between the three groups allowed us to compare their volumetric profiles against a common phase domain. Thus our results confirm the previous report that an 8 weeks
endurance training program in the same animal model of diabetes failed to alter the heart rate of trained animals (14). The lack of difference in baseline R-R intervals in SD and ED groups rule out the confounding effects of defective cardiac chronotropism on the cardiac cycle events evaluated in this study.

The body mass and plasma glucose characteristics of the diabetic rats in this study were reminiscent of a model of uncontrolled type 1 diabetes. The increased heart to body mass ratio in the SD and ED groups compared to the SC group were suggestive of cardiac hypertrophy in the former groups. LV hypertrophy in the diabetic groups was confirmed by direct myocardial volumetry (Table 2). Due to the loss of body mass, however, in the diabetic groups, the results suggesting cardiac hypertrophy must be interpreted with caution. The insignificant difference between the SD and ED groups in HbA1c levels reiterated previous results that plasma glucose control cannot be achieved by exercise training alone in type 1 diabetes (24, 39, 41). Exercise training also failed to significantly alter the body mass in the diabetic rats. These results suggest that the animal model used in this study was able to recapitulate the general features of type 1 diabetes however with extreme catabolic state. Hence these results can be compared to similar intervention studies that utilized in vitro or invasive in vivo methods for cardiac functional assessment in the past (4, 30, 39). Although the moderate endurance training protocol used in this study was previously shown to attenuate the myocardial structural defects in this rat model of diabetic cardiomyopathy (33), whether the exercise induced structural benefits on the diabetic myocardium were
accompanied by improvements in the cardiac cycle events in vivo was not clear until this report.

The defect of cardiac pump function in diabetes has been attributed to the deterioration of function in the active components (for example, myocardial contractile and Ca\(^{2+}\) signaling proteins) with questionable changes in the passive components (for example, myocardial collagen) (41). Meanwhile we speculate that the cardiac pump defects in our SD group might be the result of functional deterioration involving both the passive and active components (19, 20). The attenuation of cardiac pump defects in ED group was suggestive of therapeutic benefits on both the passive and active components in this study. For example, an interesting observation was noted with the exercise induced benefits on end-diastolic volume in this study that parallel the benefits conferred by the angiotensin converting enzyme inhibitor captopril in diabetes (1). The similarity between cardiac functional benefits resulting from two different modes of treatment (exercise therapy and captopril therapy) suggests a shared therapeutic mechanism, perhaps at the level of myocardium at this stage of DCM. The prevention of aberrant LV end-diastolic volume in diabetes with exercise training suggests a role for exercise in improving the compliance of diabetic left ventricle. Confirmation of identical effect on this parameter with a drug (captopril) in diabetes, and the possibility of a shared therapeutic mechanism between drug and training in the form of reduced interstitial fibrosis (1, 33) suggest that the trained group in this study might have benefited from the improvements in the passive components of cardiac pump function. Meanwhile the exercise induced conditioning effect might be speculated for the improvement in
active components of cardiac pump function in diabetes, chiefly due to the benefits of training in improving the contractility of LV in diabetic animals (25, 40). Exercise training in our study improved the systolic volume dysfunction that occurred with sedentary lifestyle in diabetes. The exercise-induced benefits on systolic function in diabetes have been shown to manifest as efficient contractility of cardiomyocytes (6) and energy mobilization from mitochondria (24, 26). These earlier results interpreted in view of our non-invasive evidence for improved cardiac pump function provide a renewed explanation for the possibility of exercise-induced benefits on both diastolic and systolic cardiac pump function in diabetes. These results also demonstrate the enormously beneficial effect of exercise on this otherwise nearly fatal catabolic condition.

All the body mass normalized parameters of the cardiac cycle events in the SD group except the end-systolic volume approached control values in our study. This was not unexpected as the SD group used in this study had uncontrolled diabetes along with body wasting. However, body mass normalized cardiac functional profiles require cautious interpretation since body mass does not satisfy the assumptions of homoscedasticity and hence fails as an appropriate scaling variable for cardiac performance parameters (5). This interpretive limitation on body mass normalized cardiac performance parameters between groups was overcome however with the employment of a treadmill running task at the termination of experiments as a means to discern the ability of metabolically active lean tissue to sustain physical challenge. If we assumed that the insignificant difference between the body mass normalized parameters of our SC and SD groups may not be
the optimal indicator of cardiac function, then a challenge that would engage the metabolically active lean tissue of the animal would not be sustained by the presumed suboptimal cardiac performance of the SD group unlike the SC group. The results of physical activity challenge indicated that the SD group (activity capacity score of 1.0) was not able to overcome the challenge while the SC group (score of 2.5) was successful although both groups were inexperienced runners. The failure of SD group on the activity challenge in the face of insignificant differences in the body mass normalized cardiac parameters between the SC and SD group is evidence for a poor overall cardiac performance in the SD group. Exercise training in diabetes was however able to overcome the physical activity challenge although the catabolic state and the severity of diabetes in the ED group would have prevented it presumably from exceeding the functional capacity of the SC group had we intensified the challenge further.

In summary, the first MRI evaluation, to our knowledge, of exercise induced benefits on the diabetic heart demonstrates that moderate endurance training, in addition to attenuating cardiac structural defects (33) also improves cardiac cycle volume and hemodynamic profiles. Interestingly, the functional benefits on the diabetic heart occurred in the absence of plasma glucose control or significant benefits on the body mass of the diabetic animals, confirming the results obtained previously via invasive approaches (4, 17, 30, 39). Taken together, these physiological results nevertheless suggest the possibility of locally occurring molecular correlates of training-induced benefits on cardiac pump function independent of systemic glucose homeostasis in diabetes mellitus. The benefits of training on the efficacy of
diabetic cardiac function derived from the use of various animal models however are not without translational limitations to human type 1 diabetes. The major limitation of this study is the acute diabetic state, including the extreme catabolic state of the animals accompanied by permanent hyperglycemia and loss of body mass, which does not represent the current clinical course of long-term diabetes that elicits impaired myocardial function. Hence future experiments will require studies in hyperglycemic animals that have reasonable maintenance of body mass to provide a valid model to address cellular and molecular mechanisms of DCM. This study was however able to demonstrate the tremendous beneficial effects of exercise, despite the catabolic state of the rats, and further verified MRI as a promising tool for the non-invasive evaluation of cardiac function.
ACKNOWLEDGMENTS

We are grateful to Dr. Yong-Yue He for excellent technical assistance. We greatly appreciate Dr. Lisa Stehno-Bittel for critical reading of the manuscript. We thank Drs. Ken Fischer, Wen Liu, and Milena Stanislavova for their valuable comments on the work. We thank Mr. Mukul Mukherjee for assistance with data analysis. This work was supported by American Heart Association Scientist Development Award, Lied Endowed Basic Science Pilot Research Grant, and the Department of Commerce SABIT Grant to IVS, and American Heart Association Fellowship to RL.
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FIGURE LEGENDS

FIGURE 1. Illustration of the LV spatial resolution used for the cine imaging procedure. LV was spatially resolved into 6 slices. The phase volume ‘Vp’ for each of the 10 cardiac cycle phases was computed by integrating the area (A(s)) of the blood disc in each of the slices with respect to slice thickness (X) from the apex (a) to the base (b) of LV as illustrated in this LV sagittal section and given by \( V_p = \int_a^b A(s) \, dx \).

FIGURE 2. Representative cardiac cycle phase images of the third LV slice (from apex) from sedentary control (A), sedentary diabetic (B) and exercised diabetic (C) groups. The cardiac cycle was temporally resolved into ten equally incremented phases indicated by the numbers above the images. The end-diastole occurred at phase 1 and end-systole occurred at phase 6 in all three groups.

FIGURE 3. The cardiac cycle phase volumetric profiles from the sedentary control (clear circles), sedentary diabetic (black circles), and exercised diabetic (grey circles) groups. The accompanying table provides the details of statistical difference in phase volume between the groups for all 10 phases.

FIGURE 4. Volumetric indices of cardiac cycle events from sedentary non-diabetic control (SC), sedentary diabetic (SD) and exercised diabetic (ED) animals. The left ventricular end-diastolic (a) and end-systolic (b) volumes of all three groups were obtained directly from the phase volumetric profiles in figure 3. The derived indices viz. left ventricular stroke volume (c), left
ventricular ejection fraction (d) and left ventricular output (e) were indicative of cardiac performance differences between groups.
TABLE 1
Glucometry and gravimetry

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Plasma glucose (mg/dL)</th>
<th>Hemoglobin A1c (%)</th>
<th>Body mass (g)</th>
<th>Heart to body mass ratio (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary control</td>
<td>117 ± 5\textsuperscript{a,b}</td>
<td>4.8 ± 0.3\textsuperscript{a,b}</td>
<td>434 ± 12\textsuperscript{a,b}</td>
<td>3.03 ± 0.16\textsuperscript{a,b}</td>
</tr>
<tr>
<td>Sedentary diabetic</td>
<td>557 ± 18\textsuperscript{c}</td>
<td>13\textsuperscript{c,#}</td>
<td>265 ± 20\textsuperscript{c}</td>
<td>3.79 ± 0.17\textsuperscript{c}</td>
</tr>
<tr>
<td>Exercised diabetic</td>
<td>569 ± 30\textsuperscript{c}</td>
<td>11.5 ± 1.0\textsuperscript{c}</td>
<td>272 ± 28\textsuperscript{c}</td>
<td>3.82 ± 0.11\textsuperscript{c}</td>
</tr>
</tbody>
</table>

Values presented as means ± SEMs. For the description of identifiers a, b, and c see methods section. # - since all sedentary diabetic rats had HbA1c levels higher than detectable by the method used, we used the highest detectable value (13%) for statistical purposes.
**TABLE 2**

Body mass normalized values of LV volume characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sedentary control</th>
<th>Sedentary diabetic</th>
<th>Exercised diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial volume (mm$^3$/g)</td>
<td>1.14 ± 0.12$^{a,b}$</td>
<td>2.06 ± 0.30$^c$</td>
<td>2.05 ± 0.10$^c$</td>
</tr>
<tr>
<td>End-diastolic volume (μl/g)</td>
<td>1.49 ± 0.06$^b$</td>
<td>1.59 ± 0.15$^b$</td>
<td>2.19 ± 0.17$^{a,c}$</td>
</tr>
<tr>
<td>End-systolic volume (μl/g)</td>
<td>0.48 ± 0.01$^{a,b}$</td>
<td>0.86 ± 0.06$^c$</td>
<td>0.75 ± 0.05$^c$</td>
</tr>
<tr>
<td>Stroke volume (μl/g)</td>
<td>0.97 ± 0.05$^b$</td>
<td>0.73 ± 0.09$^b$</td>
<td>1.44 ± 0.12$^{a,c}$</td>
</tr>
<tr>
<td>LV output (μl/min/g)</td>
<td>0.28 ± 0.02</td>
<td>0.21 ± 0.02$^b$</td>
<td>0.37 ± 0.03$^a$</td>
</tr>
</tbody>
</table>

Values presented as means ± SEMs. For the description of identifiers a, b, and c see methods section.
# Supplement table for FIGURE 3

Group differences in cardiac cycle phase volumes

<table>
<thead>
<tr>
<th>Cardiac cycle phase</th>
<th>SC</th>
<th>SD</th>
<th>ED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1</td>
<td>a, b</td>
<td>b, c</td>
<td>a, c</td>
</tr>
<tr>
<td>Phase 2</td>
<td>a</td>
<td>b, c</td>
<td>a</td>
</tr>
<tr>
<td>Phase 3</td>
<td>a</td>
<td>b, c</td>
<td>a</td>
</tr>
<tr>
<td>Phase 4</td>
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<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Phase 5</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Phase 6</td>
<td>a</td>
<td>b, c</td>
<td>a</td>
</tr>
<tr>
<td>Phase 7</td>
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</tr>
<tr>
<td>Phase 8</td>
<td>a</td>
<td>b, c</td>
<td>a</td>
</tr>
<tr>
<td>Phase 9</td>
<td>a</td>
<td>b, c</td>
<td>a</td>
</tr>
<tr>
<td>Phase 10</td>
<td>a</td>
<td>b, c</td>
<td>a</td>
</tr>
</tbody>
</table>

See methods section for the description of the identifiers a, b, and c.

ns – not significant
List of Figures

Figure 1
Figure 3

Cardiac Cycle Phase

LV Volume (µl)

Sedentary Control
Sedentary Diabetic
Exercised Diabetic
Figure 4a

End-diastolic Volume [μL]

- SC
- SD
- ED

Legend:
- a,b
- b,c
- a,c
Figure 4e

LV Output (l/min)

SC  SD  ED

a,b  b,c  a,c

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CHAPTER 5
Characterization of cardiomyopathy and exercise-induced benefits on cardiac function in an autoimmune-model of type 1 diabetes

ABSTRACT
Our previous studies have clearly demonstrated the abnormalities of cardiac structure and function in a streptozotocin (STZ) induced model of type 1 diabetes. These studies also underscored the role of exercise training in the amelioration of both structural and functional deficits that occurred as a result of cardiomyopathy in the STZ-induced diabetic model. The current investigation was performed to verify the pathological manifestations of diabetic cardiomyopathy and the effects of endurance training, in an autoimmune model of type 1 diabetes. For this purpose, the Bio-Breeding Diabetes Resistant (BBDR) rats were used. A series of physiological, microscopic and biochemical studies were performed in the following 4 groups (n=8/group) of rats: sedentary non-diabetic control (SC), sedentary diabetic (SD), exercised non-diabetic control (EC), and exercised diabetic (ED). The results obtained from left ventricular (LV) myocardium revealed compromised structure and function in SD group, reminiscent of diabetic cardiomyopathy (DCM), despite insulin treatment. Decreased ejection fraction and mitochondrial fractional area along with an increase in myocardial collagen fractional area were characteristic of DCM in SD rats. The benefits of exercise training were manifested as the a) prevention of
reduced LV ejection fraction b) prevention of decrease in LV mitochondrial fractional area and c) prevention of an increase in LV myocardial interstitial collagen accumulation. Immunoblotting for myocardial protein kinase C βII, an enzyme whose activation has been implicated in the myopathic phenotype of the diabetic heart, revealed decreased expression with exercise training. In summary, our results from these physiological, histological, ultrastructural and biochemical studies suggest that a) BBDR rats manifest cardinal features of DCM despite insulin treatment, b) exercise training attenuates structural and functional cardiac abnormalities in this model of autoimmune diabetes, and c) one of the molecular correlates of exercise induced benefits on cardiac function in type 1 diabetes might be an underexpression of PKC βII, a key protein involved in the pathology of DCM.
**KEY WORDS:** Cardiac cycle, Diabetic cardiomyopathy, Exercise training, Left ventricle, PKC βII.

**ABBREVIATIONS:**

BBDR rat – Bio-Breeding Diabetes Resistant rat
DCM – Diabetic cardiomyopathy
LV – Left ventricle
PKC βII – Protein kinase C beta II isoform
INTRODUCTION

Diabetes mellitus profoundly affects the cardiovascular homeostasis of the individual (5). The major cause of mortality and morbidity in individuals with diabetes is cardiovascular disease (12). In their 1972 report of individuals with long-standing diabetes, Rubler et al. (27), speculated on the presence of cardiomyopathy. This speculation was later extended by the Framingham study group to explain the excessive number of insulin treated diabetic individuals afflicted with cardiac failure that was not entirely due to coronary heart disease (18). Later reports independently confirmed the presence of cardiomyopathy independent of the coronary artery disease in individuals with both type 1 and type 2 diabetes (1, 31).

The cardiac effects of chronic hyperglycemia manifest as abnormalities of both the active (e.g., mitochondria) and passive (e.g., interstitial fiber accumulation) functional apparatus of the cardiac pump ultimately resulting in cardiac failure (10). The pathological condition of the myocardium preceding overt cardiac failure has been described as diabetic cardiomyopathy (DCM) (1).

Exercise training is of particular interest in both the prevention and treatment of DCM since a number of studies clearly indicate a beneficial role for exercise in the amelioration of cardiac abnormalities (4, 8, 9, 19). However interpretation of results from the abovementioned studies is limited due to the genetic heterogeneity of diabetic models, difference in the mode of diabetes induction, exercise dosage, presence or absence of insulin treatment, and differences in outcome variables. Furthermore a molecular correlate for exercise-induced benefits in DCM is yet to be uncovered.
A critical pathosignaling pathway that links hyperglycemia to diabetic complications involves the activation of protein kinase C (PKC) (21). In the diabetic heart, however, activation of PKC βII, a conventional PKC isoform, underlies the pathogenesis of DCM (16). In fact, targeted overexpression of PKC βII in the myocardium causes cardiomyopathy with interstitial fibrosis and hypertrophy (3, 32, 33), suggesting a critical role for PKC βII in the pathology of cardiomyopathy.

METHODS

Induction of type 1 diabetes

All animal procedures were approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee. Thirty two BBDR rats (Biomedical Research Models Inc., MA) aged 23-25 days were randomized to the following 4 groups (n=8/group): 1) sedentary non-diabetic control (SC), 2) sedentary diabetic (SD), 3) exercised non-diabetic control (EC), and 4) exercised diabetic (ED). Autoimmune diabetes was induced in SD and ED group by injection of anti-RT6 monoclonal antibody DS4.23 hybridoma supernatant (2 ml/day for 5 days/week), kindly donated by Dr. Dale Greiner (University of Massachusetts). The hybridoma was combined with a non-specific immune system activator polyinosinic-polycytidylic acid (Poly I:C, Sigma Chemical, St. Louis, MO), 5 μg/g body mass, 3 days/week. SC and EC animals were injected with vehicle. After confirmation of high plasma glucose levels (>200 mg/dL) for 3 consecutive days, the rats were considered diabetic and treated with a 1:1:1 mixture (100-200 μl, depending on the plasma glucose level) of regular, NPH, and ultra lente insulin (Eli Lilly.
Company, Indianapolis, IN) delivered via an implantable subcutaneous osmotic pump. Following pump depletion at the end of 4 weeks of diabetes, insulin was administered via manual injections. Plasma glucose levels were measured every day (Accucheck, Roche Diagnostics, Indianapolis, IN). Body mass was recorded twice per week in all groups.

we used the Bio-Breeding Diabetes Resistant (BBDR) rat model in order to verify both the manifestation of DCM and the molecular correlate of exercise induced benefits, if any, in the presence of DCM in this model of type 1 diabetes, the latter resulting from an induced failure of beta cell autoimmune tolerance. Unlike the chemical- induced diabetic models, the pathogenesis in BBDR rat model is reminiscent of human type 1 diabetes (26).

**Exercise training protocol**

The rats from EC and ED group were trained on a moderate endurance training protocol comparable to other reports (8, 9, 28). Training was initiated in the diabetic rats the day following insulin pump implantation surgery. Rats in the exercise groups were trained on a Weslo treadmill with custom built running track. The training intensity and duration began at 15 m·min⁻¹ for 5 minutes on day 1 and progressed to 20 m·min⁻¹ for 50 min by the end of week 2. The rats maintained the intensity and duration of 20 m·min⁻¹ for 60 min per day for the remaining 6 weeks.No electric shock was used to stimulate animals to run. Instead, uncooperative rats were encouraged to run by occasional gentle manual brushing on their backs. To rule out the confounding effects of non-training factors in the training environment, all animals in the SC and SD groups were handled everyday and subjected to the
noise of running treadmill by placing their cages next to the exercising animals.

**Cardiac physiological evaluation**

At the end of eight weeks of diabetes, LV physiological evaluation was performed via closed chest carotid arterial catheterization in all 4 groups under sodium pentobarbital anesthesia (40 mg/kg). A 4 cm long incision was made in the carotid triangle for right carotid artery exposure. The Millar microtip conductance catheter was introduced via a carotid incision secured by proximal and distal sutures. The catheter was gently advanced while continuously monitoring the pressure changes to ascertain its entry in to the LV. While the catheter was secure in the LV, the steady-state pressure and volume data were recorded with the Power Lab MPVS-400 (ADInstruments, Colorado Springs, CO). Immediately after data collection, the rats were euthanized with an overdose of sodium pentobarbital.

The physiological data were analyzed with Chart 5.1.1 software (ADInstruments, Colorado Springs, CO). The values for heart rate, maximum LV volume, minimum LV volume, end-systolic volume, end-diastolic volume, stroke volume, ejection fraction, LV output, dP/dt_{max} (+dP/dt) and dP/dt_{min} (-dP/dt) were averaged from at least 3 consecutive cardiac cycles (37).

**Histology**

In order to determine the characteristics of the passive components of the cardiac pump, histological analyses for interstitial collagen was performed. Hearts were excised and LV slices at approximately 3 mm above
the apex were removed and transferred to 4 % paraformaldehyde for fixation, followed by paraffin embedding, sectioning and finally staining with picrosirius red to detect collagen deposits with light microscopy (17, 23). Three fields of view were chosen randomly from each slide and included for analyses.

Transmission electron microscopy (TEM)

To determine the characteristics of the active components of the cardiac pump, TEM analyses of the myocardial mitochondrial distribution was performed. In order to ensure mitochondrial integrity, the LV sections were obtained from the beating heart. Sample preparation for ultrastructural analyses was performed according to a previous report (28). Briefly, ventricular sections were rinsed in cold phosphate-buffered saline (PBS) and placed in 2 % glutaraldehyde at +4°C overnight. The tissue was rinsed in buffer and post fixed with 1 % osmium tetroxide. Following a distilled water rinse the fixed tissue underwent a graded ethanol dehydration series and was infiltrated using a mixture of one-half propylene oxide and one-half resin overnight. Twenty-four hours later, the tissue was embedded in Epon 812 resin (Electron Microscopy Sciences, Ft. Washington, PA). Eighty-nanometer sections were cut on an LKB Nova Ultratome and were placed on acid treated grids, which were stained using a double lead stain technique with 0.5 % lead citrate and 7 % uranyl acetate (7). Images were captured using a JEM 100 CXII transmission electron microscope at 80 kV. Six fields of view, captured at random from each rat, were included for image analyses.
Image analyses

Images from both histology and ultrastructural studies were analyzed with Image J, a freely-downloadable Java-based environment (http://rsb.info.nih.gov/ij). In the analyses of histological profiles, the myocardial collagen fractional area was determined from the distribution of interstitial collagen as the ratio of collagen to the area of the field of view, expressed as a percentage. Meanwhile we included only viable mitochondria, as defined by intact inner and outer membranes, for the determination of myocardial mitochondrial fractional area (28).

Citrate synthase activity assay

An increase in the citrate synthase activity was used as a marker of exercise training. Left soleus homogenates were frozen under liquid nitrogen and thawed four times to disrupt the mitochondria to expose citrate synthase. Approximately 200-230 mg of soleus tissue was homogenized in ice-cold lysis buffer using a motorized glass-teflon tissue homogenizer (Arrow Engineering Co., INC, NJ) at 60 % motor setting. The lysis buffer contained the following ingredients: 0.1 M Tris, pH 8.1; 150 mM NaCl; 0.1 % Triton X-100, 1 mM ethylenediaminetetraacetate (EDTA) and 0.2 mM phenylmethylsulfonylfluoride (PMSF). A tissue mass to buffer volume ratio of 1:20 was used. Homogenates were subjected to centrifugation for 30 min at 10,000 X g at 4°C. Protein concentrations on the resulting supernatant were determined using the Protein Assay Reagent (Bio-Rad, Hercules, CA) with bovine serum albumin standards. The total protein ranged from 5 mg/ml through 8 mg/ml across the experimental groups. The citrate synthase activity
assay system contained in a total volume of 200μL: 20 μl 5, 5′-dithio-bis (2-nitrobenzoate) (DTNB), 5 mM in 1 M Tris-HCl, pH 8.1; 130 μl MilliQ H2O; 30 μl acetyl coenzyme A (acetylCoA), 10 mM in H2O; 10 μl muscle extract; and 10 μl oxaloacetic acid, 50 mM in 0.1 M Tris-HCl, pH 8.1. Final concentration of the reagents were as follows: 0.5 mM DTNB, 1.5 mM acetyl CoA and 2.5 mM oxaloacetic acid (30). The assay principle was to initiate the reaction of acetyl CoA with OAA and measure the release of free CoA-SH with a colorimetric reagent, DTNB (acetyl-CoA + OAA +H2O ↔ citrate + CoA-SH, then CoA-SH + DTNB → mercaptide ion). The rate of change of yellow color was monitored at 405 nm, every 20-s for 3 min using a Dynex MRXII plate reader (Dynex Technologies, Chantilly, VA). All measurements were performed in duplicates, at 20 – 22°C. The citrate synthase activity was normalized to the total protein in the reaction mix. Citrate synthase activity was expressed as A405/min/mg total protein.

PKC βII Immunoblotting
Approximately 50 mg of LV myocardial tissue was homogenized in ice-cold lysis buffer using a motorized glass-teflon tissue homogenizer (Arrow Engineering Co., INC, NJ). Briefly, the tissue was transferred to the homogenizer and 400 μl of the ice-cold lysis buffer containing the following was added: 20 mM Tris-HCl, pH 7.5; 2 mM EDTA; 0.5 mM EGTA; 1 mM DTT; 0.3 M sucrose; 25 μg/ml leupeptin (32). After homogenization for 45 s at 60 % motor setting, the homogenates were centrifuged at 10,000 x g for 30 min at 4°C. Protein concentration was estimated using the Protein Assay Reagent (Bio-Rad, Hercules, CA) with bovine serum albumin standards. Equal
amounts of myocardial samples (52μg total protein) per lane were electrophoresed on 8% SDS-polyacrilamide gels and transferred to polyvinylidene fluoride (PVDF) membranes overnight. The samples from all experimental groups were loaded in duplicates. Membranes were incubated with primary rabbit polyclonal antibody for PKC βII (Santa Cruz Biotechnology, CA) for 3h at room temperature. Following the incubation with primary antibody, membranes were washed three times in PBS with 0.1% Tween-20 for 10 minutes. Incubation with secondary anti-rabbit antibody (Santa Cruz Biotechnology, CA) was performed for 1h and incubation was followed by washing for three times in PBS with 0.1% Tween-20, ten minutes each. Then the membranes were rinsed with PBS once. Antibody binding was assessed by Pierce supersignal west pico chemiluminescent substrate (Pierce Biotechnology, Inc., Rockford, IL). Rat brain extract was used as a positive control. Equal loading was confirmed with Ponceau’s solution staining of membranes. Densitometric analyses were carried out with Adobe photoshop CS2.

Statistical analyses

Statistical analyses were performed with SPSS (version 15.0). Significant group differences on parameters were tested with a one-way analysis of variance (ANOVA). When prompted by group differences, post-hoc pair-wise multiple comparisons were performed using Tukey’s honestly significant difference (HSD) test with the level of significance held at P ≤ 0.05. The following identifiers were designated for pair-wise comparisons: a =
significantly different from SC, b = significantly different from SD, c = significantly different from EC and d = significantly different from ED.

RESULTS

Skeletal muscle citrate synthase (CS) activity

The soleus CS activity was increased 32 % in the ED group compared with the SD group thus marking the training effect in diabetes (Figure 1). We however noticed no significant difference in the CS activity of EC group compared to the SC group although there was an 18 % increase. Meanwhile the difference in CS activity between the ED and SC groups was not significant although the former demonstrated a 22 % increase.

Glucometry and gravimetry

Table 1 summarizes the results from glucometry and gravimetry at eight – wk diabetes duration, prior to physiological evaluation of LV function. With insulin treatment, the SD group was able to maintain 80 % of the SC group’s body mass. Insulin therapy was unable to restore the glucose homeostasis in the SD group as evidenced by their glycated hemoglobin levels (10.8 ± 1.6 %). However, with exercise training, the HbA1C levels of insulin treated diabetic group (ED) dropped 28 % compared with the SD group, illustrating the beneficial role of exercise on plasma glucose homeostasis in DRBB rats.

LV Hemodynamics

The LV hemodynamic parameters obtained via the carotid arterial catheterization procedure are summarized in Table 2. There was no significant difference in the heart rate among the SC, SD and ED groups,
reminiscent of our previous results obtained from the STZ diabetic rat model (24). However, we observed a 12 % decrease in the heart rate of EC group compared with the SC group. The maximum left ventricular volume was decreased 36 % in the SD group compared with the SC group. Meanwhile the minimum LV volume of the SD group also showed a decrease (27 %) compared with the SC group.

While the LV stroke volume decreased by 48 % in the SD group compared with the SC group, exercise training was efficient in preventing this deficit in the diabetic rats by mediating a 2 % increase over the SC group. Accordingly, the LV ejection fraction showed an 18 % decrease in the SD group compared with the SC group, and improved in the diabetics with training.

The LV output in the SD group decreased by 51 % compared with the SC group. With training, the diabetic group showed an 8 % decrease in its LV output compared with the SC group. Both the \(+dP/dt\) and \(–dP/dt\) showed a decrease (30 % and 46 %, respectively) in the SD group compared with the SC group. With training, however \(+dP/dt\) decreased 4 % and \(–dP/dt\) decreased 20 % in the SD group, compared with the SC group.

**Myocardial histology**

The myocardial collagen fractional area increased 142 % in the SD group compared with the SC group (Figure 2a and 2b). After eight weeks of training, the collagen fractional area in the diabetic myocardium returned to levels (4.1 ± 2.8 %) comparable to control myocardium (5.7 ± 2.7 %).
Myocardial ultrastructure

The myocardial mitochondrial fractional area in the SD group decreased by 65% compared with the SC group (Figure 3a and 3b). After eight weeks of exercise training, the reduction in viable mitochondrial fractional area in the diabetic myocardium was attenuated to a 36% decrease compared with the SC myocardium.

Myocardial PKC βII expression

We did not observe a significant difference in the expression levels of PKC βII between the SC and SD myocardium (Figure 4a and 4c). However, with eight weeks of endurance training, the total tissue PKC βII expression in the diabetic group showed a 42% decrease compared with SC group and 40% decrease compared with the SD group. Moreover we also observed a significant decrease in the myocardial PKC βII expression, with training, in the non-diabetic rats (24% decrease) compared with sedentary non-diabetics.

DISCUSSION

The main findings of the current series of physiological, histological, ultrastructural and biochemical studies on the BBDR autoimmune model of type 1 diabetes are: a) the manifestation of abnormal cardiac structure and function with the presentation of cardinal features of DCM and b) the prevention of many of the cardinal features of DCM with endurance training; not unlike our observations from previous investigations on the chemotoxin (STZ) induced model of type 1 diabetes (22-24, 28).
The BBDR rat model of type 1 diabetes was utilized in this series of investigations in order to address the following chief objectives: a) verification of the development and manifestations of DCM in a model of type 1 diabetes that is reminiscent of human type 1 diabetes and b) the possibility of attenuation of abnormal cardiac structure and function, if any, in the same model with endurance exercise training.

The BBDR rats circulate normal numbers of CD4+, CD8+ and ART2+ T cells and develop diabetes only under conditions that mimic an interaction of a viral perturbant (environment) with their genetic loci susceptible for diabetes through autoimmune pathogenesis, hence modeling human type 1 diabetes effectively (25, 26). Although invaluable in our understanding of diabetic complications, the chemical-induced rat models of type 1 diabetes (for e.g., the STZ induced diabetes), suffer from a disadvantage in modeling humans with type 1 diabetes for the following reasons among others: a) absence of the autoimmune diabetic pathogenesis, b) non-dependence on insulin for survival, and c) lack of body mass gain resulting from the catabolic flux of uncontrolled hyperglycemia. The aforementioned features, common to most of the generic models of type 1 diabetes, prevent the results obtained from studying those models for unreserved translational and therapeutic utilization. With the utility of BBDR rats, however, these limitations were mostly overcome.

The insulin treated diabetic rats used in this study demonstrated relatively low abnormal glucose homeostasis compared with the untreated STZ diabetic rats used in our earlier studies (22, 24). After eight weeks of diabetes, while the HbA1C level in the latter was more than 13 %, the BBDR
rats used in this study showed a 10.8 % HbA1C, suggesting more controlled hyperglycemia compared with the STZ diabetic model. Interestingly, with training, we observed a significant reduction in glycated hemoglobin levels of the diabetic rats compared with their sedentary counterparts. This finding was in contrast to our previous observations from the STZ diabetic rats that did not demonstrate a significant benefit on glucose homeostasis from endurance training compared with their sedentary counterparts (24). Taken together, these observations suggest that training when used as an adjunct to insulin therapy is more effective in benefiting plasma glucose homeostasis compared with training or insulin provided exclusively.

Unlike our previous results with STZ-induced diabetic rats, the BBDR rats belonging to the ED group demonstrated an increased activity of soleus citrate synthase with training compared with their sedentary counterparts. Interestingly, the same intensity of training was inadequate in increasing the CS activity of EC group compared with the SC group.

At the end of eight weeks of diabetes, the SD group showed a significant decrease of their LV cardiac pump function. There was a decrease in both end-systolic and end-diastolic volume of SD group compared with SC group. This was in contrast to our results obtained using MRI with the sedentary STZ diabetic rats that showed an increase of end-systolic volume despite a decrease in end-diastolic volume compared with non-diabetics. However, the stroke volume and ejection fraction of the SD group decreased compared with SC group in the BBDR rats, similar to STZ diabetes in Sprague-Dawley rats (24). It should be noted that the stroke volume and ejection fraction were derived by the Chart software from the difference
between maximum and minimum LV volumes in the current study. However, in our previous investigations, in which we used magnetic resonance imaging (MRI) technology to evaluate cardiac cycle dynamics in diabetes, we adopted the definition that maximum and minimum LV volumes during the cardiac cycle were the same as end-diastolic and end-systolic volumes respectively. Meanwhile, the working computational definition adopted by us for our MRI investigations could not be a source of confound since regardless of the choice of terminology, the ejection fraction of the SD groups were lower compared to SC groups in these studies. Interestingly, the ejection fraction functions obtained from MRI evaluation gave higher values (approximately 65%) in the non diabetics compared to those obtained via catheterization procedure (approximately 45%) in the current study. We speculate that the difference in the range of ejection fraction functions obtained through the abovementioned procedures might be due to the difference in the type of anesthetic agents. Isoflurane (used in our MRI investigations) has been shown to accentuate the ejection fraction of steady state ventricular pump whereas sodium pentobarbital has an attenuating effect (15, 36). Meanwhile the difference in strain of the rats and the approaches for cardiac evaluation (non-invasive and invasive) should also be considered as potential sources of the difference in observed hemodynamic measures.

In addition to cardiac cycle volumetry, the catheterization procedure allowed us to quantify both the instantaneous rate of change of maximum and minimum left ventricular pressure in the SD rats which was significantly diminished compared with SC rats. The latter results suggest diminution of
both systolic and diastolic LV function in BBDR diabetic rats at the end of eight weeks of diabetes and suggest the possibility of failing active and passive components of the cardiac pump. With exercise however, the diabetic rats were able to retain most of their cardiac pump function comparable to the non-diabetics.

The abnormal LV physiological parameters due to the sedentary mode of life in diabetes was both prevented (parameters returning toward EC levels, yet with a significant difference from controls), and in some cases improved (parameters indistinguishable from EC group) following exercise training. In particular the following volumetric abnormalities were prevented by exercise training in diabetic BBDR rats: maximum LV volume, minimum LV volume and LV end-diastolic volume. The following parameters benefited the most from the endurance training in diabetic rats by demonstrating an improvement of LV function: stroke volume, ejection fraction; output and +dP/dt. These results clearly underscore the role of endurance training in benefiting the diabetic heart beyond that attainable by insulin therapy alone. Moreover the results also reiterate most of the previous reports, on the beneficial role of aerobic exercise training, gathered from other models of type 1 diabetes (4, 8, 9, 19).

The physiological benefits on the heart attained with exercise training in the diabetic rats suggested attenuation of abnormalities of both the passive and active components of the cardiac pump function. The latter were further verified by histological and ultrastructural analyses of myocardium. Interstitial collagen accumulation in the myocardial tissue is one of the cardinal features of DCM (2) and serves as the marker for detrimental passive
performance of the cardiac pump (35). The passive components (fibrous proteins) of the cardiac pump confer the myocardium with resilient properties and are the major determinants of maximum LV volume (end-diastolic volume) and – dP/dt during the cardiac cycle. At the end of eight weeks of diabetes, we observed a remarkable increase in the myocardial distribution of collagen, one of the major fibrous proteins, in the SD group. The abundance of myocardial collagen might explain the drastic decrease in the maximum LV volume observed in the SD group due perhaps to compromised LV resiliency. In fact, the –dp/dt values of the SD group, along with the abundance of fractional collagen distribution, were suggestive of a non-resilient and stiff ventricular myocardium reminiscent of our structural MRI report (23). With endurance training however, both the structural defect in collagen accumulation and functional defect in maximum LV volume were attenuated in diabetes. Although there was an attenuation of – dP/dt, a measure of myocardial resiliency in trained diabetic rats, the result was not strongly suggestive of prevention or improvement of function of the diabetic myocardium. Considering the severity of deficit in the ability to generate high instantaneous pressures during ventricular relaxation in the sedentary diabetic condition, the result with training nevertheless was considered positive as it was significantly different from the SD group.

We also quantified the distribution of myocardial mitochondria, a major active component of cardiac pump function. The mitochondria have been implicated as the ultimate target of diabetic cardiovascular complications (5). Specifically, the diabetic heart manifests both structural and functional abnormalities of mitochondria (11, 14, 28). By quantifying the
viable fraction of myocardial mitochondria in our diabetic rats, we were able to verify one of the major organellar structural correlates that may underlie the LV functional deficits exhibited by the SD group. The ejection fraction and + dP/dt, both parameters representative of the functional efficacy of active cardiac pump function, were decreased in SD group compared to SC group. The significant decrease in viable myocardial mitochondrial fractional area in SD group might explain the functional deficits manifested by the diabetic LV under a sedentary life style. The endurance training was able to rescue the diabetic LV from the abovementioned functional deficits. The functional restoration occurred with the association of an apparent increase in the viable mitochondrial fractional area in trained diabetic rats. Taken together, these results verified the role of exercise mediated benefits on both functional and structural correlates of cardiac pump function. We also noted that the fraction of viable mitochondria in the SD group, although remarkably decreased compared with the non-diabetic groups, did not demonstrate a difference in the citrate synthase activity levels compared with the SC group. We speculate that the remaining numbers of viable mitochondria in the SD group might have compensated for the enzyme activity in the SD group. These later remarks, however, must be interpreted cautiously, since we have not ruled out the possibility of difference in the distribution and functional quality of mitochondria between skeletal and cardiac tissues in these animals.

In order to verify a molecular correlate of exercise induced benefits on the diabetic myocardium, PKC βII expression levels were analyzed. To our knowledge, studies to this date have provided information on exercise induced cardiac PKC signaling benefits that is limited to healthy coronary
arteries, normal (6) and senescent hearts (20) but not in diabetes (6). Carson and Korzick (6) speculated that a major source of exercise induced cardioprotection in normal Fischer 344 rats might be the very low level increase in PKC βII activity. Their results showed that LV myocardial PKC βII levels may be attenuated by acute/sub-chronic exercise training in healthy animals. We sought to determine whether similar cardioprotective effects prevail with chronic endurance training in diabetes. We outlined PKC βII as the target of exercise induced benefits in DCM because it has been clearly shown to participate in cardiac hypertrophy and fibrosis under hyperglycemic conditions (32), both features manifested by BBDR rats in this study.

Chronic hyperglycemia leads to increased diacylglycerol (DAG) accumulation mainly through the de novo synthesis mediated by glycolytic intermediates dihydroxyacetone phosphate and glycerol 3-phosphate (34). Increased DAG synthesis in diabetes promotes activation of protein kinase C isoforms in vascular tissues resulting in diabetic complications (5). According to the current model of myocardial dysfunction in diabetes (Figure 5), activation of the conventional PKC isoform PKC βII is a critical underlying factor resulting in cardiac fibrosis by downstream upregulation of pro-fibrotic proteins (13, 16, 21, 29, 32, 34). Accordingly, we pursued PKC βII in the determination of a molecular correlate of training-induced benefits on the diabetic myocardium.

Immunoblotting analyses indicated no significant change in the expression levels of myocardial PKC βII between SC and SD group. However, with training we observed a decreased expression of PKC βII in the EC
group. Meanwhile the most dramatic decrease in PKC βII expression was observed in the trained diabetic rats. Interestingly, exercise training attenuates PKC βII expression in both healthy and diabetic rats. Although the ultimate pathology of DCM might be the result of intricate interplay between the levels of expression, activation, and activity of PKC βII and its downstream substrates in diabetes (13, 16, 21, 29, 32, 34), the beneficial effects of exercise training as demonstrated by our results suggest a major cardioprotective role of exercise in the diabetic myocardium. By attenuating the PKC βII expression, exercise training might act as a “bottle neck”, controlling availability of the substrate for DAG, phosphatidyl serine and Ca$^{2+}$ (activators of PKC βII). In fact, it is plausible that by decreasing the availability of PKC βII exercise might thus reduce the probability of substrate phosphorylation (downstream effectors) that mediate the pathogenesis of DCM. Remarkably, the cardioprotection might be most effective with training on a diabetic background, as observed by the least amount of PKC βII expression in ED group among all four groups.

In conclusion, the major findings of our investigation with an autoimmune specific model of type 1 diabetes that is reminiscent of the human condition, reconcile most of the major findings from previous reports (4, 8, 9, 19, 22-24, 28) using generic models of type 1 diabetes such as the STZ chemotoxin model. The BBDR rats developed cardinal features of DCM affecting both the passive and active components of the cardiac pump function. The pathological features were verified at both structural and functional levels. Exercise–induced benefits on the diabetic myocardium were manifested in the BBDR rat model at the end of eight weeks not unlike the
benefits observed with STZ diabetic rats (24, 28). In addition, our findings suggest a role for PKC βII, a kinase implicated in the pathogenesis of DCM, as a possible correlate of exercise-induced benefits in diabetes.

ACKNOWLEDGMENTS

We would like to thank Anton Fedosyuk for exercising the rats and assistance with diabetes induction. We would also like to thank Ms. Rosetta Barkley for histological slide preparations, Ms. Eileen Roach for assistance with light microscopy and Ms. Barbara Fegley for assistance with transmission electron microscopy.
References


Figure Legends

Figure 1

Soleus citrate synthase activity. The activity of mitochondrial oxidative enzyme was measured to mark the exercise training effect. The enzyme activity was expressed as A405/min/mg protein. Soleus citrate synthase activity was significantly increased in the trained diabetic (ED) group compared with the SD group.

Figure 2

a) Collagen (arrows) specific picrosirius red – stained histological profiles of myocardium from all four experimental groups. The collagen distribution in both the SC and EC groups were minimal compared with the SD group. The SD group demonstrated large accumulations of interstitial collagen within the LV myocardium. However after eight weeks of training, the myocardial collagen distribution in ED group was comparable to the SC and EC groups. Scale bar: 50 μm.

b) Myocardial collagen fractional area expressed as percent collagen area over unit field of view area in all four experimental groups. Exercise training conferred benefits on diabetic myocardium as decreased fractional collagen area was not different from non-diabetic controls.

Figure 3

a) The presentation of myocardial mitochondria (arrows) and their distribution pattern in all four experimental groups. While the non-diabetic myocardium was enriched with an abundance of viable mitochondria, the SD
group showed a remarkable decrease in their distribution. The arrows in the SD profile mark few of the many non-viable mitochondria with damaged membranes. Transmission electron microscopy images magnification: 7200 x.

b) Quantification of myocardial mitochondrial fractional area expressed as percent viable mitochondrial area over unit field of view area in all four experimental groups. Only viable mitochondria, defined as electron dense mitochondria with intact outer and inner membranes, were considered for analysis. While the myocardial fractional mitochondrial area decreased in SD group, training was effective in attenuating this deficit in diabetes.

Figure 4

a) Immunoblotting for the 78 KDa protein, myocardial PKC βII, in all four experimental groups. The expression of PKC βII decreased with exercise training in both diabetic and non-diabetic hearts.

b) Ponceau’s solution stained membrane showing the transfer of proteins in all experimental groups from the 8% SDS-polyacrylamide gel.

c) Quantification of myocardial PKC βII expression by densitometry. Values were derived from three immunoblotting experiments. Expression of the kinase levels was not different between the SC and SD groups. However exercise had an attenuating effect on PKC βII expression in both non-diabetic and diabetic groups. Exercise produced the least expression in the diabetic group (ED).

Figure 5
A schematic representation of PKC βII signaling pathway in the diabetic myocardium depicting the chain of events leading to cardiac fibrosis.
Table 1

Glucometry and gravimetry at the end of 8 weeks of diabetes

<table>
<thead>
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<th>SC</th>
<th>SD</th>
<th>EC</th>
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<tr>
<td>Body mass (g)</td>
<td>379 ± 13&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>302 ± 32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>341 ± 19</td>
<td>311 ± 39&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Heart mass (g)</td>
<td>1.05 ± 0.05</td>
<td>1.03 ± 0.09</td>
<td>1.12 ± 0.09</td>
<td>1.07 ± 0.11</td>
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<td>Heart/Body mass</td>
<td>2.78 ± 0.09&lt;sup&gt;b,c,d&lt;/sup&gt;</td>
<td>3.43 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.27 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.44 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Plasma Glucose (mg/dl)</td>
<td>122 ± 12&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>546 ± 63&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>111 ± 21&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>482 ± 171&lt;sup&gt;a,c&lt;/sup&gt;</td>
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<tr>
<td>HbA1C (%)</td>
<td>4.8 ± 0.4&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>10.8 ± 1.6&lt;sup&gt;a,c,d&lt;/sup&gt;</td>
<td>5.2 ± 0.19&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>7.8 ± 0.4&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
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n = 8/group, a = significantly different from SC, b = significantly different from SD, c = significantly different from EC and d = significantly different from ED.
Table 2
Steady-state hemodynamic parameters at the end of 8 weeks of diabetes

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<tr>
<td>Heart rate (bpm)</td>
<td>262 ± 19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>245 ± 16</td>
<td>230 ± 21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>235 ± 25</td>
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<tr>
<td>Maximum LV volume (μl)</td>
<td>552.22 ± 23.67&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>351.65 ± 8.18&lt;sup&gt;a,c,d&lt;/sup&gt;</td>
<td>621.65 ± 13.27&lt;sup&gt;a,b,d&lt;/sup&gt;</td>
<td>564.03 ± 12.92&lt;sup&gt;b,c&lt;/sup&gt;</td>
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<td>Minimum LV volume (μl)</td>
<td>308.90 ± 4.50&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>225.12 ± 12.21&lt;sup&gt;a,c,d&lt;/sup&gt;</td>
<td>352.53 ± 16.48&lt;sup&gt;a,b,d&lt;/sup&gt;</td>
<td>316.68 ± 13.30&lt;sup&gt;b,c&lt;/sup&gt;</td>
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<td>LV End-systolic volume (μl)</td>
<td>374.83 ± 12.73&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>241.56 ± 18.00&lt;sup&gt;a,c,d&lt;/sup&gt;</td>
<td>368.20 ± 12.43&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>332.71 ± 17.68&lt;sup&gt;b,c&lt;/sup&gt;</td>
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<td>LV End-diastolic volume (μl)</td>
<td>532.81 ± 25.62&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>332.96 ± 20.57&lt;sup&gt;a,c,d&lt;/sup&gt;</td>
<td>600.07 ± 9.50&lt;sup&gt;a,b,d&lt;/sup&gt;</td>
<td>540.49 ± 13.30&lt;sup&gt;b,c&lt;/sup&gt;</td>
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<tr>
<td>LV Stroke volume (μl)</td>
<td>243.32 ± 21.65&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>126.52 ± 13.65&lt;sup&gt;a,c,d&lt;/sup&gt;</td>
<td>269.12 ± 18.74&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>LV Ejection fraction (%)</td>
<td>43.99 ± 2.08&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>LV Output (ml/min)</td>
<td>63.48 ± 3.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.11 ± 3.92&lt;sup&gt;a,c,d&lt;/sup&gt;</td>
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<td>+ dP/dt (mmHg/s)</td>
<td>7266.66 ± 337.31&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>7522.38 ± 415.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6977.83 ± 605.31&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>- dP/dt (mmHg/s)</td>
<td>6582.83 ± 498.38&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>3492.75 ± 364.97&lt;sup&gt;a,c,d&lt;/sup&gt;</td>
<td>6480.00 ± 315.70&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>5312.83 ± 317.49&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
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n = 8/group, a = significantly different from SC, b = significantly different from SD, c = significantly different from EC and d = significantly different from ED.
List of Figures

Figure 1

Soleus Citrate Synthase Activity

SC SD EC ED

(A405/min/mg protein)
Figure 2a
Figure 2b

Myocardial Collagen Fractional Area (%)

SC  SD  EC  ED

- SC: 5
- SD: 15
- EC: 5
- ED: 5

Significant differences:
- a, c, d
- b
Figure 3a

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Figure 3b

Myocardial Mitochondrial Fractional Area (%)
Figure 4a
Figure 4b
Figure 4c

Myocardial PKC betaII levels (arbitrary units)

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a, b, c, d
Figure 5

Hyperglycemia → Diacylglycerol → PKC βII activation → Upregulation of pro-fibrotic proteins → Cardiac fibrosis
Chapter 6

Implications and Future Directions

In the preceding series of investigations we have attempted to characterize the structural and functional cardiac abnormalities that occur in diabetic cardiomyopathy (DCM) using chemically induced and autoimmune rat models of type 1 diabetes. These models were also utilized to verify the effects of endurance training on the structure and function of the diabetic heart.

During the first series of investigations, we used magnetic resonance imaging (MRI) methods to characterize the structural aspects of diabetic myocardium. The MRI procedure revealed the remarkable tendency of the left ventricular (LV) myocardium to collapse in a normal non-beating heart. The diabetic myocardium failed to demonstrate a complete obliteration of LV cavity unlike non-diabetics. The failure of complete collapse of the diabetic left ventricular was taken as an indication of myocardial stiffness. By using the intrinsic behavior of cardiac tissue protons as a substrate for perturbation by radio frequency pulses, we were then able to delineate the underlying differences in tissue structural properties such as increased fiber distribution in the diabetics, in terms of tissue proton relaxation times. Following the MRI procedure, we confirmed our deductions with histological procedures. Although these investigations were performed on the non-beating heart, they nevertheless provided the first step toward structural characterization of diabetic myocardium using MRI; a tool whose applications in diagnostic cardiology has remarkably increased in recent years (17). As pointed out by Marwick (17), the application of MRI technology to obtain cardiac structural
and functional information from a broad spectrum of clinical population comprising both type 1 and type 2 diabetes holds greater promises for the future of diagnostic cardiology. Our study in particular, was able to bring in to focus the fibrotic pathology of the diabetic myocardium using MRI. However, the drawback in the utilization of non-beating heart to characterize DCM in rats was overcome with our subsequent investigation that attempted to characterize the cardiac cycle dynamics and hence the function in the diabetic heart, as the technology became available to utilize EKG gating methods with MRI.

In our second series of investigations, we characterized the functional cardiac deficits accompanying diabetes. With the use of EKG gated MRI technology, we were able to obtain data from both normal and diabetic animals on their cardiac cycle dynamics. We observed both systolic and diastolic dysfunction in the diabetic heart with uncontrolled hyperglycemia. Although diastolic dysfunction has been implicated as the prominent manifestation of the heart with DCM, the evidence for a systolic dysfunction remained controversial. The latter has been attributed to the compromised sensitivity of detection techniques that were used previously, since recent studies with echocardiography have demonstrated both systolic and diastolic dysfunction of the diabetic heart (16) in agreement with our findings. Following the successful characterization of cardiac functional defects in diabetes, we sought to verify the benefits, if any, that resulted from exercise training the rats.

In our third series of investigations, we focused our attention on the exercise induced benefits on DCM. Even though we had structural evidence
for the availability of cardiac benefits from exercise training the diabetic rats (24), a functional characterization of cardiac benefits was required as an assay to verify the consequences of structural benefits. Using MRI and techniques from vector calculus, we were able to demonstrate that the hearts of diabetic rats benefited by improved function, with the same level of endurance training that benefited cardiac structure. In fact, training was able to prevent the development of cardiac cycle deficits in diabetic rats. Upon review of these results, it was pointed out (by anonymous reviewers) that the uncontrolled hyperglycemia in the generic model of diabetes was a major limitation to translational studies in humans. Although widely used in diabetes research to understand the pathology (28) and the possibility of treatment for diabetic complications (7), the chemotoxin induced model of diabetes nevertheless limits the interpretation of findings since it does not model the complete status of the individual with type 1 diabetes. In order to overcome this limitation, we used an autoimmune model of diabetes that is reminiscent of human type 1 diabetes for our subsequent investigations.

We verified the structural and functional manifestations of DCM in an autoimmune model of type 1 diabetes in our fourth series of investigations. By employing histological, ultrastructural and physiological techniques, we verified the development of DCM in the BBDR rat model of type 1 diabetes. The manifestation of structural abnormalities in both passive (collagen) and active (mitochondria) components of the cardiac pump suggested both diastolic and systolic functional deficits in DCM. The manifestation of functional deficits were confirmed by the direct recording of pressure and volume data from the ventricular pump during the cardiac cycle. The
evidence not only corroborated similar findings from the chemotoxin induced model of type 1 diabetes, but were also reminiscent of the Framingham study results that DCM may develop in individuals despite insulin treatment (8). In addition, the possibility of exercise mediated benefits on the diabetic cardiac structure and function beyond the benefits offered by the insulin treatment was also verified in the BBDR model. Moreover, the pursuit for a possible molecular correlate for training induced benefits revealed a possible role for protein kinase c (PKC) βII isoform: a kinase implicated in the pathological signaling events associated with DCM. The decrease in the availability of PKC βII may be a critical event in preventing the structural and functional manifestations of DCM since increased activation of PKC has been implicated as one of the seminal events in the development of diabetic cardiovascular complications (3).

During the course of these investigations, we also noted the difference in absolute value of the parameters perhaps due to the difference in the techniques of cardiac evaluation. The insulin treated diabetic rats used in our investigations (chapter 5) demonstrated better glucose homeostasis compared with the untreated STZ diabetic rats used in our earlier studies (chapter 2, 3 & 4). After eight weeks of diabetes, while the HbA1C level in the latter was more than 13 %, the BBDR rats showed a 10.8 % HbA1C, suggesting more controlled hyperglycemia compared with the STZ diabetic model. Interestingly, with training, we observed a significant reduction in glycated hemoglobin levels of the diabetic BBDR rats compared with their sedentary counterparts. This finding was in contrast to our previous observations from the STZ diabetic rats that did not demonstrate a significant benefit on glucose
homeostasis from endurance training compared with their sedentary counterparts (15). Meanwhile, at the end of eight weeks of diabetes, the sedentary diabetic BBDR group showed a significant decrease of their LV cardiac pump function. There was a decrease in both end-systolic and end-diastolic volume of the sedentary diabetic group compared with the sedentary non-diabetic group. This was in contrast to our results obtained non-invasively with the sedentary STZ diabetic rats that showed an increase of end-systolic volume despite a decrease in end-diastolic volume compared with non-diabetics. However, the stroke volume and ejection fraction of the sedentary diabetic BBDR group decreased compared with sedentary non-diabetic BBDR group, similar to STZ diabetes in Sprague-Dawley rats.

Taken together, these results gathered from both chemical-induced and autoimmune models of type 1 diabetes imply the following: a) development of cardiomyopathy in diabetes either in the presence or absence of insulin treatment and b) ability of exercise to induce favorable structural and functional manifestations in DCM. The implications of the abovementioned investigations have been verified independently by other investigators recently in similar models systems of diabetes using various methodological procedures including MRI (5, 6, 30).

One of the important next steps would be to delineate the molecular details of exercise induced benefits since exercise appears to prevent both systolic and diastolic dysfunction of the diabetic heart. Our final series of investigations had already demonstrated a first step in this direction. However, it is unlikely that the exercise induced benefits on the diabetic heart
would be restricted to a single molecular event (for e.g., expression of PKC βII). Due to the complexity of the interaction of signaling systems with each other and the multitude of complications manifested by the diabetic heart in humans, a detailed map of expression and activation or inhibition patterns of signaling molecules in the diabetic heart responsible for both the manifestation of pathology and mediation of exercise induced benefits would be required.

For example, at least three physiological signaling systems have been implicated in the development of cardiomyopathy in diabetes: a) the cardiovascular PKC signaling system (27), b) the intra-cardiac renin-angiotensin-aldosterone system (2), and c) vascular endothelial growth factor mediated signaling systems (28). The extent to which these three systems cooperate or compete to propel the diabetic heart toward a myopathic phenotype is unclear. Future studies may reveal the interaction among multiple signaling pathways to produce the features of DCM.

It would be valuable to gain more information on various aspects of molecular signaling that exercise training may target to produce the benefits on the diabetic heart. Although we have demonstrated the possibility of PKC βII underexpression as one of the molecular correlates of exercise induced benefits, the possibility of multiple sites of advantage in the diabetic heart with training cannot be ruled out (18). In fact, training might influence the availability of activators or inhibitors of critical enzymes like PKC βII in order to confer its overall benefits on cardiac pump. Since its effects on cardiac function appear to be global (15), it is plausible that critical molecular elements like kinases and phosphatases that form the nodes in interacting
networks of signaling pathways (25) may be targeted by interventions such as exercise training.

Although the abovementioned future prospects from cell and molecular biological investigations have to be worked out in detail using appropriate animal models of diabetes, prior to contemplation of translational efforts, the methodological applications from the MRI investigations could be potentially tested in humans with diabetes in the near future. MRI has evolved as the most desirable non-invasive imaging modality for the evaluation of cardiac function in both humans and animal models of cardiovascular pathology (12, 26). In fact, MRI is ideally suited for cardiac volumetric measurements in heart failure as it allows versatility of use and produces results of excellent accuracy (19). The use of MRI for determination of myocardial T2 relaxation time or pixel wall intensity in humans with diabetes may provide valuable information about the extent of cardiac fibrosis (14). Coupled with functional analyses, the structural results may provide diagnostic and prognostic information for clinicians (13, 19) on individuals with diabetic cardiomyopathy.

Although we used the non-beating heart for our structural MRI investigation to delineate the role of cardiac fibrosis in the left ventricular wall motion dynamics, the results clearly implicated the role of collagen induced fibrosis in the increased stiffness, characteristic of the diabetic myocardium (14). The results also showed the feasibility of MRI technology for studying the abnormalities of the diabetic heart. In addition, they provided clues to the alteration of function in the diabetic heart, which was confirmed following the functional MRI studies (13). In contrast to our
expectation from the structural studies that the stiff myocardium in the diabetic heart may demonstrate a predominantly diastolic dysfunction, our functional MRI results provided one of the first clues to impaired systolic dysfunction in diabetes. The latter finding demonstrated the sensitivity of MRI, in detecting systolic dysfunction of the diabetic heart that clinicians have suspected in the past; given the limitations on the sensitivity of echocardiography to detect such deficits (16).

The clinical course of myocardial deficits in diabetes progresses through three stages of cardiac pump malfunction, mainly detected with echocardiography techniques: 1. impaired ventricular relaxation, 2. increased left atrial pressure to overcome the difficulty of ventricular filling and 3. restrictive filling profile. Although the clinical presentation is frequently documented as a diastolic dysfunction of the heart, this may have been due to the limitations of routine echocardiographic approaches to detect systolic dysfunction (16). In addition, the lack of ability to detect systolic dysfunction in the diabetic heart may have been due to the criteria that was used to define systolic dysfunction (less than 50 % ejection fraction). The finding of systolic dysfunction with doppler imaging techniques, by Yu et. al., in 52 % of heart failure subjects that were documented as “diastolic heart failure”, based on echocardiographic evaluation, clearly demonstrates the limitation of this widely utilized technique. Based on these results, Yu et. al. suggested that the 50 % cut-off limit for ejection fraction, to identify systolic dysfunction, may not be adequate to effectively screen individuals (29). It must be noted that the majority of studies have reported the exclusive presence of diastolic
dysfunction in individuals with type 1 diabetes based on echocardiographic evaluation (1, 10, 21, 23).

Based on the results of our structural and functional MRI studies that demonstrated both diastolic and systolic dysfunction, we speculate the presence of systolic dysfunction as well, in individuals with diabetes that are documented, based on echocardiographic evaluation, as diastolic dysfunction. Interstitial fibrosis, demonstrated in our diabetic rats, have also been documented in diabetic individuals (11). In fact, the presence of cardiac fibrosis, in diabetic individuals, in itself might suggest the possibility of both diastolic and systolic dysfunction. In addition to compromising diastolic suction and increasing the viscoelastic load of the ventricle (diastolic dysfunction), fibrosis may also contribute to systolic dysfunction by affecting the orderly transmission of force to the ventricular chambers (4), as suggested by the intricate three dimensional network formed by myocardial matrix proteins.

In addition to the detection of cardiac abnormalities, our investigations also underscore the benefits that endurance training might confer upon the diabetic myocardium. Exercise training benefited the diabetic heart to overcome the abnormalities of both systolic and diastolic dysfunction. With and without insulin supplement, the cardiac benefits of endurance training prevailed. As pointed out in chapter 1, the uncertainty of the tolerance of individuals with type 1 diabetes to exercise training has been overcome by the finding that the cardiovascular responses of individuals with uncomplicated type 1 diabetes was not different compared to their non-diabetic counterparts (20). Our results with rats demonstrated the ability of
diabetic groups to sustain the endurance training protocol, comparable to the non-diabetic groups. It was clear that training was able to mediate favorable functional changes in the diabetic heart (15). In fact, training induced benefits on the diabetic heart may have molecular substrate(s) in a major signaling pathway, responsible for the pathogenesis of diabetic cardiomyopathy. By interfering with the critical pathogenic signal, training might avert abnormalities of cardiac function in diabetes (chapter 5).

Diabetes poses a 2.4 and 5.1 fold increase for the development of heart failure in men and women respectively (9). The use of sensitive screening tools such as MRI, coupled with revised criteria for identification of individuals with cardiac dysfunction in diabetes, may provide clinicians with crucial information to implement prevention strategies. As Sander and Giles (22) propose, following the increase of evidence for the presence of cardiomyopathy in individuals with diabetes, “It becomes imperative to identify cardiac abnormalities early in the course of both type 1 and type 2 diabetes to allow early and aggressive intervention …” Our results underscore the importance of early intervention in preventing the cardiac abnormalities resulting from the hyperglycemia characteristic of type 1 diabetes.
References


15. **Loganathan R, Bilgen M, Al-Hafez B, Zhero SV, Alenezy MD, and Smirnova IV.** Exercise training improves cardiac performance in


